

POSTERS

DISEASES BY PROTOZOAN

MALARIA

Parasite Biology

Mal001- *Plasmodium falciparum*: Developmental regulation on the early intra-erythrocytic stage by lipid growth-promoting factor via copper homeostasis

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Malaria remains a devastating disease, particularly in the tropics. The emergence of resistance to conventional antimalarial drugs and insecticides necessitates new chemotherapeutic approaches with alternative targets. A better understanding of the parasite's biology is critical for allowing the development of new medications. To identify the factor controlling parasite development and the effects of growth-promoting factors on the parasite, we initially investigated growth-promoting substances to formulate a chemically defined culture medium (CDM) suitable for sustaining the complete development and intra-erythrocytic growth of *Plasmodium falciparum*. The CDM consists of paired non-esterified fatty acids (NEFA), phospholipids with specific fatty acid moieties, and specific proteins dissolved in basal medium supplemented with hypoxanthine. We demonstrated that different combinations of NEFA exerted distinct effects on the parasite's growth by sustaining development at different stages. In the present study, we investigated the functionally distinct effects of various NEFA on the early intra-erythrocytic stage of *P. falciparum*, including growth progression from rings to trophozoites and schizonts, by comparing genome-wide transcriptional responses during different growth stages. Our results implied a critical function of copper homeostasis in the early growth stage of the parasite, and complete regulation of the homeostasis and growth of the parasite by a pair of NEFA. This study sheds new light on the potential cellular functions of NEFA and copper ions in the biology of malaria parasites. **E-mail:** asahih@nih.go.jp

Mal002- Evaluation of *Plasmodium falciparum* egress from the red blood cell

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Introduction: Intracellular parasites exhibit different mechanisms for egress from their hosts cells, such as explosive lysis of the host cell membrane, extrusion of parasites or membrane fusion when they are enclosed in a parasitophorous vacuole (PV). *Plasmodium falciparum*, the agent responsible for human malaria, which final host cell in humans is the red blood cell (RBC) inhabits inside of a PV. The exit of *Plasmodium* has been extensively reviewed and explored, and at least two mechanisms for their exit have been proposed: widely accepted lysis exit and recently proposed PV and RBC plasma membrane fusion; however experimental evidence is still needed. In this work we explore these two mechanisms using fluorescent staining for membrane and cytoplasm, and also measuring liberation of cytoplasmic content. **Materials and methods:** The lipid analog FM4-64 (Invitrogen) was used to stain the plasma membrane of the RBC and the parasite-induced membranes. For staining the cytoplasm, the stains calcein-AM and CFDASE (Invitrogen) were used. Membrane and cytoplasm stains were observed using fluorescent microscopy. The release of calcein and CFDASE was measured using fluorometry on the supernatant of

P. falciparum cultures and non-infected RBC. The liberation of LDH was measured using colorimetric methods. **Results:** Using the membrane stain FM4-64 we found that the plasma membrane, the PV and other parasite-induced membranes can be tracked by fluorescent microscopy. The cytoplasmic stains, calcein and CFDASE, show a particular behavior in this parasite, as they are internalized by the parasite vacuole. Since calcein is a substrate of the MRP1 present in RBC membranes, probenecid was used to retain this molecule inside the cells allowing to obtain a measure of the liberation due just to exit of the parasite, besides the probenecid highly inhibits the liberation of these molecules, suggesting parasite modulation of the transport of RBC membrane. In the period of exit, the stain is strongly retained by the infected cells, suggesting that the process might not be explosive. Finally, the measurements of LDH release (an enzyme of the RBC cytoplasm) show an opposite behavior from the fluorescent stains liberation, but we have not been able to determine so far if it is coming from the parasite, the healthy RBC cytoplasm or from broken-RBC by the exit. **Conclusion:** In this work we provide strategies to follow the egress of intracellular parasites. In addition we present useful information regarding transportation of molecules in the infected RBC, as this transport could be modulated by *P. falciparum*. **E-mail:** mmcamachon@unal.edu.co

Mal003- Enzymatic characterization of Nicotinamide Mononucleotide Adenylyl-transferase of *Plasmodium falciparum*, a key enzyme in the biosynthesis of NAD⁺

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Introduction: Nicotinamide adenine dinucleotide (NAD⁺) has been identified as a vital molecule in all organisms; it participates as a coenzyme in many metabolic reactions involving electron transfer. However, recent findings have revealed that NAD⁺ is also used as a substrate in covalent protein modifications. This new NAD⁺ feature is essential in regulating many cellular events in several model organisms. Regulation of replication, telomere conservation, cell death, transcription, gene silencing, circadian rhythm, caloric restriction and ageing, among others, has been observed to rely on NAD⁺-based reactions. The discovery of these NAD⁺ dependent regulatory mechanisms has revealed that NAD⁺ biosynthesis is a key pathway to maintain such processes. This biosynthesis involves two pathways: a de novo pathway and a salvage pathway. Both pathways converge in a single step catalyzed by Nicotinamide/ Nicotinate Mononucleotide Adenylyltransferase (NMNAT) (EC 2.7.7.1), an essential enzyme for NAD⁺ biosynthesis. Identification and characterization of NMNAT has been achieved in a variety of organisms including bacteria, archaea, yeast, plants and animals. There have been numerous studies on the metabolism of NAD⁺ in different research models. However, only few studies have been conducted regarding intracellular protozoa; even though it is known that NAD⁺ metabolism plays an important role in the organism invasion and survival. One of the parasitic diseases of highest incidence worldwide is malaria, caused by protozoa of the genus *Plasmodium*. Therefore, understanding the basic principles of parasite metabolism is important also with regard to NAD⁺ metabolism of intracellular parasites. This knowledge is essential not only for the identification of new antimalarial targets. **Materials and Methods:** Previously, we have accomplished the identification, cloning and overexpression of a recombinant version of *P. falciparum* NMNAT. We performed the expression of recombinant 6His-PfNMNAT protein in strain BL21-CodonPlus, and its purification by affinity chromatography using a Ni-NTA resin. Enzymatic activity of the purified protein was determined by direct enzyme assays, in which the conversion of ATP and NMN to NAD⁺ was quantified by RP-HPLC. Using this assay, we evaluated the influence of pH range (5-9), temperature (20-40°C) and different divalent cations on enzyme activity. **Results:** We determined the optimum for enzymatic activity of the recombinant protein to be at pH 7.5, at 37°C and in the presence of divalent cations such as Mg²⁺. Under these conditions, the kinetic parameters of the protein with respect to each substrate, NMN and ATP; were K_m of 0,75 and 0,53 mM and V_{max} of 0,126 and 0,325 U/mg, respectively. **Conclusions:** Enzymatic characterization of *Plasmodium falciparum* NMNAT confirmed its identity as the enzyme responsible for NAD⁺ synthesis in this parasite. **E-mail:** lmsanchezme@unal.edu.co, mhramirez@unal.edu.co

Mal004- **GBP: a *Plasmodium falciparum* Telomere binding Protein**

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Introductio: Telomeres are specialized structures at the end of chromosomes made of repetitive DNA sequences and associated proteins. Such structures protect the ends of linear chromosomes from recognition as DNA double-stranded breaks, recombination and fusion. The architecture of telomeric DNA consists of G-rich double-stranded tandem arrays followed by a single-stranded G-rich overhang. There are three classes of telomeric proteins: those that bind to double-stranded DNA (dsBP), those that bind specifically to G-rich overhang (GBP), and proteins that interact with telomeric factors. Telomere maintenance is achieved through association with these specific binding proteins. *Plasmodium falciparum* chromosome ends consist of a stretch of telomeric GGGTT(T/C)A repeats, however proteins that bind single stranded G-rich overhang have not been identified in this parasite. In this work, we report the cloning, expression and DNA binding assays of a telomeric binding protein of *P. falciparum*. **Methods:** The PF10_0068 (PfGBP) complete gene was amplified from the *P.falciparum* cDNA by using specific oligonucleotides. The PCR product was inserted into the pGEX4T2 plasmid and the GST-GBP fusion protein was produced in *E. coli*. This recombinant protein was purified and used to produce specific polyclonal antibodies in mice. To investigate the DNA binding activity of GST-PfGBP, Electrophoretic Mobility Shift Assay (EMSA) was performed using four oligos: Tel G (ssG-rich DNA), Tel C (ssC-rich DNA), TSR8 (dsDNA) and PfTs (non related oligo). **Results:** *P. falciparum* genome contains a sequence (PF10_0068) that is highly similar to GBP proteins of *Chlamydomonas reinhardtii* and *Cryptosporidium parvum*. In order to investigate whether PfGBP is expressed in the parasites, western blot analysis was performed using parasite lysate obtained from mixed stage parasites and the antibodies obtained. A specific band was detected in nuclear extracts. In EMSA, recombinant GST-PfGBP binds Tel G, which bears four copies of *P. falciparum* telomeric repeat GGGTT(T/C)A, but not Tel C or double-stranded telomeric sequences. **Conclusion:** The results indicate that PfGBP is a nuclear protein that binds telomeric DNA *in vitro* and it may have a biological role in the protection of telomeres in *Plasmodium falciparum*. **E-mail:** epcalvot@unal.edu.co

Mal005- **Myosins involved in the malaria parasite *Plasmodium falciparum* invasion to red blood cells**

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Introduction: *Plasmodium falciparum* is an obligate intracellular parasite whose survival and proliferation depends on its capacity to invade host cells. The parasite exhibits an unusual form of locomotion called gliding, which is responsible for invasion and involves an actomyosin molecular motor immersed in a protein complex known as glideosome. Six myosins have been identified to date in the *P. falciparum* genome (PfMyoA-B-C-D-E and F) and one of them (PfMyoA) has been implicated in the process of erythrocyte invasion, a function carried out in concert with the myosin tail domain interacting protein (MTIP), the glideosome-associated protein 45 (GAP45) and the glideosome-associated protein 50 (GAP50). So far, only PfMyoA has been characterized functionally and nothing is known about the function of the other five myosins of the parasite. **Materials and Methods:** The objective of this study was to investigate the potential role of the *P. falciparum* myosins in the erythrocyte invasion process. First, a bioinformatic analysis was done to search for structural features that could suggest interactions. Then, an experimental search for interaction between myosins and glideosome-associated proteins was conducted through immunoprecipitation assays. Recombinant proteins were then made for MTIP, GAP45, GAP50 and for the six myosins, using different expression systems (pGEX, pET or pMAL). These recombinants were used to produce polyclonal antibodies in mice. Immunoprecipitation assays were done with anti-MTIP and the immunoprecipitate was subject to SDS-PAGE and WB with anti-myosin antibodies.

Results: In-silico analysis with AutoDock4.2 made it possible to try to find potential interaction between MTIP and the myosins of the parasite, using the docking between MyoA and MTIP as a positive pattern of interaction. Experimentally, recombinant proteins and polyclonal antibodies were obtained for the myosins of the parasite and for three glideosome-associated proteins (MTIP, GAP45, GAP50). Immunoprecipitation assays suggest that other myosins besides PfMyoA can interact with glideosome-associated proteins. **Conclusions:** The results indicate that PfMyoA might not be the only myosin implicated in the *P. falciparum* invasion of red blood cells. This research was funded by Colciencias through Project 1101-521-28729. **E-mail:** chaparrojacqueline@unbosque.edu.co

Mal006- Study on the redox metabolism of the intraerythrocytic forms of the malaria parasite *Plasmodium falciparum*

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Plasmodium falciparum is the main responsible for severe cases of malaria. Treatment of the infection based on quinoline drugs, such as chloroquine (CQ), has been one of the most important approaches to treat the disease. The mechanism of action of CQ is based on its capacity to bind to Heme and inhibit hemozoin formation (Hz). Free heme cause membrane damage and oxidative stress. The actual paradigm is that CQ binding to Heme promotes oxidative imbalance leading to parasite death. *P. falciparum* is highly dependent on antioxidant defenses since the parasite has a constant need to neutralize reactive species (RS) and to detoxify Heme by forming HZ. We intend to verify if CQ acts based on redox imbalance in the intraerythrocytic forms of two different parasite strains: the CQ-resistant, W2 and CQ-sensitive, Haiti. First we have validated the detection of RS by flow cytometry using mature parasites enriched with Percoll gradient and dihydroethidine (DHE), a fluorescent dye sensitive to RS. We used light microscopy to define sub-lethal doses of CQ and to observe the 24 hours - kinetic development of *P. falciparum* in presence and absence of CQ. Using fluorescence microscopy, we started the search for an efficient antioxidant to neutralize RS and reduce the DHE signal on parasites treated with sub-lethal doses of CQ. Our results indicate that Haiti, in the absence of CQ, has higher levels of ROS in comparison to the W2 strain. It was also noted that, despite of the strain, CQ treatment causes a significant increase in DHE fluorescence. Our preliminary conclusions are: 1. parasite with CQ-sensitive phenotype has higher levels of oxidative stress, probably due to a lower capacity to control oxidative imbalance; 2. CQ treatment intensifies RS generation, confirming the hypothesis that CQ's mechanism of action is based on oxidative stress in the malaria parasite. **Supported by:** Capes. **E-mail:** camilanunesb@gmail.com

Mal007- Limited interaction between apical membrane antigen 1 of *Plasmodium vivax* with human erythrocytes AS detected by flow cytometry or transfection

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Introduction and Objectives: The Apical Membrane Antigen 1 (AMA1) is important for the invasion of *Plasmodium* merozoites. Herein, our objective was to evaluate the binding of *Plasmodium vivax* (Pv) AMA1 to human erythrocytes using a new flow cytometry assay and the classic cytoadherence assay with transfected cells. **Material and Methods:** His-tagged recombinant proteins based on region II of Duffy Binding Protein (PvDBP-RII), the 19 kDa C-terminal region of Merozoite Surface Protein 1 (PvMSP1₁₉) and different domains of PvAMA-1 were tested at different concentrations (0, 1, 10, 25 or 50 µg/mL). Recombinant proteins were incubated with human erythrocytes, followed by incubation with anti-penta-His Alexa Fluor 647 (QIAGEN). The binding assay was performed by flow cytometry (FACSCANTO II – BD). The data expressed as mean of fluorescent intensity (MFI±SD) were compared. Cytoadherence assays were performed by transfecting COS-7 cells with plasmids encoding the same proteins in the

presence of polyethylenimine (Sigma). After 24 hour of transfection, the cells were incubated with human erythrocytes for 2 hours and the number of rosettes was evaluated by fluorescent microscope. The inhibition of binding was evaluated by previous incubation of the recombinant proteins or transfected cells with sera from animals immunized with PvDBP-RII. **Results:** The results of flow cytometry assays using 50µg/mL of the recombinant proteins showed that: i) PvDBP-RII binds to Duffy A (MFI=1997±405) and Duffy B (MFI=2155±395) human erythrocytes; ii) PvMSP1₁₉ failed to do bind to the human erythrocytes (MFI=201±47); iii) the ectodomain of PvAMA1 (MFI=536±99), as well as the contiguous domain I-II (MFI=120±12) and the domain II (MFI=179±30) bound poorly human erythrocytes. Sera from BALB/c mice immunized with PvDBP-RII in the presence of CFA/IFA inhibited up to 66% of the binding to erythrocytes at 1:400 sera dilution. These results were confirmed by cytoadherence assays since PvDBP-RII transfected cells were able to bind to Duffy A (274±144 rosettes/25 fields) and Duffy B (356±129 rosettes/25 fields) human erythrocytes. The sera from mice immunized with PvDBP-RII inhibited the cytoadherence to erythrocytes up to 99% at 1:400 sera dilution. In contrast, transfected cells expressing PvMSP1₁₉, the ectodomain of PvAMA1, the contiguous domain I-II and the domain III alone failed to bind human erythrocytes and no rosettes were observed. **Conclusion:** We developed an assay using flow cytometry which can be extremely useful to evaluate the binding of proteins to erythrocytes whose results were confirmed by classic cytoadherence assays using as control the binding of PvDBP-RII to human erythrocytes. Using both methodologies, we were not able to find a significant binding of PvMSP1₁₉ or PvAMA-1 to human erythrocytes. **E-mail:** katiafrancoso@usp.br

Mal008- Characterization of *Plasmodium knowlesi* reticulocyte binding ligands (*Pknbp_{xa}* and *Pknbp_{xb}*) from human infections in Malaysian Borneo

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Plasmodium knowlesi, a monkey malaria parasite, continues to infect humans in Southeast Asia. The parasite has the shortest erythrocytic cycle among the *Plasmodium* species of human and non-human primates. With its 24 hours erythrocytic cycle the merozoites invade host RBC's and parasitaemia increases daily. Two genes, the *P. knowlesi* normocyte binding protein xa and xb (*Pknbp_{xa}* and *Pknbp_{xb}*) which are closely related to *P. falciparum* reticulocyte homologs (*Rh2a* and *Rh2b*) and *P.vivax* (RBP2), encode merozoite proteins involved in erythrocyte invasion. *Pknbp_{xa}* and *Pknbp_{xb}* reference sequences available for *P. knowlesi* H strain were used to develop a method to characterize the genetic diversity and signatures of selection at these loci in human *P. knowlesi* infections. In the first instance *Pknbp_{xa}* and *Pknbp_{xb}* genes from five patient's isolates were sequenced to analyze for polymorphisms. An 8500bp fragment beginning at exon II of the *Pknbp_{xa}* gene and a fragment 3500bp beginning at exon I of the *Pknbp_{xb}* gene were cloned and sequenced to high stringency. Most diversity occurred at the 5' region of exon II for both genes (xa nucleotide diversity (π) = 0.024 and xb (π) = 0.0056). Using this information a 887bp fragment of *Pknbp_{xa}* and a 880bp fragment of *Pknbp_{xb}* were chosen to haplotype 147 *P. knowlesi* isolates from clinically well- characterized patients. *Pknbp_{xa}* haplotypes were obtained for 138 isolates, 7 failed to amplify and 2 failed to sequence. *Pknbp_{xb}* haplotypes were obtained for 134 isolates, 3 patients had multiple genotype infections and were excluded and 10 isolates failed to amplify. The majority of the SNPs were non-synonymous; 56 (68%) for *Pknbp_{xa}* and 28 (59%) for *Pknbp_{xb}* gene with nucleotide diversity (π) of 0.02269 and 0.00642 respectively in the larger sample set. There were 75 *Pknbp_{xa}* and 51 *Pknbp_{xb}* haplotypes in the study population with haplotype diversity (h) of 0.9729 and 0.9216 respectively. *Pknbp_{xa}* haplotypes formed two distinct dimorphic groups with 29 non-synonymous SNP which were represented in 44.2% (n=62) of patient isolates. McDonald and Kreitman (MK) test proved the evidence of negative selection among the *Pknbp_{xa}* isolates with neutrality index (NI) of 2.411 (P<0.007). Among the *Pknbp_{xb}* isolates the MK test results were insignificant. There were signatures of natural selection at these two loci. Two *Pknbp_{xa}* codons showed signals of positive selection and 11 under negative selection (P<0.05). Six *Pknbp_{xb}* codons were under negative selection (P<0.05). In conclusion

our results suggest that these invasion genes *xa* and *xb* display a high level polymorphism among the field isolates with signatures of natural selection which may be significant in human erythrocyte invasion.
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Molecular Biology

Mal009- Genotype comparison of *Plasmodium vivax* and *Plasmodium falciparum* clones from peripheral and placental blood of pregnant women in Colombia

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Introduction: In malaria endemic areas, most people are simultaneously infected with different parasite clones. In Colombia both *P. vivax* and *P. falciparum* co-circulate and cause malaria in pregnancy, which results in maternal and fetal morbidity. This study compared the genotype of *P. vivax* and *P. falciparum* clinical isolates from peripheral blood and placenta in pregnant women of Colombia. **Material and Methods:** Pregnant women in antenatal care (ANC) or at delivery with unique *P. vivax* or *P. falciparum* infection, confirmed by quantitative real-time PCR, were included. Peripheral and placental blood was stored in filter paper Whatman 3MM and the Chelex method was used for DNA extraction. Four microsatellites for each species were genotyped by nested or semi nested PCR and capillary electrophoresis. A cut off of 300 RFU was used for true amplification products. A secondary allele was scored if the peak height was greater than one third of the main peak. Haplotypes were constructed from dominant alleles. **Results:** A total of 68 samples with vivax infection and 60 with falciparum infection were analyzed. Of those samples, 49 were peripheral blood of pregnant women in ANC (30 vivax; 19 falciparum), 38 were peripheral blood of parturient women (20 vivax; 18 falciparum) and 51 were placental blood (18 vivax; 23 falciparum). All women in ANC were symptomatic and had high level of infection (mean of DNA copies/ μ L vivax infections: 1885 copies; falciparum infections: 1285 copies; $p=0.355$), while most of parturient women were asymptomatic and had very low level of infection (mean of DNA copies/ μ L vivax infections: 259 copies; falciparum infections: 37 copies; $p=0.025$). Haplotype construction was impossible in 27% of samples (34/128; 25 falciparum, 9 vivax) due to none (6 samples) or only one marker (28 samples) amplified; all those samples were from parturient women. A total of 59 samples were multiclonal (41 vivax ; 18 falciparum). Among 59 vivax infections with known haplotype, 50 different haplotypes were detected. For falciparum, the haplotype was known only in 35 samples and 19 different haplotypes were identified. There was not difference between clones detected in peripheral blood and placental blood. **Main conclusions:** In Colombia, the genetic diversity of *P. vivax* is higher than *P. falciparum* and the multiclonal infections are very common in peripheral blood as well as in placental blood. In parturient women, clones detected in maternal peripheral blood are similar to clones detected in placenta. **E-mail:** emarango@gmail.com

Mal010- Chloroquine-sensitive *Plasmodium falciparum* genotypes co-circulate with *P. malariae*, *P. ovale* sp and *P. vivax* in northern Angola.

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Background: *Plasmodium falciparum* parasites have the ability of developing mechanisms to resist antimalarial drugs by suffering mutations in specific genes. Populations of *P. falciparum* that were

previously dominated by chloroquine-resistant genotypes are now under the artemisinin-based combination drug pressure. *P. malariae*, *P. ovale curtisi* and *P. ovale wallikeri* are sympatric with *P. falciparum* and frequently presented as co-infections across the continent, but are often unreported. **Material and methods:** The prevalence of human *Plasmodium* species was determined by PCR using DNA from blood spots collected during a cross sectional survey conducted within CISA (*Health Research Center in Angola*, translated) project's Demographic Surveillance System (DSS) in northern Angola. *P. falciparum* was genotyped at resistance-associated loci in *pfprt* and *pfmdr1* by real-time PCR, or by direct sequencing of amplicons. **Results:** Microscopy failed to identify species other than *P. falciparum*. From the 3316 collected samples, 541 (16.31%) contained *Plasmodium* sp. infections; from which 477 (88.17%) were *P. falciparum* alone, 6.47% were *P. falciparum* and *P. malariae* together, 3.69% harbored *P. ovale curtisi* or *P. ovale wallikeri* alone or in combination with other species and 1.11% comprised *P. vivax* alone. Of 430 *P. falciparum* isolates genotyped for *pfprt*, 61.63% carried the wild-type allele CVMNK at codons 72 – 76, either alone or in combination with the resistant allele CVIET. No other *pfprt* allele was found. Wild-type alleles also dominated at codons 86, 184, 1034, 1042 and 1246 of the *pfmdr1* locus among the sequenced isolates. **Conclusion:** The use of molecular methods for species discrimination has provided an estimate of the prevalence of the different *Plasmodium* sp.. Although *P. falciparum* is the predominant species, *P. vivax*, *P. malariae* and both *P. ovale* types also exist, frequently in mixed infections. Contrasting to previous studies conducted in Angola, *P. falciparum* comprised an approximately equal mix of chloroquine-sensitive and chloroquine-resistant parasites, suggesting changes in the parasite population, possibly due to either lower drug pressure due to poor access to treatment in rural areas, or a rapid impact of the national drug policy change. **E-mail:** susana.nery@cisacaxito.org.

Mal011- Molecular diagnosis of natural *Plasmodium* spp. infection in anofelines (Culicidae, Anopheles), in the area of influence of the Curuá-una Hydroelectric Mill, Santarém, Pará, Brazil

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Introduction: The development of molecular biology techniques has become important information path for different biological samples. In the case of the natural *Plasmodium* spp. infection in anophelines. Polymerase Chain Reaction (PCR) is a diagnostic method with high sensitivity and specificity. **Methods:** The anophelines were collected in the region of influence of Hydroelectric mill Curuá-One (2 ° 24 'S and 54 ° 42'W), municipality of Santarém, Pará State, for the Department of Entomology of the 9th Central Regional Health Department State Public Health of Pará (SESPA) between July 2008 and December 2009. The mosquitoes had their DNA extracted using the resin Chelex ® Molecular Biology Grade Resin. The amplification for the genus *Plasmodium* was performed using primers described by Snounoun *et al.* (1993) and PCR was performed according to the Wilkerson *et al.* (1995) protocol with modifications. The minimum infection rate (MIR) was calculated according to Forattini (2002). **Results:** the two study areas - upstream and downstream of the dam, presented 12 *Anopheles* species. Out of all 6,715 specimens, 6,534 of them showed to be adults and 181 immature forms. Species belonged to three genera: *Nyrssorhynchus*, *Anopheles* and *Stethomya*. *Anopheles albitarsis* was the predominant species. *Anopheles darlingi* the second most frequent and often found in peridomestic environment. Of the 317 samples analyzed, nine were positive for *Plasmodium vivax*. The minimum infection rate was 3.5% (7:199) for *A. darlingi* and 3.4 (1:64) for *A. albitarsis* s.l. *Anopheles nuneztovari* corresponded to 1.5% (1:29). The tests were positive for *Plasmodium vivax* and negative for *Plasmodium falciparum*. **Concluding remarks:** In Curuá-una, the rate of infection for *A. darlingi* confirms the data reported in literature, which describes low infection levels, with some variations. These data enable the characterization of *A. darlingi* as an anthropophilic species in the Amazon, along with its distribution and density as being clearly related to malaria transmission, and *Plasmodium* infection susceptibility. *A. darlingi*, *A. albitarsis* s.l and *A. nuneztovari*, had earlier been reported by Alvarez *et al.* (1986) & Tadei Dutary-Thatcher (2000) and Pova et al. (2001), to be malaria vector species in certain areas of the

Amazon. Currently, *A. albicansis* s.l. is a complex formed by this group plus six species, three of which have probable importance as human malaria vectors in the Amazon, such as *A. marajoara*. For an effective malaria control program and the establishment of strategies that guide the actions, it is necessary to know the vector(s) server(s), or any side in transmission areas. **Financial support:** CNPq/FAPEAM-Rede Malária/CTPETRO/PIATAM. **E-mail:** lorenasm@gmail.com

Mal012- Genetic polymorphisms of B-cell co-stimulatory molecules among malaria vivax patients in Brazilian endemic area

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Introduction: Genetic polymorphisms in molecules associated with immune response interact in many signaling pathways. These, in turn, modulate the humoral immune response, which could contribute to abnormal antibody levels, thus influencing the susceptibility to malaria. This study aims at estimating the allelic and genotypic frequencies of variants in the *CD40L* and *BLyS* genes found in individuals from the malaria endemic area in the Brazilian Amazon rainforest. **Material and Methods:** The sample was collected from patients with vivax malaria and healthy individuals, from Macapá city, Amapá state. We extracted the DNA by using the extraction and purification kit Easy-DNATM (Invitrogen, California – USA) and identified the SNPs –726T>C in the gene *CD40L* and the -871C>T in the gene *BLyS* by the PCR-RFLP method. We analyzed the genotypic, allelic frequencies, as well as of those individuals carrying each allele, by direct counting. We also compared the observed genotypic frequencies with the expected ones, according to the Hardy-Weinberg Equilibrium. **Results:** Data showed a higher frequency of CC genotype (56,5%) for the *BLyS* gene, followed by the TC genotype (35%). The TT genotype presented the lowest frequency (8,5%). Since the *CD40L* gene is located in the X chromosome, we analyzed male and female individuals separately. The most frequent genotype for female individuals was TT (80,7%), followed by TC heterozygotic (17,3%). The CC genotype was the least frequent (2%) in the studied population. Regarding male individuals, the most frequent genotype was T (89,3%) and the least frequent was the C genotype (10,7%). No significant statistical association was observed in the allelic and genotypic frequencies among the patients with malaria and the control group. Both genes were balanced according to Hardy-Weinberg Equilibrium. **Conclusions:** The results suggest that the *CD40L* and *BLyS* genetic variability is not itself an important factor in the occurrence of vivax malaria in the studied population. **Funding:** CNPq and FAMERP **E-mail:** mpcapobianco@yahoo.com.br

Mal013- *IL1B*, *IL4R*, *IL12RB1* and *TNF* gene variants are associated with *Plasmodium vivax* malaria resistance/susceptibility in Pará State, Brazil

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Introduction: Malaria is the most prevalent parasitic disease worldwide. In Brazil, malaria is concentrated in the northern region and *P. vivax* malaria accounts for more than 80% disease incidence. The role of genetic factors in host immune system conferring resistance/susceptibility against *P. vivax* infections is still poorly understood. The present study investigates the influence of polymorphisms in 18 genes related to the immune system in *P. vivax* malaria resistance/susceptibility. **Material and Methods:** Genomic DNA was extracted from 216 patients diagnosed with malaria caused by *P. vivax* (malaria group) and 263 healthy subjects (control group) recruited in Pará State, Brazil. All subjects were genotyped by allelic discrimination with Taqman genotyping assays for 33 SNPs in *IL1B*, *IL2*, *IL4*, *IL4R*, *IL6*, *IL8*, *IL10*, *IL12A*, *IL12B*, *IL12RB1*, *SP110*, *TNF*, *TNFRSF1A*, *IFNG*, *IFNGR1*, *VDR*, *PTPN22* and *P2X7* genes. The subjects were also genotyped for 48 ancestry informative insertion-deletion polymorphisms to determine the proportion of African, European and Native American ancestry. A Poisson Regression model with age

as covariate was performed to access SNPs with differences lower than 20% between malaria and control groups. SNPs and haplotypes adjusted for age in the Regression model were tested for association between malaria and control groups correcting for population structure based on genetic ancestry information. **Results:** Thirteen SNPs in *IL1B*, *IL4R*, *IL6*, *IL10*, *IL12B*, *IL12RB1*, *SP110*, *TNF*, *IFNG* and *IFNGR1* genes were associated with malaria after age adjustment and were further tested for population structure. *IL1B* gene -5839C>T and *IL4R* gene 1902A>G were associated with malaria susceptibility after population structure correction ($p = 0.04$ and $p = 0.02$, respectively). The *IL1B* -5839C allele is 8.2% more frequent in the malaria group than in controls and *IL4R* gene 1902A allele is 6.2% more frequent in individuals with malaria. *IL12RB1* and *TNF* haplotypes were also associated with malaria susceptibility ($p = 0.01$ and 0.01 , respectively). The AG (-1094/-611) *IL12RB1* haplotype is only present in the malaria group whereas the TATGG (-1031/-863/-857/-308/-238) *TNF* haplotype is 2.5% more frequent in these subjects. **Main conclusions:** The present work demonstrated the association of *IL1B* -5839C>T and *IL4R* 1902A>G SNPs and *IL12RB1* AG (-1094/-611) and *TNF* TATGG (-1031/-863/-857/-308/-238) haplotypes with *P. vivax* malaria susceptibility in Pará state, Brazil. **E-mail:** vsortica@hotmail.com

Mal014- *Plasmodium vivax*: reverse transcriptase real-time PCR for gametocyte detection and quantitation in clinical samples

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The proportion of *Plasmodium vivax*-infected subjects that carry mature gametocytes and are potentially infectious remains poorly characterized in endemic settings. Here, we describe a quantitative reverse transcriptase (RT) real-time PCR (qRT-PCR) that targets transcripts of the mature gametocyte-specific *pvs25* gene. We found mature gametocytes in nearly all (95.4%) *P. vivax* infections diagnosed during an ongoing cohort study in northwestern Brazil. SYBR green qRT-PCR was more sensitive than a conventional RT-PCR that targets the same gene. Molecular detection of gametocytes failed, however, when dried bloodspots were used for RNA isolation and cDNA synthesis. Estimating the number of *pvs25* gene transcripts allowed for examining the potential infectiousness of gametocyte carriers in a quantitative way. We found that most (61.9%) gametocyte carriers were either asymptomatic or had subpatent parasitaemias and would have been missed by routine malaria control strategies. However, potentially undiagnosed gametocyte carriers usually had low-density infections and contributed a small fraction (up to 4%) to the overall gametocyte burden in the community. Further studies are required to determine the relative contribution to malaria transmission of long-lasting but low-density gametocytemias in asymptomatic carriers that are left undiagnosed and untreated. **E-mail:** nathflima@gmail.com

Mal015- Analysis of the polymorphism in exon 1 gene of the mannose binding lectin (MBL) in subjects infected by *Plasmodium vivax*

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Introduction: The mannose binding lectin (MBL) is the protein responsible for the activation of the complement which occurs when the lectin binds to oligosaccharides residues (mannose and N-acetyl glucosamida) present on the surface of various microorganisms as well as in oligosaccharides waste of parasites of the genus *Plasmodium* sp. The binding process leads to the activation of MBL associated with serine protease (MASP) which are capable of cleaving the components C4, C2 and C3 leading to the formation of C3 converted C4b2a. This cascade reaction initiated by MBL, rather than its binding to carbohydrates triggers the formation of the Membrane Attack Complex (MAC) which causes lysis and death of the pathogen. However, there are described polymorphisms in exon 1 of MBL gene that can alter the serum and/or the involvement of this pathway in the defense mechanisms against microorganisms.

Material and Methods: We analyzed 81 blood samples from individuals who sought care at the Clinical

Trials Program on Malaria at the Institute Evandro Chagas (PECM / IEC), from February 2002 to March 2003, diagnosed with the parasite *P. vivax* and reporting as the first episode of malaria. DNA extraction from blood samples was carried out using phenol/chloroform and genotyping was performed after amplification 349pb by polymerase chain reaction (PCR) followed by identifying the wild-type allele *MBL**A and the *MBL**B and *MBL**C mutations. Using the restriction enzymes *Ban*I and *Mbo*II. Subsequently, two other polymerase chain reactions were performed (PCR and PCR-SSP, respectively) for the identification of another mutant allele *MBL**D and confirmation of its homo or heterozygous. **Results:** The frequency of the alleles A, B and D were 64.20%, 19.75% and 16.05%, respectively. The allele C was not found. We identified five genotypes: AA, AB, AD, BB and BD. The frequencies for the genotypes varied from 1.23% to 34.57%, and for AA (34.57%), AB (28.40%), AD (30.86%), BB (4.94%) and BD (1.23%). **Conclusion:** In individuals who never had malaria infected by *P. vivax* was identified the following genotypes AA, AB, AD, BB and BD, and the most frequent were the AA (wild) and AD (mutant) genotypes. **Funding Agency:** CNPq e FUNTEC-SECTAM **E-mail:** rafaelathias@hotmail.com

Mal016- Cloning and Expression of *Plasmodium vivax* Glutamate Dehydrogenase and Production of Monoclonal Antibody against PvGDH

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Introduction: Glutamate dehydrogenase (GDH) is an essential enzyme for glycolysis in *Plasmodium*, is a potential diagnostic target as well, the established immunology diagnosis test based on *Plasmodium falciparum* glutamate dehydrogenase (PfGDH) shows satisfactory results, however, no report revealed about *Plasmodium vivax* glutamate dehydrogenase (PvGDH) so far. Therefore, the PvGDH was cloned and expressed, and monoclonal antibody against recombinant PvGDH was raised for further *Plasmodium vivax* specific diagnosis test development. **Material and Methods:** The primers were designed based on putative *Plasmodium vivax* Glutamate Dehydrogenase gene, then PvGDH coding sequence was amplified from emulsion library, then the full-length cDNA library of erythrocytic stage *Plasmodium vivax*, and inserted into pMD18-T vector. The sequence was confirmed by sequencing after identification using PCR and restriction enzyme digestion, then sub-cloned into pCOLD II vector for expression. The immunoreactivity of the serum from *vivax* malaria patients, *falciparum* malaria patients and health volunteers to recombinant PvGDH (rPvGDH) was tested using ELISA and Western-blot. The serum was collected from mice after immunization using rPvGDH, and its immunoreactivity to rPvGDH was tested using ELISA, as well as the immunoreactivity to antigen of *Plasmodium vivax*, *Plasmodium falciparum*, and normal human RBC was tested using Western-blot. Monoclonal antibodies against PvGDH were produced using recombinant PvGDH. The titer and subtype of monoclonal antibodies were tested; the immunoreactivity of monoclonal antibodies to rPvGDH was tested using ELISA and Western-blot. **Results:** A 1442 bp gene was amplified by PCR, and cloned into vector, the sequence has 3 bp difference with the coding sequence of PvGDH from sal-I strain genomic database, but without encoding amino acid changing. The recombinant PvGDH was expressed as soluble form, and could be recognized by the serum from *vivax* malaria patients or *falciparum* malaria patients, but not be recognized by the serum from health volunteers. The serum was collected from mice after immunization using rPvGDH could recognize rPvGDH, both antigens of *Plasmodium vivax* and *Plasmodium falciparum*, but not normal human RBC. 4 monoclonal antibodies were produced respectively using rPvGDH. All of them can specifically recognize rPvGDH with titer higher than 1: 2430. All monoclonal antibodies are IgG2b. **Main Conclusion:** PvGDH was successfully cloned and expressed with immunogenicity. And 4 monoclonal antibodies against PvGDH were successfully produced using rPvGDH. **Supported by** the Key Project of Chinese Ministry of Education.(No. 211079) and Key Laboratory on Technology for Parasitic Diseases Prevention and Control, Ministry of Health (No. WK008-002) **E-mail:** FQ333LTT@hotmail.com

Mal017- Molecular genetic epidemiology on *pvmdr1* mutation of *Plasmodium vivax*

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Introduction: A mutation in *Plasmodium vivax* multidrug resistance 1 gene (*pvmdr1*) in codon 976 (Y976F) is associated with chloroquine (CQ) resistance and is recently used to monitor the distribution and frequency of the CQ resistant vivax malaria. The aim of this study is to determine the prevalence of this mutation in *P. vivax* isolates collected in University of the Philippines Manila, the Philippines (2009, 2012), Inje University, South Korea (1994-2001, 2007), and National Center for Global Health and Medicine, Japan (1999-2011). **Materials and Methods:** We examined the mutation in codon 976 in the *pvmdr1*, using 101 *P. vivax* field isolates: from Palawan island, the Philippines (15 isolates), South Korea (56 isolates), Papua New Guinea (PNG) (6 isolates), India (4 isolates), Indonesia (2 isolates), Malaysia (1 isolate), Thailand (1 isolate), Bangladesh (1 isolate), Nepal (1 isolate), China (1 isolate), Pakistan (2 isolates), Iran (1 isolate), Uganda (1 isolate), Rwanda (1 isolate), Sudan (1 isolate), Niger (1 isolate), Mauritania (1 isolate), Comoros (1 isolate), Brazil (2 isolates), Ecuador (1 isolate) and Guyane française (1 isolate). **Results:** The Y976F mutation was observed in the 28 isolates out of the 101 isolates (27.7%): 15 from the Philippines, 4 from PNG, 1 from India, 1 from Malaysia, 1 from Thailand, 1 from Uganda, 1 from Rwanda, 1 from Sudan, 1 from Niger, 1 from Mauritania and 1 from Comoros. The other 73 isolates possessed the wild type (Y976). In the Philippines, CQ has been used for treatment of vivax malaria of which resistance against CQ has never been reported so far. However, all the Philippine isolates acquired the Y976F mutation. In the African continent, CQ resistance was reported only from Ethiopia so far. However, all the African isolates we examined also showed the Y976F mutation. These findings from the Philippines and Africa suggest that CQ resistant vivax malaria will be emerging in the each endemic area in the near future. On the other hand, in South Korea, 2 cases of CQ resistant vivax malaria were reported in 2003 and 2007, but all the 56 South Korean isolates showed the wild type in the gene. **Conclusions:** The Y976F mutation has already been widely distributed in the endemic areas and a careful monitoring is needed for control and containment of CQ resistant *P. vivax*. **E-mail:** miwagami@ri.ncgm.go.jp

Mal018- Mutations in *SOD-1* gene are associated with different protein expression and susceptibility to malaria.

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Introduction: During malaria, intense inflammation results in oxidative stress, and superoxide anions are the main reactive oxygen species produced. Cu/Zn superoxide dismutase (SOD1) is a key enzyme that, in together with catalase and glutathione peroxidase, scavenges superoxide anions and protects cells from oxidative damage. We have previously shown that SOD1 plasma levels are elevated in vivax malaria and that this enzyme can be used as a reliable biomarker for severe disease. Here, we test whether mutations in *SOD1* gene (exon 1) and a Single Nucleotide Polymorphism (SNP) -308 (G>A) in the *TNFalpha* gene are associated with susceptibility to vivax malaria. **Material and Methods:** We studied 124 subjects, including 49 with symptomatic vivax malaria, 43 with asymptomatic vivax malaria and 32 uninfected individuals from the Brazilian Amazon. The first exon of *SOD-1* and *TNF-alpha* -308 (G>A) SNP were amplified by PCR. *SOD1* PCR products were sequenced on an ABI Prism 3100 automated

DNA sequencer and *TNF-alpha* SNP were analyzed by NcoI restriction enzyme. SOD1 and TNF-alpha plasma levels were measured by ELISA and Cytometric Bead Array respectively. **Results:** SOD1 and TNF-alpha plasma levels were increased in symptomatic malaria ($44.73 \pm 31.35 \text{ ng/mL}$ and $39.07 \pm 30.25 \text{ pg/mL}$, respectively) when compared with asymptomatic ($8.49 \pm 5.82 \text{ ng/mL}$ and $5.88 \pm 10.27 \text{ pg/mL}$, respectively) and uninfected individuals ($6.99 \pm 7.42 \text{ ng/mL}$ and $6.35 \pm 19.97 \text{ pg/mL}$, respectively) ($P < 0.0001$ for each comparison). We did not find any association between the *TNF-alpha* -308 (G>A) SNP and susceptibility to malaria ($p = 0.7446$) and the TNF-alpha plasma levels were not significantly different between the subjects homozygous wild-type (GG), mutant homozygous (AA) and heterozygous (GA) ($p = 0.6892$). We found 8 novel SNPs from *SOD1* gene in 6 individuals, 4 with symptomatic malaria and 2 uninfected. The patient with the -5217 (G>T; stop codon) SNP was symptomatic and had the highest SOD1 plasma level when compared with all individuals analyzed (125.9 ng/mL). Furthermore, an individual with two SNPs side by side (-5206 A>T, -5207 A>T; Gln>Leu) was symptomatic and had also a high systemic concentration of SOD-1 (95.4 ng/mL). In contrast, a patient presenting with three different SNPs (-5207 A>T Gln>His; -5210 T>C Arg>Gli; -5211 C>G Arg>Gli) was also symptomatic but had undetectable levels of SOD1. **Main Conclusions:** This is the first study to describe functional SNPs in the *SOD1* gene among subjects with *vivax malaria*. Our findings argue that single mutations in *SOD1* gene are associated with differences in the plasma levels of this enzyme and may influence the malaria clinical outcome. These results are preliminary and we are currently performing experiments to increase the sample and to screen for polymorphisms in other exons (a total of five) of SOD1. **E-mail:** manael.barral@gmail.com

Mal019- Genetic variability in platelet integrin $\alpha 2\beta 1$ density: possible contributor to *Plasmodium vivax*-induced severe thrombocytopenia

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Clinical descriptions of *severe Plasmodium vivax infection* seem to be increasing, and there is a paucity of data on the mechanism leading to *vivax* morbidity. Based on previous evidence in favor of a role of platelets in the pathogenesis of *vivax* malaria, we hypothesized that gene polymorphisms on functionally relevant platelet glycoprotein receptors could be associated with *vivax* thrombocytopenia. The study involved 151 well-characterized Brazilian *P. vivax* patients, and the impact of two polymorphisms of platelet membrane integrins, the C807T of integrin $\alpha 2$ and the T1565C of integrin $\beta 3$, were analyzed by polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP). While the integrin $\beta 3$ T1565C polymorphism was not related with *vivax* clinical disease, patients carrying the variant integrin $\alpha 2$ allele (807T) - which is associated with high surface levels of integrin $\alpha 2\beta 1$ - had a significantly higher probability for severe thrombocytopenia (platelets $\leq 50,000$ per mm^3) than those carrying the wild-type allele (adjusted odds ratio = 4.44, 95% CI = 1.23-15.99, $p = 0.023$). This finding was further sustained by a correlation between clinical disease and surface levels of the integrin $\alpha 2\beta 1$ (Spearman $r = 0.2$, $p = 0.011$). This first evidence into the association of integrin polymorphism and *vivax* pathogenesis requests further evaluation of this pathway in human malaria. **Supported by:** FAPEMIG, CNPq, FIOCRUZ, Pronex Malaria, DECIT/MS **Email:** lhcarvalho@cpqrr.fiocruz.br

Mal020- Genetic variability of *Plasmodium vivax* from primary-relapses paired samples

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Human malaria is caused by protozoa from the genus *Plasmodium*. Approximately 40% of world population is at risk of infection. *Plasmodium vivax* is the most worldwide distributed human *Plasmodium* species. In Brazil, more than 300,000 cases of the disease were recorded in 2010 and 86% were caused

by *Plasmodium vivax*. *Plasmodium vivax* infections are characterized by hepatic dormant forms, the hypnozoites and those are activated in varying intervals of time resulting in the typical relapses. Little is known about the mechanism of latency and activation of hypnozoites associated to relapses. The scarcity of genetic markers for *P. vivax* has hampered the analysis of important parasite phenotypes, such as patterns of relapse. In this context, the aim of this work was to study the variability of the parasites from primary infections and relapses episodes. Primary-Relapse paired samples of 30 patients were genotyped using 10 molecular markers (8 microsatellites and blocks 2 and 10 of MSP-1) by capillary electrophoresis on an automated DNA sequencer. Moreover, the presence of multiple infections and rare clones was confirmed by cloning of amplicons and genotyping of different colonies. It was demonstrated a high rate of multiple infections both in primary infection and relapse. The allele's combination of all loci in haplotypes identified mainly heterologous relapsing parasites. However, there were a limited number of alleles per locus and was observed a fluctuation of predominant alleles among distinct markers in different malaria recidives of the individual. The presence of recurrent relapses after treatment with primaquine may suggest the presence of resistant parasites. Altogether our findings suggest that mechanisms involved in hypnozoites activation might be based on host/environment factors and not because of parasites genetic programming. **E-mail:** cristiana@cpqrr.fiocruz.br

Mal021- Tracking for geographic origin of imported *Plasmodium vivax* infections with mitochondrial genome analysis

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Plasmodium vivax, a relatively neglected human malaria parasite, is a major public health challenge for Central and South America, the Middle East, Central, South and Southeast Asia, Oceania and East Africa, where 3.3 billion people are currently at risk of infection and 70-80 million clinical cases are reported each year. In areas where it was eradicated, malaria is the most prominent imported pathogen. Cases of imported malaria, which means malaria contracted in an endemic region but diagnosed in a non-endemic country, are a rare event but may have a fatal outcome. Molecular techniques such as amplification of DNA fragment in conventional PCR, real-time PCR and sequencing were published in the literature as a tool available to detect the parasite in blood samples and it can also identify the geographic origin of imported cases of malaria. We aim to standardize genetic markers from mitochondrial genome of *P. vivax* and apply these markers to determine the geographic origin of imported *vivax* malaria infections. We sequenced the complete mitochondrial DNA (mtDNA) from 56 samples collected at the Centers for Disease Control and Prevention (CDC, Atlanta), between 2004 and 2008, from imported, laboratory-confirmed *P. vivax* samples diagnosed in the US. Of the 56 strains tested, 80% (45) had a presumed geographical origin determined by patient's interview. The network of mtDNA haplotypes was constructed using the program Network 4.6.2.0, combining 336 genome sequences available in the GenBank database with 56 sequences from CDC samples. We found 218 nucleotide substitutions in the 392 sequences analyzed. We were able to classify 95% of isolates with presumed geographical origin, according to the clustering pattern determined by Network 4.6.2.0. We are now standardizing TaqMan assays for typing eight informative mtDNA single-nucleotide polymorphisms for determining the origin of imported cases of malaria. **Financial support:** NIAID/NIH, FAPESP. **E-mail:** priscilathihara@yahoo.com.br

Mal022- Determination of variants of genotype Duffy in patients with severe and non-severe malaria by *Plasmodium vivax*

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Introduction: Malaria is an important problem of public health, also in Brazil, where the *Plasmodium vivax* is more prevalent. Severe malaria is defined as an infection with potentially fatal manifestations to the man and has always been associated with complications caused by *P. falciparum*; however, an uncommon standard of clinical complications associated with clinical cases of malaria by *P. vivax* has been observed. Studies have shown that the Duffy glycoprotein can act as a facilitator of invasion of erythrocytes by *P. vivax*, but little is known about the association of Duffy genotypes with protection or risk of infection by the parasite. Likewise, there are no descriptions in the literature about the Duffy genotypes and a possible association with severe malaria by *P. vivax*. This study determined the variants of the Duffy genotype in patients with severe malaria and non-severe malaria by *Plasmodium vivax*. **Material and Methods:** Samples of total blood of 160 patients diagnosed with severe and non-severe malaria vivax, collected during the period March 2009 to april 2010, of patients treated in FMT-HVD for the genotyping of blood group Duffy. Was performed extraction of DNA from whole blood and then, the genotyping Duffy, using the technique of PCR-RFLP, following the standard protocol by the institution. Some clinical and laboratory data of the patients in the study were also included in the analysis of the results, which was carried out using the statistical software Stata v. 11 (College Station, TX) and Epi Info, version 6.04 (CDC). **Results:** Considering the total number of patients and patients with non-severe malaria, the most frequent genotype was FYA / FYB (36.1% and 41%, respectively). The study results showed no significant association between a specific genotype or phenotype and the occurrence of severe malaria (p valor= 0,265 and 0,140). The association between parasite density and genotypes was not statistically significant (p value = 0.055). **Conclusion:** Through this study, it was possible to correlate clinical data, parasitaemia and phenotype of patients and compare them with results obtained in similar studies in northern Brazil. More studies are necessary to confirm the absence of association between genotype frequency of the Duffy and severe malaria by *P. vivax*. **E-mail:** anne.cg.almeida@hotmail.com

Mal023- Plasmepsin IX and X: Targets for the Anti-Malaria Activity of Selected Antiretroviral Protease Inhibitors and the Search for Specific Inhibitors

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Introduction: Each year malaria parasites infect 300-500 million people and cause ~800,000 deaths. A significant setback in the fight against malaria is the spread of drug resistant parasites. Parasite resistance to all of the currently used antimalarial agents has been detected, leading to a desperate need for the development of new drugs to right this deadly disease. Our laboratories have shown that selected antiretroviral protease inhibitors (PIs) can inhibit the growth of malaria parasites at clinically relevant concentrations against a range of parasite developmental stages. While the modest activity of the PIs against parasites (0.4-21µM IC₅₀) is likely to limit the use of these agents as first-line antimalarial drugs, identifying their antiplasmodial target may lead to the development of more potent inhibitors with activity against multiple life cycle stages. We have been investigating the target of the PIs in *P. falciparum* and have determined that these drugs inhibit the malaria parasite aspartic proteases, plasmepsins (PMs) IX and X. The role of these enzymes in *P. falciparum* is not known and neither has been exploited as a drug target. Here we discuss our structural and functional studies together with data from studies designed to identify specific inhibitors of PM IX and X. **Materials and Methods:** Molecular biology and transfection techniques were used to investigate the location, expression and function of PMs IX and X in *P. falciparum*. In separate experiments online computational structural prediction systems and molecular modeling and simulation programs were used to examine the structure of PM IX and X and to perform in silico docking studies with chemical compound libraries. Inhibitors of PM X were identified and investigated further in vitro. **Results:** PM IX is expressed in ring, trophozoite and schizont stages of the asexual *P. falciparum* life cycle. It is localized both within the parasite and exported to punctate structures in the host red cell cytoplasm, but does not co-localize with Maurer's Clefts. PM X is only located within the parasite but not within the digestive vacuole (DV) and is not exported. Techniques to knock-down or knock-out these PMs indicate that they serve essential functions within the parasite. An *in silico* model of

PM X was generated. This structure demonstrates features typical of an aspartic protease. In silico docking studies using this model have identified potential inhibitors that are being characterized in vitro against *P. falciparum*. **Main Conclusions:** The localization of PM IX and X outside of the DV of the parasite indicates these enzymes have functions different from the already well characterized DV PMs I - IV. The location of PM IX and X in different parasite compartments together with our accumulated knock-down-knock-out and over-expression data also suggest that each enzyme has different yet essential roles within the parasite. **E-mail:** tina.skinner- adams@gimr.eciu_au

Mal024- *In vitro* antimalarial susceptibility and molecular markers of drug resistance in Franceville, Gabon

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Introduction: Malaria remains a major public health problem, due to emergence and widespread *P. falciparum* drug resistance. WHO recommends artemisinin combination based therapy (ACT) to overcome *P. falciparum* drug resistance, but reports of declining ACT efficacy have been published. A thorough understanding of the molecular bases of *P. falciparum* resistance to existing drugs is therefore needed. The aims of this study were to analyze the *in vitro* sensitivity of *P. falciparum* field isolates from Franceville, Gabon, to chloroquine (CQ), mefloquine (MF), dihydroartemisinin (DHA) and monodesethylamodiaquine (MDAQ), and to investigate polymorphisms associated with drug resistance.

Material and Methods: we conducted a cross-sectional study including 53 field isolates. The sensitivity of isolates against CQ, MF, DHA and MDAQ was assessed using colorimetric test DELI-test. The *Pfmdr1* codons 86 and 1246, *Pfcr1* (haplotype codon 72 to 76) and *PfATPase6* codons 110 and 2694 were analysed by PCR-RFLP. Associations between drug sensitivity and parasite gene polymorphisms were evaluated with the Chi square test, and routine hematological parameters were analyzed with Fisher's exact test implemented with Epiinfo software. In all statistical tests, significance was assumed at $p < 0.05$.

Results: A total of 46 *P. falciparum* isolates were successfully cultured *in vitro* and their sensitivity was tested. The proportions of isolates resistant to CQ, MF and MDAQ were 43.5%, 23.4% and 56.5%, respectively. Some isolates (23.9%) had DHA IC₅₀ values higher than 10 nM. The median IC₅₀ values were 71.67 (interquartile range (IQR, 1-438.2), 6.59 (IQR, 0.08-96), 64.79 (IQR, 0.09-448) and 6.45 nM (IQR, 0.09-23) for CQ, MF, MDAQ and DHA, respectively. The highest correlation between diminished DHA sensitivity and MF resistance was observed ($r^2=0.73$), followed by diminished DHA sensitivity and CQ; A cross-resistance between CQ and MF was also observed. The prevalence of the 86Y and 1246Y mutations in *Pfmdr1*, 76T in *Pfcr1*, and 110A and 2694T in *PfATPase6* were 42% and 17.1%, 97.8%, and 0% and 22.2%, respectively. **Main conclusions:** These high levels of antimalarial drug resistance in Franceville, Gabon, call for reinforced drug efficacy surveillance. **E-mail:** lekana_jb@yahoo.fr

Mal025- Phenotypic and genotypic analyses of invasion of *P. falciparum* from South America

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Introduction: Invasion of RBCs by *P. falciparum* (Pf) involves multiple pathways including those utilizing ligands of the Erythrocyte-Binding Ligand (EBL) and the Reticulocyte-Binding protein homolog (PfRh) families. Notably, the invasion ligands also exhibit heterogeneity in their sequences with frequent clustering of the polymorphisms suggesting that different domains may be evolving under different selection and functional pressures. The invasion and ligand polymorphisms of 29 South American (SA)

field isolates from Colombia (Antioquia), Peru (Iquitos) and Brazil (Pará) was studied. **Materials and Methods:** Invasion profiles in fresh and *in vitro* adapted Pf field isolates were determined using wild type and NTC enzyme-treated RBCs. Polymorphisms in the invasion ligands was determined by PCR and sequencing. **Results:** Eight different invasion profiles were found, one of which is independent of neuraminidase (N), trypsin (T) and chymotrypsin (C) sensitive receptors (NrTrCr), and which was not previously reported. This pathway was used predominantly by Colombian and Peruvian field isolates with varying levels of resistance (58-93%) to the three enzyme treatments. Moreover, comparison of invasion profiles of field isolates from the three regions have clearly indicated that the invasion profiles of the Peruvian and Colombian isolates are similar and both are very distinct from those present in the Brazilian isolates; the Peruvian and Colombian isolates use mostly sialic acid-independent (Nr) pathways, while the Brazilian isolates use mostly sialic acid-dependent (Ns) pathways. Notably, 3/5 of the Ns-dependent Peruvian isolates might have originated in Brazil. Analyses of ligand polymorphisms in the newly collected SA field isolates further confirmed that the Peruvian parasites are admixed populations of the Colombian and Brazilian parasites with a limited repertoire of known PfRh variants and several novel PfRh variants with some being region specific. The impact these EBA and PfRh variants have on the invasion pathways utilized by field isolates is not fully known. Preliminary analysis of the Peruvian isolates found significant association between % invasion into NTC-treated cells and particular ligand variants: PfRh2a pepC or PfRh2b pepC* variants and the EBA-181 RVNKN/RVNQN variants were associated with successful invasion into N-treated RBCs while pepB in both PfRh2 ligands and EBA-181 RVIQN variant were associated with sialic acid-dependent invasion. **Main Conclusions:** It appears that in SA malaria endemic areas, where the population is highly heterogeneous, both host and parasite genetic factors may drive the emergence of distinct invasion pathways. Future studies will validate the importance of a given polymorphism within a ligand to its preferential invasion profile and thus its potential differing ability to bind to varied RBCs. **E-mail:** slustigman@nybloodcenter.org

Mal026- Investigation of polymorphisms in *Plasmodium falciparum* hrp2, hrp3, aldolase and pldh genes and their impact on the performance of malaria rapid diagnostic tests in Papua New Guinea

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Introduction: The World Health Organization (WHO) has strongly advised that a proper clinical diagnosis of malaria be performed before anti-malarial treatment is administered. Malaria rapid diagnostic tests (RDTs) can represent a valuable tool for prompt and efficient diagnosis of malaria in settings where microscopic diagnosis is unavailable or unreliable. Concerns remain, however, that some strains of *Plasmodium falciparum* may have variability in the genes coding the antigens detected in RDTs and that this could impact on their performance. This study aimed to characterise genetic variability in histidine rich protein-2 (PfHRP-2), PfHRP-3, aldolase and plasmodium lactate dehydrogenase (pLDH) genes and to evaluate their impact on the performance on RDTs. **Material and methods:** Using isolates of *Plasmodium falciparum* obtained from a number of field studies previously conducted in PNG from the East Sepik and Madang provinces. *Pfhrp-2*, *Pfhrp-3*, *aldolase* and *pldh* were amplified using a polymerase chain reaction (PCR) and sequenced to identify polymorphisms. **Results:** Sequence length polymorphisms for the histidine rich proteins were found in a wide range of samples. Aldolase and pLDH displayed more conservation in their sequences. **Conclusion:** This study confirms the presence of different sequence repeat type genetic polymorphisms in *P. falciparum* hrp2/3 isolates and the conservation of *pldh* and *ald* genes. It also noted a few important implications for RDT performance. **E-mail:** elisheba.malau@pngimr.org.pg

Mal027- Using next-generation sequencing technologies to identify *Plasmodium falciparum* genomic variation

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Introduction: Structural variations (SVs), such as point mutations, insertions and deletions (indels) and copy number variations (CNVs), in the *Plasmodium falciparum* (Pf) genome have been associated with important phenotypes, including anti-malarial drug resistance. For example, amplifications in the multi-drug resistance locus (PfMDR1) are associated with mefloquine resistance in southeast Asia. There is a great interest in detecting and cataloguing SVs across the Pf genome. Genotyping array platforms, such as comparative genomic hybridization (CGH), have been used to detect a limited set of variants. However, the application of next-generation sequencing technologies (NGTs) has the potential to characterize many types of variant across the whole genome. NGTs yield many millions of short sequences per sample, and data from many hundreds of isolates are publically available. We are using this whole genome sequencing data to identify SVs in the Pf genome, and develop a catalogue for the malaria research community. **Material and Methods:** Statistical methods, including Poisson-gamma models, were developed to detect SVs using paired-end mapped sequence alignments and the resulting genomic coverage. These approaches can adjust results for the high AT-content of Pf, which can lead to uneven genomic coverage. Application of our methods to 3D7 (reference) sequence data was used to control the false positive rate, and implementation to 5 well-characterized strains across three continents allowed for comparison with the literature. **Results:** As expected, we detect no deletions in 3D7 but detect strong signals for important amplifications (e.g., GTP cyclohydrolase I - PFL1155w). On average, 82.6 deletions and 126.6 amplifications were inferred per sample, across 5 strains. We confirmed loci identified by CGH technologies across multiple strains, including a large amplified region in chromosome 12 comprising the PFL1130c and PFL1160c. When SV diversity across strains was explored and quantified, the geographical origin of the samples was recovered. We confirm the existence of the PfMDR1 copy number in Southeast Asian strains. **Main Conclusions:** We have developed robust statistical approaches to characterize structural variation from whole genome sequencing data. The catalogue of these variants is of great interest as it may assist in the identification of new candidate genes for drug resistance as well as pinpointing regions under selection. Ongoing work is identifying variation in field isolates, complemented by experimental validation, and the development of a web-based exploratory tool for the research community. **E-mail:** nunosep@gmail.com

Mal028- Genetic Profile of *Plasmodium falciparum* Populations Resistance to Sulfadoxine-Pyrimethamine in the Amazon Basin

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Introduction: Most Brazilian malaria cases occur in the Amazon region and *Plasmodium falciparum* accounts for approximately 15% of those with a focal distribution. In Brazil, *P. falciparum* isolates had showed resistance to Sulfadoxine and Pyrimethamine in combination (SP), first time, in Goiás in 1970 and six years later in Maranhão. SP resistance is caused by point mutations in *P. falciparum* dihydrofolate reductase (*pf dhfr*) and *P. falciparum* dihydropteroate synthase (*pf dhps*). The main objective of this study was to characterize the genetic profile of *P. falciparum* population resistance to Sulfadoxine-Pyrimethamine in the Amazon basin. **Material and Methods:** For the purpose of the research, it was examined 190 *P. falciparum* Brazilian samples collected during the 80s and 90s, in Amapá, Pará and Rondonia states. Direct sequencing was used to determine the presence of mutations in *pf dhfr* (codon 50, 51, 59, 108 e 164) and *pf dhps* (codon 436, 437, 540, 581 e 613) and analyze the microsatellites loci around *pf dhfr* and *pf dhps*. The estimated or expected heterozygosity (He) was calculated for each locus by the formula $[n / (n - 1)] [1 - \pi]$, where n is the number of isolates analyzed or sample size, and π is

the frequency of each allele. The sample variance was calculated for H_e [$2(n-1)/n^3$] [$2(n-2)$] [$PI_3 - (pi_2)^2$]. The Excel Microsatellite tool kit (Microsoft Office Excel® - Version 2007) was used to calculate the number of alleles per locus and allele frequencies. The program eBurst was used to examine the haplotypes in this study. **Results:** To the *pf dhfr* gene, parasites carried one of the three *pf dhfr* alleles (511/108N, 511/108N/164L or 50R/511/108N), and the triple mutant 50R/511/108N was the most frequent. The three alleles found had two haplotypes which suggests independent origins. The collected parasites carried the *pf dhps* 437G/540E/581G, the most prevalent, and 437G/581G. Related to the origin of these alleles, the two alleles found shared a common haplotype. Based on Network Diagram, all isolates shared connectivity, which implies that there is internal migration between sites and that parasite populations were conserved over the period studied. Furthermore, the F_{ST} analyses were low in comparison to earlier values reported in other studies. **Conclusions:** These findings suggest that strong SP selective pressure have fixed resistant alleles to *pf dhfr* and *pf dhps* in these Brazilian states. **Key words:** Malaria, Amazon basin, *P. falciparum*, resistance, *pf dhfr*, *pf dhps*. **Financial support:** IEC/SVS/MS; RAVREDA-AMI/USAID/PAHO/MS, CDC, Atlanta, GA, USA; CNPq. **E-mail:** giselleviana@iec.pa.gov.br

Mal029- Phenotypic and genotypic study of artemisinin's derivatives susceptibility in plasmodium falciparum strains

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Plasmodium falciparum resistance to commonly used antimalarials still remains is a major problem for malaria control. Therefore, the use of artemisinin-combination therapies as first line treatment of non-severe malaria was recommended. Studies in Cambodia showed an increase in the rate of recrudescence after the treatment with artesunate-mefloquine. The decrease in susceptibility to these drugs is an alert to the emergence of *P. falciparum* resistant to artemisinin. Thus, the study of mutations and differential expression of genes that are associated with drug resistance can identify novel molecular targets and, eventually, ways to avoid this major problem in malaria treatment. This study aims to analyze genetic and epigenetic changes in *P. falciparum* to different pressures of artemether. Thus, the field sample from the State of Amazonas RMS, was subjected to artemether pressure in vitro and were later identified polymorphisms, gene copy number and expression of *pfatp6*, *pfmdr1* and *pfe0775c* genes by direct sequencing and real-time PCR, respectively. The results allowed to evaluate the process pressure in vitro artemether associated with the molecular markers *pfatp6* and *pfmdr1*. The genotyping results demonstrate that only the analysis of gene polymorphisms was not sufficient for association with reduced susceptibility to antimalarial. In the other hand, copy number and gene expression of these genes showed a significant increase due to the response of the drug pressure in vitro. In conclusion, epigenetics factors and gene amplification appear to have a significant influence in reducing the susceptibility to Artemether. **Keywords:** *Plasmodium falciparum*; artemisinin; artemether; *pfatp6*; *pfmdr1*; reduced susceptibility; polymorphism; increase gene copies; gene expression. **E-mail:** andreluiz.areas@gmail.com

Mal030- Blood Transcriptome of Childhood Malaria

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Introduction: Understanding malarial immunopathology in the human host represents an enormous challenge for transcriptomic research. In this work, we used microarray and real-time RT-PCR technology to pursue deeper knowledge about the mechanisms underlying this disease in African children. **Material and Methods:** We investigated the genomic transcriptional profiles in whole blood of healthy children and children with asymptomatic infection, uncomplicated malaria, malaria associated with severe anemia

and cerebral malaria using microarray and real-time RT-PCR assays and compared them with previously published microarray results. **Results:** We were able to discriminate between the different presentations of *Plasmodium falciparum* infection using supervised and unsupervised clustering of microarray data and unsupervised double-hierarchical clustering of real-time RT-PCR results of a set of 22 genes known to be expressed in at least one of the principal blood cell lineages. We further found considerable overlap between genes regulated in Kenyan and Gabonese children with symptomatic malaria, in contrast to adults with acute malaria from Cameroon. Immunoglobulin production, complement regulation and IFN beta signaling emerged as most discrepant features between uncomplicated malaria and all other investigated presentations, correlating with IRF7 and ISRE binding signatures in the corresponding genes. Down-regulation of several genes in cerebral malaria seems instead to be a response to hypoxia orchestrated by AhRF, GABP and HIF1 transcription factors. *ARG1*, *BPI*, *CD163*, *IFI27*, *HP* and *TNFAIP6* transcript levels correlated positively with lactatemia and inversely with hemoglobin concentration. **Conclusions:** In this work, a relevant part of the changes which occur in gene expression when different blood cells interact simultaneously with themselves and with the parasite during *P. falciparum* infection could be uncovered. Several markers and associations in the transcriptional network should be further validated in order to improve therapeutic measures and prevent malarial disease evolution and death. **Financial support:** CAPES/CNPq. **E-mail:** angelicaboldt@gmail.com

Mal031- Genetic associations studies of single nucleotides polymorphism (SNPs) in the promoter region of TNF- α , IL-10 and INF- γ cytokines genes with clinical malaria outcomes in a Brazilian Amazonian population

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Introduction: Malaria is a complex disease with many genetic and environmental determinants influencing the clinical spectrum of disease. Host genetic factors contribute to the variability of malaria phenotypes and thus should help to determine some of the mechanisms involved in the susceptibility to *Plasmodium* infection. A variety of genetic polymorphisms, particularly in erythrocyte receptors and immune response related genes, have been described to be associated with susceptibility and resistance to malaria. We investigated the association of polymorphisms in the promoter region of the cytokines genes TNF- α , IL-10 and INF- γ in order to establish the impact of these host genetic variations in susceptibility/resistance to *Plasmodium* infection and clinical (mild) malaria in a population of the Amazon Basin in Brazil. **Methodology:** DNA samples were isolated from whole blood cells of 702 individuals from Santa Isabel do Rio Negro and Barcelos and the promoter regions of TNF, INF and IL-10 were partially amplified using PCR. Automatic sequencing was performed using 48 capillary ABI-3730 sequencer and sequence analysis performed with SeqScape® v2.6 software (Applied Biosystems). The association tests included four groups of individuals: 1) *never_malaria* group as controls; 2) *clinical_malaria* group; 3) *asymptomatic_Plasmodium_infection* group and 4) *any_malaria* group (individuals with history of previous malaria and malaria and asymptomatic *Plasmodium* infection at the moment of the blood collection). **Results:** After data analysis we have identified a significant association ($P < 0.05$) of the IL10 -1117GG genotype (-1082GG) with a reduced risk to malaria *per se* ($p = 0,0351$, OR=0,34, IC=0,12-094) and an association of the TNF- α -308AA genotype with an increased risk of malaria *per se* ($p = 0,0213$, OR=4,69, IC=1,07-28,77). No association between SNPs in the INF locus and susceptibility to malaria were found. **Discussion:** We conducted a genetic association study investigating the role of host cytokine gene polymorphisms on malaria clinical outcomes in an Amazonian population. Our results corroborate previous results in other populations; more studies are needed to show the relevance of the highly polymorphic nature of cytokine genes in terms of serum cytokine levels and their association with disease susceptibility. Further studies should include a larger number of individuals, refined parameters and a fine-scale map obtained through DNA sequencing to increase the knowledge of the Amazonian population genetic diversity. **Funding:** CNPq/DECIT and PAPES/FIOCRUZ **E-mail:** simone@ioc.fiocruz.br

Mal032- Duffy polymorphism Fy- genetic association in two western amazon populations supports the use of retrospective account of malarial episodes as an epidemiological tool

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Introduction: The present results are part of a research that aims to investigate the genetic mechanisms related to human response to malaria infection. One of the challenges of the study of the genetics of complex traits is the choice of the phenotype, the trait itself. An appropriated phenotype should provide genetic information about the trait, detectable by researchers using genetic epidemiology methods such as complex segregation, association and linkage analysis. Environmental effects should also be taken on account and minimized if possible. It is widely accepted in the scientific literature that Duffy blood-group antigen Fy(-) phenotype provides protection against vivax malaria, which was responsible for about 60% of malaria cases at the studied population during the study period. In the present communication it is evaluated whether the retrospective account of malarial episodes can provide useful information to investigate the genetic basis of the malarial infection in two western Amazon populations, Portuchuelo and Monte Negro, Rondonia state, Brazil. **Material and Methods:** About 178 individuals from Portuchuelo population and 850 individuals from Monte Negro (Rondonia state, Brazil) were included in the samples after agreement to a consentient term. These individuals were subjected to an epidemiological survey, followed by blood collection and characterization of classic blood groups markers. Association between phenotype and genotype was tested using the non-parametric Kruskal-Wallis test. **Results:** The observed distribution of the retrospective account of malaria episodes is highly dispersed (Portuchuelo: mean=5.56 s.d.=9.83 and Monte Negro: mean=8.85; s.d.=23.59). Logarithm transformation was applied. The effects of age and sex were also corrected by regression analysis and normalized. The retrospective account of malaria episodes was significantly smaller in Duffy (Fy-) genotype individuals in both populations (Portuchuelo: $X^2=6.15$; 1 d.f., $p=0.013$ and Monte Negro: $X^2=6.73$, 1 d.f. $p=0.009$, both combined: $X^2=10.95$, 1 d.f., $p=0.001$), without significant heterogeneity ($X^2=1.934$, 1 d.f., $p=0.164$). **Main Conclusions:** The expected association between retrospective account of malaria episodes and Duffy (Fy-) genotype supports the use of this phenotype to investigate the genetic mechanisms related to human response to malaria infection. **E-mail:** hkrieger@icb.usp.br

Mal033- Genetic characterization of populations in malaria endemic region of the western Brazilian Amazon: description of gene and genotypic frequencies of the CCR5 LOCI, ACP1 and enzymes of metabolism of xenobiotics GSTT1, GSTP1 and CYP2E1.

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Infectious diseases have been considered as a strong selective factor in the modeling of human genetic response to xenobiotics in recent decades. Many genes may be involved in this process of expression regulation, but despite technological advances, few genes have been identified in this modeling process of genetic individuality. The aim of our study was to analyze the allelic's distribution of CCR5, ACP1, GSTT1, GSTP1 and CYP2E1 genes in five population samples collected from two localities in Rondônia state, located in the Western Brazilian Amazon region, where malaria is endemic, as well as the possible association polymorphisms associated with clinical phenotypes detected by Plasmodium infection in the five population samples. Four were collected in Porto Velho, the state capital and the site of several waves of migration, since the seventeenth century. Of these, two, from the Hospital de Base were comprised of HB Mothers and HB Newborns, a third from the peri-urban neighborhoods of

Candelária/Bate-Estaca and the fourth, from the Research Center on Tropical Medicine/CEPEM that was composed of malaria patients under treatment. The fifth sample came from the inland Quilombola village of Pedras Negras, situated in the Southwestern region of Rondonia in the Guaporé Valley; a region considered as isolated quilombola and suffered little migrational influence. DNA was extracted according to the methodology by digestion with proteinase K. The samples were genotyped by PCR amplification of target regions according to the protocol indicated in the region gene. Visualization took place in 10% PAGE stained with silver nitrate 10%. Statistical analyzes were performed using the programs GENEPOP (version 3.4). The significance level was 5%. The results indicate that the gene and genotypic distributions of these gene loci are similar to other Brazilian populations and are associated with ethnic pattern of the populations studied. Rondônia was colonized by several waves of migratory, since the XVII century, causing various population groups contribute to the formation of their population. In the CCR5 locus, the CCR5 Δ 32 allele, considered Caucasoid marker, was detected in all samples, including sample quilombola village of Pedras Negras, indicating the introduction of European genes in a quilombo. The presence of the CCR5 Δ 32 allele may be a modulating agent spread of HIV-1 in this region, because of the increasing number of people infected by the virus. The ACP1*C allele was observed in the HB parturients sample associated with all phenotypes of malaria reported, except for women who reported falciparum malaria. This parameter is indicative of the protective effect conferred on individuals infected with *P. falciparum*. It was not possible to establish a biological basis to explain the observed associations between allelic frequencies of the loci studied and the clinical phenotype of malaria, vivax and falciparum, except for related malaria and ACP1*C. Even if there were no statistically significant associations in our analyses, the possibility of genetic causes must be considered in future new approaches. **Keywords:** Genetic Polymorphism, Populational Characterization, Rondônia, Infectious Diseases, Malaria. **E-mail:** engracia.oliveira@uol.com.br

Mal034- Investigation of host candidate malaria-associated risk/protective SNPs in a Brazilian Amazonian population

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Introduction: Malaria is a complex disease with many genetic and environmental determinants influencing the clinical spectrum of disease. A variety of genetic polymorphisms, particularly in erythrocyte receptors and immune response related genes, have been described to be associated with susceptibility and resistance to malaria. **Methodology:** We investigated the association of 64 human single nucleotide polymorphisms (SNPs) in 37 genes using a Sequenom massARRAY iPLEX platform. A total of 648 individuals from two malaria endemic areas were studied, including 535 malaria cases (113 individuals with clinical mild malaria, 122 individuals with asymptomatic infection and 300 individuals with history of previous mild malaria) and 113 health controls with no history of malaria. **Results:** The data revealed significant associations ($p < 0.003$) between one SNP in the IL10 gene (rs1800896/-1082A/G), one SNP in the IRF1 gene (rs2706384) and one SNP in the TLR4 gene (rs4986790) with reduced risk for clinical malaria; one SNP in the LTA gene (rs909253/LTA+252) with protection from clinical malaria and one SNP in the TNF gene (rs1800750/TNF-376) associated with susceptibility to clinical malaria. A haplotype analysis of three polymorphisms in the IL-10 gene (rs3024500, rs1800896, rs1800890) revealed that those with the GCT allelic combination (~10% frequency in population) were at a lower risk of any form of malaria (OR: 0.40-0.63, 95% CI: 0.2-0.9) when compared to the common ATA combination (>83% frequency in population). Also, a new association was found between a SNP in the CTL4 gene (rs2242665), located at the major histocompatibility complex III region, and a reduced risk for clinical malaria. **Main conclusion:** This study represents the first association study from an Amazonian population involving a large number of host genetic polymorphisms with susceptibility or resistance to Plasmodium infection and malaria outcomes. Further studies should include a larger number of individuals, refined parameters and a fine-scale map obtained through DNA sequencing to increase the

knowledge of the Amazonian population genetic diversity. **Funding:** CNPq-DECIT, PAPES IV-FIOCRUZ, the Wellcome Trust Sanger Institute. **E-mail:** simone@ioc.fiocruz.br

Diagnostic

Mal035- Rapid diagnostics tests for malaria diagnosis in the Peruvian Amazon: impact of *Plasmodium falciparum* *pfhrp2* gene deletions and cross-reactions

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Introduction: In Peru, malaria is mainly endemic in rural areas in the Amazon region, where (reliable) microscopy is often not available. Malaria rapid diagnostic tests (RDTs) are an alternative, providing quick and accurate diagnosis. In Peru, species involved are *Plasmodium falciparum* and *Plasmodium vivax*. For *P. falciparum*, the main target antigen used by RDTs is histidine-rich protein-2 (HRP-2), which is often thought to perform better than the other target, *P. falciparum*-specific parasite lactate dehydrogenase (Pf-pLDH). In Peru however, *P. falciparum* strains with *pfhrp2* gene deletions, encoding HRP-2, have recently been found. They may impair the use of HRP-2 detecting RDTs. For *P. vivax*, - the most prevalent species - two target antigens exist: *P. vivax*-specific pLDH (Pv-pLDH) and pan-pLDH. Moreover, the occurrence of cross-reactions between the different *Plasmodium* species and target antigens may impede diagnostic specificity, leading to incorrect species diagnosis and treatment. **Materials and Methods:** Thirteen RDT products, 10 detecting HRP-2 and 4 Pf-pLDH (one HRP-2/Pf-pLDH combination test), were assessed with 179 prospectively collected malaria positive samples: 74 *P. falciparum*, 101 *P. vivax* and 4 mixed infections. Samples were collected at different health centers around Iquitos, within a range of 16km and in Atalaya, Alto Nanay. Species and parasite density were determined by microscopy, species diagnosis was confirmed by PCR. Presence of the *pfhrp2* gene was assessed by PCR, and presence of HRP-2 protein by ELISA. **Results:** For *P. falciparum* diagnosis, sensitivity was significantly lower for HRP-2 based RDTs compared to Pf-pLDH based RDTs: one quarter (21/74, 28.4%) of *P. falciparum* samples was missed by all 10 HRP-2 based RDTs but correctly diagnosed by all 4 Pf-pLDH based RDTs. In 19 of these samples the *pfhrp2* gene was lacking, in 18/19 absence of HRP-2 was confirmed by ELISA, while one had a weak positive result. For the two remaining HRP-2 RDT negative samples, ELISA results were negative while presence of *pfhrp2* was confirmed by PCR. For *P. vivax* diagnosis, there was no overall difference in diagnostic sensitivity between Pv-pLDH and pan-pLDH-based RDTs, but two RDT products (one targeting Pv-pLDH, the other pan-pLDH) scored significant lower. Cross-reactions of *P. vivax* samples with the HRP-2 or Pf-pLDH lines were noted in 27/101 (26.7%) of *P. vivax* samples, at a median frequency of 2.5% (range 0%-10.9%) per RDT product. Three *P. falciparum* samples cross-reacted with the Pv-pLDH line in 2 RDT products. **Main conclusion:** HRP-2 based RDTs are not suitable for *P. falciparum* diagnosis in the Peruvian Amazon due to *pfhrp2* gene deletions, which are frequently encountered. The Pf-pLDH based RDTs performed excellent for both *P. falciparum* and *P. vivax* diagnosis and are promising RDTs for this region. Development of a Pf-pLDH/Pv-pLDH based RDT would have the advantage of detecting mixed infections as well. **E-mail:** jmaltha@itg.be

Mal036- Differential diagnosis to detect *Plasmodium vivax* and *P. falciparum* in pregnant women of endemic coastal Colombian areas

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Introduction: In Colombia, *P. falciparum* and *P. vivax* infections are highly endemic and there is no official information on the prevalence of infection among the pregnant population (Campos et al. 2011), however, the effects of this infection are well known ; malaria in non-immune pregnant women produce high rates of abortion (up to 60% in the case of infection with *P. falciparum*) and maternal mortality rates between 10% and 50%, while semi-immune pregnant in malaria can cause abortions and low birth weight (WHO, 2011). Malaria in pregnancy poses a diagnostic challenge due to several factors: the low specificity of signs and symptoms and submicroscopic infections are very common during pregnancy. In addition to the above limitations affect certain techniques to detect infection and difficulties in identifying episodes of malaria (Bardaji et al, 2008). This study compares the sensitivity and specificity of three techniques: thick blood smear, PCR and qPCR to diagnose infections with *Plasmodium* spp in pregnant women. **Materials and Methods:** The thick film was made after the blood sampling. Also we used the subunit gene 18S ribosomal RNA was selected as a target amplification for both the nested and real-time PCR (qPCR). This gene contains highly conserved regions and at least 5 copies are distributed on different chromosomes in the genome of *Plasmodium* (Mangold et al 2005). The evaluation of diagnostic tests were performed by calculating the percentages of the levels of sensitivity, specificity, positive predictive value, negative predictive value and agreement between direct and comprehensive diagnostic testing through the kappa (Montoya et al.2008.) **Results:** This study determined the sensitivity and specificity of the thick blood smear, nested PCR and real-time PCR tests for the diagnosis of malaria in pregnancy. The 40 pregnant patients belonging from Antioquia, Corboba, Choco and Nariño Departments, were successfully differentially diagnosed concerning the presence of *Plasmodium vivax* and *P. falciparum*, both in peripheral blood and in umbilical cord and placenta. The molecular tests were more sensitive and specific when used species specific oligonucleotides for both species, but less sensitive than thick blood smears when the oligonucleotides used were those for genre. In conclusion, molecular tests such as nested PCR and real-time PCR were more efficient for detecting subclinical cases, which was not possible to detect by thick blood smears. The real-time PCR has advantages compared with nested PCR since its standardization is shorter, requires less infrastructure and the reaction is now increasingly more economical. **Email:** mcorredor@matematicas.udea.edu.co

Mal037- Early experiences on the feasibility, acceptability, and use of malaria rapid diagnostic tests at peripheral health centres in Uganda - insights into some barriers and facilitators

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Introduction: While feasibility of new health technologies in well-resourced healthcare settings is extensively documented, it is largely unknown in low-resourced settings. Uganda's decision to deploy and scale up malaria rapid diagnostic tests (mRDTs) in public health facilities and at the community level provides a useful entry point for documenting field experience, acceptance, and predictive variables for technology acceptance and use. These findings are important in informing implementation of new health technologies, plans, and budgets in low-resourced national disease control programmes. **Methods:** A cross-sectional qualitative descriptive study at 21 health centres in Uganda was undertaken in 2007 to elucidate the barriers and facilitators in the introduction of mRDTs as a new diagnostic technology at lower-level health facilities. Pre-tested interview questionnaires were administered through pre-structured patient exit interviews and semi-structured health worker interviews to gain an understanding of the response to this implementation. A conceptual framework on technology acceptance and use was adapted for this study and used to prepare the questionnaires. Thematic analysis was used to generate themes from the data. **Results:** A total of 52 of 57 health workers (92%) reported a belief that a positive mRDT result was true, although only 41 of 57 (64%) believed that treatment with anti-malarials was justified for every positive mRDT case. Of the same health workers, only 49% believed that a negative mRDT result was truly negative. Factors linked to these findings were related to mRDT acceptance and use, including the design and characteristics of the device, availability and quality of mRDT ancillary supplies, health worker capacity to investigate febrile cases testing negative with the device and provide appropriate treatment, availability of effective malaria treatments, reliability of the health commodity supply chain, existing national policy recommendations, individual health worker dynamism, and vitality of

supervision. **Conclusions:** mRDTs were found to be acceptable to and used by the target users, provided clear policy guidelines exist, ancillary tools are easy to use and health supplies beyond the diagnostic tools are met. Based on our results, health workers' needs for comprehensive case management should be met, and specific guidance for managing febrile patients with negative test outcomes should be provided alongside the new health technology. The extent, to which the implementation process of mRDT-led, parasite-based diagnosis accommodates end user beliefs, attitudes, perceptions, and satisfaction, as well as technology learnability and suitability, influences the level of acceptance and use of mRDTs. The effectiveness of the health system in providing the enabling environment and the integration of the diagnostic tool into routine service delivery is critical. **E-mail:** caroline.asiimwe@finddiagnostics.org

Mal038- Low prevalence of *pfhrp2* deletion in French Guiana: implications for malaria diagnosis.

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Introduction: For five years, the rapid diagnostic tests (RDTs) for malaria are increasingly used and improve care in endemic areas. Because of its abundance and its heat stability, most RDTs target the PfHRP₂ antigen to detect *P. falciparum*. However, their use in South America has been largely questioned since the publication of a high prevalence (41%) of field isolates in Peru lacking the gene encoding this protein. In French Guiana, more than one thousand cases are identified each year, mostly along rivers. The main encountered species are *P. falciparum* and *P. vivax*, 31% and 69% of the reported malaria cases in 2011, respectively. Therefore, reliable and sensitive malaria RDTs are essential for some of the remote health centers. **Material and Methods:** The *pfhrp2* gene of 140 isolates circulating in French Guiana in 2009 was amplified. Additionally, an evaluation study of SD Malaria Ag test P.f/Pan[®] (HRP₂ RDT) and OptiMAL-IT[®] (LDH RDT) kits were conducted. The RDTs performances were determined in comparison with microscopy as gold standard. **Results:** The retrospective study identified one isolate lacking the exon 2 of the *pfhrp2* gene. Between January 2010 and August 2011, 960 suspected cases of malaria have been analyzed using microscopy and RDTs. The SD Malaria Ag test P.f/Pan[®] performances were better than the OptiMAL-IT[®] kit. Sensitivities to diagnose *P. falciparum* (n=93) or *P. vivax* (n=129) were 96.8% [CI95 90.9 - 99.3] vs 83.9% [CI95 74.8 - 90.7], p<0.001 and 86.0% [CI95 78.8 - 91.5] vs 53.5% [CI95 44.5 - 62.3], p<0.001, respectively. No isolates lacking the *pfhrp2* exon 2 was identified among the 81 *P. falciparum* isolates analyzed during this evaluation study. **Main conclusions:** Field isolates lacking the *pfhrp2* gene are rare in French Guiana (<0.4%) and the SD Malaria Ag test P.f/Pan[®] kit exhibited high performances. Therefore, this kit could be a satisfying alternative to microscopy in remote health center where it is difficult to ensure the presence of highly skilled microscopists and to maintain the equipment. **E-mail:** lmusset@gmail.com

Mal039- A comparison of four diagnostic tests for malaria treatment and follow-up in São Tomé

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Introduction: A reliable and rapid diagnosis of malaria is crucial in providing effective and timely treatment for the disease. Various molecular techniques have been developed based on polymerase chain reaction (PCR). A comparison of loop-mediated isothermal amplification (LAMP) and nested PCR using for clinical malaria diagnosis and follow-up was made in Sao Tome and Principe. **Materials and methods:** During the period of Sept-Nov 2009, blood samples from 128 children (5-14 years old) with

fever (>38°C, tympan) in the District of Agua Grande were examined by use of four different methods, i.e., RDT, microscopy, PCR, and LAMP. **Results:** Numbers of the positive result for RDT, microscopy, PCR, and LAMP, were 68, 47, 64, and 65, respectively. Seventy-nine patients were diagnosed as uncomplicated falciparum malaria based on the positive result from any one of the four tests. All were treated with 3-day Coartem and followed up one week after treatment. Follow up for these 79 cases, numbers of the positive result for RDT, microscopy, PCR, and LAMP, were 33, 7, 12, and 11, respectively. **Conclusion:** RDT is useful for diagnosis of acute malaria which has similar sensitivity as microscopy but less specificity. However, the low specificity of RDT (60%) with extreme low positive predictive value (20%) which cannot be applied for follow-up after treatment (over-estimation). For both malaria diagnosis and follow-up, results of LAMP are comparable to those of PCR. Therefore, LAMP can be applied for follow up after malaria treatment. **E-mail:** shaio22@yahoo.com.tw

Mal040- Evaluation of the Rapid Diagnostic Test Malaria Quick Test versus microscopy method, among HIV positive and unknown serology patients in Ouagadougou, Burkina Faso

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Background: Malaria and HIV show an overlapping geographical distribution in tropical areas, with a higher risk of severe malaria in HIV-infected patients. A key of effective management of malaria is an early and accurate diagnosis necessary to reduce morbidity, mortality and to differentiate malaria from non malarial fever thus avoiding the unnecessary use of anti-malarial drugs. This is particularly important in HIV positive patients because the interaction between HIV and malaria is bidirectional and synergic. Moreover in HIV infected patients febrile illness might have several causes other than malaria. The use of Rapid Diagnostic Tests (RDT) has an important role in the expansion of rapid access to malaria diagnosis helping to successfully control malaria. **Materials and methods:** This study was carried out from August to December 2011, during and right after the rainy season. We report the performance of a malaria rapid diagnostic test Malaria Quick test (Cypress Diagnostic) compared with the microscopy method, tested on samples of HIV infected individuals and HIV unknown serology individuals in Ouagadougou, Burkina Faso. **Results:** In total 114 HIV infected patients were included in the study: 48/114 (42.1%) were RDT and thick smear concordantly positive and 63/114 (55.3%) concordantly negative. None of the samples were thick film positive and RDT negative, while 3/114 (2.6%) were thick film negative and TDR positive. The sensitivity and the specificity of the test were respectively 100.0% (95% CI: 92.6-100.0) and 95.4% (CI: 87.3-99.1) with a 5.8% of false-positive results and a total agreement of 97.3%. A sample of 127 patients with unknown serology was analyzed: 52/127 (40.9%) samples were RDT and thick smear concordantly positive and 59/127 (46.4%) concordantly negative. None of the samples were thick film positive and RDT negative while 16/127 (12.5%) samples were thick film negative and RDT positive. The sensitivity and the specificity of the test in this population were 100.0% (CI: 93.2-100.0) and 78.6% (95% CI: 67.7-87.3) with a 23.5% of false-positive results and a total agreement of 87.4%. **Main Conclusions:** Malaria Quick test is rapid and effective for the diagnosis of malaria and has a high sensitivity, which allows detecting also cases with low parasitaemia. The high agreement between the Quick malaria test and microscopy method reported in the HIV-infected population, suggest that the HIV virus does not interfere or corrupt the performance of the test, confirming the utility of the rapid diagnostic test in general and in HIV patients in particular. **E-mail:** ariannakim@libero.it

Mal041- The role of fish farming in the maintenance of urban malaria transmission, in Mâncio Lima, Acre

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Introduction: Strong economic incentives for the development of fish farming in the State of Acre, northern Brazil, has led to a rapid change of the urban landscape, with a direct impact on the risk of malaria introduction and maintenance in the region. Several factors may contribute for the high malaria incidence observed in this region, among them, the several fish tanks that may act as potential reservoirs for the malaria vector, *Anopheles darlingi*. The city of Mâncio Lima, the westernmost of Brazil, has a complex landscape characterized by the presence of wetlands and streams crossing the urban space. A large number of fish tanks was created in the urban area in recent years, and more are planned by the Government and by the residents themselves, to support their own subsistence. The objective of this study was to investigate the spatial distribution of immatures of *Anopheles sp*, mainly *An. darlingi*, in the different water bodies of urban Mâncio Lima, Acre, and their association with fish tanks. These results will contribute for the proposition of risk reduction management strategies. **Material and methods:** A survey of 90 water bodies in the city of Mâncio Lima was carried out in February 2012, during the rainy season. Each water body was physically and chemically characterized, as well as georeferenced, and basic ecologic observations were made. Variables included: type of water body (fish tanks x wetlands), overall characteristics (border's vegetation density, surface area, degree of shadow, presence of fluctuating macrophytes, and type of use), water variables (turbidity, pH, temperature, dissolved oxygen, conductivity, nitrate, chlorophyll, ammonia). Immature anophelines were collected in the early morning or late afternoon, using standard procedures. The number of sampling points per water body was proportional to its perimeter. Larvae, kept in plastic bags, were transported to the Centro de Endemias de Cruzeiro do Sul, Acre, for identification to the level of species. Water variables were measured at two points, using a limnological probe. **Results:** A total of 655 immatures of *Anopheles sp* were collected, being 180 *An. trianulatus*, 169 *An. albitarsis*, 140 *An. darlingi*, 111 *An. rangeli*, 5 *An. peryassui*, 4 *An. argyritarsis*, 3 *An. oswaldoi*, and 2 *An. brasiliensis*. From the 90 water bodies investigated, 52 (58%) were positive for *Anopheles sp* and 27(30%) for *An. darlingi*. Presence of *Anopheles sp* and *An. darlingi* were significantly associated with fish tanks (OR =4.34, 95% IC: 1.60-11.7) as compared to other water bodies (χ^2 = 8.9418, df=1, p <0.003). Among the 45 fish tanks, turbidity was a significant protective factor (OR =0.97, 95% IC :0.95-0.99) while mildly or heavily dense vegetation at the borders were risk factors (OR =1.013, IC: 0.99-1.03) for the presence of *An. darlingi*. Among the natural water bodies, only 15% were positive for *An. darlingi* and none of the investigated variables was significantly associated with its presence. Geographically, the distribution of *Anopheles* was heterogeneous, with greater density in the peripheric neighborhoods, the same presenting the highest Annual Parasite Index in January and February 2012. **Conclusion:** This study suggests that the urban landscape of Mâncio Lima induces a spatial heterogeneity in the distribution of the malaria vector and that fish farming is an important component of this landscape. This landscape interacts with the immunological, behavioral and economical profile of the resident population to determine the spatial distribution of malaria in this region. **E-mail:** izabio2005@gmail.com

Mal042- Prevalence of anemia in malaria-endemic region (Mâncio Lima, Acre, Brazil)

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Introduction: According to World Health Organization (2008), anemia is defined as a state in which the concentration of hemoglobin in the blood is abnormally low as a result of the lack of one or more essential nutrients, whatever the origin of this deficiency. There are many causes of anemia, and malaria episodes can be one of them. Brazil is the country with the highest morbidity attributed to malaria in the Americas. Urban populations of Amazonian cities are in a context of possibly overlapping of several important factors in the etiology of anemia, being malaria one of them. **Material and Methods:** The study area comprises the urban area of Mâncio Lima (7 districts), in the state of Acre. This cross-sectional study involved 1198 individuals (of the total of 15 206 inhabitants) distributed in 347 households, selected randomly from the register of PSFs to ensure a proportional random probabilistic sampling by district. Individual and household questionnaires were applied and venous or fingertip blood was collected of each individual subject for quantification of hemoglobin by HemoCue® hemoglobinometer (Ängelholm, Sweden) and thick smear preparation. For the definition of anemia the WHO definition (2008) was used. Data on malaria in the year 2011 were taken from Epidemiological Information System. The prevalence of anemia and malaria was stratified by district and compared using the Pearson correlation test. The frequencies and association between anemia and neighborhood, age, sex and type of home was tested using the Pearson's chi-square test or Fisher's exact test. **Results:** The general prevalence of anemia in this population was 6.8%, distributed in 20, 17% of the households), being higher in children under 5 years (21.21%) and those over 65 years (17.8%). The prevalence of anemia in the districts ranged from 3.77% (Bandeirantes district) to 8.27% (Cobal district); the frequency of malaria ranged from 14.22 cases / 100 inhabitants (Bandeirantes district) to 91.68 cases /100 inhabitants (Iracema district). The data suggest no association between anemia and malaria in the 7 districts studied. No association was found between anemia and gender ($p = 0.43$), type of household ($p = 0.076$) or neighborhood ($p = 0.76$). However there was a strong association between anemia and age ($p < 0.001$). **Main Conclusion:** The results showed that malaria isn't the main cause of anemia in the study area, and other factors such as helminthic infections and nutritional deficiency of micronutrients, especially iron, may be the cause of anemia. The higher prevalence in children of preschool age may indicate a strong nutritional deficiency and high prevalence of parasitic infections. On the other hand, the high prevalence in the elderly may be caused by common comorbidities in this age group, in addition to nutritional deficiencies. **E-mail:** alcant_junior@hotmail.com

Mal043- The importance of shared and decentralized management of surveillance and control of malaria in the municipality Lima Mâncio/AC, involving the three spheres of government

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Introduction: The city of Lima is enabled Mâncio full management of health care SUS, since certification in 2005, however the management and decision making is done in an integrated manner involving all three levels of management. Malaria is a major public health problem among the municipalities of Juruá Valley, a region that contributes 85% of the state, but this reality has been changed thanks to the commitment of managers at the federal, state and municipal levels, provided by actions that involve

planning, monitoring and evaluation. In this sense it has been to demonstrate the experience of the strengthening of local management for malaria control actions in a shared manner. **Material and Methods:** Situated in the western state of Acre, Mâncio Lima has 15,785 inhabitants and is 658 km from Rio Branco. **Results:** We conducted a descriptive analysis of the functioning of PCM in the municipality's actions are performed by a team of 44 Agents Health Surveillance (AVS), 20 microscopists and a manager. It has 14 laboratories for diagnosis and treatment distributed among the inland areas, urban, indigenous and rural areas. The bond is split between the professional city employees, state and Ministry of Health (MOH). There was a great advance in physical structure and organization of work processes from the outbreak experienced in 2006. Expansion of network diagnostic and treatment provision in a timely manner, in less than 24 hours of collection of the blade, is one of the highlights in this process of integration and qualification of the shares. The decision is based on routine analysis of epidemiological information system and other data generated locally. The action planning is based on physical capacity and operational and adjusted to the needs of each locality in order to focus the actions and optimization of time and resources. The continuous monitoring of the actions of early detection of cases and appropriate treatment for each type of malaria, monitoring the patient for verification blades healing, thorough investigation of the suspected infection site in conjunction with the municipal boundary, an active search for patients to detect asymptomatic patients and suspects, residual spraying in areas of high risk stocks allied health education and social mobilization are performed routinely. Supervision and evaluation promoted by the State Department of Health and MS are also made periodically. Recently the city was selected to receive funding from the Global Fund. It is noteworthy that in 2007 the city was a pilot for the study of the use and implementation of bed nets impregnated with insecticides of long duration (Milds) **Conclusion** With these measures Mâncio Lima has shown a gradual reduction of cases. Therefore, the model of decentralized management and shared favors all involved actions in addition to the improvement of the NHS and therefore the quality of services provided to people affected by the disease. **Email:** neilsonmelo2010@hotmail.com

Mal044- Extension of measures for prevention and control af malaria among three integrated management nives, in Rodrigues Alves, AC.

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Introduction: Malaria is a major public health problem in some municipalities of Acre State, including Rodrigues Alves is considered by the Ministry of Health (MOH) for scaling up priority actions that are under the responsibility of the State Secretariat of Health of Acre, because the municipality is not yet certified, however, the municipal and federal governments has contributed in an integrated way in these actions. The objective is to analyze the expansion of prevention and control of malaria and the interference of three levels of NHS management in the results of actions in the city. **Material and Methods:** We analyzed epidemiological information system www.saude.gov.br/sivep_malaria, and indicators of structure, process and results of the Malaria Control Program (MCP) in the city which lies in the Vale with the Juruá 14,100 inhabitants. **Results:** in 2011 were identified 3383 cases of autochthonous malaria with 22% reduction compared to 2010. The risk of infection is still considered too high, however this indicator decreased, reaching 245 cases per thousand inhabitants. Over 90% of cases are in rural areas and disease control in settlements represents a major challenge, since more than 70% of cases occur in this category. There are two species of malaria, *Plasmodium vivax* the most frequent (81.5%) and *P. falciparum* (18.5%), of greater concern because it is more serious and more difficult to control, especially in pregnant women who are most affected by falciparum (24%). Active surveillance (BA) can reduce the time between onset of symptoms, diagnosis and treatment is directed to high-risk localities. BA is represented with 67.7% of the total 81,101 slides examined by the network composed of two diagnostic laboratories in the urban area and 14 in rural areas. The percentage of 84% of cases were diagnosed and treated within 48 hours of symptom onset. With the deployment of about eight thousand mosquito nets impregnated with long-term (Milds) in 2010 the shares were reduced residual spraying, however the actions of environmental management on vector breeding sites, in order to prevent the

development of adults were conducted with support from City Hall and population. The actions of health education were conducted through partnerships between institutions using materials flip charts, data and video addressing the issues surrounding the disease contributes significantly to the effective membership of milds. **Conclusions:** The main control strategies were defined and differentiated according to local characteristics. The success can be attributed to reaching strengthening of local capacity of health services to understand the dynamics of transmission and thus guide, more effectively, program interventions and actions of the regular PCM. To achieve good results in controlling this disease is important that responsibilities are shared between the three levels of government and the population. **E-mail:** helio.cameli@gmail.com

Mal045- *Plasmodium falciparum* malaria in the municipality of Cruzeiro do Sul (Acre state, Brazil) in 2010: the use of control chart at the districts level.

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Introduction: Despite advances in malaria control programs, the incidence of the disease remains high in some municipalities of the Brazilian Amazon Region and improvements in surveillance strategies are needed. This study aimed to analyze the usefulness of the control chart, as a malaria surveillance tool, to improve the early detection of variation in the *Plasmodium falciparum* incidence rates in the municipality of Cruzeiro do Sul in 2010. **Method:** The control chart was based on quartiles of the *P. falciparum* incidence rates from 2003 to 2009, in Cruzeiro do Sul municipal districts (n = 14). Based on the results, districts were classified into three groups: 1) those with *P. falciparum* incidence rates lower than expected; 2) those with *P. falciparum* incidence rates within the expected range; and 3) those with *P. falciparum* malaria epidemics (incidence rates higher than expected). **Results:** The incidence of *P. falciparum* malaria was higher than expected in two districts (4 and 5). In District 4, twelve months of the year a epidemic condition was detected. In District 5, the epidemic started in October of 2010, although during months of May and June high incidence rates of the disease were also observed (above the expected values). These results indicate that *P. falciparum* malaria is not responding, as expected, to the control measures in these areas. District 2 is also noteworthy, as the malaria incidence rates remained above the expected values in December of 2010, possibly announcing a resurgence of the epidemic in 2011, already seen in previous months (from January to August of 2010). On the other hand, values below the upper limit were registered in November and December in the remaining 10 districts (1, 3, 6, 7, 8, 9, 10, 11, 12 and 14), revealing absence of *P. falciparum* malaria epidemics. Among those, Districts 6, 8, 9 and 11 should be noted, as the incidence rates of the disease presented values below the estimated lower limit in the last months of the year. This indicates a better-than-expected response to control measures or environmental conditions. The district 13 had no case recorded in December of 2010, and the estimated lower control limit equals to zero, did not allow for registration of reduction in the incidence of the disease. **Conclusion:** The magnitude of the *P. falciparum* malaria rates and the observation of periods of epidemic caused by this *Plasmodium* specie in 10 of the 14 districts of the municipality in 2010 disclosed the need for better understanding of the epidemiological situation and control measures of *P. falciparum* malaria in this region. To improve the epidemiological surveillance of malaria, it is recommended the use of the control chart as a routine activity undertaken in the municipality. **E-mail:** rui.braz @ saude.gov.br

Mal046- Malaria in the state of Amapá: the epidemiological position in the periods of 2007 to 2011

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Malaria remains a severe Public Health issue and is still endemic in tropical regions in Africa, Asia and the Americas. In Brazil, 500 thousand people contract the disease every year and 99,9% of the cases happen in Legal Amazon. The state of Amapá is located in this region, thus standing out as an area of endemic transmission. In this context, this study has the objective of analysing the Malaria distribution in the state of Amapá, having as a temporal reference the periods of 2007 to 2011. The exploratory descriptive study was used to support the data analysis obtained by the Epidemiological Vigilance System (SIVEP). The program Excel version 2007 was chosen to perform the treatment of the data and the results were used to generate the evaluation graphs. The results have shown that in the period of the study the cities of Porto Grande, Pedra Branca do Amapari, Serra do Navio e Oiapoque presented the highest incidence of Annual Parasitic(IPA), where the autoctonia was predominant and the species that affects this population is the *P. vivax*. It was noted that the age zone most inflicted was the 10-29 years old, being prevalent in the male sex. September and November were the most occurring months, which coincides with the drought season favoring the formation of anopheles breeding. It can be concluded that even with the policies aiming towards the Malaria control, it still presents alarming growth rates and the residing population is exposed to the risk of being struck by the disease due to the unfavorable social conditions and the environmental factors. **Keywords:** Amapa. Epidemiology, Malaria **E-mail:** agro.paulo.vieira@gmail.com

Mal047- Descriptive analysis of the impact of malaria in the indian villages along the municipality of Oiapoque in the BR156-AP 2008/11

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Introduction: The study aim epidemiological analysis of malaria in the period of 2008/11 based in Índice Parasitário Anual (IPA), registered in Indian villages in the municipality of Oiapoque, situated along BR156, observing and correlating this indicator depending of the location of Indian village in relation to proximity or distance of the highway that links Macapá to municipality of Oiapoque, area bordering with French Guiana **Material and Methods:** Data was obtained with Fundação Nacional de Saúde (FUNASA) / Distrito Sanitário Especial Indígena do Amapá – DSEI-AP and Sistema de Informação de Vigilância Epidemiológica – SIVEP-MALARIA, related to the period of 2008 to 2011. The Indian villages, object of study, are located in municipality of Oiapoque, in the geographical mesoregion of northern state. Was performed descriptive analysis from the tabulation sheets in *Microsoft Excel* and translated into graphics with statistical analysis using *BioEstat 5.0* **Results:** The IPA of Indian villages in the region according to an annual distribution, was: in 2008 (268,8/1000hab.), 2009 (299,7/1000hab.), 2010 (236,4/1000hab.) and 2011 (334,0/1000hab.), corresponding to a total of 7.429 examined plates with 7.488 (99,5%) positives, with prevalence of *P. vivax* (80%), *P. falciparum* (19%), mixed malaria (F+V) (1%) and *P. malariae* (0,01%). The average of (IPA/1000 hab.) observed in Indian villages located near BR156: Cariá (832,3) Curupí (513,2) Santa Izabel (180,0), Galibi (435,6) Piquiá (296,7) Arumã (242,0) Samaumá (119,5), Estrela (204,0) e Manga (318,0); from remotes villages of the highway : Flexa (50,8), Encruzo (66,2), Açaizal (97,6), Kumenê (379,3) e Kumarumã (153,7). Evidencing, in principle, an increase of cases with high risk by the IPA standard, considering the period of study **Conclusion:** It is noted that villages along BR156 have an elevated IPA considering the ones that are more internalized, perhaps associated with greater exposure due to intensity of road mobilization population, considering that the majority of the cases of malaria are originating from the villages along the highway. The most internalized presents IPA with classification (low and medium), emphasizing that two villages (Encruzo and Flexa) do not presented any case of malaria in 2008 and 2011. Therefore, it reflects the profile of malaria in the state that in the biennium 2010/11 had an increase of 34% of cases. **E-mail:** ericoliveira_351@hotmail.com

Mal048- Study about cases of malaria registered in the indigenous area of the city of Oiapoque - Amapá

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Malaria is a complex disease, because for it to occur is necessary the interaction of three elements: the parasite, vector and the parasite. Since the socio-environmental dynamics is present and exerts a strong influence on the relationship between the vector and the man, whom can define if an area or region is endemic or not. The area of the Amazon is considered an endemic region because it concentrates approximately 99% of registered cases in Brazil, where the socio-economic and environmental situation favor the proliferation of the *Anopheles* mosquito, vector of the disease. On indigenous lands (IT) of the State of Amapá, located in Oiapoque, this endemic remains with high incidence, accounting for 57.75% of notifications of malaria recorded in 2009. In this context, this study turned to this area in order to describe the distribution of cases in the period of 2003 to 2010, using the available data supported by the Epidemiological Surveillance System for Malaria (SIVEP). The Geographic Information System (GIS) was used to spread the autochthonous cases detected in the field in the period of 2010 to 2011. To support the research, the method used was descriptive observational study with qualitative and quantitative approach. From the selection of villages using the Annual Parasite Incidence (API) and geographical location, the villages chosen were Caria, Kumarumã, Kamuywá, Uahá and Holy Spirit. Thus, the data obtained about these villages contributed 35.80% of the autochthony and *Plasmodium Vivax* was present in 88% of cases. It was observed a reduction of the IPA in the period of 2009 to 2010, however, the village Kamuywá continued growth of this indicator with 1035.29/1000 inhabitants. For the genre, the field survey showed that the male was most commonly reported in the villages Kamuywá and Kumarumã with 83.33% and 75% respectively and that the female was more present in the village Caria with 70% of cases. It is noteworthy that most reports were of male patients aged between three and 15 years old. The results showed that subsistence activities and leisure time conducive to the blood repast of the *Anophelinos* and the shy performance of the Indian Health Agents (ISA), in community outreach to promote health prevention were determining factors for the maintenance of malaria in these areas. It was also found that the imported cases identified "in loco" referred to other villages with restricted location of the IT of Oiapoque, which confirms the autochthony in the region. **Keywords:** Amapá, Indigenous Area of Oiapoque, Malaria, spatial spread. **E-mail:** agro.paulo.vieira@gmail.com

Mal049- HIV & Malaria co-infection in the Brazilian Amazonia: the effect of HIV on malaria clinical case

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Introduction: HIV infection causes a severe deterioration in the immune response of man and its effect in patients with malaria affects the premonition in adolescents and adults, represents a higher risk of infection, higher parasite density in infected, severity of the clinical picture and less therapeutic response to antimalarials. **Materials and methods:** in order to determine the effect of HIV/AIDS on the clinical picture of malaria, clinical, parasitological and laboratory data were compared among people living with HIV/AIDS with malaria and Malaria patients seen at the Foundation of Tropical Medicine (FMT -HVD) in Manaus. **Results:** The mean of the number of episodes among people living with HIV (PLH) was 1.7 ± 1.5 , very close to the average of episodes among people living with AIDS (PLA) that was 1.7 ± 1.1 . Parasitic recurrence occurred in 26.7% of people living with HIV / AIDS (PLHA) being of 28.3% among the PLH and 40.17% for PLA. The episodes occurred in PLHA were compared with those occurring in 228 patients with malaria who accepted the invitation to participate in the study. Regarding gender, there was a high prevalence of malaria cases in men in both the sample of PLHA (68.2%) as in patients with malaria (68.4%). The mean age was 38.70 ± 10.9 years among PLHA and 36.74 ± 12.22 among patients with malaria. *Plasmodium vivax* was the cause of 83.3% of malaria cases in PLHA and 96.5% of patients

not coinfecting. With regard to hematological parameters, Hb and Hto were very close between the two groups of patients (12.15 ± 1.97 vs. 12.29 ± 4.4 and 33.2 ± 5.06 vs. $36, 46 \pm 12.91$ in PLHA when compared with malaria patients not coinfecting, respectively). Thrombocytopenia was a common event in both groups and its intensity was higher among patients not coinfecting when compared to PLHA ($108.389,73 \pm 77.255,04$ vs. $115.877,78 \pm 77.492,3$, respectively). **Main Conclusion:** Except to the difference in the frequency of *P. falciparum* more frequent among co infected patients, but very similar to that observed in spontaneous demand of the FMT-HVD, and a small difference in platelet count slightly lower among patients with malaria, no other differences could be established in patients co-infected when compared to patients with malaria. **Financial Support:** UNESCO 296/06; FAPEAM. **E-mail:** florespinosa@gmail.com

Mal050- The relationship between anthropometric indicators of nutritional status and malaria infection among children in a rural community in the Brazilian Amazon

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Introduction: Malaria is probably the infectious disease of greatest impact in the Amazon Region. Over the past five years, the Amazonas state had the highest number of reported cases in Brazil. Little is known in Latin America about the relationship between malaria infection and nutritional status. The objective of this project was to understand the relationship between malaria infection and nutritional status in children from an endemic area in the outskirts of Manaus (Brazil), in the Municipality of Careiro (Amazonas state). **Materials and Methods:** We conducted a three-year prospective follow-up of children aged 0 to 14 years residing in the study area, with four cross-sectionals for active detection of malaria infection by thick blood smear and blood samples on filter paper to perform molecular diagnosis (PCR). Nutritional status yearly evaluation was performed by anthropometric measurements, with the parameters of height/age (H/A) and BMI/age (BMI/A) using the “z score” values. Between the cross-sectionals passive detection of malaria by thick blood smear of symptomatic children was regularly performed. The thick blood smears were reviewed twice and data were recorded on standardized questionnaires. **Results:** We followed 161 children from 0-14 years old. Eighty-nine children (52.3%) were male and 72 (47.7%) female. Forty-two children (26.1%) were under five years old. Regarding nutritional status, 65 (40.4%) were malnourished. During the study period, 42 (26.1%) had at least one episode of malaria. In evaluating the parameter H/A, 120 (74.5%) had worsening of nutritional status, 26 (21.6%) were under 5 years and 94 (78.4%) were between 6 to 14 years. By using the BMI/A, children over five years also had a greater deterioration of nutritional status. When analyzing the deterioration of the nutritional status according to the occurrence of at least one episode of malaria, there was a decrease on the H/A among children aged 6 to 14 years (0.067) and no difference among children below 5 years age. **Conclusions:** Children affected with malaria have worsening of nutritional status and in this study this situation was more frequently observed in children over five years. **Funding:** PRONEX (CNPq&FAPEAM) **E-mail:** marciaalexandre@gmail.com

Mal051- The Quantiles Technique in the relation between Malaria X Precipitation in four Legal Amazon cities: from 1970 to 2005

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Malaria, as a disease found in tropical and subtropical areas, is a matter of public health, once it is one of the most important parasitic diseases around the world. Brazil contributes to this statistics by having the highest number of incidences. In 1999, 637.474 cases in this country were notified, 99% of them occurred inside the Legal Amazon Region. However, in 2004, it was noticed a reduction in Malaria cases, as a result of health policies, especially in cities classified as high-risk ones (Annual Parasitic Incidences – API

- above 49,9 cases/1000 inhabitants). The state of Pará, located in the Brazilian northern region, contributes to the highest incidence within this country. In this paper, it was focused the categorization of Malaria range occurrence and precipitation by the Quantil technique to four cities in Pará. **Material and Method:** This study was retrospective and ecological. The Quantil Method was applied to Malaria and precipitation data. A series of 35 years of annual data (1970 – 2005) was used to validate the theory, and five categories of Malaria incidence rate and precipitation for each city were established. Four representative cities from differentiated rainfall regions in the state of Pará were selected. Selection criteria were the following: high Malaria incidence, the fact the cities are located in relatively distinct rainfall regions, and their available historical data. According to Malaria Data from the State Department of Health, the cities of Anajás, Itaituba, Santana do Araguaia and Viseu were selected. Precipitation data were supplied by The National Water Agency (ANA: Agência Nacional de Águas) and by the National Institute of Meteorology (INMET: Instituto Nacional de Meteorologia INMET). **Results:** By applying such method, a new epidemiological classification to five different categories in each of the four studied cities was obtained. By comparing these values to the FUNASA classification (2001), significant differences in the cities' epidemiological index were found. Anajás presents the higher value in "very high" API category (>391). In "high risk" and "very high risk" categories, Anajás and Santana do Araguaia present the highest values. Viseu is the city which presents the lowest API categorized values. In precipitation series, Viseu showed precipitation around 1990-2392 mm/year, being the second in highest annual precipitation volume, exceeded by Anajás. In Santana do Araguaia and Viseu, the highest Malaria incidence index occur in water deficit years. **Conclusions:** Understanding Malaria incidence and control in the Amazon Region is a challenge to all political and social fields. Among all environmental variables, precipitation alone does not explain fluctuation in the number of cases. Multifactorial investigation must be applied to a better enlightenment to the factors that contribute to the endemic disease. **E-mail:** adressatp@ufpa.br

Mal052- Cost of illness attributable to malaria in the city of Manaus, Amazonas.

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Introduction: In the last decade, endemic malaria framework has intensified in the west, north and east areas in Manaus. The survey estimated the monetary cost attributable to malaria in Manaus, capital of Amazonas. **Material and Methods:** The cost of illness was estimated by the sum of two components: (i) hospital costs collected in the website of the Ministry of Health, (ii) values of lost workdays resulting from the stay in hospital bed calculated based on data from the worker population average earnings with more than 14 years of age available on the website of the Ministry of Labor and Employment. In this calculation, it was also considered, for each year studied, the coverage of the population with private health insurance, plus the study population between the years 1998 to 2009. The datum was obtained on the site of the National Health Insurance. **Results:** In that period, 2,864 hospitalizations were recorded, representing 1.4% of hospital admissions due to malaria. In 12 years there was negative variation of 28.1%. In 2009 malaria was responsible for 0.3% of lost workdays and 0.2% of the costs of diseases attributable to environmental factors in Manaus, which in monetary values corresponds to US\$ 52,963.18 (accumulated in 12 years the figure was US\$ 807,173.76, which represents a positive variation 49.8%). **Main Conclusions:** The hospitalization is indicated for patients with malaria caused by *Plasmodium falciparum* or with signs and symptoms of serious disease. The observed reduction is just the tip of the iceberg concerning the economic impact of this disease in society, because it estimates only the monetary impact of those individuals who needed medical care within the hospital environment. The recorded cases in the city ranged from 8,057 in 2001 to 80,009 in 2005. These oscillations show the difficulty of controlling malaria. In summary, we can say that the increase in cost of illness attributable to malaria in Manaus was due to: (i) the increase in population, due to the attractor characteristics of the resumption model of economic growth, initiated by free zone in the years 1979 and today the dizzying momentum of the Industrial Pole of Manaus, (ii) the low coverage of primary health care, (iii) and changes in risk factors of the city, linked to spatial nature problems explicit by socio-spatial inequalities of housing, reflected in the expansion of the city on the edge of the forest either by spontaneous occupations or

targeted occupations of the government designed into new housing. E mail: muribeka@amazonia.fiocruz.br

Mal053- Urban malaria time-space analysis in the state of Amazonas Brazil [2003-2011]

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Introduction: Urban Malaria transmission controlling, in the Amazonian region, has been one of the great challenges facing both technicians and managers in state or municipal malaria control programs. The forest occupation process lacking both guidelines and official consent, coupled with the exposed population's socioeconomic conditions has been one of the great catalysts for the persistence of malaria transmission in these areas. **Objective:** To demonstrate urban malaria transmission dynamics by means of time space analysis from 2003 to 2011. **Methods** Epidemiological data were available in site www.saude.gov.br/sivep_malaria - referring to 2003- 2011. The used population data and cartographic basis were made available by IBGE. ArcGis 10.0 software was used for risk areas spacing as well as their aggregation into High Risk [IPA>50.00]; Medium Risk [IPA>10.00≤50.00], Low Risk [IPA≤10.00>0] and No Transmission [IPA=0]. **Results:** From 2003 to 2011, 225,020 urban malaria cases, were recorded and showed the following rates: 107,274 [47.30%] in Manaus; followed by Borba, with 16,803 [7. 41%]. However, Borba Township presents the highest epidemiological risk with mean IPA equal to 129.57‰, followed by Barcelos [115.07‰], São Gabriel da Cachoeira [77.73‰], Careiro [74.46‰] and Novo Ayrão [74.14‰]. Barcelos Township stands out among the rest them with IPAs equal to 358.69‰ [2006] and 270.43‰ [2007]. In 2011, only the townships of Borba and São Gabriel da Cachoeira persisted with IPA>50.00‰. When analyzing, falling hill with malaria in Amazonian urban areas, thematic maps, High Risk clusters (Hot Spots) stand out in Guajará [2005], Rio Preto da Eva [2005-2007], Novo Airão [2003-2005], Careiro [2004-2007], Uarini [2005-2009], Barcelos [2003-2007 and 2008], Borba [2004-2009 and 2011], Manicoré [2006], Ipixuna [2008-2009], Eirunepé and Borba [2008 and 2009]. **Conclusion:** Time space visualization of epidemiological events like malaria in the Amazonian region, becomes a major management decision making tool to be used by Health Care managers and Field workers since it enables them to understand the disease's transmission dynamics, by identifying what townships present higher risk for the population to fall hill from the *sirease*. Yet, when dealing with urban malaria transmission, one must space data into higher scales thoroughly detailing neighborhoods, hydrographic basin and any other space attributes, which may facilitate the demonstration of whatever harmful to heath events are being analyzed. **FUNDING:** FVS-AM/CNPq/FAPEAM-Rede Malária. **E-mail:** wagner.terrazas@hotmail.com

Mal054- Malaria in Rural Settlement of Rio Pardo, Amazonas, from 2006 to 2010

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Introduction: Malaria in Brazil is concentrated in the Amazon, where 99.5% of all cases occur. Despite the low mortality among adults, the morbidity of malaria caused by *Plasmodium vivax* is high, accounting for 85.3% of these cases reported in 2010. Usually it is the first disease to emerge due to the disorderly occupation of land affecting populations in areas of invasion at the periphery of cities, People River, agricultural settlements and gold miners. This summary aims to describe the transmission of malaria through a retrospective study of prevalence in a rural settlement in the Central Amazon. **Material and Methods:** The study area was the Rural Settlement of Rio Pardo which is located in the municipality of Presidente Figueiredo/AM, where dwellings are located on five dirt roads and a stream. We analyzed data from the Information System of Epidemiological Surveillance SIVEP-Malaria between 2006 and 2010. **Results:** Come from a total of 1,277 cases were reported from 582 individuals, distributed in 228 homes. The region is at high risk of transmission with an Annual Parasite Incidence (API) over 104/1.000 inhabitants in 2010, and 2006 of 866/1.000 inhabitants. The largest number of cases was reported between the months of September to November, coinciding with the onset of drought and rainfall in the

region, 88% caused by *P. vivax*. A total of, 44% of cases occurred in people aged between 15 and 45 years and 60% of reported cases were from males. The highest number of cases occurred in residents of stream, suggesting an association with possible breeding places of the malaria vector. Among the dirt roads, the more populous regions of Samuel and Cachacinha recorded fewer cases than stream, but Samuel, which is the nearest to stream of the two, had more cases than Cachacinha. There was a gradual decrease in the absolute number of malaria cases in the study period, with the most falls between 2006 and 2007, when there was a reduction of 45.4% of cases. **Main conclusions:** The data show a decrease in the number of malaria cases which was also observed in Brazil and worldwide, especially in the period considered. This can be partially attributed to the control actions, and especially that early diagnosis reduces the source of infection. It is concluded that although the malaria situation has substantially improved in the Settlement of the Rio Pardo, it remains a highly endemic area. **E-mail:** sergioluz@amazonia.fiocruz.br

Mal055- The burden of imported malaria in the municipalities of Brazilian Amazonian frontiers

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Introduction: Amazonian international borders are highly vulnerable areas for endemic diseases. Two elements are essential as determinants of this vulnerability: information and access to health services do not pass international borders, but pathogens, mosquitoes and diseases do it. Malaria is an important public health problem in these areas and one of the most important determinants of the disease is the long range of frontier with endemic countries. The aim of this study was to establish the burden of malaria in the Amazon Brazilian borders focusing in the imported malaria cases from the neighboring countries. **Methodology:** from 98 municipalities that form the Amazonian border areas, we selected the higher epidemiology risk ones. Using data from Ministry of Health, the variables studied were number of malaria cases, annual parasitic index (API), number and percent of imported cases, probably origin country of the infection, and parasitic specie. Data of 2003 and 2010 were used. In order to understand the malaria problem, was used the Machado's classification of Brazilian frontiers: North arch: Oiapoque-Tucumaque, Campos do Rio Branco, Parima-Alto rio Negro, Alto Solimões, Alto Juruá, e Vale do Acre-Puru and central arch Madeira-Mamoré. Maps were built using SIG. Statistical analysis for category variables were done using χ^2 . Statistical significance was considered if $p < 0.05$. **Results:** There were 105.471 malaria cases in 2003 and 123.895 in 2010 in the 98 Amazonian borders municipalities; comparing these two years, there was an increment of 17.5%. The epidemiologic situation was worse in the Parima-Alto rio Negro, Campos de rio Branco, Alto Solimões and Alto Juruá regions. The prioritized municipalities were responsible by 60% of malaria cases in the Amazonian borders. In total, in the prioritary municipalities for malaria only 2.8% cases were imported from other countries. Important regional differences were found: 26.5% of Oiapoque, 25.9% of Plácido de Castro and 13.7% do Atalaia do Norte were imported from other countries. These differences were statistically significant comparing with the mean of other borders municipalities. French Guyana (1.412 cases, 60.5%), Peru (533 cases, 22.9%), Bolivia (299 cases, 12.8%) were the countries that exported cases to Brazil. *P. falciparum* was the parasitic specie in 30.9% of malaria imported cases. **Conclusions:** Different transmission intensity was found in the municipalities of Amazonian international borders. Contrary to Brazil, which had a reduction in 18.4% in the number of malaria cases between 2003 and 2010, the cases in the borders had increased in 17.5% on the same period. Surprisingly, only 2.8% of malaria cases were imported from other countries but some municipalities received high number of imported cases, especially from French Guyana, Peru and Bolivia. It is necessary to strengthen the international cooperation in these frontiers areas in order to elaborate programs to control and elimination of malaria. **E-mail:** marmutis@ioc.fiocruz.br

Mal056- Deployment of screens and treated nets as a tool for control of malaria in a community of the countryside of the state of Amazonas

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Introduction: The strategy to control malaria in the State of Amazonas is based on the incorporation of multiple control measures that aim to identify and adapt to the peculiarities of socioeconomic, cultural, political and ecological each region. The deployment of mosquito nets has proven to be an effective tool for malaria control; however, the effectiveness of this strategy is closely related to socio-cultural population. **Objectives:** The objective of this study was to evaluate the impact of the use of screens and bed nets impregnated with insecticide for malaria control. **Material and Methods:** The selected communities were divided into two groups: Group Intervention, consisting Jabote community with 172 residents, were suspended all vector control activities, keeping only the actions of diagnosis and treatment, were installed and impregnated mosquito nets and screens and specific actions of health education and social mobilization; the control group, formed by the communities of Old Lake, and Bio Miuá, which together have 159 inhabitants, were kept all routine activities recommended by the National Malaria Control, and (diagnosis and treatment, indoor residual spraying, insecticide application space, as well as actions of health education). During the execution of the project were monitored indicators entomo-epidemiological and socio-economic characteristics of the population, through the questionnaires CAP. **Results:** We found that the population knows the ways of transmission of malaria, since only 64.5% answered that the mode of transmission is by mosquito bites and 22.5% admit to not knowing the treatment of disease. It was also observed that only 19.7% of people use personal protection and that 94.5% of homes do not provide safeguards against the vector, being built with wood, with cracks and unfinished walls. In the first year of the project was an observed 92% reduction in the number of malaria cases in the Community Jabote, where in the control communities, the reduction was 45%. **Conclusion:** The implementation of the deployment of treated mosquito nets and screens associated with the actions of health education is presented as an alternative to the inadequacies of rural households with indoor residual spraying in malaria control functioning as a physical and chemical barrier that reduces the exposure of humans to the mosquito vector. **Keywords:** Malaria, PPACM, treated nets. **E-mail:** camila.fisio1@gmail.com

Mal057- Multiannual plan of integrated control of malaria prevention - PPACM: a successful strategy

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Introduction: Malaria is considered a major public health problem in the state of Amazonas. Due the gravity of the situation, the State Government, through the Foundation for Health Surveillance of the Amazon / FVS-AM developed the Multi-Year Plan for Prevention and Control Integrated Malaria - PPACM, which aims, in partnership, provides the implementation of a policy of prevention and control of malaria in Amazonas, integrated state and local governments for socioeconomic development, able to develop the technical reference and operational capacity, intensifying the support to municipalities, promoting and creating technical and administrative conditions for a sustainable, reduce and control the transmission of malaria. **Materials and Methods:** The project covers 62 municipalities, prioritizing 41 cities selected by epidemiological criteria, social and economic. The priority municipalities formed a stratum corresponding to 83.64% of the population, accounting for 94% of the incidence of malaria in the state. Once defined the priority municipalities, preceded to the elaboration of municipal plans, with stratification, identification and characterization of malarious areas. The financing of the actions agreed in three ways, especially among federal, state and municipal levels. The program is monitored and evaluated continuously at both the municipal and state level, primarily through a federal program computerized SIVEP-Malaria, available to all municipalities. **Results:** The actions foreseen in PPACM is a macro policy of the Government of the State of Amazonas, impacted in a sustainable manner in the transmission of malaria by redirecting and implementing the policy of surveillance, prevention and control

of malaria. The project followed the guidelines of the National Malaria Control strategies adapted to the epidemiological profile of the state. In 2011, were trained 7339 professionals in prevention and control of malaria, there were 29 workshops insertion/ integration of surveillance activities in health in primary care teams, involving more than 1,400 health care professionals were deployed 231 new laboratories, expanding about 33% of network diagnosis of malaria, in addition to the restructuring of 269 laboratories, and were distributed and installed 75,000 treated nets, etc. **Conclusions:** Considering the transmission of malaria and the impact of product development policy on the environment and the population is that much of the conditioning factors of the disease outside the governance of the health sector, this plan focused its main strategy in the interaction and intersectoral institutional. Focusing therefore on the expansion of governance in the health sector over the control of malaria transmission, continuously mobilizing sectors of government, private enterprise, as well as its own population, and promoting decentralization of the implementation of actions to municipalities, with a strong support for logistics and technical structuring of local health departments. **E-mail:** camila.fisio1@gmail.com

Mal058- Technical assistance in shares of surveillance and control of malaria as a key component in reducing transmission of malaria in the Amazon in the period 2008/2011

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Introduction: The State of Amazonas, the largest Brazilian state by area, where the biggest health problems is Malaria, reported 202,954 cases (43% of the notifications in Brazil) In 2007, IFA of 18.2% and 35 municipalities (56%) at high risk position, with IPAs ranging from 50 to 737 cases /inhabitant. Given the seriousness, FVS-AM implemented a successful plan - Multi Annual Plan for Prevention and Control of Malaria/PPACM - from 2008 to 2010, and an improved second edition of PPACM 2011/2015 is still being implanted. **Materials and Methods:** According to the needs of FVS, a service provider named Bioamazonas Imp. and Exp Ltda, hired and made available to FVS 41 technicians and after technical training in surveillance and control of malaria, they were allocated in prior municipalities, at first during a period of 1 year and renewable for another 4 years, with advisory assignments to Municipal Health Office, development and implementation of municipal PPACMs, and technical support to empowerment of the municipalities, seeking to fill a gap left by the enactment of the ordinance of the MS No.1399/99. In general, each technician is at the municipality and can be relocated to any county of the state financed by the service provider. This outsourcing system might work till a public tender be launched by the State Government. **Results:** That strategy linked to others, became fundamental to achieve the established targets set by PPACM, with 70% of reduction in malaria cases in the period from 2008 to 2011 compared to 2007, only 14 municipalities are at high risk rate, in the segment 50 to 182 cases/inhabitant with IFA decreased to 8%. The strategy has contributed to the growth of technical empowerment of the municipalities which have developed greater governance in solving the determinants and constraints of malaria and growth of institutional participation and intersectoral, providing to the labor market technicians trained in surveillance and control of malaria, able to meet the demand from the private sector. **Conclusion:** In 1999, the responsibility for implementation and coordination of control actions is assigned to the states and municipalities. Even the qualified and experienced FUNASA teams in the eradication strategy, were not enough to train the municipalities. Therefore, there are needs to find strategies for technical and technological empowerment of the municipalities, important to the development of local programs, consistent with National and State guidelines, capable of facing and sustainably the challenge of malaria control under the risk of seeing in a short time the Malaria Control Program in Brazil, aimless and completely mischaracterized. **E-mail:** romeo_fialho@yahoo.com.br

Mal059- Experience of malaria control in Careiro city/ Amazonas from 2007 to 2010

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Introduction: The Careiro city, with a population of 31,000 inhabitants, in 2007 reported 10,512 cases, being a priority for the deployment of the Multi-Year Plan for Integrated Prevention and Control of Malaria (PPACM)- 2007 to 2010. In 2010, there were only 1,134 cases of malaria, being a reduction of 89.2%. This reduction resulted of many strategies of work schedules, according to the local reality, like: the Annual Operational Plans (POA), the epidemiological objectives, structuring and operational planning and rational management of strategic raw materials, etc. **Objectives:** of this study was made to describe the lived experience of the city of Careiro developing PPACM by: Plan, support, propose, implement, monitor and evaluate the technical and administrative structures of the municipality, aimed at meeting the goals and objectives established by the program; Streamline feasibility of logistics for the proper performance of surveillance activities and control of endemic diseases resulting in a reduction of malaria cases in the city of Careiro. **Material and Methods:** The study was conducted in the Municipality of Careiro the period 2008 to 2010. Was used as a data source Information System for Epidemiological Surveillance of Malaria (SIVEP_malaria) Bulletins and Field team municipal control of endemic diseases. From the data collected were designed strategies such as stratification of transmission areas, the program targeted vector control actions; Expansion and decentralization of diagnosis, and optimization of logistics. **Results:** The implementation of PPACM provided a hiring an outside company which has allocated top-level technicians with expertise in planning and implementing programs for public health and vector control, where the logistics strengthened through monthly supply of basic inputs and allowed the city manages has acquired greater support and better development in prevention, surveillance and control of malaria, clearly observed through the epidemiological indicators of the Plan, with a reduction of cases compared to previous years (2007,11%; 2008,17%, 2009, - 63.5% and 2010, with-40.5%). **Conclusion:** We can observe that despite some difficulties such as inadequate transportation, lack of breeding interventions in rural, low coverage of indoor residual spraying of insecticide used and the refusal to have interfered directly in the shares was possible to achieve the goals set PPACM. Moreover, it is note worthy partnerships through institutions such as (FMT, INCRA, USAID, the Municipality of Careiro) that contributed to the better performance of the municipality of PPACM Careiro. **Keywords:** PPACM; Malaria; control. **E-mail:** camila.fisio1@gmail.com

Mal060- Survey of *Plasmodium* sp. in monkeys native to the Western Amazon region

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Introduction: Of the five species that cause human malaria, four have counterparts in monkey. Questions have been lingering for decades if there is a reservoir role of monkeys harboring the human parasites sisters. Answering these questions stumble upon in the difficulties of collecting animal blood samples. Peculiarities in the region of Rondonia, with forested areas of ecological instability and in consequence, with human and monkey living in proximity, are allowing us to study these parasites and verify the hypothesis of their role as reservoir of human malaria. **Materials & Methods:** We analyzed monkey blood samples for the presence of the parasite using a nested PCR followed by sequencing. One hundred and forty-seven blood samples were collected from wild monkeys found in areas in Rondônia, of diverse environmental conditions and with humans living in proximity to the forest. We classified the different areas as follows: (1) areas with ongoing environmental impact, e.g., wood extraction (Manoa), construction of hydroelectric plants (Jiraú and Sto Antônio) and land occupation (Sto Antônio do

Canutama and Machadinho D'Oeste); (2) areas that have suffered environmental impact, e.g., tin mining (Flona do Jamari) and consolidated hydroelectric dams (Samuel and Rondon II); (3) areas in which monkeys are kept in captivity and close to urban areas, e.g., the Ecological Park and animals seized by IBAMA. **Results:** The frequency of *Plasmodium brasilianum* as identified by PCR and sequencing, was 8.8%, of which 2% showed an apparent mixed infection of *P. brasilianum* and yet to be identified parasite species. Positive samples were from monkeys of the family Cebidae (*Ateles chamek*, *Callicebus brunneus*, *Cebus apella*, *Chiropotes albimanus*, *Lagothrix cana*, *Pithecia irrorata* and *Saimiri sciureus*). *Chiropotes albimanus* was the monkey species with the highest infection rate (33.3%) and *Cebus apella* was the species with the lowest (7.1%). All positive samples were from animals in the wild, except one kept by IBAMA. Further analysis of the samples using the *P. brasilianum* CSP gene based PCR and sequencing, resulted in the detection of at least two different parasite strains, each strain from animals at two different areas. We are further analyzing these results and building a database of gene sequences of malaria parasites found in monkeys native to the region. To this database we are adding gene sequences from parasites found in human malaria patients as well as in mosquitoes collected in the same areas. **Conclusion:** We have detected *P. brasilianum* in monkeys found in forests close to human dwellings. This proximity creates an ideal situation for verifying the hypothesis of monkeys as reservoir of human malaria. We are creating a database of parasite gene sequences detected in both hosts, which will serve as the basis for studying the transmission dynamics of the malaria in these hosts and verify the reservoir hypothesis. **E-mail:** maisaraujo@gmail.com

Mal061- **Comparison of asymptomatic *Plasmodium* spp. infection in two malaria endemic Colombian locations**

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Introduction: The study of asymptomatic infection by *Plasmodium* spp, could help to understand the transmission dynamics in regions normally having low transmission. The objective of this research was to compare the prevalence of asymptomatic infection by *Plasmodium* spp. in two Colombian locations with different transmission patterns, ecological and epidemiological characteristics, and their potentially associated factors. **Materials and Methods:** Cross-sectional studies were carried out with random probabilistic sampling in two endemic areas. Infection by *Plasmodium* spp was determined using blood thick smear and Polymerase Chain Reaction (PCR) on 212 persons from Tierralta (Atlantic Coast) and 207 from Tumaco (Pacific Coast). All patients answered a validated questionnaire in order to establish risk associated factors to asymptomatic infection. **Results:** In Tierralta, the prevalence of asymptomatic infection was 11.3% (95% CI 7.2-16.8) by blood thick smear and 16.5% (95% CI 11.5-22.9) using PCR, while in Tumaco, the corresponding values were 2.4% (95% CI 0.7-5.5) and 5.8% (95% CI 2.3-9.2) respectively. The predominant species in Tierralta was *Plasmodium vivax* and in Tumaco *Plasmodium falciparum*. PCR detected 61% more infections than microscopy. Potential risk factors for the presence of asymptomatic infection by *Plasmodium* spp by PCR, were being male [adjusted odds ratio (aOR) = 2.52, 95% CI 1.28-4.56], and the number of prior malaria events ($p = 0.01$). **Main conclusions:** There are important variations in the epidemiology of asymptomatic plasmodium infection in these two endemic locations. These results could help to explain the differences in dynamics of transmission in two Colombian endemic regions (Atlantic and Pacific Coast). Considering the importance of submicroscopic infections in transmission, the measurement of the prevalence of asymptomatic *Plasmodium* spp infection is suggested as part of the epidemiologic evaluation for malaria in areas of low intensity of transmission as Colombia. **E-mail:** zcucunuba@gmail.com

Mal062- Epidemiologic Aspects of Malaria in a Non Endemic Area, Distrito Federal, Brazil

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Introduction: Distrito Federal (DF) is currently considered a malaria non endemic area in Brazil. However, there is the potential of its transmission due to *Anopheles spp* mosquito presence and to existence of malaria cases originated from Amazonic Area that migrated to DF. Then, adequate epidemiological data and surveillance are necessary to early identification and treatment of malaria patients, preventing its transmission. **Material and methods:** This was a prospective study with malaria patients diagnosed by Distrito Federal Epidemiologic Surveillance Service in 2009 and 2010. They were interviewed using a pre-formulated questionnaire and underwent medical physical examination. **Results:** 33 patients were interviewed. About 79% of them were males and their mean age was 37 years (5-69). For habitation conditions, 61% inhabited complete walls houses; 67% lived nearby rivers, lakes and wells; 70% lived nearby woodlands. Malaria prevention methods used were repellent (65%), insecticide spray indoor (33%), mosquito net (30%) or chemoprophylaxis (18%). The disease was acquired in other countries in 11 (33%) cases and, in the other 22 cases (67%), it was acquired on the North Region of Brazil. A total of 11 (33%) patients reported a previous episode of malaria. Eighteen (55%) had been vaccinated against hepatitis B and 30 (91%) had been vaccinated against yellow fever, other common regional preventable diseases. Main activities being performed during malaria exposition were working (58%) or tourism (27%). **Conclusion:** Malaria patients profile is mainly young adult males coming from working travels from Amazonic Region - or from malaria endemic African countries - exposed to woodlands and water reservoirs nearby areas in conjunction with low attempt rates of malaria prevention. These features can help in clinical suspicion of malaria and hence in its early diagnosis, thus avoiding its transmission within DF. **E-mail:** joaobarberino@yahoo.com.br

Mal063- Clinical Aspects, Diagnosis and Treatment of Malaria in a Non Endemic Area, Distrito Federal, Brazil

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Introduction: Malaria affects around 50% of the population of 109 countries, causing a large economic impact. In Brazil, 99% of cases occur within Amazonic Area. About 80% of Extra-Amazonic cases were imported and come from Legal Amazonia, Paraguay, Amazonian and African countries. Distrito Federal is considered a malaria-free area; however, there is a potential risk of diffusion because of the presence of *Anopheles sp*. **Materials and methods:** This is a prospective study of malaria cases diagnosed by Distrito Federal Health Surveillance Service in 2009 and 2010. Patients were asked about symptoms, investigated about signs, and verified about diagnosis and treatment. **Results:** 33 patients were interviewed. The most common symptoms were fever (100%), shivering (29 cases - 88%), asthenia (29 cases - 88%), headache (27 cases - 82%), sweating (27 cases - 82%) and anorexia (27 cases - 82%). Other symptoms reported were myalgia (25 cases - 76%), dizziness (23 cases - 70%), weight loss (23 cases - 70%), nausea (21 cases - 64%), abdominal pain (21 cases - 64%), arthralgia (21 cases - 64%) and vomiting (15 cases - 45%). The most common signs were pallor (19 cases - 58%), dehydration (13 cases - 39%), jaundice (13 cases - 39%), hemorrhage (8 cases - 24%), splenomegaly (7 cases - 21%) and hepatomegaly (5 cases - 15%). The only complication observed was hypoglycemia. The most commonly suspected diseases in these patients before definite diagnosis were malaria (45%), dengue (36%), and influenza (15%). *Plasmodium vivax* accounted for 70% cases and *Plasmodium falciparum* accounted for 27% of cases; 3% had both etiological agents. The mean time between symptom onset and diagnosis was 8.84 days and the time interval between diagnosis and treatment ranged from immediately to up to 24 hours. All patients were diagnosed by thick blood film (TBF). The patients were treated with Chloroquine and Primaquine (vivax malaria) and Artesunate and Lumefantrine (falciparum malaria). There was no evidence of therapeutic resistance. **Conclusion:** There was an important

prevalence of symptoms highly suggestive of malaria clinical setting. Probably because of early diagnosis, there was not a high prevalence of splenomegaly, hepatomegaly or any other complications. Despite both quick diagnostic methods and treatment, clinicians must be aware of malaria occurrence in non-endemic areas in order to an early disease suspicion. **E-mail:** joabarberino@yahoo.com.br

Mal064- Frequency of asymptomatic carriers of *Plasmodium vivax* or *Plasmodium malariae* as determined by PCR in an area of very low incidence in Brazil

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Introduction: Almost all malaria cases in Brazil are reported from the Amazon region, with 307,000 cases in 2009. However, Extra-Amazonian autochthonous *Plasmodium vivax* infections have been reported in mountainous regions surrounded by the Atlantic Forest in Espírito Santo state. A follow-up of the incident cases coupled to blood sampling of dwellers in an area of 2 Km around the residence of each case, performed from 2001 to 2004, disclosed a prevalence of 1.5% and 0.9% of asymptomatic carriers of *P. vivax* and *Plasmodium malariae*, respectively. It is difficult to determine where the reservoir of these parasites is when one considers the broad area of transmission (5,343 Km²) and the low frequency of symptomatic cases (around 40 each year). In this study, we present the preliminary results of a 2 years follow-up of a dynamic cohort of dwellers in one of the Municipalities of transmission aiming to determine the frequency of asymptomatic carriers as a way to establish their role in transmission. **Material and Methods:** All the dwellers in an area of 2 Km around the residence of the first case of autochthonous malaria reported in 2010 were invited to take part in the study. After signing the informed consent form, each dweller who accepted to participate was submitted to an interview with questions about socio demographic data and travel history, and to the collection of a blood sample for smears and Multiplex PCR. Abdominal examination was also performed to check up for splenomegaly. After the first visit, visits of follow-up were performed at each quarter, with revision of the initial information, new blood sampling and new abdominal examinations, comprising the months of March, June, September and December. PCR was performed by the technique of Rubio for the initial screening followed by the technique of Snounou and Kimura for confirmation. **Results:** Ninety-two dwellers were initially included. Mean age plus/minus standard deviation was 36.7 ± 23.8 years, with a median of 32 years and interquartile range of 14.25 to 54.75. There were 49 males (53.3%). An initial prevalence of 3.4% was detected for both species (2.27% *P. vivax* and 1.13% *P. malariae*). In the follow-up visits, an incidence rate of 7/156.5 persons-years of observation was detected for both species, comprising 4/156.5 persons-years for *P. vivax* and 3/156.5 persons-years for *P. malariae*. For the eight dwellers that had positive results in any time (either prevalence or incidence), three had persistence of the carrier state for several visits (consecutive or not) while the other five became negative spontaneously. **Main conclusions:** It is possible that the transmission of malaria in the residual areas of Atlantic Forest outside the Amazon Region have been maintained by the asymptomatic circulation of parasites among the residents of such areas. In such a scenario, the clinical cases would be only the tip of broader contingent of affected individuals, justifying their apparent low incidence. **Grants by:** FAPES (45617600/2009) and FAPESP (10/50707-5). **E-mail:** fil.cris@terra.com.br

Mal065- Epidemiological and entomological aspects of malaria in the municipality of Aripuanã in the Amazon region of Mato Grosso - Brazil

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The present study aimed to describe the epidemiological and entomological aspects of malaria in an Amazonian municipality of Mato Grosso State, Aripuanã. **Materials and Methods:** This is a descriptive study of malaria cases and entomological surveys conducted in the municipality of Aripuanã, Mato

Grosso - Brazil between the years 2009 to 2010. Epidemiological datas were collected through the Information System for Epidemiological Surveillance of Malaria and entomological datas. Entomological survey was conducted in the municipality on the months: December of 2009, June and October 2010.

Results and Discussion: There were reported 412 cases of malaria in the municipality of Aripuanã on the years worked, with an Annual Parasitic Index (API) of 11.26 in 2009 and 9.7 in 2010, classified as medium and low risk of transmission, respectively. In epidemiological analysis stand out 68.20% of malaria cases were reported in males, 71.36% aged 15 to 59 years. In relation to the parasitic form, 83.74% of cases of malaria were *Plasmodium vivax* infections, 10.44% of cases caused by *Plasmodium falciparum*. Entomological found in surveys: *Anopheles darlingi* (9.15%), *An triannulatus* (15.57%), *An rangeli* (5.23%), *An oswaldoi* (11.19%), *Anopheles benarrochi* (1.72%), *An minor* (1.12%), *An deaneorum* (0.46%), *An albitarsis* (44.76%), *An peryassiu* (0.14%) and *Anopheles sp* (10.66%). The main vector species, *Anopheles darlingi*, presented females active through all night biting and that of 261 females dissected 63.60% were parous females (females that had at most one oviposition in life). **Conclusion:** The municipality of Aripuanã is considered endemic for malaria, precisely because it has vulnerability, caused by the presence, density and longevity of the main vector species, *Anopheles darlingi*, and because of the arrival of carriers of malaria from endemic regions. Therefore, it is necessary to detach efforts of malaria control programs to develop activities that ensure reduction of cases. **Financial Support:** State Department of Health of Mato Grosso. **E-mail:** sftgies@hotmail.com.

Mal066- Evaluation of different methods for capturing the main malaria vector *Anopheles darlingi* in a rural community in the city of Cuiabá - Mato Grosso - Brazil.

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Introduction: Malaria transmitters are represented by the genus *Anopheles* in Brazil and the main vector of this disease is *Anopheles darlingi*. The most common method and more efficient for capturing mosquitoes is the human bait, however, this work provides great exposure of the risk to contracting this disease. The objective of this study was to evaluate three different methods of capture the *A. darlingi* in a rural community in the city of Cuiabá - Mato Grosso - Brazil. **Materials and Methods:** The study was conducted in a rural location near Cuiabá city, Community of Rio dos Peixes, with three different methods of capture: Shannon trap, Mosquito Magnet Defender (MMD) and protected human bait with Castro manual suction. Sampling occurred from May (2011) to February (2012) for three consecutive nights each month, at the margin of the Rio dos Peixes (S 15 ° 25 '16.9 "W 55 ° 59' 10.6"); all sites were equidistant. For the larval survey was carried out the method to sample 30 times near on the river's margin. The insects were collected and stored for specific identification that occur in the Medical Entomology laboratory - UFMT, and was incorporated to the collection of the same laboratory. **RESULTS:** Until February of 2012 a total of 896 mosquitoes of the *Anopheles* genus have been captured: from this total 882 are *A. darlingi*. The months with the highest catch rates were May, June and July of 2011 with 560, 93 and 188 respectively captured insects. It was observed in this specific period warmer temperature in comparison to other months ranging from 23.3 to 26.3 °C and is apparently associated with this high density of mosquitoes. There is a positive significant statistical correlation between the Shannon trap and protected human bait ($r = 0.9942$, $p < 0.0001$) which captured 159 against 673 *A. darlingi* in human bait, coincidentally in the months of highest vector density. Although the same result in relation to the MMD and protected human bait ($r = 0.9632$, $p = 0.0001$) we observe that the MMD had only a total of 50 individuals captured. As for the immature observed only larvae of *A. argyritarsis*. **Conclusion:** According to our results, the Shannon traps may become an alternative tool for capturing *A. darlingi* when there is a high population density of this vector, but offers exposure to the professional at the time of capture and the MMD does not offer this risk to get results despite the lower number of insects. **E-mail:** adaianejacobina@gmail.com.

Mal067- Epidemiological evaluation of the profile of patients treated with malaria in a public health service, during the period of 2005 to 2010

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Introduction: Malaria is a potentially severe tropical infectious disease caused by parasites (protozoa of the genus *Plasmodium*). It is an endemic disease that causes more social and economic problems in the world. In Brazil, the Legal Amazon concentrates 99.9% of reported cases and their wide dissemination in the region, high incidence, morbidity and mortality impacts and the difficulty to control represents a serious public health problem. **Objective:** This study aim to evaluate epidemiological variables of patients with malaria treated at the Clinical Malaria Trials Program in the Evandro Chagas Institute, Health Surveillance Secretariat, Ministry of Health (PECEM/SEPAR/IEC/SVS/MS). **Material and Methods:** It was effected updates informations through review of medical register of patients with positive thick blood smear for malaria during the period of 2005 to 2010, which were stored in the EPIINFO 2007 database for analysis of gender, age and age group, *plasmodium* species, infections site, origin, number of cases per year, parasitological response in the eighth day after the starting treatment (D7) and negative control of parasitemia during the treatment. **Results:** The epidemiological assessment indicated that 56,3% (799/1420) of patients were male; 29,1% (414/1420) of people aged between 21 to 30 years. Highest number of incidents (25.8%, 367/1420) was in 2010. Most served treated proceeded from rural area reaching 85.3% (1211/1420). The mainly state where individuals acquired malaria was Pará (70.8%; 1005/1420), followed by other states or others countries, respectively 18,9% (269/1420) and 10.3% (146 / 1420). *Plasmodium vivax* was the predominant species (84.6%, 1202/1420), followed by *Plasmodium falciparum* (11.1%, 157/1420) and mixed infections (*P. vivax* + *P. falciparum*) in 3.8% (54/1420); information on control of cure in D7 showed that 72% of the subjects had gout thick negative for plasmodium research, however, in 27.8% (393/1420) of patients had no negative information on date of parasitemia during the treatment period (up to D7). **Conclusion:** The higher incidence of malaria acquired in rural areas is related to residence and / or work activities performed Amazon areas or other countries (Guyana) in which man is exposed to permanently contact with the infected vector. *P. vivax* remains the predominant species in the Amazon region. Educational policies on mode of transmission, treatment and cure consolidation need to be maintained and increased, aiming at reducing the number of malaria cases in rural areas and consequently in the country. **E-mail:** soniarodrigues@iec.pa.gov.br

Mal068- The epidemiology of malaria in Anajás, Pará, Brazil, between 2001 and 2010, in relation to the sixth millennium development goal

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Malaria is one of the diseases with biggest population impact on the planet, being a serious public health problem, especially in developing countries. This study analyzes the epidemiology of malaria in the city of Anajás, Marajó Island, PA, Brazil, to identify the factors associated with the potential accomplishment or not of the sixth Millennium Development Goal (MDG). Pará occupies a high place in malaria prevalence among the Amazonian States, being Anajás one of the greatest responsible for this. Between 2001 and 2010 over 99% of the population was affected, being this one of the highest indexes in the world. To be fully attained, the MDGs should be transformed in practice in each city, town, state and region of the country. Therefore, understanding the epidemiological situation and the reality of health politics in cities like Anajás is fundamental to know if Pará, and Brazil, will reach the proposed goals until 2015. It was observed that malaria is not only a public health problem, but also a political, social, economic, and ecological issue; and its complex nature makes its management impossible without an integrated action of agents from the three governmental spheres, the civil society and the health workers. From an epidemiological point of view, the city will not fulfill the sixth MDG by 2015. Opposed to what was expected, available official data indicate that there has been an increase in annual rates during the study period. Among the factors potentially associated with the findings are difficulty of accessibility to health

services, small investment in local public policies such as environmental sanitation and the Family Health Strategy, and absence of specific projects for malaria eradication, developed in accordance with the local reality. Other factors that contribute to this situation include a low HDI (0,592), elevated illiteracy, and accelerated and disorganized urbanization (38%). This reality, unfortunately, is not limited to the studied city. Brazil is the only country in the Amazon region that fulfilled the goal of reducing hunger by 50%, also reduced children malnutrition to below the average of other Latin-American countries. However, it made small advances in the control of malaria, particularly in Amazonia. Not only in Anajás, but in Marajó Island, and all Pará State, malaria will continue to be a grave threat to public health for many decades. E-mail: robertoenf21@yahoo.com.br

Mal069- Spatial-temporal analysis of environmental factors associated malaria incidence in the influence area of Belo Monte hydroelectric, Pará, Brazil, in the period of 2004 to 2009

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Malaria is a tropical disease of great epidemiological importance in the Brazilian Amazon. Its incidence have been related to great development projects, in the last decades, that affect environmental and socioeconomics relationships, that increase the occurrence of risk factors. In this context, the main propose of this study was to analyze the spatial distribution of malaria in the localities of Aldeia Paquiçamba (03°22'46.67"s and 51°45'16.54"w), Belo Monte (03°07'34.62"s and 51°42'03.40"w), Santo Antônio (03°07'23.79"s and 51°47'25.73"w) and Terra Arroz Cru (03°22'11.4"s and 51°57'15.0"w) in the direct influence area of Belo Monte's Hydroelectric at Vitória do Xingu (02°52'48"s and 52°00'36"w), Pará, Brazil. Those study places were selected by its environmental, socioeconomical and epidemiological differents characteristics, which will change after the project development. For this purpose were used databases obtained at SIVEP-Malária/Ministério da Saúde (MS), Amazon Deforestation Monitoring Project (PRODES)/National Institute for Space Research (INPE) and through georeferencing of the localities, where there were notifications of the disease. The database was created using all variables above. The results obtained showed that the location of Aldeia Paquiçamba had the lowest percentage rate of deforestation due it is a preserving indigenous area, as well a low incidence of malaria; Belo Monte, located near the BR-230, presented around 50% of deforestation caused to an uncontrolled urban expansion, and a high incidence of the disease; Santo Antônio had the highest rate of deforestation, by constant activities of cattle raising, but has the lowest number of confirmed cases due to low existing population; and Terra Arroz Cru that presented around 21% of deforestation rate and more than 60% notifications, this deforestation rate was considered low in compares to other study areas. Considering the analysis it was observed a direct relationship between the correlated variables in following study localities Aldeia Paquiçamba, Belo Monte and Santo Antonio, however in the Terra Arroz Cru locality this direct relationship was not observed may be due to the population's socioeconomic characteristics whose housing conditions favored more contact with the vectors of malaria, increasing its local risk factors. Finally, it was considered that the spatial-temporal analysis techniques used were satisfactory to understand the process of the disease establishment at the study area and also a new perception of the relation between the incidence of malaria and deforestation that is a very recurrent process at the Amazon, whose mitigation should be placed. E-mail: nelsoncg2009@gmail.com

Mal070- The Importance of Entomological Survey on Monitoring and Control of Malaria in Assentamento Sombra Santa, Placas, Para, Brazil

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Introduction: Based on the transmission cycle of malaria, *Anopheles* mosquitoes, especially *An. darlingi*, presents itself as an important entomological indicators are reported in areas where indigenous cases of the disease. For the Ministry of Health (MOH), the Information System of Epidemiological Surveillance - SIVEP / malaria, based on data obtained through the analysis of the system, there is the oscillation of the Annual Parasitic Index (IPA) and so the estimated risk of this disease in an area and / or locality specified time. **Methods:** Analysis of SIVEP/malaria within 4 years (2008-2011) linked to information from the survey carried out in the Assentamento Sombra Santa (S 03 ° 28,557 'and 54 ° 27,774'), city of Placas, state of Pará from 22 to 26/08/2011. All material collected was identified on home support in the settlement, with record of the location, date, time of capture and number of copies. **Results:** We collected 46 fourth instar larvae of the species *An. oswaldoi* (44), *An. nuneztovari* (01) and *An. mattogrossensis* (01). The (06) breeding sites had water temperatures around 26 ° C with pH variation 4-6, current moderate, cloudy water, shading at the margins, organic detritus on the surface, submerged vegetation and floating, and are classified mostly such as permanent breeding pond. Were captured in four residencies 1,169 specimens of *An. darlingi*, with 627 catches in two of four hours (18h to 22h) and 542 in the capture of 12 hours (18h to 06h). In catches of four hours were collected in 122 copies and 505 in indoor environment and peridomestic capture 12 hours were 193 specimens collected in indoor environment and 349 in peridomestic. **Conclusion:** Based on data obtained from the catches of winged it was found that the darlingi has dominance of territory with a high degree of anthropophilic, and is possibly correlated with high rates of malaria infection in Assentamento Sombra Santa, which is attributed the high susceptibility to infection by *P. vivax* and *P. falciparum*. In larval surveys not found the presence of this vector, but the existence of other species of *Anopheles*, which are not expressed in human dwellings in their adulthood. Therefore, it is important to the development and implementation of projects entomological monitoring to characterize the actual settlement. **E-mail:** elizangela991@gmail.com

Mal071- Cases of malaria diagnosed in Lacen Pernambuco – five years in the period

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Introduction: Malaria is an acute febrile infectious disease of transmission vector caused by protozoa of the genus Plasmodium. It is recognized as a serious public health problem in the world, reaching approximately 40% of the population of more than 100 countries. According to World Health Organization (WHO) about 300 to 500 million and approximately 1 million deaths occur each year. In Brazil nearly 500,000 cases of malaria are reported each year. They are distributed in two geographic regions: Legal Amazonia and the extra-Amazon region. Although most reported cases are from Legal Amazonia, some events drawn attention to the occurrence of cases in the extra-Amazon region, due to the possibility of reintroduction of disease in unaffected areas or where the damage has been eradicated. The state of Pernambuco, endemic in the 40's, presents today a comfortable epidemiological situation with malaria control; however it is a state with a massive influx of people from endemic regions presenting areas of susceptibility to the vector. **Material and Methods:** We conducted a survey in LACEN-PE database regarding the parasitological diagnosis of Plasmodium using thick and distended smear in patients with suspected malaria, sent to Central Laboratory by hospitals or by spontaneous demand, between 2007 and 2011. **Results:** In 2007, 58 (31,02%) of 187 suspected patients attended were positive; in 2008, 74 (28,69%) of 258; in 2009, 53 (13,22%) of 401; in 2010, 27 (17,65%) of 153 and in 2011, 25 (26,04%) of 96, totaling 237 (21,64%) positives of 1095 patients analyzed in five years. **Conclusion:** Considering the massive influx of people from endemic areas, and also the presence of *Anopheles* mosquito that transmits Plasmodium, we can say that Pernambuco is a vulnerable state to occurrence of malaria. The execution of early and precise diagnosis, as a tool in identifying a new case, provides adequate and timely treatment, prevents death, human suffering and elimination of infection sources, discontinues the transmission chain and blocks the occurrence of new cases, thus making the monitoring indispensable to avoid the state rewinds about disease endemicity. **Keywords:** Malária, Pernambuco, LACEN, Diagnose. **E-mail:** fgarnier@ig.com.br

Mal072- Public politics to face malaria in Piauí, Brazil

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Introduction: Piauí is the second state of northeast Brazil in the incidence of Malaria, losing only to Maranhão. The State Secretariat of Health of the State of Piauí (SESAPI) reported, recently, cases in towns like Uruçuí and Buriti dos Lopes where the focus of infected individuals were coming from regions near the north. Currently, the policy adopted by SESAPI in relation to these tropical diseases, has been based on a model of preventive and therapeutic health. The Institute for Tropical Diseases Natan Portela has been the only alternative to the treatment of this parasitic in the state, and is currently the only hospital with specialized services in this area and who treats by the Unified Health System (UHS). In this context, this work purpose to approach the epidemiological profile of malaria in Piauí and the influences he suffered against the actions of government in health care. **Material and Methods:** Qualitative and quantitative research, exploratory and retrospective by active search in publications of the Ministry of Health and SESAPI, and data collected for notifiable Diseases Information System for the years 2010 and 2011. **Results:** Malaria has significant prevalence in the State of Piauí, in view of the increasing levels of local focus, mainly in small towns, where prevention and drug therapy are inefficient or absent. In 2010 were 443 confirmed cases of the disease, and only the first half of 2011, there were 78 cases of malaria, which represents 17% of all cases presented in the previous year. It is noteworthy that the large influx of people migrating to Maranhão constitutes a factor of great importance when considering the recent bouts of this disease. The activities performed by health managers have summarized the practices that encourage prevention, by the control of the vector, as well as provide the necessary treatment, which is mainly carried out in IDTNP, specializing in the treatment of infectious and parasitic diseases, the only alternative of attendance for the majority of SUS users in these specialties. **Conclusion:** Despite the expanded vision of health that virtually permeates the practice of UHS in the field of infectious diseases just reverberates into practice on these matters. The public policies to face malaria in Piauí reflect a national policy that ignores the social aspects in developing proposals, focusing on the identification and retrieval of cases diagnosed. Preventive actions are rare, strictly focused on combating mosquito vector rather than a social situation predisposing to risk of disease and death from severe malaria. For these reasons, public health policies in the state did not succeed in fighting this disease, when considering the stability of its epidemiology. **Email:** paulomed7@gmail.com

Mal073- Epidemiological and entomological aspects of autochthonous malaria in the cities of non- endemic area - Piauí - Brazil

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Introduction: Malaria is an infectious disease caused by protozoa of the genus Plasmodium. In Brazil presents almost all the cases in the legal Amazon. Piauí regarded as an area free of disease transmission, introduced in 2010 an occasional outbreak with 26 cases distributed in six locations in the city of Buriti dos Lopes, located in the northern state to 302 km away from the capital Teresina, is the etiologic agent Plasmodium vivax. **Objective:** To analyze the occurrence of autochthonous cases of malaria due to Plasmodium vivax in the Buriti dos Lopes city occurred in 2010, and propose measures to control the disease in the area. **Material and Methods:** We analyzed data from reporting and investigation of Malaria Information System for Notifiable Diseases (SINAN), technical reports of the Malaria Control Program of the Department of Health of the Piauí State (SESAPI), and I entomology team of the Regional Coordination of Health Parnaíba. **Results:** It was found that the 26 malaria cases, the male was the most affected, with 61.5%, the age groups with a majority of cases were in the 20 to 24 years for males, and 01 to 09 years for females. The farm work represents 50% of cases, followed by the student with 30.8%. The malaria cases reported using the existing catch basins in their localities for

bathing and fishing activity mainly from 18 to 20 hours, time of greatest activity vector. The entomological surveys detected the presence of 06 species of Anopheles vectors of malaria in the city, among them two of primary importance in malaria transmission in Brazil, Anopheles darlingi and Anopheles albitarsis, either as an adult, immature, or both, in locations with disease transmission. The time of greatest biting activity of anophelines occurs in the period from 18 to 19 hours. **Conclusion:** The research shows that the infections occurred away from home, especially along the river Pirangi, where people who contracted the disease flocked to bathe or fish. The presence of dispersion and endofilia primary vectors of malaria in the villages make clear the vulnerability of those for the transmission of this disease, making it necessary to intensify the actions of epidemiological and entomological surveillance, aiming to prevent the occurrence of new outbreaks. It is recommended that actions be developed for health education in the city, aiming to inform the population about malaria, with prioritization of prevention, using for this purpose the Family Health Teams. **Keywords:** Entomology, malaria, epidemiology. **E-mail:** mauroapiaui@yahoo.com.br telmaevangelista@gmail.com

Mal074- *Plasmodium* spp infection in Guapimirim, a municipality of Rio de Janeiro State

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Introduction: Although malaria was an important health problem in Rio de Janeiro at the first part of century XX, currently in Brazil, malaria is a disease that occurs predominantly in the Amazon region. In extra-Amazon region, malaria is occasionally reported particularly in the Atlantic forest States. In these areas, the most part of cases are imported from endemic areas of Brazil or other countries. However, the Health Surveillance System of Rio de Janeiro State have notified 35 cases of autochthonous malaria in some municipalities from 2001-2010. From 2008, five malaria cases have been diagnosed in the municipality of Guapimirim, Rio de Janeiro. None of these had traveled to endemic areas and anybody had been submitted to blood transfusion. The present study aimed to investigate the presence of malaria cases and asymptomatic infection in the peri-urban and rural areas of Guapimirim in order to identify the main factors associated with autochthonous occurrence of *Plasmodium* infection in this non-endemic area. **Materials and methods:** A transversal study was conducted in the peri-urban and rural area of Guapimirim in the first semester of 2010. A systematic sample was selected and only volunteers with 5 or more years were interviewed for risk determinants. Blood for parasitological, molecular and serological tests was collected of each participant. **Results:** Overall, 324 volunteers were recruited. Of them, 3,5% had IgG antibodies against *P. falciparum* and 7,7% IgG-anti *P. vivax*. Individuals who usually enter into the forest (for several activities) were more reactive for *P. vivax* than individuals who never go into the forest ($p=0.0137$). The number of reactive samples for both (*P. falciparum* and *P. vivax*) was higher in Caneca Fina, Orindi and Paraiso localities of the municipality. Nine persons (2.8%) were positive in the PCR for *Plasmodium* (six for *P. malariae*, two for *P. vivax* and one for *P. falciparum*). These individuals did not have malaria symptoms in the follow up. None thick smear was positive. **Conclusions:** The study showed evidence of circulation of *Plasmodium* spp. in Guapimirim, a municipality of Rio de Janeiro State. We did not find malaria cases, but asymptomatic *Plasmodium*, infection as other studies in the Atlantic forest had shown. Two important findings enlightened in future studies: 1) individuals who enter into the forest have more risk of contact with the *Plasmodium* spp. and 2) Caneca Fina, Orindi and Paraiso localities (the poorest areas in the municipality) have more proportion of positive results by PCR and serological test. We concluded that malaria transmission is not totally interrupted in the state and the area must be classified as residual malaria area. More studies are necessary in order to evaluate the geographic, social and ecologic conditions of re-introduction of malaria in this state. We discuss three different scenarios for malaria transmission present at the Rio de Janeiro State. **E-mail:** renatab_miguel@yahoo.com.br; marmutis@ioc.fiocruz.br

Mal075- Etiological profile of NO – Amazonian malaria in Brazil in 2011

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Introduction: Malaria is a potentially serious infectious disease caused by parasites of the genus *Plasmodium* unequally distributed in space, determining transmission standards differentiated geographically. Three species of the protozoa are related to the disease in Brazil, *P. falciparum* (F), *P. vivax* (V), and occasionally *P. Malari* (M), the first being more severe. When it is considered the fragmentation of healthcare for the control and identification of this disease in these areas, knowledge of local epidemiology adds to the clinical diagnosis of malaria. Furthermore, changes in the prevalence of the parasite in reported cases is virtually predictive of flows and tool for control the outbreaks, mobilizing politics and actions of health systems. The present study aims to approach the geo-etiological profile of malaria in Brazil. **Materials and Methods:** Retrospective documentary study based on publications of the Ministry of Health and Information System for Notifiable Diseases for the year 2011 in Brazil. **Results:** During the study period, there were no cases of malaria by *P. Malari*. Moreover, there are two geographic areas in domain of *P. falciparum* and *P. Vivax*. The first comprises the states of Northeast Brazil, from hot and dry weather, torrential rain and vegetation, which highlight the Piauí and Ceará, with 42.72% and 30% (N = 1894) positive for F, respectively. The other, for its part, composed of the southern states, especially Paraná (69.40%), cold climate and vegetation conifer, has a predominance of V (7.08%). The Southeastern States, in turn, have positive parasitological F + Gametocyte of *P. Falciparum* (FG) (26.08%), especially São Paulo (38.86%), Rio de Janeiro (24.50%) and Minas Gerais (23.07%), while cases of malaria diagnosed in the States of the Midwest have parasitological positive for F + V (6.60%), with higher prevalence in Goiás (47.20%) and Distrito Federal (32.0%). **Conclusion:** The geographical distribution of *P. falciparum* and *P. Vivax* in Brazil is marked, assuming greater importance for guiding health policies aimed at the epidemiological surveillance, especially in non-Amazonian areas. However Malaria not limited to geopolitical, structures, a larger number of cases by *P. falciparum* in areas of low Human Development Index also reflect socio-cultural aspects related to livelihoods, such as housing in proximity to possible sources. In these areas, interventions focused strictly on the fight against mosquitoes tend to fail. **E-mail:** manael.medufpi@gmail.com

Mal076- Description of autochthonous malaria cases diagnosed in a research center in Rio de Janeiro/Brazil

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Malaria in Brazil is concentrated in the Amazon region, with approximately 313,000 cases/year. However, despite its predominantly focal character, an average of 913 cases of imported and autochthonous malaria is reported each year outside the endemic area near the Atlantic Forest. Although the transmission of malaria has been considered eliminated in the late 1968 in the state of Rio de Janeiro (RJ), about 79 cases are still reported annually, most of them having the diagnosis delayed due to low diagnostic suspicion and unfamiliarity of health teams with the disease. Eight cases of malaria diagnosed in the state of RJ with evidences of indigenous transmission are presented here in a descriptive study of autochthonous malaria patients treated at the Clinical Research Institute Evandro Chagas, Fiocruz from January 2006 to December 2011. The definition of autochthony of the cases was established by the absence of movement of individuals to endemic areas, or blood transfusions, organ transplantation or sharing of syringes. Imported cases were dropped for lack of evidence of entry of any individual with malaria from a transmission area. The diagnosis was made through direct study of *Plasmodium* (thick and thin blood smears) and molecular test (PCR). Eight cases of malaria with evidence of indigenous transmission were identified. The probable site of infection were: Lumiar (one case) Guapimirim (five cases), Cachoeiras de Macacu (one case) and Macaé (one case), all in region of the Atlantic forest

approximate altitude of 600 meters localized at the state, 140 km far from the city of Rio de Janeiro, most cases were diagnosed between the months of January to May, during the summer, season most frequented by tourists. The time elapsed between the onset of symptoms and diagnosis ranged from 10 to 25 days, with an average of 14.6 days and median of 14 days. There was a predominance of males (87.5%), and the mean age was 50 years (27-79). All patients had *Plasmodium vivax* infection, with low parasites count, and have had a clinical diagnosis of other infections before laboratory confirmation of malaria. In all cases the clinical characteristics were similar: initially nonspecific, with daily fever, which after a few days was presented as the classic triad: tertian fever (75%), preceded by chills (87.5%) and then sweating (87.5%). Three individuals with comorbid conditions required hospitalization. After characterized the presence of malaria in the region of the Atlantic Forest of Rio de Janeiro it is necessary to investigate its magnitude, forms of transmission and the risk of re-introduction of the disease in the state. The prevalence of *P. vivax* malaria with low parasitaemia could explain the mild clinical course of malaria in these patients which, associated to the unfamiliarity of the disease in the area, justify the average of 14 days required for the diagnosis and the presentation of complications in patients with co morbidities. **E-mail:** anielle.pina@ipecc.fiocruz.br

Mal077- Current considerations about malaria in Northern Brazil

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Abstract: The knowledge that malaria is endemic in the Northern of Brazil is not new, therefore, it's intriguing to understand the reasons for the high incidence of this pathology remain so significant, even after a long time of the first manifestations. To be considered a rural disease, it reaches similar socioeconomic profiles, generally, people with low education, which lives in transmitter mosquito's natural habitat. **Material and Methods:** The study was realized based on a critical review of the specialized literature like articles and manuals dated since 2006 to 2012. **Results:** After careful analysis, became clear that malaria remains a challenge in the Northern of Brazil supported by a problematic tripod: deforestation; failed conventional treatment and failed training of many professionals involved in combat, especially in interior areas. **Main conclusions:** One of the most observed failures in this study was the low adherence of treatment because it's very long, complex, with many side effects, which make people, especially the less educated one, abandon the combative measures. Among the main solutions, investing on nanotechnology, which focuses would be the most viable way, because encompasses efficiency, reaching a large proportion of population. **E-mail:** keily_nery@hotmail.com

Mal078- Overview epidemiological of malaria in Ariquemes, Rondônia, western Amazon: a survey of six years (2005-2010)

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Introduction: The state of Rondonia, in particular the Jamari Valley region, home of the city of Ariquemes has high rates of malaria. Based on this information becomes fundamentally important the epidemiological survey of the disease to develop prevention and control measures of the same. From this perspective, this study aimed to evaluate the epidemiological picture of malaria in the municipality of Ariquemes, Rondônia, Western Amazon, in the years 2005 to 2010. **Material and Methods:** Data were obtained from the database available through the National Health Foundation (FUNASA) and the Information System of Epidemiological Surveillance (SIVEP), where we calculated the annual number of cases, seasonality, Annual Parasitological Index (IPA), age and agent. **Results:** It was found that the city of Ariquemes 2005 to 2010, 13,444 cases were reported positive for malaria, with the highest incidence in 2005, where he also found the largest Annual Parasitological Index (IPA) (61.1). It was found that the

highest average of seasonality was observed in July to 217.3 cases, and every month the age group most affected was 20 to 29 years. Notified that the city of Ariquemes are recorded the occurrence of 15 species of Anopheles, having a higher prevalence of food contamination by the etiologic agent *P. vivax*, which is reflected in the number of sick caused by even reaches (77.1%) of all cases, addition of mixed infections. **Conclusions:** It is necessary to better control the disease by application of measures of prevention, early diagnosis and treatment, with emphasis on strengthening the capacity of local professionals, in order to improve public policy, improving practices of health education, intending to draw attention of the rulers and leaders of private institutions for the problem caused by malaria until these days, which is the major neglected disease in the world. **Keywords:** Epidemiology, Malaria, Vectors and Western Amazon. **E-mail:** dionatas@icbusp.org

Mal079- Relationship between malaria and dengue in a capital of the legal Amazon

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Introduction: Malaria is an acute febrile infectious disease transmitted by mosquitoes of the Anopheles genus, whose etiologic agent is a parasite of the genus Plasmodium. Of malaria cases reported annually, 98% are restricted to the Amazon region. In addition, the Brazilian Amazon is home to about 200 types of Arboviruses, of which the most prevalent are the flaviviruses, dengue causes. Dengue is a viral disease, acute and systemic, which is primarily transmitted by the mosquito Aedes aegypti. Both diseases have in common a high prevalence in the north of the country and symptoms of acute fever, and such characteristics favor a constant confusion between both. Although co-infection can occur, resulting in worse clinical condition. **Materials and Methods:** A retrospective descriptive study conducted in Porto Velho, Rondônia, which correlates the period of incidence of malaria and dengue. The data collected is for the period 2009 to 2011 and from the Information System of Epidemiological Surveillance (SIVEP) of the National Notifiable Diseases (SINAN), and the Department of the Unified Health System (DATASUS). We used data on the number of tests for malaria, the number of negative slides, distribution of cases throughout the year, and reported cases of dengue in the region. **Results:** In the city of Porto Velho is conducting a large number of tests for malaria research, however, a large portion has a negative result. During 2009 141,469 tests were carried out microscopy to search for malaria in Porto Velho. Of these, 120,878 (85%) were negative. In 2010, there were 152,190 sheets, of which 128,933 (84%) were negative and in 2011, of 122,268 tests, 103,019 (84%) were negative. The average negative in the months June, July and August, between the years 2009 and 2011 are 82%, 80% and 82.6%, respectively. As the months December, January and February had an average negative slide 88.4%, 87.7% and 90% respectively. During this period, were reported in 2009 Porto Velho dengue cases in 2009, 6,542 in 2010 and 351 in 2011. In 2009, most reported cases of dengue occurred in the months November and December in 2010 and 2011 occurred in January and February. Note that the highest rates of negative slides for malaria occur just at the time of greater reporting of dengue cases in the region. This situation can be partly explained by the confusion of symptoms between the two diseases. **Conclusions:** The municipality of Porto Velho is the theater of transmission of dengue and malaria, which favors the occurrence of clinical confusion between these two frames acute febrile. One sign that an outbreak of dengue fever is starting in the region may be the increase of negative slides for malaria. In addition to alerting the medical community that, in the presence of clinical signs to a more severe disease, you should think about possible cases of co-infection in the period from the end and beginning of the year. **E-mail:** dheliopereira@yahoo.com.br

Mal080- Malaria caused by chloroquine-resistant *Plasmodium vivax* , a case report.

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Introduction: Malaria continues to be one of the most frequent causes of disease in the Amazon Region, and *Plasmodium vivax* has been responsible for the majority (73%) of the cases. This case-report aims to present a patient who travelled to Porto Velho (Rondonia State, Brazil) with malaria caused by a chloroquine-resistant *P. vivax*. **Case report:** A 49-year-old truck driver travelled to Porto Velho on August 29, 2011, where he stayed for two days; he went back to Lençóis Paulista (São Paulo State, Brazil), arriving on September 3, 2011. Few days after going back home he presented malaise, chills, fever, profuse sweats, and rigors. One day later he observed conjunctival jaundice, which has been progressive. Three days after the symptomatology started, he asked for medical attention and received antibiotics to treat a possible pneumonia. On the second day of treatment he was examined by an infectious disease specialist who suspected of malaria and referred him to our Service. He denied drug addiction, alcohol intake, and smoke use. Physical examination showed a patient in good general condition, pale (2+/4+), BP=110x60mmHg, RR=24/min, PR=110/min, and conjunctival jaundice (2+/4+); pain at abdominal palpation of the right and left hypochondrium, liver palpable at 1.0cm below the right costal margin, spleen percussible 3.0cm below the left costal margin and painful at palpation. Lower limbs without alterations. Complementary exams showed anemia (Ht=37.5%, Hb=12.8 g/dL), platelets count = 42,000/mm³, leukocytes = 4,300/mm³ (neutrophils=18%, eosinophils=0.1%, basophils=1.0%, lymphocytes=77.5%, and monocytes=3.4%). Thick and thin peripheral blood smears Giemsa-stained showed typical *P. vivax* trophozoites and gametocytes. Chloroquine phosphate, 4 tablets in the first day and 3 tablets every day in the following two days, associated with primaquine phosphate 15 mg/day for 14 days were orally administered. Daily clinical and parasitological evaluation showed a complete treatment failure. The patient worsened, presenting respiratory insufficiency with indication of endotracheal intubation, and was transferred to the ICU. Clinical and parasitological cure were observed only after introduction of quinine sulfate 650 mg every 8 hours for 3 days *plus* doxycycline 100mg twice daily for 7 days. **Comments:** This case-report confirms that chloroquine-resistant *P. vivax* can be a problem for individual patients and suggests an association between resistance and severity. **Email:** tietemendes@terra.com.br

Mal081- Malaria control with Long Lasting Insecticidal Nets - LLINs in the areas of influence of the Jirau Dam, Porto Velho, Rondônia, Brazil

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Introduction: Malaria is a disease endemic of the Amazonian region. Porto Velho is one of the Brazilian municipalities with more cases in the last three decades. The control of this endemic consists in the use of chemical and biological insecticides against the vectors and in the treatment of the patients with antimalarial medicines. Both measures are costly and difficult to implement due to the Amazonian dimensions and cultural characteristics. This study had the objective of evaluating the effectiveness of g Lasting Insecticidal Nets - LLINs in controlling the disease in the areas of influence of the Jirau Hydroelectric Dam, in Porto Velho, Rondônia, Brazil. **Material and methods:** In the beginning of 2011, 8,083 LLINs were gratuitously installed in the houses of miners and settlers. Lectures were given to them on preventive measures, diagnosis, and treatment of the disease, and how to use the mosquito nets. Vector densities inside and outside the houses and epidemiological data pertaining to the localities studied were evaluated by sampling. **Results:** The quantity of mosquitoes inside the houses was significantly reduced after installation from 70±2 (before installation) to 14±2 (after installation). In the immediate vicinity of the houses, no statistical difference was detected; variation was from 35±1.3 (before installation) to 31±1.5 (after installation). Malaria cases in settlements were reduced by 25.3%, from 2,658 cases in 2010 to 1,986 in 2011. Malaria caused by *Plasmodium falciparum* was reduced by 55.5%, 398 cases in 2010 and 177 in 2011. In mines, the disease was reduced by 33%, from 433 cases in 2010 to

290 in 2011. Cases of *P. falciparum* were reduced by 62.7%, from 161 in 2010 to 60 in 2011. **Main Conclusions:** LLINs were considered effective, since they concurred to reduce the number of mosquitoes biting inside the houses and the number of cases of the disease in the studied localities. However, transmission risk in the vicinity of the houses was apparent. It is a simple measure, of low cost, environmentally safe, and efficient, for the protection that it affords and its residual effect. We believe that one of the factors that concurred to the effectiveness of the LLINs was the education of the population. **E-mail:** fabiologocosta@gmail.com

Mal082- A retrospect review of malaria cases seen in non-endemic area of Sao Paulo State-Brazil

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Introduction: Malaria is a risk for travelers to endemic areas, but sometimes the disease flourish when the patient go back home, in non-endemic areas. We describe here, the diagnosis and the profile of all the patients who were seen in a hospital which is the reference for a population of 3.5 million people in the Northeast of Sao Paulo State, which is a non-endemic area for malaria, but endemic area for dengue in Brazil. **Material and Methods:** The archived notification forms and the records of specimens submitted to the medical microbiology laboratory for malaria investigations over five years (from Jan 2007 to March 2012) were reviewed. Clinical records were analyzed to extract demographic data, history of travel to endemic areas for malaria, and platelets count at the moment of admission in the hospital. The laboratory performed malaria smears and only the first specimen for each patient was included in this study. All the patients were followed in the infectious diseases outpatient clinic (ID) and treatment mainly followed Brazilian guidelines for malaria. **Results:** A total of 220 patients were referred to the ID clinic with suspicion of malaria. Of those, 69 were confirmed, resulting in an average frequency of 13.8 cases per year in the studied period. Males were 7.6 times more frequently than females (61/8). The mean age of the infected people was 36.7 yr old, (from 8 to 72), and there were 4 children (<18 yr old). The probable exposure area for the 69 patients, were endemic areas of Brazil in 61%; South America: 10%; Africa: 13%, and not reported in 16%. The most frequent organism detected was *P. vivax* (52/69); followed by *P. falciparum* (14/69), and co-infection of both (3/69) in patients who had been travelling to French Guiana. Eight out of nine (89%) of the patients who had been travelling to Africa had *Plasmodium falciparum* diagnosis. Platelet counts under 150.000/mm³ were found in 65% of the cases (45/69), normal counts in 25% (17/69) and not this information was not available for 10% (7/69). **Main Conclusions:** When the exposure occurs in the Brazilian territory the most frequent agent detected was *P. vivax*, while *P. falciparum* is predominant in the patients who travelled to Africa. Thrombocytopenia is one of the most common complications of both *P. vivax* and *falciparum*, as well as for dengue hemorrhagic fever, which is a very common disease in this area. This highlights the importance of history taking, especially related to travel or exposure to endemic areas for malaria. **E-mail:** vbollela@fmrp.usp.br

Mal083- Implementation plan for systematic screening and treatment with artemether-lumefantrine of *Plasmodium falciparum* asymptomatic carriers in a community setting in Africa

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Introduction: Complementary interventions to reduce the burden of malaria are required despite nationwide adoption of artemisinin-based combination therapy and associated decline in malaria-related deaths. **Methods:** This 18-cluster (9 intervention clusters or villages; 9 control), randomized, single-center, controlled, parallel, prospective study will evaluate in a community setting in Africa the impact of systematic screening and treatment of asymptomatic carriers (ACs) of asexual forms of *P. falciparum* with artemether 20 mg-lumefantrine 120 mg (AL, Coartem/Coartem Dispersible, BID for 3 consecutive days) in

approximately 9000-14000 subjects (male/female adults, children, and infants). The primary objectives are to evaluate whether treatment of *P. falciparum* ACs is associated with a lower number of symptomatic malaria episodes, RDT confirmed, per person-year over a 12-month follow-up period in children aged <5 years, and improvement in hemoglobin levels in treated ACs after 28 days. Subjects were excluded from receiving AL in case of severe malaria, known disturbances of electrolyte balance, history of congenital QTc prolongation or sudden death, body weight <5 kg, hypersensitivity to AL, or if they were in the first trimester of pregnancy. Those subjects were treated with alternative drugs per current national guidelines. Responsibilities of the investigator's central site included microscopy, data entry, source data archiving, and supervision of the Health and Demographic Surveillance System (DSS). HDSS monitored each cluster population every 2 months during the study for births, deaths, and in-/out-migrations; and provided an up-to-date demographic status of the study population. A mobile team, supervised by the principal investigator and supported by community healthcare workers performed 3 campaigns of systematic screening and treatment in the intervention group. Incident malaria episodes in the whole study population were diagnosed and treated at the local healthcare facilities. **Results:** Results will be available in 2012. **Conclusions:** If the impact of treatment of ACs on disease burden is confirmed, policymakers may consider this approach in the surveillance strategies being implemented by malaria control programs across Africa. **E-mail:** kamal.hamed@novartis.com

Mal084- Cross-border Malaria Control: Impact and Need for Sustainability

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It has long been recognised that malaria cannot be viewed as a country specific problem, especially in regions where many countries share a common border. Vectors and parasites are able to move with ease from one country to another, taking with them genes that may confer resistance to the insecticides and drugs in that country. It is for this reason that the Lubombo Spatial Development Initiative (LSDI), incorporating the high transmission areas of Mozambique, Swaziland and South Africa was established. The main objective of the LSDI was the implementation of malaria control measures in a region where malaria transmission had remained high due to ineffective insecticide and chemotherapy protocols. Since there were established malaria control programmes in both South Africa and Swaziland, the focus of attention was southern Mozambique. Baseline and annual malaria prevalence were determined in order to assess the impact of the indoor residual spraying (IRS) programme in southern Mozambique. The coverage with IRS was monitored as a goal of a minimum of 80% spray coverage was required for IRS to be effective. Since 1999-2000 was a period of intense malaria transmission in southern Africa, the baseline malaria prevalence in all three participating countries was quite high with the average prevalence in Mozambique being 70%. Over a decade, from 2000-2010, sustained vector control has resulted in the dramatic decrease of malaria in all three countries with Mozambique, South Africa and Swaziland reporting decreases of 82%, 99% and 98% respectively. However, in 2009-2011 when optimum spray coverage was not achieved, an increase of malaria cases numbers was observed in Mozambique together with an increase in the number of imported malaria cases reported in both in Swaziland and the adjacent provinces of South Africa. As seen from the LSDI experience, harmonising insecticide and treatment protocols have an enormous impact on reducing malaria transmission. Through the sharing of technical and financial resources in a cross-border setting, unstable malaria can be brought under control. The gains in malaria control achieved in southern Mozambique had a ripple effect which saw substantial decreases in malaria case numbers in South Africa and Swaziland to such an extent that these two countries have now adopted an elimination agenda. However, failing to ensure the sustainability of these cross-border malaria control initiatives can result in the resurgence of malaria in areas where malaria had been reduced from being high transmission to low transmission. **E-mail:** rmaharaj@mrc.ac.za

Mal085- Aspects of demographic and health-seeking characteristics of clinical malaria among New Bakassi Resettles in Ekpiri-Ikang, Nigeria

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Introduction: Nigerians in Bakassi were evacuated after International Court of Arbitration awarded Bakassi to Cameroon. This study, carried out after two years of resettlement, was aimed at determining the incidence, health-seeking occurrences and spatial clustering of clinical malaria among New Bakassi resettles at Ekpiri-Ikang, Nigeria. **Materials and Methods:** Records from approved hospitals and structured questionnaires were employed. **Results:** Overall, 25.4% of resettles seeking medical assistance were diagnosed with clinical malaria. Prevalence was highest among the 0-9 years age group; higher among females of reproductive age than among their male counterparts (χ^2 -test; $p < 0.05$); comparable between sexes in all other age brackets, (χ^2 -test; $p > 0.05$). The risk of having malaria was six times as high among children (0 – 9 years) than among adults (OR 5.99; 95% CI 1.738 TO 1.842); twice as high among females than among males (OR 1.79; 95% CI 0.531 to 0.627); five times as high among females of reproductive age than among their male counterparts (OR 4.55; 95% CI 1.349 to 1.581). Malaria incidence was 2.4 episodes/person/year (2.2 for males; 2.6 for females). Generally, females had higher number of episodes than males. Overall, 6.5% of respondents had zero malaria episode per year, and were from 8.3% of the total households. From the latter, 15.4% had two residents each with zero malaria episode. **Main Conclusions:** The occurrence of clinical malaria was high among health-seeking resettles generally, especially among children and women of reproductive age. Very few households (8.3%) had zero malaria episodes. **E-mail:** drecuttah@yahoo.com

Mal086- Is malaria illness among young children a cause or a consequence of low socioeconomic status? Evidence from the United Republic of Tanzania

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Background: Malaria is commonly considered a disease of the poor, but there is very little evidence of a possible two-way causality in the association between malaria and poverty. Until now, limitations to examine that dual relationship were the availability of representative data on confirmed malaria cases, the use of a good proxy for poverty, and accounting for endogeneity in regression models. The observed negative correlation between malaria and socioeconomic status (SES) may indicate that malaria infections cause low SES (e.g. ill workers are less productive), or that poverty increases the risk of malaria transmission (e.g. the poor are less able to afford malaria preventative measures). Also, there may be incidental associations that could simultaneously increase household incomes and reduce malaria incidence. Understanding whether the malaria-poverty correlation implies causality and, if it does, the direction of causality, has crucial implications for malaria control efforts. **Materials and Methods:** A conceptual framework of the bi-directional link between malaria and SES, utilizing a multi-disciplinary and multi-scale approach was proposed and tested empirically with Demographic and Health Survey (DHS) data for Tanzania, which is nationally representative, and included malaria parasite testing with RDTs for young children (6-59 months). A simultaneous equation model was estimated, and accounted for environmental variables assembled with the aid of GIS. A wealth index based on assets, access to utilities/infrastructure, and housing characteristics was used as a proxy for socioeconomic status. Model estimation was done with instrumental variables regression. **Results:** The wealth model suggests that malaria illness among young children (6-59 months) was a contributing factor for low household wealth in Tanzania. Controlling for other factors that influence wealth, results show that households with a child who tested positive for malaria at the time of the survey had a wealth index that was, on average, 1.9 units lower (p -value < 0.001). However, malaria prevalence among young children was unrelated to the household's wealth position. The coefficient of the wealth index had the anticipated negative sign, but it was not statistically significant (p -value = 0.677).and that an increase in the wealth index did not reveal

significant effects on malaria. **Main Conclusions:** If malaria is indeed a cause of poverty, as the findings of this study suggest, then malaria control activities, and particularly the current efforts to eliminate/eradicate malaria, are much more than just a public health policy, but also a poverty alleviation strategy. However, if poverty has no causal effect on malaria, then poverty alleviation policies should not be advertised as having the potential additional effect of reducing the prevalence of malaria. **E-mail:** mcastro@hsph.harvard.edu

Mal087- Trends of malaria incidence over a decade in endemic and non-endemic areas of South Africa, 2000 – 2009

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Introduction: It is estimated that globally, the number of malaria cases decreased between 2000 and 2009. However, the sub-Saharan African region still harbors the largest proportion of the malaria cases. This study aimed at identifying trends of laboratory-confirmed malaria in both malaria endemic and non-endemic areas of South Africa over a period of ten years (January 2000 to December 2009). **Methods:** An exploratory analysis of secondary malaria surveillance data from the National Health Laboratory Service was conducted to analyze trends of malaria incidence over time as well as to determine demographic risk factors for confirmed malaria in endemic and non-endemic areas of South Africa. Chi-square test for trends was used to test differences in the distribution of demographic and temporal characteristics. Annual parasite incidence (API) of malaria was calculated to estimate malaria incidence. **Results:** The overall malaria positivity rate was 21.4%. There were more malaria cases among males (65.4%) than females; those aged 25-44 years (49.4%) than in any other age group and among those living in non-endemic provinces (61.7%) than those living in endemic provinces. A linear downward trend in malaria incidence was observed over the ten-year period ($p < 0.001$) from 64 per 100,000 population in 2000 to 50 per 100,000 population in 2009. The incidence of malaria was highest in the month of January (15 and 24 per 100,000 per respectively) in both endemic and non-endemic provinces. **Conclusion:** A high burden of laboratory-confirmed malaria cases in non-endemic areas of South Africa was observed. Targeted information, education and communication (IEC) to all travelers during the annual festive and holiday season should be reinforced to minimize malaria importation into these non-endemic areas. **E-mail:** peter.nyasulu@wits.ac.za

Mal088- Malaria case management by community health workers, in recollection zones of Castana (*Bertolletia excelsa*), implementation services in tropical rural regions in Bolivia

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Introduction: In Bolivia malaria is still a public health problem, principally in the north border with Brazil, where we have cases due to a *Plasmodium vivax* 85 % and *Plasmodium falciparum* 15 %, and the main vector is *Anopheles darlingi*. More recently under support of Global Fund, the Adventist Development and Relief Agency (ADRA) are implementing services for malaria cases by the community health workers (CHW) in recollection zones of Castana, by using of malaria rapid tests, and treatment according with the national policy, all CHW are placed along the rivers, in locations where regular public health services are not offered to the population. **Material and Methods:** Initial implementation was developed during February to December in 2010 with Optimal^R, and during February to December 2011 with CareStart^R, in each of them, training 160 CHW in diagnosis and treatment. Two visits were developed, first in January-February 2011 and the second January-February 2012, doing assessment to 44 and 87 CHW respectively. During the first assessment we used a written test with 4 main aspects: general information

about CHW, register use, malaria rapid tests and treatment to 44 CHW, in the second, we used the same instrument and additionally we made direct observations of rapid test procedures to 87 CHW. **Results:** The average age of CHW was 36.5 y., where 35 % are women, and 47 % have secondary instruction (more than 6 years), in the first assessed group of 44 people, all received training in a workshop and in the second group of 87, a total 66 participate in a similar way and 21 received training in service at community level. Both groups at the assessment time in 2011 and 2012 had some job aid material 87 and 91 %, and almost all CHW had a basic information about malaria 96 % and 93 % respectively, about how we interpret questions and use of register form, we have still the same 64 % and 65 %, about written and pictoric interpretation also a 88 % and 89 %. In relation to a direct observation of rapid test, 51 % did not have verify expiration date, 30 % did not write the name of patient, 20 % did not collect adequately the blood with the device, 9 % did not put the blood in the right place, 17 % made a lecture before recommended time, 25 % did not write the results in the form, 36 % did not dispose properly material used, and 18 % did not give any oral message to patients about results, and how CHW will be given a treatment to children, we observed an improvement since 64 to 86 % in *P. vivax* cases and 66 to 76 % in *P. falciparum*, in treatment to adults the values for 2010 and 2011 are 93 % and 87 % respectively. Finally, reviewing data available in relation to cases due to *P. vivax*, with diagnosis and treatment in the same day are 97 and 96 %. **Conclusion:** CHW had at the time of visits job aid materials or guidelines, they knew basic information, so besides that they did not use properly the register, particularly place of infection and dates of fever started. Some aspects remaining for improve, in relation to rapid tests, particularly in context to change the brand (Optimal^R to CareStart^R), and whereas CHW should be care malaria patients adequately, it is important also verify others aspects, like rapid test and drugs availability at community level and treatment adherence. **E-mail:** mlopez@adra.org.bo

Mal089- Enhancing malaria diagnosis and surveillance through portable devices in an endemic area in Colombia

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Introduction: Malaria surveillance in endemic countries is limited by geographical access, low diagnosis offer in the rural context, and delayed and unreliable data availability to public health officials. Fio Corporation has developed a technology designed and validated to address these issues with a portable device that integrates automated interpretation of RDTs with cell phone network and cloud information systems (the Fio System), to increase diagnosis accuracy and to provide close to real-time high quality data availability. In association with the National Malaria Program (NMP) and the Malaria Colombia Project funded by GFATM, Fio Corporation carried out a pilot to test the performance of the system under real life conditions. **Materials and Methods:** Ten community health workers (CHW) performing diagnosis and treatment in remote areas were selected to collect malaria events information through the Fio device, using the National Malaria Reporting Form in electronic format. Three of them used a device enabled to collect RDT images for QC purposes. In all cases the CHWs also filled out the paper based form and read the RDTs to decide the management conduct. The follow up was performed using the Fio Portal (a web based data management solution). Two evaluations were conducted with CHWs, information system staff and decision makers on the utility of the Fio System. **Results:** All 10 CHWs reported malaria cases from remote malaria endemic areas. The data collection was complete and accurate and included geo-location, date and time. For the first time in the country negative diagnosis events were reported along with the positive cases, providing a denominator to the NMP performance indicators. A total of 1.523 reports have been collected to date: 30.1 % positive, 83.6% *P. vivax*. Program managers monitored the progress of the pilot at the costumer built Fio Portal. Software and Survey forms updates were installed remotely in the devices. Compatibility between the Fio system and the National Public Health Surveillance System (SIVIGILA) was granted. System users (CHWs, information system staff and decision makers) were satisfied with the benefits and results of the system. **Main conclusions:** The results indicate that the use of portable devices provides benefits to patients, health care workers and health program managers in a setting of malaria endemicity in Colombia. The availability of accurate and timely information to make not only everyday decisions (e.g.: to ensure the continued existence of supplies and medications), but also to conduct monitoring & evaluation of public health programs

activities, can be accomplished using mobile technology to collect diagnosis and treatment information in the field. Readily available, accurate and reliable data should facilitate program managers decision making process. **E-mail:** edisoto@yahoo.com

Mal090- Comparison of asymptomatic *Plasmodium* spp. infection in two malaria endemic Colombian locations

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Introduction: The study of asymptomatic infection by *Plasmodium* spp, could help to understand the transmission dynamics in regions normally having low transmission. The objective of this research was to compare the prevalence of asymptomatic infection by *Plasmodium* spp. in two Colombian locations with different transmission patterns, ecological and epidemiological characteristics, and their potentially associated factors. **Materials and Methods:** Cross-sectional studies were carried out with random probabilistic sampling in two endemic areas. Infection by *Plasmodium* spp was determined using blood thick smear and Polymerase Chain Reaction (PCR) on 212 persons from Tierralta (Atlantic Coast) and 207 from Tumaco (Pacific Coast). All patients answered a validated questionnaire in order to establish risk associated factors to asymptomatic infection. **Results:** In Tierralta, the prevalence of asymptomatic infection was 11.3% (95% CI 7.2-16.8) by blood thick smear and 16.5% (95% CI 11.5-22.9) using PCR, while in Tumaco, the corresponding values were 2.4% (95% CI 0.7-5.5) and 5.8% (95% CI 2.3-9.2) respectively. The predominant species in Tierralta was *Plasmodium vivax* and in Tumaco *Plasmodium falciparum*. PCR detected 61% more infections than microscopy. Potential risk factors for the presence of asymptomatic infection by *Plasmodium* spp by PCR, were being male [adjusted odds ratio (aOR) = 2.52, 95% CI 1.28-4.56], and the number of prior malaria events ($p = 0.01$). **Main conclusions:** There are important variations in the epidemiology of asymptomatic plasmodium infection in these two endemic locations. These results could help to explain the differences in dynamics of transmission in two Colombian endemic regions (Atlantic and Pacific Coast). Considering the importance of submicroscopic infections in transmission, the measurement of the prevalence of asymptomatic *Plasmodium* spp infection is suggested as part of the epidemiologic evaluation for malaria in areas of low intensity of transmission as Colombia. **E-mail:** zcucunuba@gmail.com

Mal091- *Plasmodium cynomolgi*: first case of natural acquired malaria infection in Malaysia

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Introduction: *Plasmodium cynomolgi* is a natural parasite of macaques and it infects a variety of anopheline mosquitoes. Its closest relative is *P. vivax* that resides in the same subclade in the ancestral tree of the simian malaria parasites. Similarities include a 48-hour asexual cycle, parasitaemias within the same range and presence of a hypnozoite stage. It is capable of infecting Old and New World monkeys in laboratory conditions, also accidental infections of man were reported as early as 1960. However, this is the first report, to our best understanding, of a natural human malaria infection due to *P. cynomolgi*. To describe the first case of a naturally acquired *P. cynomolgi* malaria in a Malaysian resident. **Material and Methods:** Patient is a 39-year-old woman from East Coast, Malaysia with no previous history of malaria. She lived in a modern housing area not known to be endemic for malaria. There was a small forested area behind her house with occasional sightings of the long-tailed macaques. Fever started gradually and she was admitted to the hospital 10 days later when her symptoms worsened. Malaria parasites were identified by microscopic examination as *P. malariae* or *P. knowlesi*. Blood was taken for molecular confirmation of malaria species by nested PCR (Singh et al, 1999) in IMR and multiplex PCR (Rubio et al, 2002) together with universal malaria PCR plus sequencing in the ISCIII. The patient was treated

immediately with standard antimalarial drugs, recovered and discharged one week later. **Results:** Re-examination of blood film showed characteristics of *P. vivax*. The nested PCR assay also reported *P. vivax*. Meanwhile multiplex PCR was negative for the four human *Plasmodium* species. Universal malaria PCR product was sequenced and homology searching into GenBank achieved maximum homology with *P. cynomolgi*. Clustal-W software alignment confirmed that the sample sequence was closest to the rest of *P. cynomolgi* and was also markedly different than the *P. vivax* sequence in the multiplex *vivax*-specific PCR primer. **Conclusion:** This is the first report of human *P. cynomolgi* infection acquired in a natural way but probably there are more cases undiagnosed or misdiagnosed due to lack of specific diagnostic method. Morphologically *P. cynomolgi* is indistinguishable from *P. vivax*, and as shown in this report some PCR methods could characterize those infections as *P. vivax*. The importance of zoonotic malaria transmissible by non-human primates cannot be ignored in view of closer interaction between man and the wild animals in the process of urbanization. **E-mail:** jmrubio@isciii.es

Mal092- Dramatic decrease in malaria after repeated rounds of mosquito net distribution in Papua New Guinea

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Introduction: Papua New Guinea (PNG) is known to have one of the highest malaria transmissions outside of sub-Saharan Africa. The country's malaria epidemiology is also one of the most complexes with four endemic malaria species present and a variety of anopheline vectors filling the diverse ecological niches. Only recently, the national malaria control program was strengthened by two consecutive grants from the Global Fund supporting the large-scale free distribution of insecticide treated mosquito nets. **Methods:** Two cross-sectional household surveys carried out in 2008/09 and 2010/11 in randomly selected villages across PNG investigated changes in malaria control intervention coverage and population prevalence of malaria infection. Malaria surveillance in sentinel sites documented trends in the incidence of clinical cases and the prevalence of malaria infection among fever cases in health facilities. Prevalence of *Plasmodium* spp. was assessed by rapid diagnostic test (RDT) and light microscopy. **Results:** Country-wide household ownership of long-lasting insecticide treated nets (LLIN) reached 65% (n=1958) in 2009 and over 80% (n=1986) in 2011; usage in the target group of children under five years amounted to 40% (n=1599) and over 55% (n=1768) in the respective years. Data from sentinel sites suggest that prior to the first large scale LLIN distribution (2005-2009) both ownership and usage of LLIN were below 10%. No other malaria control interventions were introduced on a large scale during the mentioned period. Simultaneously, *Plasmodium* spp. prevalence in the general population decreased from 14% (n=6442) in 2009 to below 7% in 2011 (n=7978). While the decrease was significant for *P. falciparum*, *P. vivax* parasite rates remained virtually unchanged resulting in a shift from *P. falciparum* to *P. vivax* dominance in all regions. A significant decrease was also noted in malaria cases in sentinel health facilities where the proportion of fever cases with a positive RDT dropped from 56% pre-distribution (n=1330) to 18% post-distribution (n=681). **Conclusions:** This dramatic effect of the Global Fund supported LLIN distribution on malaria in PNG poses new challenges to the national malaria control program. Implications for surveillance, prevention and treatment choices are discussed in consideration of experiences from comparable settings. **E-mail:** manuel.hetzel@pngimr.org.pg

Mal093- Changing pattern of severe imported malaria in France: the experience of a Paris teaching hospital

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France is the non-endemic country with the highest number of annual imported malaria cases. We present our experience of severe imported malaria in Tenon Hospital, Paris. **Patients and methods:** All malaria cases observed from 2002 to 2011 were retrospectively examined. Severe malaria was defined according 2000 WHO criteria. **Results:** During the study period 563 malaria cases were diagnosed, 336 during the first five years and 227 during the last. *Plasmodium falciparum* was isolated in 524 (93%), associated with another species in 9. Forty-nine (9, 3%) presented with severe malaria, in 30 more than one criteria were present. All patients except one were contaminated in sub-Saharan Africa. Most of them, 29 (59, 2%) were African migrants who have lived in France for more than 8 years. Ages were ranging 29-67 with mean age 45.2. They contracted malaria returning to their country of origin to visit relatives and friends (VRF). Only 7 took a chemoprophylaxis but without compliance (3) or with a inadequate product (4). Seventeen had one or two underlying conditions: HIV infection (7), diabetes mellitus (4), obesity (4), hypertension (2), renal transplantation (1), renal insufficiency (1) and lepromatous leprosy (1). Eleven patients were second generation African migrants; they had a median age of 25.9 (range 19-35). Only two had taken chemoprophylaxis not adapted or stopped prematurely. Two patients were Asian immigrants recently arrived. They came from non-endemic areas and contracted the infection during the travel to Europe via Asia and sub-Saharan Africa. The other patients were two African residents coming to France for medical treatment and five French travelers who went to endemic countries for tourism. Forty six patient were hospitalized, sixteen of them in UCI. First line treatment was intravenous quinine in 39 and oral antimalarial therapy in 10. One African migrant, a woman of 33, VRF traveler, died of cerebral malaria. **Conclusion:** Till the end of the twentieth century migrants has appeared as the major risk group for imported malaria in Europe. VRF travelers were considered as semi-immune presenting mostly with uncomplicated malaria. Because of a longer stay outside the endemic zone with loss of malaria immunity they became a group with similar risk for severe malaria than European travelers. We identified last years another population at high risk for severe malaria: non-immune young people of the second generation of African migrants, born in France or arrived during childhood, also VRF travelers. **E-mail:** michel.develoux@sat.aphp.fr

Mal094- Prevalence of *Plasmodium falciparum* infection in the community; Guéckédou Prefecture, the Republic of Guinea

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Introduction: Malaria is hyper-endemic in the Republic of Guinea however there is little information on the actual malaria burden; the most recent demographic health survey was conducted in 2005 and incidence estimates are derived from hospital and health centre records. In 2010 Médecins Sans Frontières began a multi-component malaria intervention in Guéckédou Prefecture with the objective of reducing malaria morbidity and mortality. MSF conducted a cross sectional survey to ascertain community specific information on the prevalence of individuals infected with the malaria parasite and determine the proportion of asymptomatic carriers among them. **Materials and Methods:** A two stage cluster survey was conducted in one district and three sous-prefectures of south-western Guinea. One hundred and twenty clusters, 30 in each sous-prefecture, were selected by population proportionate to size sampling and stratified according to area. Within each cluster households were randomly selected using the EPI method and at least 55 people sampled per day. Informed consent was obtained for each participant and a structured questionnaire was administered. Participants were tested with SD Bioline HRP2 rapid test (RDT) to determine the presence of *Pl. falciparum* parasites in peripheral blood and both a thick and thin blood smear were made for laboratory analysis. All RDT positive participants were treated with the national first line ACT treatment or referred to the hospital when necessary. **Results:** Overall, 6,947 people participated in this cross-sectional survey. Malaria parasitemia confirmed by RDT was found in 63% (4435/6947) of the survey sample while laboratory analysis of the thick blood smears available detected the malaria parasite in 69.5% of participants. Thirty-three percent of laboratory positive participants were children <5 years of age and 67% were ≥5 years of age. In 99% of the laboratory positive samples available *Pl. falciparum* was the most frequently detected parasite species. Asymptomatic individuals constituted 18% of laboratory positive cases (95% CI:17.1-18.9%) reporting

neither having an episode of malaria within the month prior to their test nor having a self-reported history of fever 24 hours prior to being tested and were not febrile. Chi squared analysis showed a statistically significant difference ($p=0.000$) in malaria prevalence when stratified by area, lower prevalence in the urban district compared to the 3 more rural sous-prefectures. **Main Conclusions:** This is the first survey of its type in Guéckédou Prefecture where despite investments in malaria control and a focus on reducing malaria burden malaria remains a major cause of morbidity. Malaria prevalence as confirmed by this survey is high and has until now been an estimation. Asymptomatic individuals constitute a large proportion of the population indicating that in order for intervention strategies in this region to be effective they also need to target this parasite reservoir. This survey will be used to guide intervention strategies in south-western Guinea and as a baseline to monitor the impact of our program. **E-mail:** amanda.tiffany@geneva.msf.org

Mal095- Imported malaria: epidemiological and clinical analysis of cases diagnosed in University General Hospital of Alicante (Spain) during 1995-2012

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Introduction: Malaria is the main imported disease in Spain. The aim of the present study is to describe epidemiological and clinical pattern of imported malaria diagnosed in the University General Hospital of Alicante (UGHA) and to know his temporal tendency. **Material and Methods:** retrospective review of clinical records of patients with malaria (B50-B54 categories ICD-10) diagnosed between 1/1/1995 until 29/02/2012 in the UGHA. Information was introduced in a protocol of data collection. **Results:** We diagnosed 109 cases of malaria, 16 (14,7%) in the period of 1995-2000, 54 (49,5%) between 2001-2006 and 39 cases (35,8%) between 2007-2012. Age (mean \pm SD): $31,9 \pm 15,5$; 17 pediatric cases (15,6%). Women: 48,6%; immigrants: 37 (33,9%), European travellers: 31 (28,4%), VFR (visiting friends and relatives) 41 (37,6%). 56,1% (60 cases) had history of previous malaria and the 91,7% (100 cases) came from Sub-Saharan Africa, mainly of Equatorial Guinea (56 cases). 68,4% of the travellers/VFR not made any malaria chemoprophylaxis. *Plasmodium falciparum* was detected in 76,5% of the cases. In 10 (9,2%) cases *Plasmodium* species was not detected; one case of mixed *falciparum-vivax* malaria. In 19 patients (17,4%) thin and thick blood smears was negative and the diagnosis was made by Multiplex-PCR in 8, by rapid test antigens in 7 and by serology in 2. Median time between clinical onset and diagnosis: 4 days. 81% of the patients was admitted to the hospital. Median time of hospitalization: 5 days. Symptoms/signs more frequents: fever 96.3%, chills 75%, headache 53.7%, swelling 51,9%, arthromyalgias 47.2%, splenomegaly 29,4%, vomits 28.7%, hepatomegaly 26,9%, diarrhoea 25.9%, cough 18,5% and jaundice 13%. More frequents laboratory alterations was: LDH > 250 UI/l (89%), platelets counts $< 150000/\text{mm}^3$ (68,6%), AST > 40 UI/l (62,5%), total bilirrubin > 1 mg/dl (53%), haemoglobin < 12 g/dl (42,9%), leukocytes counts $< 4000/\text{mm}^3$ (26,7%). Mean of leukocytes counts ($5242/\text{mm}^3$ vs $7062/\text{mm}^3$; $p= 0,006$) and platelets counts ($10258/\text{mm}^3$ vs $173267/\text{mm}^3$; $p< 0,001$) was minor in travellers/VFR than in immigrants. Headache (61,4% vs 39,5%; $p=0,028$) was more frequent in travellers/VFR and enlarged spleen (21,4% vs 43,6%, $p=0,014$) in immigrants. Fifteen patients (13,7%) presented complications, without differences between travellers/VFR and immigrants. Evolution: cure in 107 cases (98.2%), dead in 1 case (0.93%), severe neurology saequels in 1 case (0.93%) and *vivax* malaria relapse in 1 case. **Conclusion:** 1. After an increment of cases of malaria in the period 2001 - 2006 due to of the great increase of the immigration and the intercontinental travels, there is a descendent tendency from 2007. 2. Its must be to remark on the importance of a correct compliance of malaria chemoprophylaxis in travellers/VFR. 3. Differences were observed in malaria clinical and biological pictures between travellers/VFR and immigrants. 4. Clinical evolution is similar in travellers/VFR and immigrants. **E-mail:** torrus_die@gva.es

Mal096- *Plasmodium falciparum* malaria with unusually long incubation period about 3 cases

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Introduction: Clinical symptoms of *Plasmodium falciparum* malaria normally appear within 2 months of transmission. The incubation period is important for deciding whether a febrile illness is associated with a previous stay in a malarial region. This cases report shows that *P. falciparum* malaria can have a prolonged incubation period. **Material and Methods:** Series of cases and revision of the medical literature. **Results:** CASE 1: Woman of 56 years old with intermittent fever episodes (2-3/year) since 11-12 years ago. She lived in Equatorial Guinea during 14 years and come back to Spain in 1996, without travels to malaria endemic countries since then. Personal history of malaria, onchocerciasis and loasis. She consults in 2008 January because since her return from Africa has intermittent fever episodes that she self treat with chloroquine. Its make thin and thick blood smears and rapid malaria antigen test that was negatives. *Plasmodium* serology IFA 1/32. *Plasmodium* Multiplex-PCR: positive for *P. falciparum*. She was treated with quinine sulphate + doxycycline for 5 days. Multiplex- PCR *Plasmodium* negative after this treatment. No fever episodes since then. CASE 2: Woman of 30 years old of nigerian origen, with 8 weeks gestation. She was admitted to the hospital in 2007 October because one month of fever and pancitopenia. She lives in Spain for 7 years ago; no travels outside of Spain since then. Physical examination: T^a 38,2°C, pale conjunctives, systolic cardiac murmur and enlarged spleen. Hb 6 g/dl, leukocytes count 1710/mm³, lymphocytes count 700/mm³, platelets 84000/mm³. Thin and thick blood smears: presence of *P. falciparum* trophozoites (parasitemia 3%); rapid malaria antigen test positive for *P. falciparum*. VIH serology positive; CD4 lymphocytes count 35/mm³; *Plasmodium* serology (IFA) negative. She receive treatment with quinine sulphate + clindamicin for 7 days. Fever disappear. Thin and thick blood smears became negative and the platelets return to normal values. No relapses since then. CASE 3: Spanish man of 66 years old was admitted to the hospital in the Haematology department in 2009-01-31 because fever and neutropenia of 2 days evolution. He was diagnosed of chronic lymphocytic leukemia in 2008 december; in 2009-01-19 receive first cycle of QT (cyclophosphamide and fludaribine) and rituximab for rapid progression. Treatment with antibiotics and GM-CSF start with fever and neutropenia resolution. Hemocultures were negatives. In 2009-02-07 is admitted other time because high fever (T^a 39°C). Leukocytes 2750/mm³; neutrophiles 2320/mm³; lymphocytes 120/mm³; platelets 44000/mm³; Hb 10,4 g/dl. Hemocultures negatives. Treatment with meropenem and amikacin was started and discharge without fever in 2009-02-13. In 2009-04-19 is admitted other time for high fever (T^a 39,3°C), without neutropenia. Broad spectrum antibiotic and antifungal treatment was initiated. Hemocultures negatives. Study of unknown origin fever was initiated. Patient report travel to Colombia 1 year before (2008 april-may) without malaria chemoprophylaxis. Thin and thick blood smears: negative; rapid malaria antigen test positive for *P. falciparum*. *Plasmodium* serology (IFA) negative. Treatment with quinine sulphate + doxycycline for 7 days with fever disappearance since then. **Conclusions:** 1. *Plasmodium falciparum* malaria can appear years after to exit of endemics areas. 2. We not find *falciparum* malaria cases with incubation period of 10 years or more in the medical literature. **E-mail:** torrus_die@gva.es

Mal097- Field research: Micronutrient status and malaria infection among rural villagers of Lao PDR.

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Introduction: Malaria and malnutrition are serious public health concerns in Lao PDR. To observe the impact of malaria on the serological indicators of micronutrient status among the rural villagers, we conducted 1) research on asymptomatic malaria infection, growth status, and anemia among the children and 2) serum zinc concentration and malaria antibody titer among rural villagers. **Methods:** **Asymptomatic malaria and anemia:** This cross-sectional study was conducted In December 2010 and March 2011 in Savannakhet province. Malaria infection was detected by polymerase chain reaction (PCR) on *Plasmodium falciparum* (Pf). Underweight, stunting, and anemia were defined according to WHO standards. Odds Ratio (OR) of anemia from asymptomatic malaria infection was calculated. **Serum zinc concentration and anti-Pf IgG titer:** This study was conducted in Attapeu Province, in November 2006. We collected blood samples from villagers. An enzyme-linked immunosorbent assay was used to measure anti-Pf IgG titer. Each serum sample was measured the concentration of zinc. **Results:** **Asymptomatic malaria and anemia:** We analyzed samples from 319 children aged 2-9 years. We found 20 subjects (6.3%) had asymptomatic malaria infection. Anemia was in 90 (28.2%). Asymptomatic malaria infection was associated with the risk of anemia. OR were 5.35 (95% confidence interval (CI): 2.06-13.91). Among the underweight children (n=123), OR of anemia with asymptomatic malaria were 5.60 (95% CI: 1.32-23.81). Among children who were not underweight, OR was 5.14 (95% CI: 1.44-18.34). Among stunted children (n=137), the OR of anemia was 4.18 (95% CI:1.31-13.34). Among those without stunting, the OR of anemia with asymptomatic infection was 6.80 (95% CI: 1.20-38.50). **Serum zinc concentration and anti-Pf IgG titer:** Of 71 blood samples (aged 4-55 years old), 41 were Pf positive and 30 were negative. The median serum zinc concentrations were 56.0 µg/dl in the Pf-positive group and 62.5 µg/dl in the Pf-negative group. The median anti-Pf titers were 833.4 in the positive group and 1237.2 in the negative group. **Main conclusions:** Asymptomatic malaria infection was associated with anemia among the children in Lao PDR. Further, the results indicated low zinc status but sustained antibody responses among the villagers. **E-mail:** watanabe@comb.u-ryukyu.ac.jp

Mal098- Long latent period of relapse *Plasmodium ovale* in a malaria non-endemic area

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An imported *Plasmodium ovale* relapse in a man at least 13 months after initial infection in sub-Saharan Africa is reported. The suspicion of malaria relapse is important, especially on travelers arriving from endemic areas. *P.ovale* is one of four *Plasmodium* species, transmitted by the bite of infective female *Anopheles* mosquito. In Brazil, there is no autochthone's cases caused by this species. On *P. ovale* and *P. vivax* infections, hypnozoites can remain dormant in the liver before infecting red blood cells and causing relapse, which can occur even after appropriate treatment of a blood-stage infection. Reports describe relapses of 7 days to 45 months after treatment of the primary attack. Case report: a 48 year-old man who had lived in Equatorial Guinea for 18 months, presented with 2-week history of intermittent fever, chills, headache, and joint pain, starting 12 months after leaving the endemic area. These symptoms would recur approximately every 48 hours. He had no other complains and was asymptomatic between febrile episodes. While in Africa, he had been treated with artemether-lumafantrine for malaria on two occasions, most recently one month before traveling back to Brazil. He didn't perform treatment-control blood films. Physical examination was normal. The current malaria thin blood smear demonstrated *Plasmodium ovale*, confirmed by genomic amplification. Initial evaluation showed 4735 parasites/mm³ by microscopic examination of Giemsa-stained thick film. Cell blood count showed mild anemia and thrombocytopenia with normal white cell count. He was treated with chloroquine 600 mg base PO, followed by 450 mg daily for 2 days combined with 45 mg base primaquine PO daily for 7 days, after a negative glucose-6-phosphate dehydrogenase (G6PD) deficiency test. He had a clinical response with parasitaemia resolved within 72 hours and no febrile episodes after treatment. Discussion: a history of living in a non-endemic malaria area associated to arthralgia and thrombocytopenia, suggests a viral illness, like Dengue, endemic in Rio de Janeiro, more likely than malaria. However, it is crucial to consider

malaria in differential diagnoses on unexplained fever in subjects who have a history of previous malaria episodes or travel to endemic areas. When this patient took malaria drugs during the previous infections he eliminated blood-stage parasites and left *P. ovale* hypnozoites. Certainly, this case showed the need to considering co-infection, in patients who was exposed to *P. ovale*, founded of previous malaria and geographic area of travelling. It also highlights the importance of considering even rare or unusual etiologies. Finally, this report emphasizes the importance of requesting malaria testing in febrile patients coming from endemic areas, even after a long asymptomatic period. **E-mail:** edwigesmotta@yahoo.com.br

Methodology

Mal099- Efficient extraction of *Plasmodium falciparum* DNA from dried blood spots on filter paper.

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Introduction: Single-nucleotide polymorphism (SNP) detection is the most often used method to examine in a large number of field samples the genetic variation of *Plasmodium falciparum* associated with resistance to antimalarial drugs. Dried blood spots on filter paper are the samples generally used for such tracking, as this form of sampling avoids difficulties associated with collecting, transporting and processing the isolates. However, DNA extraction is inefficient when the samples have been stored for a long period of time and some methods produce DNA with a short half-life. **Materials and Methods:** Four of the most reported methods to extract DNA from *Plasmodium* using dried blood spots on filter paper were modified and their efficiency was tested by using them to extract DNA from clinical samples that were collected in previous studies. To monitor the success of the procedure, DNA obtained from each sample, with each of the four methods, was used as a template in PCR assays to amplify the *Plasmodium falciparum* chloroquine-resistance transporter (*pfcr*t) gene. **Results:** The four modified extraction methods were efficient in extracting DNA from clinical samples collected up to 10 years earlier. The methanol-based method was the least efficient and the least reproducible, while the NaOH-based method was the one that produced the least amount of material. The modified methods based on saponin/chelex-100 and Tween/chelex-100 were the most efficient, particularly the last one. **Conclusions:** In this study, we were able to standardize a method to extract DNA from *P. falciparum* from dried blood spots on filter paper, which was efficient irrespective of the parasitaemia and the storage time of the samples. PCR assays done two (2) years after extraction showed the quality of the DNA remained adequate for use in genotyping assays. The standardized method made it possible to extract DNA from clinical samples from which DNA could not be extracted using traditionally reported methods. This research was funded by Universidad El Bosque (El Bosque University) through Project PCI 2010-177. **E-mail:** chaparrojacqueline@unbosque.edu.co

Mal100- Preservation of wild isolates of human malaria parasites in wet ice and adaptation efficacy to *in vitro* culture

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Wild isolates of the human malaria parasites were preserved at fields in wet ice for 2-12 days, and cultivated at a laboratory in Indonesia by a candle jar method. In four isolates of *Plasmodium falciparum* collected from Myanmar and preserved for 12 days, parasitaemias were much decreased from the original values, and all isolates failed to grow. In 31 isolates preserved for 5-10 days, nine were

transformed to young gametocytes within Day 6, and stopped asexual growth. However, other 22 isolates grew well. Particularly, 14 isolates grew well for a month or more, and stocked as culture-adapted isolates. From Ranong, Thailand, nine isolates were cultivated after preserved for 7 days, and six isolates grew well. On the other hand, all of 59 isolates collected from eastern Indonesian islands (Buru, Halmahera and Flores) failed to establish as culture-adapted isolates, even though most of them were preserved only for 2-3 days: 49 isolates were transformed to sexual stages within Day 5-10, and ten isolates stopped to grow on Day 3-5 by unknown reason. These results indicated that a great different characteristic on adaptation to in vitro culture may exist between wild isolates distributing in continental Southeast Asia (Myanmar and Thailand) and in eastern Indonesia, and gametocytogenesis might be easily switched-on in Indonesian isolates after new ring forms appeared in culture. In wild isolates of *P. vivax*, *P. malariae* and *P. ovale* collected from Myanmar and Indonesia and preserved for 2-9 days, ring forms or young trophozoites were survived, but adaptation to in vitro culture was failed. These results may indicate that wild isolates of the four human malaria parasites could be preserved in wet ice for around 10 days. **E-mail:** hiko@oita-u.ac.jp

Mal101- *Plasmodium falciparum* malaria: proteomic analysis

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Introduction: The infection by *Plasmodium falciparum* is responsible for severe forms of malaria, characterized by multisystemic alterations with high mortality rates. The proteomic studies have allowed identifying forms of the parasite with the highest power of infectivity by analysis of expressed proteins. From the mapping of these proteins is possible to envision new prophylactic and therapeutic approaches, including the prospects for vaccine development. The application of proteomic techniques has also allowed the development of biomarkers of malaria due to *P. falciparum*, for diagnostic purposes. **Material and Methods:** The objective was to present current applications of proteomics techniques in *P. falciparum* malaria. The text has been constructed from the review of the literature search strategy set (descriptors: malaria, proteome, *Plasmodium falciparum*). We used the databases Scientific Electronic Library Online (SciELO) and U.S. National Library of Medicine (PubMed). **Results:** Of the total of 95 citations were selected 18 articles for the composition of the review, choosing texts that are directed to the application of proteomic techniques in malaria by *P. falciparum*, conducted in humans. **Conclusions:** A proteomic analysis reveals new possibilities for improving the diagnosis, treatment and prophylaxis of malaria due to *P. falciparum*. In this context, new experimental studies are sorely needed. **E-mail:** rsiqueirabatista@yahoo.com.br

Mal102- Microscopic enumeration of *Plasmodium* using an assumed standard white blood cell value or a blood volume in a microscopic area overestimates parasitaemia in malaria patients

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Introduction: Although several methods for estimation of densities of blood-stage malaria parasites by microscopy are in use, the most common is to count the number of asexual parasites seen relative to a given count of white blood cell (WBC) and then to multiply the parasite: WBC ratio by 8000, the assumed number of WBC per μL of blood. This assumed standard value most often does not give the true value instead, it under or overestimates it. If an accurate WBC count is known, this can be used to give a more accurate figure with appropriate adjustment of the multiplication factor. Among 656 malaria patients attending at our School Hospital from 2003 to date, 50% and 75% had WBC counts less than 4300 and less than 6800 cells/ μL , respectively. This study aims to correlate parasite loads obtained by

different methods of parasitaemia quantification, in order to guide health professionals and researchers to adequately monitor their malaria patients. **Material and Methods:** 5mL of venous blood was drawn from 50 malaria patients into an EDTA-filled tube, to be used for blood films and for an automated WBC count. Parasite density was calculated by counting only blood stage parasites per 200 WBC and converting to a parasite/ μ L by knowing the patient cells count. We assumed this method as gold standard. Alternatively, the parasite density was determined according to the following methods: i) converting to an assumption of a standard 8000 WBC/ μ L; and ii) counting of parasites in 200 microscope fields in a standard thick film, that is equivalent to approximately 0.4 μ L blood, and convert parasites/ μ L simply multiplying the value found by 2.5. To analyze the results we tested the correlations between parasite density obtained by the alternative methods and that obtained in the gold standard. Different rates of assumed white blood cell value (4000, 5000, 6000 and 7000 per μ L) were further used to test the WBC cut-off that provide a best correlation with the parasitemia obtained by the method using automatic cell count. **Results:** The box plot analysis of the parasite load determined by all alternative methods using different assumed values of WBC or by examined microscopic area revealed a negative skewed distribution. However, only the assumed cut-off point of 5000 WBC/ μ L provided a boxplot similar to that found when parasitaemia was based on the patient WBC automatic counting ($p=0,776$ – Wilcoxon-Mann-Whitney test). Moreover, only the parasite density obtained by the 5000/ μ L cut-off showed distribution values statistically similar to that obtained by the automatic counting of WBC. **Conclusion:** Enumeration of malaria parasites assuming a standard value of 8000 WBC/ μ L or by a estimated blood volume in a microscopic area overestimates parasitaemia. A WBC count of 5000 cells/ μ L as a reference to estimate the parasite load is best suited to determine the parasitaemia of malaria patients. **E-mail:** eduardo.expert@gmail.com

Pathogeny

Mal103- Severe malaria by *Plasmodium vivax* and *Plasmodium falciparum* in Cordoba, Colombia

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Introduction: The clinical spectrum of complications of infection by *Plasmodium* spp. is very wide due to the number of systems affecting and complex pathophysiological mechanisms. The WHO considers malaria complicated the clinical case presented with confusion, cerebral malaria (defined as the presence of coma not attributable to another cause), severe anemia, renal failure, pulmonary edema and hypoglycemia, shock, bleeding or disseminated intravascular coagulation, liver damage and jaundice, generalized convulsions, hemoglobinuria, hyperparasitemia, hyperpyrexia, and hyperbilirubinemia. Severe malaria for decades has been attributed to that caused by *P. falciparum*, though in the last 5 years there have been reports in different countries cases of severe malaria by *P. vivax*. **Objective:** The aim of this study was to determine the frequency of severe malaria and the clinical characterizing of these patients in Cordoba, Colombia. **Methodology:** For this descriptive, retrospective and transversal study, we was reviewed medical records of 127 patients hospitalized in the Hospital San Jerónimo de Montería, Cordoba during the period January 2009 to June 2010, which found that 60% of cases of malaria due to *P. vivax* 31% *P. falciparum* malaria 9% were mixed, by microscopic diagnosis. **Results:** We found thrombocytopenia, anemia and acute respiratory distress syndrome as the most frequent complications and hemoglobin of 4.4mg/dL to 11mg/dL, hematocrit less than 33.5% and platelets under 150.000/ μ L, as most important laboratory indicators. *P. vivax* was the cause major cases of severe malaria, this is an important finding that government health agencies and the team physician must consider in order to initiate new prevention and control strategies for this species of parasite. There were 4 cases of death; these patients were diagnosed by microscopy as *P. vivax*. **Conclusions:** We conclude that there is a high frequency of severe malaria cases in the department of Córdoba. Prospective studies are necessary to confirm the species of the parasite by molecular methods and to characterize the patients clinically. In

addition to molecular epidemiology is very important to characterize the parasites, to know the possible circulating strains, their virulence and the continuous monitoring and directed to the possible reduction of the treatment failure. **E-mail:** mfab203@hotmail.com

Mal104- Evaluation of serum levels of nitric oxide in acute and convalescent phases of malaria patients in Brazilian Amazon

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Introduction: Malaria remains one of the most important public health problems in the world resulting in over 200 million cases each year. Several mechanisms involved in pathogenesis and pathophysiology of malaria remains unclear. It is known that Nitric Oxide (NO) is thought to be an important mediator and critical signaling molecule in this process, which have been conflicting reports concerning the clinical significance of NO in development/protection of severe malaria, however the role of NO in *P. vivax* infections and non-severe malaria patients is poorly understood. Therefore, the **objective** of this study was to evaluate the levels of NO in uncomplicated malaria caused by *Plasmodium vivax* and *Plasmodium falciparum* in 72 patients from a population living in the Amazon region. **Material and methods:** Plasma samples of infected individuals were collected in acute phase (Day 0 – diagnosis) and convalescent phase (after complete treatment – day 15) of malaria infection. The levels of NO were assessed by Griess reaction with a standard curve to assess the plasma concentrations. **Results:** Among the patients evaluated in our study, the overall concentration of NO ranged from 0,86 – 19,75 μ M. However, NO levels detected were similar in acute ($4,34 \pm 3,95 \mu$ M) and convalescent ($3,84 \pm 3,05 \mu$ M) phases. Moreover, the levels of both phases were significantly lower ($P < 0,0001$) than the control group ($7,75 \pm 1,21 \mu$ M), suggesting a limited production of NO in uncomplicated malaria infection. Furthermore, patients infected with *P. falciparum* presented significantly higher ($P < 0,0001$) levels of NO ($5,53 \pm 4,42 \mu$ M) when compared with patients infected by *P. vivax* ($3,15 \pm 2,46 \mu$ M), which suggest that NO levels are related to the plasmodial species. We did not find relationship between NO production and epidemiological data obtained during the study, such as length of residence in an endemic area, time since last infection, number of previous infections and co-infection with intestinal parasites. **Preliminary conclusions:** In uncomplicated malaria the production of NO is limited and a *P. falciparum* infection stimulates a higher production when compared with *P. vivax*. However, genetic variations in gene promoters related to NO production could also influence the observed production of NO in our patients. Therefore, studies are in progress to evaluate the frequency of such polymorphisms in individuals living in areas of unstable transmission of malaria. **E-mail:** virginia@ioc.fiocruz.br

Mal105- Platelet count and platelet parameters in malaria patients from Colombia

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Introduction: The purpose of this study was to characterize the platelet profile of Colombian patients with malaria and explore the relationship with the clinical presentation of the disease. **Material and methods:** **Patients and study design:** The population included patients of all ages with clinical symptoms suggesting malaria, attending to malaria diagnosis post and rural hospitals in 4 endemic regions in Colombia. Were analyzed retrospectively clinical and laboratory data of 862 patients enrolled with positive test for *Plasmodium*, with previous informed consent. **Laboratory testing:** Thick blood smear was made following WHO standards and parasitemia was estimated based on counting 200 WBC. A sample of blood was taken on admission during the acute stage; an automated hemogram was done in fourth or fifth-generation cell counters which included the analysis of platelet parameters –PP-. **Data analysis:** The normal reference values to classify the PP were: **1)** platelet count –PC- 150.000-400.000

platelet/ μ L; **2)** Mean platelet volume –**MPV**- 6,5-13,5 fL; **3)** Platelet distribution width –**PDW**- 15,4-16,8%; **4)** Plateletcrit 0,085-0,287%. Thrombocytopenia was defined as mild (50.000- 149.999 platelet/ μ L, moderate 25.000-50.000 and severe <25.000); severe malaria was established by the WHO criteria adjusted for Colombia. The Kolomogorov-Smirnov test was used for test normality. The median of the platelet parameters were compared with Mann-Whitney U test and proportion analysis were done with Chi-squared test for qualitative variables. **Results:** Were analyzed the PP 862 patients, 542 male (63%) with a median age of 23 years and a median of 5 days time evolution of the disease. The specie diagnosed was *P. falciparum* in 556 patients (63%), 311 were infected with *P. vivax* (36%) and 13 (2%) had mixed infection with both species. The median of PC was 121.000 platelets/ μ L, the count was low in 564 (65%) with moderate thrombocytopenia in 73 (9%) and severe in 20 (2%). The MPV was obtained for 720 patients and was normal in 83% of them and low in 17%. The PDW was obtained in 654 cases, was normal in 32% and high in 65%. While not significant difference was established in the platelet count by species of plasmodium, in the *P. falciparum* cases was higher values for plateletcrit, MP and PDW (Mann-Whitney U Test, $p < 0,005$). Presented clinical complications 247 patients (29%) and among these severe or moderate thrombocytopenia was presented as the only complication in 44 (5%). In the complicated cases (excluding thrombocytopenia) were found lower median values for platelet count and plateletcrit and higher value for PDW, with statistically significant differences ($P < 0,002$). **Main conclusions:** Thrombocytopenia was a common disorder in patients with malaria, characterized by normal platelet volume and moderate variability in platelet size. However there were a significant number of severe and moderate thrombocytopenia cases. On the other hand, the lowest platelet count and high PDW were more frequent in patients with complicated malaria, suggesting platelet sequestration and platelet proliferative activity. **E-mail:** albertobon@saludpublica.udea.edu.co

Mal106- White blood cells count abnormalities in patients with malaria: relationship to clinical status in patients from Colombia.

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Introduction: The hematological effects of *Plasmodium* on red blood cells and platelets are spreadly known, however those on white blood cells –WBC- have not been completely determined. WBC counts –WBCC- alterations in malaria have been reported, however are their importance in the early diagnostic or as outcomes predictors is unknown. The purpose of this study was to characterize the WBCC profile of Colombian patients with malaria and study the relationship with the clinical presentation of disease.

Material and methods: The population included patients of all ages who consulted to rural hospitals with clinical symptoms suggesting malaria in 4 endemic regions of Colombia. Clinical and laboratory data of 880 patients with positive test for *Plasmodium*, previous informed consent, were analyzed retrospectively. Thick blood smear was made following WHO standards and parasitemia was estimated based on counting 200 WBC. A sample of blood was taken the enrollment day and a complete automated hemogram was done in automatized cell counters, when appropriate, manual cell count data was added. According the age groups (<1, 1–5, 6–15, >15 year) and sex, the hematological parameters were classified using reference values for Colombian population. The WBCC were qualitatively classified as high, normal or low. Severe malaria was established by the WHO criteria adjusted for Colombia. The Kolomogorov-Smirnov test was used for test normality. The median WBCC were compared with Mann-Whitney U test and proportion analysis were done with Chi-squared test for qualitative variables. **Results:** The WBCC from 880 patients were analyzed, 551 male (63%). The patients over 15 years were 71% ($n=617$), with mean age of 23 years. The specie diagnosed was *P. falciparum* in 556 patients (63%), 311 were infected with *P. vivax* (36%) and 13 (2%) had mixed infection. The WBCC was normal in 691 (79%) at moment of enrollment; leucopenia present in 157 patients (18%). Other abnormalities includes neutropenia (5%), neutrophilia (6%), eosinophilia (13%), eosinopenia (4,5%) and basofilia (8%). In the mononuclear cells analysis, were found lymphocytopenia (54%), lymphocytosis (2%), monocytosis (10%) and monocytopenia (0,2%). Association between the abnormalities in the WBCC and the clinical outcome were found, showing that patients with severe malaria had more probability of having leucopenia, lymphocytopenia, eosinopenia, monocytosis and basophilia (Chi-squared test $p = < 0,05$) and neutrophilia was more likely in patients with advanced stages of complication ($P < 0,05$). In severe malaria cases the

median cell count was lower for lymphocytes and eosinophils compared with patients with non-complicated infection (Mann-Whitney U test $p=0,000$). **Main conclusions:** Our results show the importance of the WBC in malaria, reporting that lymphocytopenia and eosinopenia were present at the moment of diagnosis in patient who more likely had severe malaria and lymphocytopenia was a constant finding present in patients in hyperparasitemia; a well-known poor prognostic marker in malaria patients. We hope that WBCC become taken into account in the early diagnosis and prediction of severe infection in malaria patients. **E-mail::** albertobon@saludpublica.udea.edu.co

Mal107- Evaluation of lipid profile in pregnancy women with malaria and without malaria in a hyperendemic area of malaria in the state of Para

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Introduction: Changes in the serum level of lipids, especially cholesterol, its fractions and triglycerides have been observed in many parasitic infections, including malaria. Few studies have addressed the lipid profile in pregnant women with malaria or living in endemic areas of malaria, objectives of this study. **Material and Methods:** A cross-sectional study was conducted from May/2010 to January/2012 in which 223 pregnant women were enrolled, the majority living in an Anajás, a hyperendemic area of malaria in the state of Pará. Pregnant women were distributed into groups: Group A (n=23) - pregnant with malaria from Anajás; Group B (n=144) - pregnant without malaria from Anajás; Group C (n= 56) - pregnant without malaria from Belém, the capital of Pará, considered area of low risk for malaria. All pregnant women drawn venous blood to perform hemogram, cholesterol, HDL-c, LDL-c and triglycerides and did thick blood film to identify the plasmodia. **Results:** Pregnant women without malaria resident in Anajás (Group B) had lower significant levels of total cholesterol, HDL-c, LDL-c than the pregnant women from Belém (Group C) ($p<0.05$). Pregnant with malaria (Group A) had the lowest levels of total cholesterol and its fraction among all groups. Triglycerides serum levels had different patterns according to gestational trimester: the maximum level of triglycerides were observed in second trimester in pregnant women resident in Anajás, regardless malaria (groups A, B). In group C, triglycerides levels increase progressively up to third trimester. No lipids changes were associated with previous history of malaria. Parasitaemia showed a significant inverse correlation with HDL and LDL serum levels ($r= -0.45$; $r= -0.5$; Spearman correlation, $p<0.05$). The cut-off of 47mg/dL of HDL-c had 95.5% of sensitivity, 82.2% of specificity and 83.4% of accuracy and was a biomarker to distinguish between pregnant women with malaria (Group A) from pregnant women without malaria. **Conclusions:** Total cholesterol, HDL-c, LDL-c had different dynamic behavior in pregnant with malaria or in pregnant living in hyperendemic area of malaria, even accounting for the broad physiological values of the lipids that occur in pregnancy. The gestational age may influence the profile of triglycerides. Parasitaemia may influence the levels of HDL-c and LDL-c. The behavior of lipids, especially the HDL-c may act as a biomarker of malaria in pregnancy. Further studies enrolling great number of pregnant women are necessary to address these questions. **E-mail:** jmdesouza2@hotmail.com

Mal108- Concurrent severe *P. vivax* malaria and dengue infection: case report

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Introduction: Despite a wide overlap between malaria- and dengue- endemic areas, published reports of co-infections are rare. Malaria and dengue co-infection can result in overlapping symptoms. Therefore, diagnosis and treatment may become difficult for a physician. Herein we report the case of a 32-year-old Brazilian man, living in malaria endemic area, who presented concurrent infection of *P. vivax* malaria and dengue. **Case Report:** A 32-year-old man coming from Rio Branco (AC), Brazil, was working in Cuiabá (MT), Brazil, for thirty days, when was admitted to the Hospital São Matheus because of high grade fever,

chills, prostration, severe headache, dyspnea, dry cough, cutaneous rash and abdominal pain. He had been well until seven days earlier, when fever, myalgia, headache, fatigue and thrombocytopenia= $130.000/\text{mm}^3$ developed. At the admission, the patient complained worsening of symptoms. The temperature was 39.1°C , the blood pressure was 110×60 mmHg and respiratory rate=28 breaths per minute. There was no evidence of *Plasmodium* at the thick blood smear (TBS) performed on admission. Since he had thrombocytopenia ($89.000/\text{mm}^3$) and a positive IgM ELISA for dengue on day seven, the diagnosis of dengue fever was made. He was treated symptomatically with antipyretics. Two days after the admission, the cutaneous rash subsided but the platelet count progressively decreased ($46.000/\text{mm}^3$) and the fever spikes persisted. The chest X-ray of the lungs was performed showing interstitial pneumonia and bilateral pleural effusion. Ultrasonography revealed hepatosplenomegaly and moderate ascites. Laboratory investigations revealed: hemoglobin=9.2 g/dL, AST= 82.5 U/L, ALT=67.9 U/L and serum creatinine=0.7 mg/dL. Total serum bilirubin was 7.14 mg/dL. Three days after the admission, *P. vivax* malaria was diagnosed by TBS and confirmed through PCR. Parasitemia at admission was ++/++++. He was treated with artesunate, clindamycin and primaquine. Parasitemia and fever cleared after 72 h of treatment. The patient showed progressive clinical improvement and was discharged eleven days after admission. **Discussion:** Malaria and dengue co-infection must be suspected in febrile patients living in or returning from endemic areas. Both diseases cause similar symptoms and concurrent infection may result in overlapping clinical manifestations. Although the incubation phase is longer for malaria, it is important to remember that dengue and malaria co-infection requires special attention because delayed diagnosis can result in fatal complications. **Conclusion:** Our case suggests that the clinical suspicion of malaria and dengue concurrent infections can be easily forgotten. When a disease can be diagnosed in acute febrile illness, the concern on the existence of other infections is usually not taken. Therefore, it is important to remember that both diseases have similar clinical findings and diagnosis can be made concomitantly in patients living or returning from areas where both diseases are endemic or during dengue outbreaks. **E-mail:** andreia_nery@yahoo.com.br

Mal109- Association between α^+ thalassemia and *Plasmodium vivax* blood infection levels in Brazilian Amazon region

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Introduction: A number of mechanisms of α^+ thalassemia protection against malaria disease have been proposed. However, the most clinical and epidemiological studies has focused on *Plasmodium falciparum* and much less is known about the role of α^+ thalassemia in *vivax* malaria. Since parasite density has been recognized as important factor in the outcome in malaria infections, we investigated whether α^+ thalassemia is associated with *P. vivax* parasitemia in malaria patients from Brazilian Amazon Region. **Material and Methods:** To evaluate this hypothesis, *P. vivax* parasitemia was assessed in 236 malaria patients. α^+ thalassemia was determined by electrophoretic analysis at pH 7.0 and microscopic visualization of Hb H inclusions in unfixed cells with Brilliant cresyl blue which is an oxidative dye. The subjects were matched with respect to age (± 5 years), sex, and ethnicity. All the control subjects were genetically independent. Thick blood films were confirmed by two independent experienced microscopists who were unaware of each result according to the World Health Organization recommended procedures. Association of parasitemia with α^+ thalassemia was examined using model based and model free approaches. **Results:** The parasitaemia on the thick blood films ranged from 5 to 16,680 parasites/ μL (mean $1,540 \pm 1,915$). The frequency of α^+ thalassemia was 47,8% (113/236). Alpha thalassemic carriers have lower parasitemia count compared with non-alpha thalassemic carriers ($p < 0.01$). **Conclusion:** Our results show that α^+ thalassemia reduces *P. vivax* parasite density in Brazilian malaria patients which suggests that this mutation may confer selective advantage against *vivax* malaria. **Funding:** CNPq and FAPESP **E-mail:** gcapatti@hotmail.com

Mal110- Rosette formation in *vivax* malaria

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Introduction: Malaria still remains one of the most important infection diseases. In *falciparum* malaria the severity of the infection can be associated to the capability of the parasite to form rosettes. Rosetting is the ability of the infected erythrocyte to adhere non-infected cells. While many rosetting studies have focused in *P. falciparum* little is known about rosetting in *vivax* malaria. So far, only three studies had reported rosetting formation in *vivax* malaria, all of them in isolated originated from Thailand. Different from *P. falciparum*, rosetting in *P. vivax* has not been associated with severity neither with parasitaemia or any other parameter. To date, there is no information about rosetting formation with isolates from other localities where *vivax* malaria is prevalent, and the rosetting biological meaning is still unknown.

Methods: To investigate *Plasmodium vivax*-infected erythrocytes (Pv-iE) rosetting capability, blood samples were harvested from infected patients originated from Manaus - Amazonas, Brazil. Following leukocyte depletion by *Plasmodipur*TM columns and enrichment by *Percoll*TM gradient, rosetting formation of Pv-iE (4% parasitaemia and 4% hematocrit) was analyzed after 40 min incubation at 37C in the presence of homologous plasma or controls, using parasites collected immediately post enrichment or after 16 hour s in culture (maturation). **Results:** Of those 22 Pv-iE isolates analyzed, 50% had more than 5% rosetting rates and 18% no rosettes were observed. Most important and in sharp contrast, after maturation almost half of the isolates (42%) had more than 50% of rosetting rates. Although a small data sample was tested, no association between rosetting rates and blood group type were observed.

Conclusions: Here we have shown that isolates of *P. vivax* from Brazil are able to form rosettes, especially after maturation. Our data suggest that rosetting formation of *P. vivax* is dependent on parasite late forms (schizonts) and blood group type might not play a role. However, studies looking for the role of rosetting using worldwide *P. vivax* isolates are needed and careful analysis regarding rosetting invasion and severity is urged. **E-mail:** letusaa@gmail.com

Mal111- Acute Respiratory Distress Syndrome (ARDS) As Initial Clinical Presentation of Severe *vivax* Malaria: Case Report

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Introduction: Although often regarded as causing a benign infection, there is recent increasing evidence that the overall burden, economic impact, and severity of *P. vivax* have been underestimated, in part due to a bias in the scientific literature which traditionally devoted most of its attention to the more lethal parasite *Plasmodium falciparum*. ARDS has been described as a complication of *P. vivax*. However, this complication is most reported after starting antimalarial treatment. Herein we report the case of a 14-year-old Brazilian girl living in a malaria endemic area, who presented ARDS caused by *P. vivax* before starting anti-malarial treatment. **Case Report:** A 14-year-old girl was admitted to the Hospital Regional de Cacoal (Cacoal, Rondonia, Brazil) because of fever, chills, prostration and severe dyspnea. She had been well until seven days earlier, when fever, myalgia, headache, fatigue, dry cough, tachypnea and decreased in the consciousness developed. Two days before the admission, the patient complained of worsening of symptoms. On the admission, the temperature was 38.9°C, the blood pressure was 78x56 mmHg, hypoxemia (SaO₂ below 90%) and heart rate=125 beats per minute. *P. vivax* malaria was diagnosed by thick blood smear and confirmed through PCR. Parasitemia at admission was +++/++++. The APACHE II score was 26, with death estimated risk of 55%. The patient was sent to the Intensive Care Unit (ICU), where invasive mechanical ventilation was initiated. Arterial gasometry showed metabolic acidosis (pH = 7,2) and FiO₂/PaO₂ ratio was < 200, consistent with a diagnosis of ARDS. The chest X-ray of the lungs were performed showing defined heterogeneous densification and consistent with interstitial pneumonia. Laboratory investigations revealed: hemoglobin=6.8 g/dL, platelet

count=67,000/mm³ and serum creatinine=0.4 mg/dL. Total serum bilirubin was 2.84 g/dL. She was treated with artesunate, clindamycin and primaquine. Parasitemia became undetectable after 48 hr of treatment. Ceftriaxone and azitromycin was initiated 24 hours after the admission considering the possibility of bacterial superinfection. The patient showed progressive clinical improvement, with progressive reductions of the levels mechanical ventilation and was discharged from the ICU ten days after admission. **Discussion:** ARDS as complication of vivax malaria usually appear from six hours to eight days after the initiation of anti-malarial treatment. These findings are consistent with what is observed in cases of falciparum malaria, and they could correspond to an exacerbation of the post-treatment inflammatory response. Nevertheless, our patient presented severe pulmonary symptoms before the initiation of anti-malarial treatment, which was previously related and characterized as interstitial pneumonia. **Conclusion:** Our case suggests that severe pulmonary involvement is not necessarily a consequence of an inflammatory response produced after anti-malarial treatment. **E-mail:** andrea_nery@yahoo.com.br

Mal112- Cytoadhesion of *P. vivax*: the involvement of host receptors and parasite stage-forms

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Introduction: The higher pathogenicity of falciparum malaria is ascribed to cytoadhesion of the *P. falciparum*-infected erythrocytes (Pf-iE) in the microvasculature of several organs (e.g. brain, lungs and placenta). Although clinical complications similar to those observed for severe falciparum malaria are now described to *P. vivax*, the cytoadhesion phenomenon is only recently noted for *P. vivax*. We have recently shown that *P. vivax*-infected erythrocytes (Pv-iE) harvested from several patients were able to cytoadhere under static and flow conditions to lung and brain endothelial cells and to placenta cryosections. However, Pv-iE cytoadhesion were 15-fold lower than Pf-iE. As only a small proportion of mature forms, especially schizonts, are detected in peripheral circulation in *P. vivax*-infected patients, we questioned about the ability of this parasite stage-form to cytoadhere to host receptors. **Methods:** To investigate *P. vivax* schizonts adhesive capacity in endothelial cells, we established an in vitro technique as follow. After leukocyte depletion by CF-11 columns, Pv-iE obtained from several patients were enriched by *Percol*TM gradient. Part of parasites obtained in enrichment was used immediately and the other part was cultivated for 18-22h to maturation before been used in cytoadhesion assays. Briefly, 5x10⁴ Pv-iE before or after maturation were added to human lung endothelial cells (HLEC) or CHO (Chinese Hamster Ovary) cells monolayer. After 1 hour of incubation in the presence or not of different inhibitors, the non-adhered Pv-iE were removed, slides were stained and cytoadhesion evaluated. **Results:** Maturation of Pv-iEs for 18-22h after *Percol*TM significantly improved cytoadhesion in HLEC up to 6-fold. Moreover, a positive correlation between schizonts percentage and cytoadhesion rate was noticed. The search for receptors involved in cytoadhesion before and after maturation, using transfected CHO cells or specific inhibitors, showed that ICAM-1 and CD36 were both involved in Pv-IE cytoadhesion, whereas the involvement of CSA (Chondroitin sulfate A) remains to be elucidated. Also, while ICAM-1 seems to be involved in all stage-forms of Pv-IE (trophozoites and schizonts), CD36 seems to be strictly involved in mature forms (schizonts). **Conclusions:** Here we have shown that *P. vivax* schizonts are able to cytoadhere to endothelial cells and, different endothelial receptors could be involved in specific parasite stage adhesion. Collectively these data open new avenues to investigate parasite and host molecules involved in *P. vivax* cytoadhesion and highlight the need for research focusing on *P. vivax* pathophysiology. **E-mail:** stefzinha@gmail.com; costafm@unicamp.br

Mal113- Assessment of oxidative stress, genotoxic damage and apoptosis in mononuclear cells from patients with Malaria vivax

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Malaria vivax is an endemic disease in Amazon region, corresponding to more than 80% of registered cases. The infection increases the production of reactive oxygen species (ROS), a harmful agent to macromolecules such as DNA, which may lead cells to apoptosis induction. The aim of this study was to evaluate the basal level of DNA damage, as well as apoptosis frequency, antioxidant enzymes activity and the presence of an oxidative stress marker in samples of periphery blood of patients with malaria vivax and healthy controls. Samples of BU patients and 76 controls were submitted to the comet assay to evaluate genotoxicity, having tail moment as a parameter. Apoptosis (measured by caspase 3 activity) and the activity of glutathione peroxidase (GPx) and superoxide dismutase (SOD) enzymes were performed using commercial kits. Malondialdehyde (MDA) was detected by thiobarbituric acid reaction (TBA). The results showed that mononuclear cells of malaria patients presented higher levels of basal DNA damage (TM=26,71) when compared to controls (TM=t0,05) ($p<0,0001$). The frequency of apoptotic cells also was significantly increased ($p<0,0001$) in patients (39,3 %) in comparison to controls (21,6%), as well as of MDA levels (2,48 μM e 1.93 μM , respectively). There was no difference between both groups in relationship to SOD activity; however, GPx activity was significantly higher ($p= 10005$) in patients (76669 U/L) when compared to controls (63865 LI/L). There was no significant correlation between DNA damage and SOD, GPx and MDA; however, there was a significant negative correlation between DNA damage and apoptosis ($r= -0,30$, $p=0,006$). The results demonstrate that malaria vivax infection increases the oxidative stress, which has significative genotoxic and cytotoxic effects on mononuclear cells of malaria patients. **E-mail:** mccoandrey@gmail.com, carlubassi@yahoo.com.br

Mal114- Clinical study of the levels of methemoglobinemia in a subject of G6PD deficiency

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Malaria vivax is a major public health problem in Brazilian Amazon. The treatment is based on the association between chloroquine with primaquine. There are several adverse effects of these drugs on neurological, cardiac and gastrointestinal systems, with emphasis to the hemotoxicity of primaquine and its metabolites, causing methemoglobinemia and severe hemolysis in subjects with glucose-6-phosphate dehydrogenase deficiency, which is considered the most prevalent enzyme deficiency in the world. In this study, we described the levels of methemoglobin of a subject with glucose-6-phosphate dehydrogenase deficiency during the treatment with primaquine to *P. vivax* malaria. The levels of methemoglobin were determined according Hegesh *et al.* 1970 and the activity of glucose-6-phosphate dehydrogenase by electrophoresis. The initial parasitaemia was 11.000 asexual forms /mm³. The levels of methemoglobin ranged from 0,85% on D0 to 4,45% on D14 1,1%. The highest level was 11,95% on D7 ($p<0,001$). No signal or symptoms of methemoglobinemia was reported. This finding corroborates previous reports in which the levels of methemoglobin enhanced after therapeutic doses of primaquine, but the glucose-6-phosphate dehydrogenase deficiency do not promote a clinically relevant impact on the levels of methemoglobin. **E-mail:** luizrod65@hotmail.com, jvieira@ufpa.br

Mal115- Effect of a fucosylated chondroitin sulfate on *Plasmodium falciparum*-infected erythrocytes

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Introduction: Severe malaria is characterized by the sequestration of *Plasmodium falciparum*-infected erythrocytes (Pf-iEs) in the microvasculature of vital organs. Pf-iE cytoadhesion has a key role in the pathogenesis of life-threatening malaria and could be targeted by anti-adhesion therapy. Accordingly, sulfated polysaccharides such as heparin and chondroitin sulfate A (CSA) have shown ability to inhibit the cytoadherence of Pf-iEs to endothelial receptors. Although heparin was used in the past as treatment for severe malaria, its use was discontinued due to the occurrence of serious side effects such as bleeding. Moreover, given that these compounds are obtained from mammals, potential risk of contamination has to be considered. In fact, although many compounds have been tested, none demonstrated unequivocal evidence of improvement in clinical trials for prevention and treatment of severe malaria. That said, we investigated the effect of fucosylated chondroitin sulfate (FucCS), a highly sulfated polysaccharide isolated from sea cucumber, *Ludwigothurea grisea*, on *Plasmodium falciparum*-infected erythrocytes. **Methods:** To investigate the effect of FucCS on Pf-iEs (FCR3-strain) cytoadhesion, 5×10^4 Pf-iEs were added to human lung endothelial cells (HLEC) monolayer and incubated with increasing concentrations of FucCS for 1h. Cytoadhesion assays under flow conditions were also performed in the presence of 100 ug/ml of FucCS. To evaluate the FucCS effect on parasite reinvasion, Pf-iEs were incubated with increasing concentrations of FucCS at 37°C for 24h. Rosette disruption was assessed using *P. falciparum* FCR3S1.2. **Results:** FucCS showed to be effective in inhibiting cytoadherence in human lung endothelial cells (HLEC) of different parasite phenotypes (FCR3-strain), previously panned for CD36, ICAM-1 and CSA receptors. Removal of the sulfated fucose branches on the FucCS practically abolished the inhibitory effect, suggesting a central role played by these branches in the occurrence of the inhibitory process. Furthermore, in assays conducted under flow conditions, FucCS reversed significantly parasite cytoadhesion. Also, FucCS blocked parasite reinvasion and disrupted rosettes efficiently. **Conclusion:** Collectively, these findings open perspectives to use FucCS for adjunct therapy against severe malaria. **E-mail:** costatfm@unicamp.br

Mal116- Inflammatory Response Associated with Malaria-induced Anemia

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Introduction: In Sub-Saharan Africa falciparum malaria is a major contributor to childhood anemia, which affects nearly two thirds of all children. While mild and moderate forms of anemia may impair cognitive and motor development, growth, and immune function its severe form is associated with an increased risk of death. **Materials and Methods:** To investigate the development of anemia with regard to malaria-associated, clinical parameter a cohort of 465 children from 129 families was surveyed weekly over eight months during the raining season in rural Ghana, West Africa. Population-averaged models were applied to establish a multivariate regression model for the median hematocrit observed over time. **Results:** Of all parameters studied, temperature was found to be the strongest correlate of hematocrit level, followed by individual *Plasmodium falciparum* infection rate, and C-reactive protein plasma concentration. Notably, individual mild malaria attack rates and parasite densities did not provide better predictive measures. **Conclusion:** These findings suggest that inflammatory responses induced by repeated *P. falciparum* infections rather than pathophysiological processes during acute malaria episodes are responsible for promoting chronic anemia in African children. **E-mail:** schuldt@bni-hamburg.de

Mal117- Maternal anaemia in pregnancy: assessing the impact of preventive measures in a malaria endemic area

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Introduction: In sub-Saharan Africa, malaria, helminth infestations and iron and folic acid deficiencies are the main preventable causes of gestational anaemia. However, there is a lack of information on the effectiveness of anaemia preventive measures in this area, although they are widely implemented. Our study aimed to assess the effectiveness of intermittent preventive treatment in pregnancy (IPTp), anti-helminthic and haematinics on maternal anaemia at different time points of gestation. **Material and methods:** 1005 pregnant women participating in a clinical trial of IPTp (MiPPAD) were followed from early pregnancy until delivery. The study was conducted between 2010 and 2012 in Allada (southern Benin) where malaria transmission is perennial. Women had to be HIV negative and less than 28 weeks of gestational age on inclusion (ANV1). At ANV1, baseline characteristics of the women were recorded. At ANV1, the second antenatal visit (ANV2) and delivery, gestational age was assessed and anthropometric measurements were made. The first and second intakes of IPTp (either SP or MQ) were given on ANV1 and ANV2 under supervision. A treatment dose of albendazole and haematinics were given at ANV1 to be taken home. At all-time points, haemoglobin (Hb) concentrations, malaria and helminth infections were determined. Serum iron, folic acid, vitamin B12, CRP concentrations were also measured. The effectiveness of preventive measures on the risk of anaemia and Hb concentrations was assessed at ANV2 and delivery by comparing the risk factors between ANV1 and after interventions (ANV2 and delivery). Multivariate linear and logistic regressions were used as appropriate. **Results:** 63.8% of the women were anaemic at ANV1, 64.7% at ANV2 and 40.6% at delivery. The prevalence of malaria decreased from 15.1% at ANV1 to 4.0% at ANV2, and increased again at delivery to 9.6%, malaria infection being associated with a lower mean Hb at ANV1 and delivery. Helminth prevalence decreased from 11.1% at ANV1, to 7.2% at ANV2 and 2.4% at delivery. Iron deficiency stayed high throughout pregnancy (33.3% at ANV1, 36.3% at ANV2 and 30.7% at delivery). **Conclusion:** IPTp and anti-helminthic treatments were efficacious to clear parasitic infections and to improve haematologic status, whereas the effectiveness of daily iron and folic acid supplements to correct iron and folate deficiencies and to decrease anaemia was less marked. A lower compliance to this strategy seems the most likely explanation. **E-mail:** smaila.ouedraogo@ird.fr

Mal118- Anemia in Malaria: A Case Report

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Introduction: Anemia in malaria is one of several pathogenic forms of clinical disease, especially of severe morbidity. The causes may be multifactorial due to intra-erythrocyte parasitism with mechanical destruction of erythrocytes, humoral and cellular immune response mediated by cytokines, formation of autoantibodies and changes in erythropoiesis by inadequate production of erythropoietin induced by addition of antimalarials. **Case Report:** EVVC female, 15 years old, coming from Luanda, with previous treated malaria, had fever, chills and headache. Malaria by *Plasmodium falciparum* (3+) was diagnosed at IPEC associated to anemia (Hb=7.7g/dL), reason for hospitalization. Parasitological clearance occurred in 48 h after *iv* treatment with artesunate. She was transfused with two units of packed red blood cells (PRBCs) but anemia remained. Five days later she was discharged after a clinical improvement, but returned complaining of vomiting, fever, dizziness, hematuria three days after. Total bilirubin, direct and indirect LDH was increased and a severe anemia (Hb= 4.3 g/dL) was observed. The Intravascular and extravascular antibody screening were positive for the presence of irregular cold nonspecific antibodies. She remained hospitalized for more seven days and was transfused with two units of previously heated PRBCs. At the end of this period she showed general improvement and restoring of eritropenia. **Discussion:** It was observed that the persistence and severity of anemia was not directly related to parasitaemia and subsequent lysis of erythrocytes, because even after the absence of circulating parasites, anemia continued and intensified probably due to other mechanisms, particularly immune. Immunohematological study concluded that there was not immune hemolysis induced by autoantibodies formation. The potential mechanisms involved were: lack of preheating of PRBC and agglutination reactions with the clumps of red blood cells that favored hemolysis; imbalance reactions as the overproduction of TNF with suppression of erythropoietin and erythroid aplasia observed by the low production of reticulocytes and inadequate reticulocytosis. **Main Conclusions:** Anemia in malaria transfusion should be performed only in cases of imminent risk of death because eritropenia restoration is not immediate and erythroid aplasia of the sector may persist even after the clearance of circulating

parasites. Immunohematological parameters must be carefully evaluated considering the patient's potential individually for the development of cold antibodies and possible transfusion reactions. Therefore blood transfusions will be performed efficiently and configure not risk the safety of the patient. **E-mail:** patricia.brasil@ipef.fiocruz.br

Mal119- Genetic regulation of nitric oxide bioavailability and the risk of cerebral malaria

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Introduction: Inducible nitric oxide (NO), synthesized by the NO-synthase isoform NOS-II, is an anti-pathogen. In clinical malaria infection, it has been proposed that NO is produced at high levels to kill Plasmodium parasites. Polymorphisms of the nitric oxide synthase 2, inducible gene (NOS2A) promoter has been found associated to cerebral malaria (CM) in African children and increasing amounts of evidence support the alternate hypothesis that NO production is limited during severe malaria. However, its role in clinical disease remains controversial; it is believed that the low bioavailability of NO play a significant role for malarial pathogenesis both in human and experimental CM contributing to pathologic activation of the immune system, the endothelium and the coagulation system. We sought to determine whether NO plasma levels were different in distinct clinical groups of malaria patients and whether genetic NOS2A variants correlate with NO plasma levels, parasitaemia levels and risk to developing CM syndrome. **Material and methods:** We carried out a case-control study in Angolan children testing differences in NO plasma levels by comparing two groups of CM patients: one with low and other with high parasitaemia levels, respectively to low parasitaemia; one group of uncomplicated malaria (UM); and one group of hiperparasitaemic patients exempt of CM malaria (HP). **Results:** CM phenotype, independent from parasitaemia levels, was associated to low NO. The genotyping data of all the children for 22 SNPs in the NOS2 gene were used to explore their association either to NO levels, either to the risk of progressing to the cerebral malaria syndrome. **Main Conclusions:** Genetic factors may contribute by different mechanisms to the risk of progression to CM syndrome either through the NO bioavailability, either altering the individual susceptibility to severe disease. **E-mail:** cpenha@igc.gulbenkian.pt

Mal120- Gametocyte Carriage and G6PD Deficiency Prevalence in Malaria Symptomatic and Asymptomatic Patients Attending Health Facilities in Limpopo Province, South Africa

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Introduction: South Africa has embarked on the ambitious goal of eliminating malaria within its borders by 2018. If this goal is to be attained then all malaria cases must be detected and effectively treated. To ensure onwards transmission is halted completely, it has been suggested that primaquine become standard first line treatment together with artemether-lumfantrine. This suggestion has raised some health concerns as primaquine has been associated with haemolysis in glucose-6-phosphate dehydrogenase (G6PD) deficient individuals. **Methods:** To address this issue a pilot study to assess both G6PD deficiency levels and gametocyte carriage was conducted during 2011/2012 malaria season. The Vembe District of Limpopo Province, South Africa was selected for the study as it had a malaria incidence above 5. All patients presenting at the selected health facilities were tested for malaria and G6PD activity with a combo rapid diagnostic test kit. In addition a filter blood paper sample was collected from

all patients. Parasite mRNA extracted from the filter paper samples was subjected to reverse transcriptase PCR to determine the presence of gametocytes in both asymptomatic and symptomatic patients. **Results:** Preliminary results suggest G6PD deficiency levels in the region are below 1% with gametocyte carriage in population very low. **Main Conclusion:** The low prevalence of the gametocyte carriage amongst asymptomatic patients seems to imply in Vembe District there is no need to supplement the standard ACT treatment for uncomplicated malaria with primaquine. However if primaquine is used, the risk of haemolysis appears to low given the low prevalence of the G6PD deficiency in the region. A wider study needs to be conducted to see if this is true for all the malaria endemic regions of South Africa. **E-mail:** jaishree.raman@mrc.ac.za

Mal121- Macrophages and neutrophils are involved in the pathogenesis of malaria associated acute lung injury/acute respiratory distress syndrome

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Introduction: Malaria impinges a huge burden on global health. In 2010, this parasitic disease was shown to be the underlying cause of death for 1,24 million individuals, including 714 000 children younger than 5 years and 524 000 individuals aged 5 years or older (Murray, et al, 2012). Severe malaria disease can manifest in the lungs, an illness known as acute lung injury/acute respiratory distress syndrome (ALI/ARDS). We previously established a murine model that mimics various human ALI/ARDS aspects, such as pulmonary edema, hemorrhages, pleural effusion and hypoxemia. Using this model we evaluated the lungs cellular profile as well as other inflammation aspects that could be involved in the pathogenesis of malaria associated ALI/ARDS. **Methods and Results:** DBA/2 mice were infected via intraperitoneal injection with 10⁶ *Plasmodium berghei* ANKA iRBC. Lungs and bronchoalveolar lavage were collected and analyzed at different time-points during infection. Through a mathematical model, and making use of different parameters of a control group (our gold standard survival group), such as breathing pattern (enhance pause and respiratory frequency) and parasitaemia, we established a cut-off using a ROC curve (Receiver Operating Characteristic) and classified experimental animals euthanized at the different time-points as ALI/ARDS or HP (hyperparasitemia). We observed an increase in neutrophils, measured by increase of Ncf2 expression (qRT-PCR) and flow cytometry (Gr-1⁺ expression) in the lungs of the ALI/ARDS group (p <0.05). A significant increase of neutrophils was also observed in the bronchoalveolar lavage. Moreover, by flow cytometry we observed larger numbers of alveolar macrophages (F4/80⁺/CD11c⁺) in the ALI/ARDS group (p <0.05), and of interstitial macrophages (F480⁺) in HP mice (p <0.05). No differences in mRNA expression of TNF- α were observed in the lungs of animals with ALI/ARDS compared to HP animals. Moreover, there were also no differences regarding mRNA expression of IFN- γ , TGF- β , ICAM-1, VCAM, iNOS, IL-6 and KC. Meanwhile, expression levels of *Plasmodium berghei* ANKA mRNA in the lungs of DBA mice on days 7 and 9 after infection were higher in the ALI/ARDS group compared to the HP group. **Conclusion:** Our data leads us to infer that neutrophils and alveolar macrophages are the main inflammatory cells involved in the pathogenesis of malaria associated ALI/ARDS and that the accumulation of *P. berghei* in the lungs may be related to an increase in both the inflammatory response and ALI/ARDS development. **E-mail:** luana_ortolan@yahoo.com.br

Mal122- Leukotriene B4 is essential for the development of experimental cerebral malaria

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Introduction: Cerebral malaria (CM) is a life-threatening complication of *P. falciparum* infection. CM physiopathogenesis is incompletely understood, but inflammatory mediators are known to participate in the process. Murine models of CM have been used to understand the mediators involved in the disease and unravel hypotheses about its genesis. Leukotrienes are pro-inflammatory molecules known to play a role in asthma, sepsis, and fungal infections. We studied the role of leukotrienes in murine models of CM induced by *P. berghei* ANKA infection. **Methods:** Survival curve, course of parasitaemia, and histopathology were determined in *P. berghei* ANKA infected mice knockout (KO) mice for the 5-lipoxygenase (5-LO) enzyme, the BLT-1 receptor, or treated with zileuton, montelukast, or LY2552833. All treatments were started on day 0 of infection and continued until day 12. **Results:** 5-LO KO mice in a 129sv background were resistant to the development of CM and presented only mild adhesion of leukocytes in the brain microvasculature on day 6 of infection, when compared with 129sv wild-type mice. Pharmacologic inhibition of 5-LO with zileuton also protected C57Bl/6 mice from CM development and did not interfere with the course of parasitaemia. The inhibition of LTB-1 and LTB-2 receptors with the non-selective inhibitor LY2552833 also prevented CM. This data is corroborated by the high resistance of BLT-1 KO mice in a C57Bl/6 background to develop CM (60% vs 20% survival). On the contrary, the inhibition of CysLT-1 receptors did not prevent CM. The course of parasitaemia did not vary between treated and untreated mice, or between KO and wild-type mice with the same background. **Conclusions:** These data show that Leukotriene B4 is essential for the development of experimental CM. This mediator may act mainly through BLT-1 receptors as BLT-1 KO mice are highly resistant to the development of CM, but the role of BLT-2 was not ruled out. In addition, cysteinyl leukotrienes are not involved in the development of experimental CM. **E-mail:** yuricmartins2004@yahoo.com.br

Mal123- Effect of *agaricus blazei murrill* in the immune response and development of experimental cerebral malaria

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Introduction: The *Agaricus blazei* Murrill (AbM) is a Brazilian originated mushroom, and it is being used as an alternative medicine and functional food, which preventing various diseases, such as infection, allergy, and cancer. AbM was described to contain bioactive compounds related to an immunomodulatory activity. Malaria is a disease caused by *Plasmodium* species reaching 106 countries, affecting approximately 216 million people and leading to deaths of 655,000, and that has been exacerbated by the emergence of drug-resistant parasites. The most important complication of *Plasmodium falciparum*-infected human is cerebral malaria (CM). Here, we investigated the effect of AbM against cerebral malaria (CM) in mice infected with *P. berghei* ANKA (PbA), this parasite strain faithfully recapitulate many of the characteristics of human CM. **Material and Methods:** C57Bl/6 mice were pretreated (3 days) with aqueous extract or fraction of AbM and then infected with 1×10^5 infected red cells, followed by treatment with AbM (aqueous extract or fraction) or chloroquine, and the parasitaemia, survival, body weight and development of CM were monitored periodically. **Results:** Mice treated with aqueous extract or fraction of AbM demonstrated lower parasitaemia, longer survival and reduced weight lost when compared with untreated PbA-infected mice. Both treatments prevented the development of CM in mice, which was confirmed by the Rapid Murine Coma and Behavior Scale test. **Main Conclusions:** These findings indicate, for the first time, that AbM has an important protective role in the development of experimental cerebral malaria. **Financial support:** CNPq and FAPEMIG. **E-mail:** cynthia_honorato7@yahoo.com.br

Mal124- Modulation of immune response and development of experimental cerebral malaria: role of AhR

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Introduction: The cerebral malaria (CM) is the major complication found in the individuals infected by *Plasmodium falciparum*. However, the events that result in the development of CM are multi-factorial and could not be explained by only one mechanism. Mice infected with *Plasmodium berghei* ANKA (PbA) faithfully recapitulate many of the characteristics of human CM and it has been an important tool to investigate the disease pathogenesis. The Aryl Hydrocarbon receptor (AhR) is an intracellular receptor activated by several ligands and is important to modulate the inflammatory response. However, the involvement of AhR in CM is not known. **Objective:** Investigate the role of AhR in the regulation of immune response and development of CM. **Methods:** C57Bl/6 (WT) and AhR^{-/-} mice were infected with PbA and the parasitemia, survival and body weight were monitored periodically. The production of cytokines (TNF- α , IL-1 β , TGF- β , IL-6, IFN- γ , IL-12, IL-17 and IL-10) in the brain and spleen was assessed by ELISA and flow cytometry. The expression of SOCS1, SOCS2 and SOCS3 was assessed by Reverse Transcriptase and Real Time PCR. Leukocyte recruitment in the brain was evaluated by intravital microscopy. Nitric oxide (NO) was assessed by the Griess method in the brain. Histopathology analysis was also performed in brain. **Results:** The parasitemia was significantly dramatically higher in PbA-infected AhR^{-/-} mice. In the brain and spleen of PbA-infected AhR^{-/-} mice there was a significant decreased expression of TNF- α , IL-1 β , IFN- γ , IL-12 and IL-10 when compared with WT counterparts. Additionally, there was an increased levels of TGF- β , IL-6, IL-17 and high expression of FOXP3 in the brain of infected AhR^{-/-} mice when compared with WT infected mice. AhR deficiency also resulted in an increased level of transaminases, especially ALT, evident liver damage, as well as increase iron concentration in serum during the infection. Moreover, PbA-infected AhR^{-/-} mice had an increased BBB permeability. **Conclusions:** These findings indicate, for the first time, the role for AhR in the immunopathogenesis of PbA-associated CM. **Financial support:** CAPES, CNPq, FAPEMIG **E-mail:** fatimacbrant@gmail.com

Mal125- Neurological signs in adult Wistar rats submitted to early protein malnutrition infected with *Plasmodium berghei*

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Human malnutrition and some infectious diseases like malaria can produce alterations in brain development of infants and children, with pathophysiological effects that persist up to adulthood. The aim of this study is to verify the effect of the early protein malnutrition in the neurological status of rats Wistar during the course of infection with *Plasmodium berghei* (Pb). Four experimental groups were assembled, each one composed by a lactating female and 6-8 pups, performing the control group (C), malnourished (M), parasitized (P) and malnourished and parasitized (MP). The dam of group C was fed with normal diet (22% of protein) and her offspring received intraperitoneal (ip) saline inoculum. The dam of group M received a low protein modified diet (4% of protein) during the first 10 days of life, and her offspring received ip saline inoculum. The dam of group P received normal diet and her offspring were inoculated ip with Pb and the dam of group MP received a low protein diet and Pb in equal conditions. The animals were inoculated after reaching the adulthood (81th day) with 5x10⁷ red blood cells parasitized with Pb (NK65) and were followed on alternate days until the 30th day post infection. The pre-patent period (PPP), parasitaemia, mortality and neurological status were monitored. The assessment of neurological signs was performed by SHIRPA protocol: initial evaluation (IE), general assessment (GA), the reflexive

behavior (RB) and strength/coordination (SC) categories. For each assessment item was assigned a negative, zero or positive score, indicating weak, normal or exaggerated response. The values were added within each class of functional categories and was calculated a mean score for each group of animals. The PPP between groups P and MP was similar, occurring on day 5±0, the parasitaemia peak was ranging 1,7% to 3% and there was no mortality. In the parameters of the IE category, the group M showed a similar behavior to C group; the P group had higher scores, indicating an exaggerated response, and the MP group showed inhibited responses (negative scores). In the GA category, the M group showed higher scores than the C group (exaggerated response); the P group had similar behavior to C group and MP group showed a trend line in the course of infection from negative scores to positive scores at the end of follow-up time. In the RB and SC categories, the P group showed similar results to C group, M and MP group had negative scores, with inhibited response. Early protein malnutrition in the Wistar rats induced permanent effects in neurobehavioral patterns that can be modulated by infection with PbNK65. **E-mail:** nanda_emilly@hotmail.com

Mal126- Long-term memory impairment and hippocampal damage during experimental cerebral malaria

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Introduction: Cerebral malaria (CM) is a clinical syndrome resulting from *Plasmodium falciparum* infection. A wide range of clinical manifestations follows the disease including cognitive dysfunction, seizures and coma. CM pathogenesis remains incompletely understood although vascular, immunological and metabolic changes have been described. **Objectives:** The aim of the present study was to investigate cognitive impairment and hippocampal changes in a murine model of CM. **Methods:** Six week-old, female wild-type C57Bl/6 mice were used in this study. Mice were infected with *Plasmodium berghei* ANKA by the intraperitoneal route, using a standardized inoculation of 10⁶ parasitized red blood cells. Memory was evaluated using the object recognition test. The open field task was performed to assess locomotor and exploratory activities. The mRNA expression of IL-6, TNF-α, IL-1β, IFN-γ and IFN-α in the hippocampus of control and infected mice were estimated by quantitative real time PCR. The percentage of neuronal death in the CA1 region of the hippocampus was analyzed by confocal microscopy. Apoptosis was measured in the hippocampus by the expression of cleaved caspase-3 in western blot. All experiments were conducted on day 5 post-infection. **Results:** CM mice presented a significant impairment of long-term memory compared to controls (p<0.05). No differences in locomotor (p=0.88) and exploratory activities (p=0.90) were found between groups. A higher mRNA expression of IL-6, TNF-α, IL-1β, IFN-γ and IFN-α was found in the hippocampus of infected mice (p<0.05). A significant increase in the percentage of neuronal death in the hippocampus of CM mice was also observed along with enhanced levels of cleaved caspase-3 (p<0.05). **Conclusion:** In this study, we found that CM mice presented cognitive deficits associated with inflammatory and apoptotic changes in the hippocampus. These findings suggest a role for immune system in the pathogenesis of cognitive outcome in CM. **Financial support:** CNPq, CAPES, FAPEMIG, PRPq/UFMG. **E-mail:** alines.miranda@hotmail.com

Mal127- Antiinflammatory and antioxidant effect of lovastatin prevents cognitive impairment due to experimental cerebral malaria

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Introduction: Cerebral malaria (CM) is the worst complication of infection by *Plasmodium falciparum* and is a major cause of acute encephalopathy in tropical countries. These neurological injuries are more

common and severe in children who survive CM, and persistence of cognitive deficits after cure of infection suggests areas for further research. Several studies have been shown that statins present anti-inflammatory and antioxidant effects due to several mechanisms, as well as alteration on geranylgeranylation of proteins. The aim of this study was to investigate the anti-inflammatory effect of lovastatin during experimental cerebral malaria development and the consequent prevention of cognitive impairment development. **Methodology:** C57BL/6 mice were inoculated with 10⁶ *Plasmodium berghei* ANKA (PbA) infected or healthy (RBC) erythrocytes. On days 6 and 7 post infection they were treated with saline or lovastatin (20 mg/kg, per oral). 2 hours later, mice brains were excised and cytokines levels were measured by Bio-Plex system, and malondialdehyde (MDA) and free thiols amounts were evaluated. Alterations in blood brain barrier permeability were evaluated by blue Evans dye. In another set of experiments, mice were anesthetized (xylazine 10 mg/kg and ketamine 200 mg/kg) and the jugular vein was cannulated to administer rhodamine 6G or FITC dextran. Intravital fluorescence videomicroscopy was performed on pial vasculature through a cranial window. To access the cognitive function, infected mice were treated with chloroquine on day 6 to 12 post-infection and on day 15 and 16 post-infection, mice were submitted to open-field and inhibitory avoidance tasks. **Results:** Lovastatin therapy markedly reduced MCP-1, TNF- α , IL-1 and IL-12 levels on days 6 post infection. Brain edema was reduced on lovastatin-treated group on day 7-post infection. Increased MDA levels and reduction of free thiols (representative of oxidative stress) were observed in PbA-infected mice and the treatment with lovastatin reverses both phenomena. Lovastatin therapy markedly reduced the leukocyte rolling and adhesion induced by CM, and reversed brain functional capillary rarefaction observed in infected mice. Finally, infected mice healed from parasitic disease presented persistent cognitive impairment in all behavioral tasks, which was reversed by lovastatin treatment. **Main conclusion:** Our results suggest that treatment with lovastatin is able to prevent the inflammatory events during CM and may protect the brain oxidative damage by free radicals, which prevents cognitive sequel due to experimental cerebral malaria. **E-mail:** reispa@gmail.com.

Mal128- *Plasmodium berghei* triggers differential inflammasome adaptor protein ASC and caspase-1 expression in resistant and susceptible mice to cerebral malaria infection.

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Introduction: Malaria remains a major global problem, infecting millions of people and resulting in almost 2 million deaths per year. Cerebral malaria is the most severe complication of *Plasmodium falciparum* infection in humans and the pathogenesis is still unclear. The pro-inflammatory cytokines are involved in pathogenesis of severe forms of the disease. The pro-inflammatory cytokine IL-1 β is produced by cleavage of inactive precursors by caspase-1 within a large multiprotein complex named inflammasome. The NLRP1 inflammasome is composed of the NLR protein Nalp1, the adaptor protein ASC and caspase-1. This work aims to investigate the activation of NALP1 inflammasome components NALP1, ASC and Caspase-1 in resistant (BALB/c) or susceptible (CBA and C57BL/6) mice to cerebral disease caused by *P. berghei* ANKA. **Material and Methods:** C57BL/6, CBA and BALB/c mice were infected or not with *P. berghei* ANKA and after six days of infection, peritoneal macrophages were obtained. The expression of NALP1, the adaptor protein ASC and caspase-1 in those macrophages was accessed by immunostaining and the fluorescence images were captured and processed using Leica SP5 laser-scanning microscope. **Results:** The susceptible CBA and C57BL6 mice infected with *P. berghei*-ANKA showed an increased expression of ASC compared to the resistant BALB/c mice. The pattern expression of ASC in resistant mice was similar to non-infected mice. The expression of caspase1 protein was increased in all infected mice strains compared to non-infected mice. Likewise, macrophages from *P. berghei*-ANKA infected C57BL/6 mice showed higher caspase-1 expression compared to macrophages from *P. berghei*-ANKA infected BALB/c mice which showed a slight caspase-1 expression. Surprisingly, there was an absent Nalp1 expression in macrophages from all studied *P. berghei*-ANKA infected or non-infected mice strains. **Conclusions:** Our data showed that the inflammasome adaptor protein ASC and caspase-1 are

differentially expressed in cerebral malaria resistant and susceptible mice infected with *P.berghei*-ANKA. Our results suggest that inflammasome components ASC and caspase-1, but not Nalp1, may be involved in resistance and susceptibility phenomenon in experimental cerebral malaria infection. **E-mail:** tatianakarl@br.ibm.com

Mal129- *Plasmodium (Novyella) nucleophilum* from Egyptian Goose in São Paulo Zoo, Brazil: microscopic confirmation and barcoding

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Using microscopy and PCR, we identified infection of *Plasmodium (Novyella) nucleophilum*, Manwell, 1935, in an Egyptian Goose (*Alopochen aegyptiaca*) who died in São Paulo Zoo, Brazil. This parasite is characterized by the following main characters: i) small (less than erythrocyte nuclei) meronts with scanty cytoplasm; ii) ≤ 8 merozoites in meronts, iii) small (length $< 10 \mu\text{m}$ on average) elongate gametocytes possessing < 10 pigment granules; iv) trophozoites, meronts and gametocytes closely appressed to erythrocyte nuclei (nucleophilic). Additionally, *P. (Haemamoeba)* sp. was also identified by microscopy in the same blood sample. The latter parasite lacks nucleophilic blood stages and is characterized by i) large (size greater than erythrocyte nuclei) meronts possessing plentiful cytoplasm and large nuclei, ii) large (greater than erythrocyte nuclei) gametocytes and iii) large roundish trophozoites, each with a prominent centrally collated vacuole. The latter parasite can be readily distinguished from *P. nucleophilum* even on early blood stages, primarily due to absence of nucleophilic parasites. Unfortunately, this co-infection was not confirmed by PCR amplification of *cytb* gene and sequencing; only one *Plasmodium cytb* sequence was detected in the blood sample. Because parasitaemia of *P. nucleophilum* (2,38%) was much higher than that of *P. (Haemamoeba)* sp. (0,19%), PCR may have favoured the amplification of *cytb* sequence of the former parasite. Phylogenetic analysis is in agreement to this conclusion because the reported *cytb* sequence was positioned in the same branch of sequences of several *Novyella* species. When the obtained sequence was compared with GenBank sequences, the most similar *Plasmodium* sequence was # AF254962, with 94% of identity. However, this sequence, formerly attributed to *P. nucleophilum*, currently has been assigned to *P. (Novyella) ashfordi*. Natural infection of *P. nucleophilum* has been recorded in > 60 species of birds belonging to different families and orders, including anseriform birds, in all zoogeographical regions, except the Australian and Antarctic. *Haemamoeba* subgenus contains 11 malaria parasite species, and *P. relictum*, the type species, has been reported by our group in penguins from São Paulo Zoo. However, *P. (Haemamoeba)* parasite recorded in this study is more similar to *P. (H.) tejeraei* because its' each advanced trophozoite and young erythrocytic meront possesses a large vacuole with pigment granules arranged around it, the characteristic features of this species. Mature meronts and gametocytes are absent from our blood sample, thus final identification of the *Haemamoeba* parasite is currently impossible. Interestingly, until now, *P. tejeraei* has been recorded only from *Meleagris gallopavo* (common turkey) and some experimentally infected birds, including domestic geese in Venezuela. This is the first assignment of mitochondrial *cytb* gene sequence to *P. nucleophilum*. Representative images of the blood stages of *P. nucleophilum* and *P. (Haemamoeba)* sp. are given. **E-mail:** cchagas@sp.gov.br.

Mal130- Malaria and leishmaniasis co-infection in mice: evidences of simultaneous modulatory effects

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Introduction: Neglected tropical diseases such as Malaria and American Tegumentary Leishmaniasis are highly endemic in several regions of the world, including Brazil. The co-infection by multiple parasite

species is commonly observed in nature and recently epidemiological studies indicates that this interaction represents a complex challenge in the ecology of parasites and human health. At present time, there are no data on prevalence of co-infection between Malaria and Leishmaniasis and no data reporting the occurrence of changes in the natural course of these diseases when they are associated in Brazil. Considering that populations are naturally exposed to different pathogens, it is extremely important to study the interaction of these parasites and possible changes in the balance between immune response and clinical progression of the disease. **Objectives:** determine if concurrent infection with *Leishmania* spp. and *P. yoelii* influences the course and load of parasites in each disease. **Material and Methods:** BALB/C mice were divided into eight groups, called G1 to G7 (G1: *P. yoelii*; G2: *L. brasiliensis*; G3: *L. major*; G4: *L. amazonensis*; G5: *L. brasiliensis* and *P. yoelii*; G6: *L. major* and *P. yoelii* and G7: *L. amazonensis* and *P. yoelii*). Firstly, BALB/c mice of G2 to G7 were infected with one of the three *Leishmania* spp. (*L. brasiliensis*: inoculum of 1 X 10⁵ parasites; *L. major* and *L. amazonensis*: inoculum of 1 X 10⁴ parasites). *P. yoelii* 17XNL infection (inoculum of 1 X 10⁶ infected erythrocytes) was performed three days later on groups G1, G5-G7. Lesions sizes were monitored weekly with a digital caliper for a period of 11 weeks. Additionally, the time of appearance and the number of ulcers in lesions was observed. Parasite load of infected ears was determined using a quantitative limiting-dilution assay at days 5, 10, 17, 25 and 77 post-infection. *P. yoelii* infection was monitored through blood strains stained with Giemsa every two days. **Results:** Lesions of co-infected mice had a slower development compared to those of *Leishmania* spp. only infected mice. As a result, ulceration occurred at a later date and in a less number of lesions on these groups. The load of *Leishmania* spp. parasites was slower or stable in co-infected mice during malaria course. *P. yoelii* infection was similar in only-infected and co-infected groups. Of note, the mean peak parasitaemia was slower on G5 and G6 and a more persistence of parasites was observed in G7. Additionally, we observed a significant increase in mortality on groups G6 and G7 during malaria infection. **Conclusions:** Our data demonstrates that malaria infection influences the course of leishmaniasis in BALB/c mice, especially during parasitemic stages, presumably in consequence of systemic immune response elicited. In a minor grade, leishmaniasis is also capable of influence the course of malaria in co-infected mice as seen in *L. major* and *L. amazonensis* infections in which the lethality was increased. **E-mail:** banic@ioc.fiocruz.br

Immune response

Mal131- Lymphocytic apoptosis in human *P.falciparum* and *P.vivax* malaria: relationship with immune response to the parasites

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To evaluate the role of lymphocytic apoptosis during a non-complicated malaria attack, blood samples were collected from 35 individuals living in malaria endemic area with positive blood smears, as well as from 17 individuals without previous reported malaria episodes. Cellular apoptosis - CD4⁺, CD8⁺ and B - were analyzed ex-vivo and after 96 hour-culture in presence or absence of plasmodial antigens by cytometry flow. Once malaria apoptosis could be regulated by Fas/Fas L system, Fas/APO-1 (CD95) molecular expression was also examined in these cells. Cellular and humoral immune responses after antigenic stimulation, absolute numbers of CD4⁺, CD8⁺ and B as well as B cells polyclonal activation and the auto-immune response against components of erythrocytes membrane, cardiolipin, actin and DNA were also investigated. In ex-vivo analyses low levels of apoptosis and high levels of cellular activation in CD4⁺ cells were observed in malaria patients, although increased levels of early apoptosis in CD4⁺ and CD8⁺ cells had been detected. Still in ex-vivo analyses high levels of IFN- γ and IL-10 and low levels of TNF were also verified when compared to clinical healthy individuals. No difference was observed between TNF and IL-10 plasmatic levels between *P. vivax* and *P. falciparum* infections, although high levels of IFN- γ was registered in *P. vivax* malaria patients. No relationship was observed between cytokines levels and activation, viability or peripheral blood mononuclear cells (PBMC) apoptosis. After 96

hour-culture apoptosis levels in malaria patients were increased in CD4+, CD8⁺ and B cells, and even in the presence of stimuli the cellular proliferation was lower than in healthy individuals. Antibodies against membrane erythrocyte components, cardiolipin and DNA were similarly detected in malaria patients and healthy individuals, while antibodies against actin were more frequently detected in malaria patients. We conclude that the higher levels of apoptosis observed in cells from *P. vivax* and *P. falciparum* malaria patients could contribute to lymphopenia associated to malaria. However, the lack of correlation among apoptosis and parasitaemia, number of past malaria attacks and low cellular response, especially against malaria antigens, suggest that apoptosis associated to uncomplicated malaria could be a physiological reaction of the immune system to control polyclonal activation and maintain the balance of these cellular population densities. **E-mail:** ericcio@ioc.fiocruz.br

Mal132- Malaria clinical immunity in asymptomatic infected individuals from the Barcelos Municipality (Amazonas, Brazil) is not reflected by a corresponding anti-Plasmodium falciparum glycosylphosphatidylinositol antibody response

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Malaria, caused by protozoa of the genus *Plasmodium*, remains one of the most prevalent endemic diseases worldwide, especially in Africa. In the erythrocytic phase of the *Plasmodium* life cycle, at the time of rupture of infected erythrocytes the release of merozoites in the circulation, signs and symptoms revealing the pathogenesis of the disease (fever, chills, headache...) occur following the release of toxins by the parasite and the resulting macrophage activation with subsequent induction of pro-inflammatory cytokines (TNF alpha, IL-1, etc.). One of the most important toxins involved is a phospholipid, glycosylphosphatidylinositol (GPI), which allows the anchoring of several plasmodial proteins responsible for invasion of the parasite in the erythrocyte. The clinical manifestations resulting from the excessive production of these mediators in response to toxins like GPI can accompany severe systemic effects (such as anemia, cerebral malaria and involvement of various organs and systems). In areas of high malaria transmission, as those of Africa, the development of clinical immunity (premunition) - that results in the decrease of the symptoms and of the severity of disease, but cannot clear the parasitaemia or prevent reinfection - is usually gradual and increases with age and exposure to plasmodial antigens. It is classically accepted that the antibody (Ab) response induced by the GPI of *Plasmodium falciparum* can reflect and, at least partially, mediate the clinical immunity in malaria. In this study, we aimed to assess whether the acquisition of clinical immunity could be associated to the production of anti-GPI antibodies in an area of high risk of transmission in the Brazilian Amazon. We investigated the response to the GPI, whole extract, and the merozoite surface protein 3 (PfMSP-3) antigens of *P. falciparum*; as well as to the PVVISP1-19 of *P. vivax*, in Brazilian asymptomatic carriers of plasmodial infection and controls (with no infection or history of disease), comparatively to African patients with acute malaria. Our data indicate that anti-*P. falciparum* GPI immune response did not reflect the probable clinical immunity of asymptomatic individuals chronically exposed to malaria in the Brazilian Amazon and that the PVVISP1-19 could represent a good antigen to reflect previous exposure to *Plasmodium* in areas of high transmission of *P. vivax*. **Keywords:** *Plasmodium falciparum*, *P. vivax*, Glycosylphosphatidylinositol, Clinical immunity, Brazilian Amazon, Angola.

Mal133- Profile of inflammatory, anti-inflammatory cytokines and chemokines in pregnant woman of a malaria endemic area of Colombia, Latinamerica

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Introduction: Malaria during pregnancy is a major cause of maternal and child morbidity can cause anemia to pregnant women, premature birth and low birth weight. During pregnancy, maternal immune system adapts to accept the implant fetal therefore pregnancy is characterized by the predominance of an inflammatory type immune response. The role of proinflammatory cytokines is to decrease the production

of inflammatory cytokines that may be harmful to the fetus. In Latin America there are few studies conducted to study the immune response in pregnant women with malaria, especially in infections caused by *P. vivax*. **Objective:** The aim was to evaluate the regulation of immune responses during gestational malaria, through the effect on the balance of pro-inflammatory cytokines (TNF α , IFN γ , IL1 β), anti-inflammatory (IL4, IL6, IL10, TGF- β) and chemokines (MIP1, MCP1, IL8) in pregnant women with different histories of malaria during pregnancy, Urabá-South Córdoba, Colombia. **Methods:** We measured the expression of cytokines/chemokines in maternal peripheral blood, cord blood and placental tissue from 20 pregnant women with malaria at the time of delivery (MG +) and 20 without malaria at any time during gestation (MG-). The diagnosis was made through thick and thin smear from peripheral blood and the species was confirmed by nested PCR. The expression of cytokines and chemokines was determined by real-time PCR (TaqMan). Data were analyzed by testing "Mann Whitney". **Results:** We found that pro-inflammatory cytokines were increased in the group of MG + in placental tissue. The same cytokines in the placentas of pregnant women with *P. vivax* (n = 10) showed a significant increase TNF α (p = 0.019), IFN γ (p = 0.047) and IL1 β (p = 0.038). On the other hand, placentas of pregnant women with *P. falciparum* (n = 10) only IFN γ (p = 0.017) showed a significant increase. About of the anti-inflammatory cytokines (IL10, IL6, TGF β), none of samples showed significant increase of these molecules. Furthermore, the observed increased MCP1 in placental tissue of both pregnant women with *P. vivax* in pregnant women with *P. falciparum*. **Conclusions:** The results show a localized inflammatory response in the placentas of pregnant women with *P. vivax*. The pro-inflammatory cytokines responses may prove an effective immune regulated against the parasite, but this response may have serious consequences in pregnant women and the product of gestation. Furthermore, it is suggested that the chemokine MCP1 is a promoter of the inflammation in placenta, both in *P. vivax* as *P. falciparum*. **E-mail:** mfab203@gmail.com

Mal134- Cross-reactive anti-PfCLAG9 antibodies in the sera of asymptomatic parasite carriers of *Plasmodium falciparum* and *Plasmodium vivax*

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Introduction: Previous studies performed in Papua New Guinea (1) showed that asymptomatic parasite carriers (APC) of *P. falciparum* present high titer of antibodies against the product of the *clag9* gene (cytoadherence linked asexual gene) that indicate the possible interest of including CLAG 9 protein among vaccine candidates against *falciparum* malaria. Clinical epidemiological and parasitological studies identified asymptomatic malaria carriers in suburban and rural localities of Porto Velho in the Western Brazilian Amazon. PCR genotyping identified unique or mixed infections by *P. falciparum* and *P. vivax*. **Methods and procedures:** Three synthetic peptides representing different segments of the CLAG9 protein were prepared and tested in ELISA assays using sera from parasite-positive patients with or without clinic symptomatic infections. MSP1-₁₉ recombinant antigens of *P. falciparum* and *P. vivax* were also used to analyze the species specificity of antibodies present in patients' sera and eventual persistence of antibodies from previous heterologous infections. **Results:** High titers of antibodies against synthetic peptides corresponding to the *P. falciparum* antigen PfCLAG9 were found in sera of semi-immune and immune *falciparum* malaria patients from the Brazilian Amazon, confirming observations in Papua Guinea (1). However, it was also observed that sera of *vivax* patients, in particular asymptomatic parasite carriers, strongly cross-reacted with the same peptides. Complementary bioinformatics analyses demonstrated highly homologous amino acid sequences encoded by PfCLAG 9 and *vivax* ortholog PvCLAG 7, which may explain the antibody cross reactivity. **Conclusions:** The observation that both *P. falciparum* and exclusively *P. vivax* infected patients reacted against the tested PfCLAG 9 peptides calls for an in-depth reevaluation of the potential of CLAG9 as a target of protective immune responses and an eventual role of cross-reactive antibodies in the regulation of interactions of *Plasmodium* parasites in mixed infections. CLAG9 protein could represent the variant antigen responsible for previous

observations (2,3,4) on biological interactions and possible cross-species immunity by *P. falciparum* and *P. vivax* **E-mail:** joanadarcneves@hotmail.com

Mal135- Clinical spectrum of non-complicated malaria in semi-immune patients of the Brazilian Amazon

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Introduction: The clinical presentation of plasmodial infection in semi-immune subjects can vary. Usually, one expects to find fever, chills and sweating, but these symptoms may not be present all the time. This study evaluated the clinical presentation of non-complicated plasmodial infections in an area of urban transmission in the Western Brazilian Amazon. **Material and Methods:** A clinical questionnaire containing 18 symptoms (fever, chills, sweating, headache, retro orbicular pain, myalgia, arthralgia, lumbar pain, abdominal pain, nausea, vomiting, diarrhea, weakness, cough, coryza, lack of appetite, dizziness and bitter mouth) was applied in patients from the rural and urban area of Mancio Lima, in the state of Acre. All patients included in the analysis had a positive thick smear. **Results:** Fifty-three (53) patients were interviewed. Three had asymptomatic infection, 2 had mixed-species infection (*P. vivax* and *P. falciparum*) and 48 had symptomatic single-species infection (34 with vivax malaria and 14 with falciparum malaria). Of these, 58.3% had low parasitaemia and 41.7% had high parasitaemia. Only those presenting symptomatic single-species infection were included in the analysis. The average age was 29 years (range 11-68); 68.8% were males and 31.3% were females. The average previous malaria episodes were 12, with a median of 7 episodes (range 0 -50). The number of symptoms per patient varied from 4 to 21, being the median number 13 symptoms. Symptoms that appeared in the majority of patients were headache (95.8%), fever (91.7%), chills (83.3%), arthralgia (77.1%), weakness (75.0%), lumbar pain (68.8%), dizziness (62.5%), sweating (60.4%), bitter mouth (56.3%), nausea (54.2%) and lack of appetite (54.2%). The less frequent symptoms were myalgia (47.9%), retro orbicular pain (33.3%), abdominal pain (29.2%), vomiting (25.0%), cough (22.9%), coryza (12.5%), diarrhea (12.5%) and dyspnea (8.3%). There was no association between age, sex, previous malaria episodes, parasitaemia and number of symptoms/frequency of symptoms. However, a difference in number and frequency of symptoms was found between infecting species. The average number of symptoms for vivax malaria was 11.71, and for falciparum malaria was 15.12 ($P = 0.005$, Anova test). The clinical presentation differed by species: myalgia, nausea, cough and lack of appetite were significantly more frequent in falciparum malaria (71.4%, 78.6%, 42.9% and 78.6%, respectively) than vivax malaria (38.2%, 44.1%, 14.7%, 44.1% respectively). **Main conclusions:** This study details the clinical spectrum of non-complicated malaria in semi-immune patients, showing that malaria has to be considered as a possible diagnosis even in non-febrile patients in the Amazon. What is more, even in semi-immune patients, non-complicated falciparum malaria presents a more intense clinical presentation than vivax malaria. This study will be continued in order to elaborate an algorithm to differentiate clinically both species. **E-mail:** antonio_camargo0@yahoo.com.br

Mal136- Gestational malaria in Colombia: current epidemiology and immune modulation in naturally infected populations

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Introduction: Gestational malaria has deleterious effects on both the mother and fetus. These have been best characterized in *Plasmodium falciparum* infections in Africa. However, *Plasmodium vivax* infection might also result in damage to placental tissues and congenital malaria. Recent work in the Northwest region of Colombia, where *P. vivax* is predominant, confirmed rates of infection in the mother ranging from 10-14% by microscopy and placental infection by histopathology of around 19%. To further contribute to our understanding of the physiopathology of pregnant malaria, immune variables have been explored in infected subjects, resident in the Uraba and Bajo Cauca regions of Colombia. **Methods:** Incidence and prevalence of gestational and placental malaria were determined by microscopy and nested PCR. Selected markers of the Th1/Th2 balance were monitored to explore the association between pathology and malarial infection. In addition, apoptosis in infected and uninfected placentas was assessed. Samples were analyzed for the expression of genes encoding IFN- γ , TNF, IL1- β , IL-6, IL-10, TGF- β , MCP1, Fas, FasL and Caspase 3 by RT-qPCR. The apoptotic index was evaluated by Tunel. Placental histopathology was used to assess malaria-related tissue changes. **Results:** Nested PCR identified 1.5 fold more maternal infections and 5 fold more placental infections as compared by microscopy. The significance of submicroscopic parasitaemia in cases of either *P. vivax* or *P. falciparum* infection in the pregnant population and the neonate remains unknown. However, when in the placenta, these infections correlate with inflammation of the intervillous space, decidua and villi. These results confirm the significant predominance of pro-inflammatory cytokines in infected placentas, as well as increased apoptosis, regardless of the infecting species. **Conclusions:** The efficacy of the different diagnostic tools applied to the diagnosis of pregnancy malaria and placental infection in the Colombian context is discussed, including microscopy, histopathology, nested PCR and qPCR. *P. falciparum* and, to a lesser degree, *P. vivax* invade placenta. Both species of *Plasmodium* associate with increased inflammation and apoptosis in placental tissues. **E-mail:** aemaestre@gmail.com

Mal137- Lymphopenia in vivax malaria: relative contribution of apoptosis and T-lymphocyte reallocation

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Apoptosis and leukocyte reallocation are major hypotheses to explain the lymphopenia commonly found in *Plasmodium falciparum* infection, but little is known about the causes of lymphopenia in the relatively benign vivax malaria. We sought to analyze the relative contribution of apoptosis and lymphocyte reallocation to lymphopenia in uncomplicated vivax malaria. Compared with healthy controls (n = 8), Brazilians acutely infected with *P. vivax* (n = 14) had lower T-lymphocyte counts and a 3-fold reduction in the median number of CD4⁺ (441.4 [median], 196.9-605.5 [interquartile range] vs. 1262.6, 909.8-2147.9) and CD8⁺ cells per microliter of blood (211.8, 127.5-354.4 vs. 806.0, 303.2-1267.8), with no change in the CD4/CD8 ratio. Four weeks after starting chloroquine treatment, 13 patients had a partial recovery in T lymphocyte numbers, with a median of 793.9 (483.1-915.4) CD4⁺ T lymphocytes and 435.2 (324.2-482.6) CD8⁺ T lymphocytes per microliter of blood. To investigate whether apoptosis contributes to decreased T-cell counts in acute infection, we used flow cytometry to quantify peripheral blood mononuclear cells labeled with anti-CD3, anti-CD4 and anti-CD8 antibodies followed by double staining with annexin V and propidium iodide (PI). We found similar proportions of annexin V-positive, PI-negative CD3⁺, CD3⁺CD4⁺ and CD3⁺CD8⁺ cells in acute and convalescent vivax malaria patients and healthy controls. To investigate whether T-cell reallocation to sites of inflammation is a major contributor to lymphopenia, we next labeled CD3⁺, CD3⁺CD4⁺ and CD3⁺CD8⁺ cells with antibodies to CXCR3 and CCR5, chemokine receptors involved in lymphocyte trafficking during inflammation. We observed increased proportions of CCR5⁺ cells among CD3⁺ (33.7%, 28.4-47.7%) and CD4⁺ lymphocytes (26.3%, 22.2-36.5%) from acute vivax malaria patients, compared to healthy controls (29.2; 25.6-44.8%, and 21.1%, 19.8-33.0%, respectively). In addition, we found an increased proportion of CXCR3⁺ CD4⁺ T lymphocytes in acute vivax malaria patients (54.7%, 47.1-64.8%) compared to healthy controls (44.1%, 35.1-58.2%). No differences were found in the proportions of CD8⁺ cells expressing CXCR3 and CCR5. We conclude that apoptosis is unlikely to be associated with lymphopenia in acute vivax malaria. Reallocation to sites of inflammation, however, may contribute to the remarkable decrease in the number of CD4⁺ cells (but not necessarily of

CD8⁺ cells) found in the bloodstream during acute vivax malaria infections. **Financial support:** FAPESP. **E-mail:** quelmg_biomed@yahoo.com.br

Mal138- Brazilian individuals naturally infected with *Plasmodium vivax*: acquired antibodies response versus host genotype for Duffy antigen receptor for chemokines

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Introduction: The Duffy (*Fy*) receptor for chemokines acts as a receptor for *P. vivax* on the red blood cells surface and, therefore, its polymorphisms have an important impact where vivax malaria predominates. We investigated the correlation between host Duffy genotypes and the frequency of the antibody response against sporozoite and merozoite peptides in patients with *P. vivax* malaria acquired in endemic areas of Brazil. **Material and Methods:** Peripheral blood samples were collected from 98 *P. vivax*-infected individuals from Brazilian malaria-endemic areas (Macapá, Amapá state; Novo Repartimento, Pará state; Porto Velho, Rondônia state; and Plácido de Castro, Acre state). The Duffy blood group genotypes were assessed using PCR/RFLP. IgG antibodies were detected by ELISA against four peptides of circumsporozoite protein (CSP) (amino, carboxyl, VK210 and VK247 repeats) and peptides of merozoite surface protein 1 (MSP-1), apical membrane antigen 1 (AMA-1), and Duffy-binding protein (DBP). Statistical analyses were performed using BioStat (6.0) and Graphpad prism (5.0) software. Differences in the means were assessed using Student's test and Chi-squared test. *P* values <0.05 were considered significant. **Results:** different frequencies of naturally-acquired antibodies responses against the Pv-CSP, Pv-MSP-1, Pv-AMA-1 and PvDBP among those with single (*Fy*^a/*Fy*^{b-33} and *Fy*^b/*Fy*^{b-33}) and double (*Fy*^a/*Fy*^a, *Fy*^a/*Fy*^b and *Fy*^b/*Fy*^b) positive alleles were observed. In respect to the allelic combinations of *FY* and humoral response, there was a significant difference between *Fy*^b and *Fy*^a antigen (Fischer's Exact Test, *P* < 0.0001). **Conclusion/Significance:** studies performed in the Brazilian Amazon region suggested that individuals expressing the *Fy*^b compared with *Fy*^a antigen may be more susceptible to *P. vivax* infection. Previous publication added *in vitro* and *in vivo* data from experiments using a simian malaria parasite, which invades human red cells in a Duffy-dependent manner, showing that its orthologous proteins binds preferentially *Fy*^b-expressing rather than *Fy*^a-expressing erythrocytes. Our data also suggest that immune response of antibodies specific for *P. vivax* multiple antigens varied with the Duffy genotype in Brazilian population. This mechanism may be a selective advantage in the population, reducing the rate of infection by *P. vivax* in this region. **Funding:** FAPESP and CNPQ **E-mail:** ricardomachado@famerp.br

Mal139- Evaluation of the levels of IL-10 in treated and untreated patients with malaria caused by *Plasmodium vivax*

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Introduction: Human malaria is a disease caused by parasites of five *Plasmodium* species (*P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*). In 2010, according to estimates by the World Health Organization, malaria was held responsible for approximately 216 million clinical cases and some 655,000 deaths worldwide. *P. vivax* accounts for about 80 million of those cases as well as being deemed the main responsible for malaria in Brazil. Vivax malaria was thought to be a benign and self-limited disease for a long time; currently, complications associated to this disease in the Amazon area and other places throughout the world have motivated research of the immunopathological events responsible for

these complications. Pathological changes in malaria, which determine either alleviation or exacerbation of this disease, rely on a delicate balance in the T cells cytokines expression. **Objective:** Investigate the levels of IL-10 (Interleukin-10), an anti-inflammatory cytokine, both in the plasma and in the PBMC (Peripheral Blood Mononuclear Cells) culture supernatants of treated and untreated patients with vivax malaria. **Material and Methods:** Blood samples from treated and untreated vivax malaria patients were used to provide the plasma and to isolate the PBMC, which were added to wells culture plates, incubated for 2 days (37°C, 5%CO₂) and, later, harvested. The IL-10 levels in the plasma and in the cell culture supernatants were evaluated using Cytometric Bead Array – CBA. **Results:** The IL-10 plasmatic levels were significantly higher in untreated patients, compared to treated ones ($p < 0.05$). In the cell culture supernatants, no significant difference was observed between the two groups. **Conclusion:** Plasmatic IL-10 high levels in untreated patients in opposition to treated ones suggest that this cytokine has an important regulatory role during malaria infection and that its expression may be induced by the parasites, therefore consisting of a probable escape mechanism of *P. vivax* from the host immune response. The IL-10 low levels in culture supernatants from the same group can be explained by the fact that regulatory T cells are no longer in contact with parasites' antigens, thus suggesting that low IL-10 plasmatic levels in treated patients represent clinical recovery. **Financial support:** FAPEMA/CNPq. **E-mail:** brunodpr@hotmail.com

Mal140- Phenotypic characterization of memory T lymphocytes during acute *Plasmodium vivax* infection

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Introduction: *Plasmodium vivax* malaria has an enormous socioeconomic impact and is now recognized as a cause of severe and fatal malaria in different endemic areas including the Brazilian Amazon. The development of a safe vaccine would be an important strategy to control malaria. Unfortunately, effective malaria vaccines are still not available. Understanding host protective immune mechanisms is essential to develop efficient malaria vaccines. It has been known that protective memory is mediated by effector T cells. Those cells migrate to inflamed peripheral tissues and display immediate effector function. However, memory T cell development is mediated by central memory T cells which home to T cell areas of secondary lymphoid organs and have little or no effector function, but proliferate and differentiate into effector cells in response to antigenic stimulation. Central memory T lymphocytes are CD45RO memory cells that constitutively express CCR7 and CD62L, which are required for cell extravasation through endothelial venules and migration to lymphoid organs. **Objectives:** In the present study, we assessed the frequency of central memory cells in naturally Brazilian *P. vivax* infected individuals determining the cytokine production by these cells. **Methods:** Patients with non-complicated *P. vivax* malaria (n=64) were used in the study. All of the patients were attended to and diagnosed at the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Manaus, Brazil. Healthy adult blood donors (n=22) living in Belo Horizonte, Minas Gerais State, Brazil, a non-endemic area for malaria were also recruited for the study. PBMCs were stained using monoclonal antibodies to determine the expression of T cell memory markers (CD45RA, CD45RO, CCR7, CD62L) and intracellular cytokine production (IL-10 and IFN- γ). Phenotypic analyses were performed using a Becton Dickinson FACScalibur flow cytometer and the analysis was performed using the CellQuest software (BD Biosciences, USA). **Results:** Our results revealed that malaria-vivax individuals have higher frequencies of naive (CD45RA^{high}) CD4⁺ T cells. Elevated percentages of memory (CD45RO^{high}) CD4⁺ T cells were also demonstrated in infected individuals. *P. vivax* Infected patients presented a pattern of central memory cells, demonstrated by significant expression of CCR7 and CD62L by CD4⁺CD45RO⁺ cells. Furthermore, the profile of secreted cytokines observed in patients showed a significantly higher percentage of IFN- γ and IL-10 producing cells. **Conclusions:** Infection by *P. vivax* elicits the expansion of central memory T cells during acute infection, which are responsible for production of pro-inflammatory and regulatory cytokines. **Financial support:** FAPEMIG / CNPq. **E-mail:** analuiza0201@gmail.com

Mal141- Expression of annexin-A1 in lymphocytes of patients infected with *Plasmodium vivax*

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Introduction: Malaria is a potentially serious infectious disease caused by protozoa of the genus *Plasmodium*. In Mato Grosso, the disease is predominantly focal, being endemic only in the northern region of the State. Among the cells involved in the pathogenesis of malaria, CD8+ T cells are activated during the erythrocyte stage. The CD4+ T cells and antibodies are also needed to promote the reduction of parasitaemia and mediate the elimination of parasites during the chronic phase of malaria. The activation of lymphocytes is dependent on endogenous mediators. One of these mediators is annexin-A1 (ANXA1), which modulates a positive TCR signaling in lymphocytes, influencing cell proliferation. **Materials and Methods:** We collected whole blood samples of patients diagnosed with malaria (*Plasmodium vivax*) (n = 7) in the Malaria Clinic of the University Hospital Julio Muller (HUJM) Federal University of Mato Grosso (UFMT), to perform blood count and blood smear for the parasitological diagnosis and determination of parasite density. Blood was also collected from control patients (n = 3). The quantification of endogenous ANXA1 protein expression and identity cell markers CD4+ and CD8+ was performed by immunofluorescence technique. Data were analyzed as mean \pm SEM and statistical differences were evaluated by analysis of variance one way ANOVA. **Results:** To evaluate the expression of ANXA1 in CD4+ and CD8+ cells, patients were evaluated for level of parasitaemia and platelets. In control patients, the CD4+ and CD8+ cells were positive for ANXA1 expression, and CD8+ cells presented higher expression. During infection by *Plasmodium vivax*, both CD4+ and CD8+ cells had a significant reduction in ANXA1 expression. **Conclusion:** The reduction in ANXA1 expression may indicate an increase in pro-inflammatory and pro-proliferative activity in CD4+ and CD8+ cells, indicating a possible mechanism of potentiation of action of these cells against *Plasmodium vivax*. **E-mail:** quessiborges@hotmail.com

Mal142- Host Genotype for Duffy Antigen Receptor for Chemokines (DARC) and inhibitory antibodies response against *Plasmodium vivax*

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Erythrocyte invasion by *Plasmodium vivax* is highly dependent on the red cell's surface receptor, known as the Duffy blood-group antigen (DARC). The interaction between DARC and its parasite ligand, the Duffy binding protein (PvDBP), is of critical importance for vaccine development. Notwithstanding, few studies investigated the relationship between DARC genotype and immune response to PvDBP. In the present study, we examined the influence of DARC polymorphisms in the ability of immune sera to inhibit the interaction between DARC-PvDBP. For that we used an in vitro binding assay, in which the PvDBP ligand domain (region II, DBPII) was expressed on the surface of cultivated mammalian COS-7 cells. Regardless the erythrocyte genotype, we found that the frequency of responders to PvDBP was higher among those individuals carrying one functional allele FY*B/FY*BES (ES; erythrocyte silent). The results presented here can contribute to improve our understanding of the relationship between DARC receptor, immune response and susceptibility to *P. vivax* infection. **Supported by:** CNPq, FAPEMIG, Pronex Malária, DECIT/MS. **E-mail:** praflavia@cpqrr.fiocruz.br

Mal143- Prevalence of antibodies to *Plasmodium vivax* MSP1₁₉ in samples from donors of blood banks in the states of São Paulo and Rio de Janeiro, Brazil

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Introduction: Besides the transmission by *Anopheles*, malaria can be acquired by blood transfusion, sharing of contaminated needles or congenitally at birth. In the last 10 years, 12 cases of transfusion transmitted malaria have been reported in a number of countries, as France, Italy, USA, Republic of Korea, United Kingdom and Brazil. Four cases occurred in Sao Paulo State, Brazil, where asymptomatic carriers with displacements to regions of the Atlantic forest have donated blood, resulting in contamination of recipients, including one death. According to Brazilian guidelines, in endemic regions, tests should be carried out for detection of *Plasmodium* or its antigens. In malaria free areas, this procedure is also necessary for a candidate coming from endemic regions after 30 days and up to 12 months of displacement. Donors involved in transfusional accidents are invariably semi-immune, with parasite densities below the detection threshold of the thick blood smear or rapid diagnostic tests (RDTs), indicating the importance of using other methods, as serology, particularly in non-endemic areas. In this study, we assessed the prevalence of specific IgG anti-PvMSP1₁₉ antibodies among donors of blood banks from the states of Sao Paulo and Rio de Janeiro, Brazil. **Material and Methods:** Nineteen hundred and seventy-four serum samples from 21 blood banks in Sao Paulo (N=1,309) and 10 in Rio de Janeiro (N=665) were tested, using ELISA-IgG with the recombinant antigen of *P. vivax* MSP1₁₉. These samples were collected after the donors had been screened by clinical parameters, provided they were considered fit to donate and sign the informed consent form. **Results:** In the samples from the state of Sao Paulo, the test detected a positivity of 1.99% (N = 26), and 0.53% of the results were in the gray zone. In the samples from Rio de Janeiro, the positivity was 1.35% (N = 9), and 0.75% (N = 5) results in the gray zone. The reactivity index (RI) of the positive samples ranged from 7.38 to 1.12 (Sao Paulo) and 7.13 to 1.21 (Rio de Janeiro). **Main Conclusions:** The detection of specific antibodies is not necessarily an indicator of parasitemia or disease, but the presence of IgG anti-*P. vivax* antibodies in blood bank donors of non-endemic areas suggests the need of reviewing criteria adopted in the clinical-epidemiological trial of donors. An adequate strategy for minimizing the risk of malaria transmitted by blood in non-endemic areas without losing donations may be a combination of suitable donor selection complemented by screening for malarial antibodies. **E-mail:** ariannisanchez@usp.br

Mal144- Influence of HLA-DRB1 and HLA-DQB1 alleles on IgG antibody response to the *P. vivax* MSP-1, PvMSP-3 α and PvMSP-9 in individuals from Brazilian endemic area

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Introduction: The antibody response generated during malaria infections is of particular interest, since the production of specific IgG antibodies is required for acquisition of clinical immunity. However, variations in antibody responses could result from genetic polymorphism of the HLA class II genes. Given the increasing focus on the development of subunit vaccines, studies of the influence of class II alleles on the immune response in ethnically diverse populations is important, prior to the implementation of vaccine trials. **Methods:** HLA-DRB1* and -DQB1* allelic groups were typed by PCR-SSO (Luminex) and the naturally acquired humoral response from Brazilian Amazon individuals (n=276) against *P. vivax* Merozoite Surface Protein-1 (MSP-1), MSP-3 α and MSP-9 recombinant proteins were detected by ELISA. **Results:** Our results provide information concerning these three *P. vivax* antigens, relevant for their role as immunogenic surface proteins and vaccine candidates. Firstly, the studied population was heterogeneous presenting 13 HLA-DRB1* and 5 DQB1* allelic groups with a higher frequency of HLA-

DRB1*04 and HLA-DQB1*03. The proteins studied were broadly immunogenic in a naturally exposed population with high frequency of IgG antibodies against PvMSP1-19 (86.7%), PvMSP-3 (77%) and PvMSP-9 (76%). Moreover, HLA-DRB1*04 and HLA-DQB1*03 alleles were associated with a higher frequency of IgG immune responses against five out of nine antigens tested, while HLA-DRB1*01 was associated with a high frequency of non-responders to repetitive regions of PvMSP-9, and the DRB1*16 allelic group with the low frequency of responders to PvMSP3 full length recombinant protein. **Main conclusions:** HLA-DRB1*04 alleles were associated with high frequency of antibody responses to five out of nine recombinant proteins tested in Rondonia State, Brazil. These features could increase the success rate of future clinical trials based on these vaccine candidates. **E-mail:** josue@ioc.fiocruz.br

Mal145- Duffy blood group polymorphism and naturally acquired IgG antibodies to *Plasmodium vivax* Duffy binding protein (PvDBP) in rural Amazonians

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In Brazil 99.9% of the reported cases of malaria originate from Amazon region and 90% of them are due to *Plasmodium vivax*. To invade their target cells, young erythrocytes (reticulocytes), *P. vivax* merozoites recognize specific receptors on the host cell surface. One of the major parasite's ligands is the Duffy binding protein (PvDBP), localized in micronemes, which binds to a erythrocyte membrane glycoprotein known as Duffy blood group receptor/antigen for chemokines (DARC). Interaction between domain II of PvDBP and DARC is crucial for red blood cell invasion and DARC-negative individuals are usually resistant to blood-stage infection with *P. vivax*; therefore, PvDBP is a major vaccine candidate antigen. Here, we investigated IgG antibody responses to three recombinant proteins derived from PvDBP in 344 subjects exposed to hypoendemic malaria in the Amazon Basin of Brazil. Both recombinant proteins Sal I_{II-IV}-GST and Sal I_{II}-His contain part (either domains II to IV or domain II alone) of the Sal-I variant of PvDBP fused to either GST or a histidine tail, while O_{II}-His contain the domain II of the PNG-O variant fused to a histidine tail; recombinant proteins Sal I_{II}-His and O_{II}-His (but not Sal I_{II-IV}-GST) were refolded to reproduce the native conformation of PvDBP. IgG antibodies to Sal I_{II}-His, O_{II}-His and Sal I_{II-IV}-GST were found in 43.9%, 39.2% and 14.5% of the subjects, with a strong positive correlation between antibody levels to the Sal I_{II}-His and O_{II}-His but a poor positive correlation between levels of antibodies to these refolded proteins and non-refolded Sal I_{II-IV}-GST. Current *P. vivax* infection (as detected by microscopy or PCR) and cumulative exposure to malaria (as determined by the length of residence in endemic areas) were significantly associated with increased antibody responses to all proteins. The Duffy genotypes FY*A*FY*B and FY*B^{ES}FY*B^{ES} [DARC negative] were the most and least common genotypes, respectively. As expected, FY*B^{ES}FY*B^{ES} individuals were less likely to have antibodies to PvDBP, but we found similar proportions of seropositive subjects with the FY*A*FY*A, FY*AFY*B^{ES}, FY*B*FY*B and FY*BFY*B^{ES} genotypes. Sequence analysis revealed several region II PvDBP variants in local *P. vivax* isolates. The most common of them (found in 22% isolates) differs from Sal I and PNG-O alleles in six amino acid codons (including the including N417K change, which has previously been shown to be part of a linked haplotype that alters PvDBP sensitivity to inhibitory antibody). No Sal-I-type variant, from which a PvDBP-based vaccine prototype currently under clinical testing has been derived, was found in local parasites. **Funding:** FAPESP, NIAID/NIH **E-mail:** vanessanic@gmail.com

Mal146- Evaluation of IgG antibodies against chimeric recombinant constructs derived from *Plasmodium vivax* surface proteins in individuals naturally exposed to malaria

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Introduction: Malaria remains one of the most prevalent parasitic diseases in the world, leading to death a large number of people throughout year. In Brazil, *Plasmodium vivax* is the most prevalent species, accounting for more than 87% of cases. The search for vaccines against *P. vivax* is poorly explored and the numbers of genes that encode proteins antigenically relevant are restricted. However, a recent approach in vaccine development is the production of recombinant chimeric proteins expressing different epitopes, this strategy allow the generation of a simultaneous response against multiple targets in parasite. **Objective:** The present work evaluated the reactivity of IgG antibodies from individuals naturally exposed to malaria against two chimeric proteins representing different epitopes of *P.vivax*. **Material and Methods:** Two proteins were expressed: CH27, containing the C-terminal region of Merozoite Surface Protein (PvMSP-1.19) and five T-Cell promiscuous epitopes from C-terminal region (T6, T4, T8, T19 and T53); and CH28, containing a fragment of Reticulocyte Binding Protein 1 (PvRBP-1_{431 - 748}) and three promiscuous T-Cell epitopes. To detect natural IgG antibodies against CH27 and CH28, ELISA was performed using plasma samples of 263 naturally exposed individuals. **Results:** Firstly, we observed that both the CH27 and CH28 are naturally immunogenic, being recognized respectively by 49,4% and 47,1% of IgG antibodies from studied individuals. The reactivity index (RI) of these IgG antibodies ranged from 0,07 to 12,3 on the assessing response against CH27 and 0,21 to 7,30 against CH28. However, to investigate the possible associations between immune response and the epidemiological profile of studied individuals we associated the RIs with epidemiological factors. We observed that reactivity indexes against the chimera CH28 are associated with the time (years) of residence in malaria endemic area ($r=0,2781$; $p<0,0001$) and the number of past malaria infections ($r=0,1765$; $p=0,0049$), indicating a cumulative effect on the acquired immune response. On the other hand, the response observed for the chimera CH27 was inversely correlated with the months since the last infection ($r=-0,3208$; $p<0,0001$) and directly associated with the number of malaria episodes during the collection year ($p<0,05$), indicating that high levels of antibodies is not associated with protection, but might indicate a recent infection. **Preliminary conclusions:** Thus, our data suggest that both chimeras, CH27 and CH28, appears to have retained their functional identity in the context of specific immune response and also are naturally immunogenic in populations living in malaria endemic areas. However, studies are in progress to characterize the profile of the IgG subclasses present in individuals responders and evaluate the cellular response against the T-cell epitopes, as well as the relation of the immune response observed with the protection or susceptibility to malaria. **E-mail:** josue@ioc.fiocruz.br

Mal147- Baseline study of the *Plasmodium vivax* Duffy binding protein in a well-consolidated settlement of the Amazon area

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Antibody-mediated protection against the erythrocytic stages of malaria parasites has been implicated as a critical component of naturally acquired immunity. The *P.vivax* Duffy binding protein (PvDBP), a leading malaria vaccine candidate, plays a critical role in *P. vivax* erythrocyte invasion. Here, in a well-organized population of the Amazon area, we investigated which factors contribute to the acquisition of anti-PvDBP antibodies. In the baseline study, 541 volunteers of the Rio Pardo community (77% of residents), northeast of the Amazonas state, and were interviewed by a structured questionnaire; 432 of them (79.8% of the eligible) had their blood collected. The overall anti-PvDBP IgG response (ELISA) showed 49.5% (217/432) of responders. Univariate analysis showed that the risk for having anti-PvDBP antibodies increased with age, time of residence in the Amazon area, recent malaria infection and local of dwelling (higher levels among those living along of local streams, known as Igarapé). Furthermore, people living along of Igarapé had lower symptoms when compared with people living along of unpaved roads (25.8%, 67/260 x 34.4%, 94/273; $p=0.025$). After adjusted logistic regression analysis, age was a strong predictor of the presence of IgG antibodies (aOR 1.05; CI-95%: 1.02-1.08; $p=0.005$). In other words, each additional year of age increases the probability of having PvDBP antibodies by 5%. Our results provide an additional insight by showing low levels of sequence diversity in PvDBP (region II, DBP_{II}) among local parasites (50% of infections with the same DBP_{II} haplotype). Our findings suggested

that (1) malaria transmission is a local problem and it varies within a village according to the microepidemiological factors; (2) continuous exposure to malaria may induce significant anti-clinical disease in native Amazonians; (3) PvDBP antibodies are boosting by natural infection; (4) the polymorphism of PvDBP was restricted in the study area. Aiming to investigate the protective role of this antibody response, a prospective study is in progress in the study community. **Financial support:** Fapemig; CNPq/Pronex Malária/Decit/MS; Fiocruz-MG; Fiocruz-AM; Fapeam. **E-mail:** florakano@cpqrr.fiocruz.br

Mal148- Human leucocyte antigens and antibody response against *Plasmodium falciparum* sporozoite-, liver- and blood-stage antigens in individuals living in Brazilian malaria endemic area

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Introduction: Antibodies are believed to be important in mediating both naturally acquired and artificially induced immunity generated by blood-stage vaccine candidates. Thus, efforts have been made in the search of a vaccine that is capable to induce the formation of specific antibodies in enough amounts and functionally capable to participate in the protection process against the parasite. Different proteins have been identified and selected as candidate molecules to enter in the composition of a malaria vaccine. In this context, several studies have investigated associations between naturally acquired immunity in populations living in malaria-endemic areas and the immune response to particular antigens, determining antibody and isotype titers in response to these antigens. In this context, several proteins have been identified and selected as candidate molecules to enter in the composition of a malaria vaccine. In this context, several studies have been investigating associations between naturally acquired immunity in populations living in malaria-endemic areas and the immune response to particular antigens, determining antibody and isotype titers in response to these antigens. **Objective:** In this work we evaluated the antibody response and used HLA as genetic markers in an attempt to determine the presence of genetic control of the responsiveness to *Plasmodium falciparum* sporozoite-, liver- and blood-antigens in malaria-exposed individuals from Brazilian Amazon. **Methodology:** The study was carried out in Rondonia state, located in the southwestern part of the Brazilian Amazon. Serum from 107 individuals living in rural area of Porto Velho (RO) was tested by ELISA using synthetic peptides corresponding to different regions of the CSP, LSA-1, MSP-3, EBA and SPf70. HLA-DR and DQ was determined by PCR-SSP and PCR-SSO. **Results and conclusion:** IgG antibodies to EBA were significantly more prevalent than antibodies to LSA1, MSP-3, NANP and SPf70. Not only the frequency of responders to EBA was higher but EBA also induced higher levels of IgG antibodies than did LSA-1, MSP-3, NANP and SPf70. IgM antibodies against LSA-1 were significantly more prevalent than antibodies against EBA, MSP-3 and NANP. The levels of IgG and IgM to LSA-1, EBA and MSP-3 increased with age and time of residence in malaria-endemic area. IgG1 was the IgG subclass preferentially induced by all tested antigens. The levels of IgG1 antibodies against LSA-1 and EBA and the levels of IgG3 against NANP showed a positive correlation with age. We observed significant associations between anti-EBA response and HLA-DR11 and anti-SPf70 response and HLA-DR11 and HLA-DR13. Given the increasing focus on the use of subunit vaccines in the control of malaria, the concern of the influence of class II allele frequencies in ethnically diverse populations may be important before vaccine trials are conducted among people naturally exposed to parasites.

Mal149- Natural isotypic response against potential vaccine candidate *Plasmodium falciparum* Glutamate-Rich Protein (GLURP) in individuals living in Brazilian malaria-endemic area

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Introduction and Objectives: The *Plasmodium falciparum* Glutamate-Rich Protein (GLURP) is an antigen considered to be one of the leading malaria vaccine candidates. GLURP is expressed in all stages of the parasite life cycle in humans. Studies performed in high transmission areas have shown a high prevalence of anti-GLURP antibodies in adults as well as a significant association of high levels of GLURP-specific antibodies with low parasite densities, and protection against clinical malaria. The aim of this study was to evaluate the antibody response profile induced by GLURP in naturally exposed individuals from a Brazilian endemic area, identifying the immunodominant B cell epitopes. **Methodology:** The study was carried out in the farming area of Colina (CL) and the riverside fishing community of Ribeirinha (RB), both near the capital of Porto Velho (RO). The population of CL primarily consists of migrants from non-endemic areas, and the population of RB consists of natives from the Amazon Basin. The antibody response against immunodominant regions of the GLURP (RO and R2) and synthetic peptides (RO: P3, P4, P5, P8, P9, P10, P11, S3; R22 S4) was evaluated by ELISA. **Results and discussion:** The results showed a high prevalence of individuals with anti-RO and -R2 antibodies in both CL and RB groups. Different data have been reported in an African area of low endemicity, where antibody responses to R2 were observed in only 16 % of donors. Surprisingly, similar results were reported in an African area of high endemicity. The R0-induced antibodies were predominantly IgG1 subclass and R2-induced antibodies were predominantly of cytophilic subclasses (IgG1 and IgG3). S4 and P11 epitopes were identified as immunodominant B-cell epitopes. These results are distinguishable from a previous study conducted in clinically immune Liberian adults, in whom the most frequently recognized B-cell epitopes of GLURP were P3 and P4. However, in RB group, P3 and S3 epitopes induced higher levels of cytophilic antibodies, effective in ADCC, like clinically immune Liberian adults. **Conclusions:** The GLURP protein is immunogenic in natural conditions of exposure and the seropositivity to GLURP increases with exposure. Also, GLURP protein is able to induce cytophilic antibodies that can participate of protective acquisition immunity. In conclusion, our results highlight the importance of GLURP like a malaria vaccine candidate. **E-mail:** riccio@ioc.fiocruz.br

Mal150- Specific antibody responses and immunochemical analysis of potential antigens of *Plasmodium falciparum* in patients with imported malaria

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Introduction: *Plasmodium falciparum* is the most important of the five species of *Plasmodium*, responsible for human malaria. About 300 million clinical cases occur each year, resulting in approximately two million deaths. About 40% of the world population is at risk (WHO, World Malaria Report 2010). There is no effective vaccine against malaria, and rapid and efficient diagnostic tests are crucial components in the strategy of malaria control and epidemiological surveillance of the disease. Despite the global importance of *P. falciparum*, the vast majority of its proteins have not yet been experimentally characterized. Therefore, the main objective of this study is to evaluate the serological reactivity of serum samples from individuals with imported malaria, and subsequent identification of major antigenic proteins. **Materials and methods:** The native proteins of *P. falciparum* (3D7) were produced in an *in vitro* culture system of RBCs in RPMI 1640 medium supplemented according the methods described by Trager and Jensen, 1976 (Science; 193, 4254: 673-5) and Thaithong *et al.*, 1994 (Trans. Royal Soc. Trop. Med. Hyg., 88, 4: 490), with some adjustments. We analysed sera from 336 patients

with imported malaria. As a negative control we used 23 sera from healthy Portuguese individuals who have never been in endemic malaria areas. By enzyme immunoassays (ELISA) distinguished from the positive to negative population and evaluated the prevalence of IgG and IgM antibodies anti-*P.falciparum*. Parasite antigens were separated by SDS-PAGE and sera with higher reactivity by ELISA were analysed by western blot and by mass spectrometry. **Results:** The anti-*P.falciparum* ELISA optimized and validated in this study was effective in discriminating positive from the negative population. It was observed that IgG antibody levels are higher than the levels of IgM in sera analysed. The immunoblotting analysis showed a consistent pattern of immunoreactivity of some antigens important in the range of molecular weight 30-60 kDa, which some of them were subsequently identified immunogenic proteins, including rhoptry-associated protein 3 (RAP3) and rhoptry-associated protein 2 (RAP2). **Conclusion:** In the present study, we identified immunogenic proteins essential for parasite survival in the host, of which two of them already described in the literature as proteins that play an important role in the invasion of erythrocytes by extracellular merozoites. As a final conclusion, we reiterate the importance of immunogenic proteins in the immune response by patients with imported malaria, which remains an important area of study in the development of strategies for prevention and control of malaria. **E-mail:** mssilva@ihmt.unl.pt

Mal151- Association of anti-*Plasmodium falciparum* invasion ligand antibodies with clinical immunity in a Peruvian hypoendemic region

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Introduction: Clinical immunity against symptomatic *P. falciparum* malaria has been thought to develop slowly and only after repeated infections. Merozoites invade erythrocytes using varied ligands that belong to two protein families: erythrocyte binding-like (EBL) proteins and reticulocyte binding-like proteins (RBL or *P. falciparum* RH [PfRH]). Invasion-inhibitory antibodies may mediate naturally-acquired and vaccine-induced immunity. We tested the hypothesis that IgG responses against these two *P. falciparum* invasion ligand families differ between Symptomatic (S) and Asymptomatic (A) individuals who live in the low-transmission setting of the Peruvian Amazon. **Materials and Methods:** ELISA was used to measure IgG against recombinant erythrocyte binding-like proteins: EBA-175, EBA-140, EBA-181 and EBL-1 and reticulocyte binding-like proteins: PfRH1, PfRH2a, PfRH2b and PfRH5. Responses against MSP-119 were analyzed for comparison. **Results:** The percentage of IgG responders with asymptomatic infections was much higher against almost all ligands tested than in the group with symptomatic infections: PfRH1, 100% of responders (A) vs. 86.5% (S); PfRH2a, 75% (A) vs. 75.7% (S); PfRH2b, 75% (A) vs. 21.6% (S); PfRH5, 25% (A) vs. 8.1% (S); EBA-175, 100% (A) vs. 32.4% (S); EBA-140, 25% (A) vs. 5.4% (S); EBA-181, 88% (A) vs. 21.6% (S); MSP-1, 100% (A) vs. 54% (S). The IgG response levels were significantly higher in the asymptomatic (N = 8) than the symptomatic individuals (N = 37) only against PfRH1, EBA-175, EBA-181 (P<0.01), PfRH2b (p<0.001), and MSP-1 (p<0.0001). When the antibody responses were compared based on parasitaemia, significant negative correlation was found for PfRh1, PhRh2a, PfRh2b, EBA-175, EBA-181 and MSP-119. **Main Conclusions:** The higher IgG response in the asymptomatic individuals against most of the repertoire of invasion ligand antigens tested in this pilot study might indicate that these individuals have been exposed previously to *P. falciparum* infections. Naturally acquired anti-ligand antibody responses may also reflect invasion pathways used by locally prevalent parasites, which may be dictated by the repertoire of host RBC receptors available in this specific human population or the immune evasion mechanisms the parasite employ. The contribution of these antibody responses to protective immunity needs to be confirmed by growth inhibition assays. **E-mail:** elimeli_85@yahoo.com

Mal152- Analysis of IgG responses against *Pf*GPI and kinetic of TNF α and IL-10 serum levels in Senegalese Adults with Severe Malaria

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Introduction: The induction of neutralizing immunity to *P. falciparum* Glycosyl Phosphatidyl Inositol (*Pf*GPI) by vaccination has been proposed as a preventive strategy to limit the severity of malaria. *Pf*GPI can induce TNF α production by murine macrophages *in vitro*. However, its pathogenicity in human malaria is not currently well established. **Materials and Methods:** Our study analyses the association between levels of IgG responses against *Pf*GPI, kinetic of TNF α and IL-10 serum levels evaluated by ELISA and clinical outcome, in 110 Senegalese patients with severe malaria (SM) (8-74 years), including 27 fatal cases. Sera were collected in days 0, 1 and 2 of hospitalisation when available. 72 patients with uncomplicated malaria (UCM) (10-77 years) were recruited. **Results:** From the admission to the third day, means of TNF α levels decreased: 70.29 pg/ml to 42.21 pg/ml. According to the outcome, TNF α levels were higher at the admission in death patients than in recovered cases: 92.54 vs 63.06 pg/ml (day 0), and inversely at day 1: 16.59 vs 53.25 pg/ml ($P=0.037$) and day 2: 17.38 vs 48.29 pg/ml ($P=0.032$). IL-10 levels were lower in death patients at day 0 compared to recovered cases ($P=0.011$). 24% of patients showed positive IgG responses against *Pf*GPI and antibodies levels were lowest in death patients at day 0 ($P=0.026$) and day 1 ($P=0.014$). Negative correlation is found between TNF α and IgG levels only at the day 2 ($\rho=-0.460$, $P=0.014$). IL-10 levels appears not correlated to antibodies levels during the follow.

Main Conclusions: These findings provide insights into the implication of high levels of TNF α and *Pf*GPI in the pathogenesis of SM. We find a discriminative result of anti-*Pf*GPI IgG level for fatal cases. Anti-*Pf*GPI IgG plays a role in protecting against disease progression and fatality. **Keywords:** *Plasmodium falciparum*, Severe malaria, *Pf*GPI vaccine candidate. **E-mail:** dieye@pasteur.sn

Mal153- Modulation of immune responses to a heterologous malaria parasite protein on *Lactococcus lactis* cell walls and related histopathological changes in mice

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Introduction: Non-pathogenic food grade bacteria such as Lactic acid bacteria are considered as safe vectors to deliver antigens to the mucosal immune system. Antibodies against a merozoite surface antigen (MSA2) of the human malaria parasite *Plasmodium falciparum* are associated with protection against malaria in field studies. We report on the oronasal immunization of different mouse strains with the *Plasmodium falciparum* merozoite surface antigen (MSA2), a putative vaccine candidate, displayed on *Lactococcus lactis* cell walls and related histopathological changes. We also have investigated the magnitude of immune response by co-administration of recombinant vector and nisin inducer *L. lactis* strain. **Materials and methods:** A malaria parasite protein (PfMSA2) was displayed in lactic acid bacteria in two forms viz. covalently attached to cell walls of live *Lactococcus lactis* (MSA2cP) and non-covalently attached to *L. lactis* cell wall ghosts (MSA2cA), and then used for immunisation. Effect of co-administering the inducer *L. lactis* strain together with the recombinant vector and histopathological changes in gut associated lymphoid tissue were also investigated. **Results:** Serum IgG antibodies to MSA2 were elicited by both immunogens in strain-dependent manner. Balb/c and C3H mice responded better to MSA2cP and MSA2cA on *L. lactis* respectively. The IgG isotype antibodies of these mice reflected the influence of Th1 and Th2 cells. However, the IgG response to native MSA2 on the surface of *P. falciparum* merozoites was better in outbred ICR mice. Serum and faecal IgA anti-MSA2 antibodies were also detected. Antigen specific IFN- γ producing T cells were detectable in spleens of all inbred mouse strains immunized with MSA2cA and in C57 mice immunized with MSA2cP. The co-inoculation of inducer *L. lactis* with the

MSA2cP expressing bacteria, significantly improved the antibody response to MSA2. Enlargement of mesenteric lymph nodes, increased lymphatic infiltration of the lamina propria as well as germinal centre formation in the spleen were noted in mice fed with *L. lactis*. **Conclusions:** Results suggests that mucosal immunization of *Pf* MSA2 is able to generate protective levels of systemic antibodies and cellular immunity in mice dependent on strain and anchoring method and co-administration of an inducer strain elicited better immune responses. These findings are relevant for developing better vaccine delivery systems for human use. **E-mail:** gowry@nsf.ac.lk

Mal154- Faecal antibody response, age related immunogenicity and microscopic cellular changes in immune tissues of mice immunized with *Lactococcus lactis* expressing a malaria parasite protein

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Introduction: The gram positive food grade bacterium *Lactococcus lactis* is a potential vehicle for delivering immunogens to the mucosal immune system. Mucosally delivered vaccines are known to activate the local mucosal immune system to elicit protective secretory IgA antibodies. **Materials and Methods:** Plasmodium falciparum merozoite surface antigen2 (PfMSA2) was expressed in recombinant *Lactococcus lactis* in a form that was partially covalently anchored to the peptidoglycan of the cell wall (MSA2cP). Recombinant *L. lactis* strain was delivered oro-nasally for mucosal immunization to Balb/c mice of ages 1wk (neonates), 6wk (young adults) and more than 25 wks (old adults). Non-recombinant *L.lactis* was used as control. The serum and faecal antibody response was investigated by ELISA using recombinant MSA2 as antigen and by immunofluorescence assay (IFA). Microscopic cellular changes in gut associated lymphoid tissue were investigated. **Results:** Antibodies in the faecal pellets were detectable after oro-nasal immunizations by IFA. Serum IgG anti-MSA2 response was significantly higher in young adult Balb/c mice, after oronasal delivery, compared to old mice and neonates. Antibodies elicited in young mice reacted with native MSA2 in the surface of *P. falciparum* merozoites in an immunofluorescence assay. Enlargement of mesenteric lymph nodes and increased lymphatic infiltration of the lamina propria were noted in both recombinant and non recombinant *L. lactis* immunized mice. The gastro-intestinal tract was otherwise normal in oronasally immunized mice. The spleen showed periarteriolar lymphoid aggregations in immunized mice. **Conclusions:** Recombinant *L. lactis* is a suitably safe vector for subunit vaccines. Oro-nasal immunizations give rise to detectable faecal antibodies. The foreign proteins expressed in *L. lactis* can be used in nasal or oral vaccination procedures to elicit protective secretory antibodies in the gut. The antibody responses to recombinant *L. lactis* were markedly weaker in extremes of age. The histological changes of spleens of older mice support weak antibody response seen. These findings are relevant for further developing *L. lactis* to deliver vaccines mucosally for use in humans of different ages. **E-mail:** surangiy@hotmail.com

Mal155- Killer cell immunoglobulin-like receptor gene diversity in population naturally exposed to malaria in Porto Velho, Northern Brazil

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Rational and Objectives: Killer immunoglobulin-like receptors (KIR) regulate the activity of natural killer and T cells through interactions with human leucocyte antigen (HLA) class I ligands. Some studies have been associated KIR genes and genotypes KIR/HLA ligands with incidence and progression of various infectious diseases, including AIDS, hepatitis C virus infections, tuberculosis and malaria. Within this context, our work characterized the genetic frequency of KIR receptors and their ligands HLA-I in subjects (n = 377) naturally exposed to malaria (Porto Velho - RO). **Methodology:** After DNA extraction,

genotyping of the population was performed by PCR-SSO and Luminex equipment for reading. The data were analyzed by chi-square test (χ^2) with Yates' correction or Fisher's exact test using Graphpad software. **Results:** We observed a higher frequency of the genes KIR2DL1, 3DL1, 2DS4 and 2DL3 (> 89% in all), HLA-C1, -Bw4 and -C2 (> 66% in all) and pairs KIR2DL2_3/C1, KIR3DL1/Bw4 and KIR2DL1 / C2 (> 66% in all) in the population of Porto Velho, which is similar to other Brazilian regions. In our study, we identified 48 KIR genotypes and these are shown in the database Allele frequencies (www.allele-frequencies.net). The most frequent were genotype 1 (30.8%) and 2 (15.2%). Individuals in the group of native of Porto Velho presented a greater genotypic variability (43/48) and a higher frequency of genotype 2 compared to migrants ($P < 0.01$). The native group presented a large number of KIR genes that were present only in this group (27/43). The native with genotypic profile exclusive reported less malarias in the past and longer time since the last infection. **Conclusion:** We did not observe any influence on the KIR genes and their HLA class I ligands on susceptibility to malaria. Group of native with exclusives genotypes are associated with Amerindians population and such genotypes have a higher gradient gene activating. Our data suggest that the genotypes of native exclusives confer added protection against malaria. The data obtained in this work may contribute to future studies on the functional impact of these genes in regulating the immune response in relation to the incidence and clinical course of the disease not only in malaria as well as in other infectious diseases. **E-mail:** banic@ioc.fiocruz.br

Mal156- High levels of antibodies to the N-terminal VAR2CSA early in pregnancy correlate with improved pregnancy outcomes

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Introduction: Pregnant women are preferentially infected by VAR2CSA-expressing *P. falciparum* parasites exhibiting unique adhesion properties that allow them to specifically bind Chondroitin sulphate a proteoglycan in the placenta. Acquisition of protective immunity over successive pregnancies is attributed to antibodies that block the adhesion of infected erythrocytes to CSA. **Material and Methods:** In this study we analysed plasma samples and characterized parasites from women of various parity enrolled from their first trimester of pregnancy and followed until delivery in Benin. The plasma levels of antibodies reactive with the surface of infected erythrocytes (anti-VSA) were measured by Flow cytometry and the plasma adhesion inhibitory activity was assessed on a Petri dish-based static binding assay. Two parasite lines selected for CSA binding on Bewo cells (FCR3 and HB3) were used. The plasma level of anti-VAR2CSA IgG was assessed by ELISA using a panel of recombinant proteins expressed in the baculovirus/insect cell system. The antigens used covered the extracellular part of the VAR2CSA. **Result:** The majority of primigravidae had low levels or no anti-adhesion antibodies at enrolment compared to multigravidae. Significant increase in the levels of anti-VSA, anti-VAR2CSA as well as the plasma binding-inhibitory activity, between enrolment and delivery was observed. However high antibody levels to VAR2CSA domains located at the N-terminal part of the molecule, were associated with improved birth weight. Absence of placental malaria was associated with increasing anti-adhesion ability of the plasma during pregnancy. **Main conclusions:** These data suggest that immunity to placental malaria results from high antibody levels acquired against a particular VAR2CSA region located at its N-terminal. The consistency of the data obtained here with the CSA binding site described in the N-terminal region of var2csa will be discussed. **E-mail:** nicaise.ndam@ird.fr

Mal157- Malaria: non-parasitized red blood cells apoptosis is related to parasite load, but not to immune response

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Background: Recently, through the study of erythrocytic apoptosis during *Plasmodium yoelii* infection, we observed a rise in the levels of non-parasitized red blood cells (nRBC) apoptosis that could be associated with the development of severe malaria anaemia, as premature elimination of nRBC is a relevant mechanism leading to this malaria complication. In the present study, we attempt to investigate the participation of nRBC apoptosis in malaria anaemia as well as the influence of parasite load and immune response on this cell death process. **Methods:** Balb/c mice were intraperitoneally infected with *P. yoelii* 17XL and, then, nRBC apoptosis as well as number of peripheral RBC, parasitaemia and plasmatic levels of cytokines, nitric oxide and anti-RBC antibodies were evaluated at early and late stages of infection. **Results:** Apoptosis of nRBC was increased only at the late stage of infection and it was related to parasite load, but not to the intensity of the immune response. In spite of increased percents of nRBC apoptosis observed when the anaemia degree was accentuated, this increase was not associated to the reduction of peripheral RBC counts. **Conclusions:** We conclude that nRBC apoptosis in malaria can be induced in response to high parasite load and that this apoptotic process does not significantly contribute to the anaemia observed in the malaria model studied herein. Further studies on malaria models in which acute anaemia develop under low parasite burden could help to identify the potential pathogenic role of nRBC apoptosis. **E-mail:** prtoto@ioc.fiocruz.br

Mal158- The role of integrin CD11d/CD18 differentiation and activation of macrophages: Effects of heme and synthetic hemozoin in the immune response.

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Malaria is a parasitic disease caused by protozoa of the genus *Plasmodium* and is a major public health problem. It is known that half the world population lives in endemic areas where this disease causes about 500 million clinical cases and around 1 million deaths annually. Host innate immune response to infection plays a major role in the pathophysiology of Malaria. In this process, leukointegrins are important players mediating the cell adhesion, migration and signaling. CD11d/CD18 is the latest describe leukointegrin and is involved in several pathological events such as atherosclerosis and neuronal damage. Preliminary results from our group have shown that animals deficient (CD11d^{-/-}) in this integrin have a higher survival rate after infection with *Plasmodium berghei* Anka (PbA), but the mechanism of this protection remains unknown. The present study demonstrated that CD11d/CD18 is dynamically expressed in differentiated bone marrow macrophages from animals infected with PbA. Experiments with these cells *in vitro* have demonstrated that CD11d/CD18 does not affect the proliferative capacity of these cells. Furthermore, we evaluated parameters important to cellular activation, such as production of reactive oxygen species, proinflammatory and anti-inflammatory cytokines. We observed that macrophages from CD11d^{-/-} mice produce significantly lower amounts of malondialdehyde and TNF- α , and high levels of IL-10 in relation to the macrophages from wild type controls. In addition, the production of prostaglandin E₂ was significantly reduced in macrophages from CD11d^{-/-} animals. These data indicate that CD11d/CD18 plays an important role in modulating the immune response after PbA infection. We also observed that macrophages from CD11d^{-/-} animals showed a decrease in their ability to phagocytize infected erythrocytes as compared to CD11d^{+/+} macrophages. In contrast, absence of CD11d/CD18 did not affect the ability of macrophages to phagocytize synthetic hemozoin. Together our results suggest

that CD11d/CD18 integrin is involved in cellular activation of macrophages after PbA infection, which makes this molecule a potential target for therapeutic actions. **E-mail:** acosta@ioc.fiocruz.br

Mal159- HIV&Malaria co-infection in the Brazilian Amazonia: the effect of infection by *Plasmodium vivax* on the immune status of the person living with HIV; a case report.

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Introduction: Although co-infection HIV-malaria has been studied in recent years, the data available in the literature on HIV-*Plasmodium vivax* co-infection are scarce. **Material and Methods:** at The Foundation of Tropical Medicine Dr Heitor Vieira Dourado (HVD-FMT), a tertiary reference unit for malaria and other infectious and tropical diseases like HIV in the Brazilian Amazon, a special clinic service was set up to people living with HIV / AIDS who contract malaria; we report here a case of HIV-*Plasmodium vivax* co-infection. **Results:** A 63 years old male patient, coming from the east zone of Manaus, with a diagnosis of HIV since October 2009, has been followed in the HIV/AIDS outpatient clinic service at the FMT-HVD. It has no presented opportunistic infection and has no initiated HAART therapy. Refers three days of development of fever, headache, chills, myalgia, arthralgia, back pain, jaundice and mucocutaneous pallor. At the time of evaluation is presented in good general condition, afebrile, eupneic, normotensive and at the physical examination only has herpes labialis associated with the onset of fever. A thick blood smear was performed showing malaria by *P. vivax* with 5304 parasitic forms per mmc. This is the third episode of his life. At the time of assessment shows leukopenia of 3900 and thrombocytopenia of 134,000, but no alterations in liver or kidney function was observed. The patient uses the default plasmodium vivax therapeutic schema with chloroquine-primaquine without side effects and he progressed satisfactorily in the following days. Relate of its immune status was observed: CD4 titles of 455, 129 and 414 cells one week before the onset of the symptoms of malaria, at the day of malaria diagnosis and two weeks after starting treatment, respectively; CD8 titles of 592, 191 and 519 cells, respectively, while the viral load has increased from 5753 to 9677 copies from a week before until the day of diagnosis of malaria, showing an increase of 68.7%. It was necessary to provide prophylaxis for pneumocystis pneumonia, but due to the repayment of the immune status almost two weeks after the diagnosis of malaria, that therapy has been suspended one month after. **Main conclusion:** These data, shows that there is a detrimental effect of malaria caused by *P. vivax* on the immunity of the person living with HIV that can be reversed with proper treatment but requires close observation to prevent the progression from infection to AIDS. **Support:** UNESCO 296/06; FAPEAM. **E-mail:** florespinosa@gmail.com

Mal160- HIV & Malaria co-infection in the Brazilian Amazonia: the effect of malaria on HIV infection

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Introduction: Malaria in the people living with HIV/AIDS (PLHA) can lead to increased viral load and then, with an increased risk of virus transmission. In areas of unstable malaria transmission is expected that the adult PLHA develop severe forms of malaria more frequently and with decrease in the number of CD4 cells and an increased likelihood of therapeutic failure to antimalarials. Malaria also stimulates replication by increasing viral load, In order to assess the progression of HIV infection or AIDS clinical behavior during malaria infection, a retrospective case series was conducted in co-infected patients, followed at the FMT-HVD. **Material and Methods:** The levels of CD4, CD8 and viral load in 189 PLHA who had 283 episodes of malaria were compared before and after of the malaria case in people living

with HIV (PLH) and in people living with AIDS (PVA). **Results:** PVA were on average three years younger than the PVH, although this difference was not significant (37.9 ± 9.9 vs. 40.6 ± 12.3 years, $p > 0.1$), women were on average eight years younger than men (32.7 ± 9.7 vs. 41.2 ± 10.2 $p < 0.001$). When compared, the titles of the CD4 and CD8 cells were significantly lower as well as their viral load was significantly higher among PVA compared with PVH. Malaria has most deleterious effect in PVH than in the PVH, when compared the levels of CD4, CD8 and viral load before and after the episode of malaria; This difference was statistically significant, being of 670.1 ± 437.3 vs. 491 ± 305.6 , $p < 0.05$ for CD4; 1147.5 ± 408.3 vs. 982.9 ± 397.8 , $p < 0.05$ for CD8 and $38,421 \pm 59,453$ vs. $84,777 \pm 59,951.1$, $p < 0.05$ for viral load copies. Already in the PVA, although there are differences in titles among the two moments, this difference was not statistically significant. Main Conclusion: The results shows that malaria infection plays a significant deleterious effect on immunity of PLHA, but this effect seems to be much higher in PVH than in PVA. Financial Support: UNESCO 296/06; FAPEAM. **E-mail:** florespinosa@gmail.com

Mal161- Anti-EPO antibodies levels varies in different strains of semi-immune mice infected with *Plasmodium berghei* ANKA

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Background: Malaria anemia is still a major public health problem and its pathogenesis still unclear. It has been observed that some individuals develop anaemia at low parasitaemia and are protected and others not due to complications from hyperparasitaemia. This was also observed in our animal studies model where high Hb loss, high survival rate and low parasitaemia were observed in Balb/c when compared with B6, CBA and NZW. A study has shown that treatment with exogenous anti-erythropoietin (EPO) antibodies (Ab) of infected mice gives protection, suggesting an important role for anti-EPO Ab in malaria. Thus we hypothesized that elevated levels of anti-EPO Ab is associated with Hb loss and protection in *Plasmodium* infected semi-immune individuals. **Methodology:** Semi-immune status was attained in four mice strains (Balb/c, B6, CBA and NZW) by repeated infections with 10^4 PbANKA, and treatment with chloroquine/pyrethimine. ELISA was used to measure transferrin, EPO and anti-EPO Ab, while inflammatory cytokines measurement was done using bead-based multiplex assay kit. **Results:** Anti-EPO Ab levels was similar in Balb/c and NZW ($p=0.61$), but significantly higher when compared with B6 and CBA, $p<0.0001$. Interestingly EPO levels were significantly high in NZW and least in Balb/c ($p<0.05$), with those of B6 and CBA of intermediary values. Again NZW was highly parasitaemic (17%) and others (Balb/c, B6 and CBA) between 1-3% ($p<0.05$). Anti-EPO Ab correlated positively with extent of Hb loss ($r^2=0.41$; $p=0.0009$). Significant elevated levels of IL6 and IFN γ ($p<0.0001$), both associated with erythropoiesis suppression were observed in the Balb/c. Transferrin was significantly lower in Balb/c ($p<0.0001$). **Conclusion:** Our data presented here seems to suggest that anti-EPO Ab may play a beneficial role in protecting some individuals from severe malaria anaemia but its mechanism need to be studied further. The anti-EPO Ab variation in the different strains of mice seems to implicate some host factors in this phenomenon. **Email:** kofigidi@yahoo.com

Mal162- Enhancement of dendritic cell activation via CD40L-expressing $\gamma\delta$ T cells is responsible for protective immunity against *Plasmodium berghei* XAT.

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Introduction: Previous reports have shown that $\gamma\delta$ T cells are important for the elimination of malaria parasites in humans and mice. However, how $\gamma\delta$ T cells are involved in protective immunity against blood-

stage malaria remains unknown. **Materials and Methods:** We infected $\gamma\delta$ T cell-deficient (TCR δ KO) mice and control wild-type (WT) mice with *Plasmodium berghei* XAT, which is a non-lethal strain, by intravenous injection of 104 parasitized red blood cells (RBCs). IFN- γ production by $\gamma\delta$ T cells or CD4⁺ T cells and IL-12 production by dendritic cells were detected by intracellular cytokine assay. Cytokines in supernatant of splenocyte culture were quantified by ELISA. Flow cytometry was performed for detection of cell markers and activation indicators and cytokines by FACS Calibur and FACS Canto II. **Results:** Although infected RBCs (iRBCs) in WT mice were eliminated within 30 days after infection, TCR δ KO mice could not clear the iRBCs, showed high parasitaemia, and eventually died. Therefore, $\gamma\delta$ T cells are essential for clearance of the parasites. On day 5 post-infection, dendritic cells (DCs) were activated in both WT and TCR δ KO mice. However, the activation status of DCs in TCR δ KO mice was weaker than that in WT mice. IFN- γ production of CD4⁺ T cells was decreased in TCR δ KO mice after infection as compared in WT mice. $\gamma\delta$ T cells produced IFN- γ and expressed CD40L during dendritic cell activation. Moreover, in vivo induction of CD40 signaling prevented the death of TCR δ KO mice after infection with *Plasmodium berghei* XAT. **Main Conclusions:** Here, we found that $\gamma\delta$ T cells play a key role in dendritic cell activation after Plasmodium infection. Our results suggest that $\gamma\delta$ T cells enhance dendritic cell activation via IFN- γ and CD40L–CD40 signaling. This study improves our understanding of protective immunity against malaria and provides new insights into $\gamma\delta$ T cell-mediated protective immunity against various infectious diseases. **E-mail:** fumfum@ks.kyorin-u.ac.jp

Mal163- Expression, purification and evaluation of bacterial-expressed recombinant *Plasmodium knowlesi* MSP-1₃₃, MSP-1₁₉ and SPATR

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Abstract: *Plasmodium knowlesi* is now regarded as the fifth species of *Plasmodium* causing human malaria in South East Asia. Merozoite Surface Protein (MSP) and Surface Protein Containing an Altered Thrombospondin Repeat Domain (SPATR) of *Plasmodium falciparum* and *P. vivax* has been studied as potential target for vaccination and diagnosis of malaria. However, limited studies focused on *P. knowlesi*. In this study, the recombinant *P. knowlesi* MSP-1₃₃, MSP-1₁₉ and SPATR proteins were expressed optimally after two hours of induction with 0.1 mM isopropyl thio- β -galactoside (IPTG) at 37°C using an *Escherichia coli* (*E. coli*) system. Purified pkMSP-1₃₃ reacted with patient serum samples infected with *P. knowlesi* (33/33) and Non-*P. Knowlesi* malaria (27/28) in Western Blots. Most of the non-malarial infection (51/52) and healthy donor sera (65/65) did not react with recombinant pkMSP-1₃₃. Western-blots evaluation using recombinant MSP-1₁₉ and SPATR proteins is on-going. **E-mail:** lauyeeling@um.edu.my

Therapeutics and chemoresistance

Mal164- New primaquine-thiazolidinone derivatives block gametocyte development in the mosquito vector

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Primaquine (PQ) is an 8-aminoquinoline used to prevent the *Plasmodium vivax* late relapses caused by the late sporozoite stages in the liver, the hypnozoites. PQ also interferes with gametocyte viability in the mosquito vector, being used in malaria endemic areas to interrupt transmission, by blocking oocyst development. PQ metabolites cause severe hemolysis and anemia in G6PD deficient patients, and thus it needs to be replaced by less toxic drugs. In the present work new heterocyclic thiazolidinones

synthesized from multicomponent reaction of PQ, arenealdehydes and mercaptoacetic acid (Neuenfeldt et al., Synthesis, 2012) were evaluated for their biological activity. All PQ derivatives tested exhibited low *in vitro* toxicity against a human hepatoma cell line (HepG2) (MDL₅₀ varied from 250 to > 1000 μ g/mL), unlike PQ (MDL₅₀ 106 μ g/mL); five were active against *P. falciparum* blood stages (IC₅₀ <10 μ g/mL) and four were partially active in mice with *P. berghei*. The anti-gametocytic activities of the some compounds were measured through the inhibition of oocyst formation in *Aedes fluviatilis* mosquitoes blood fed on *P.gallinaceum*-infected-birds six hours after drug treatment, in relation to control mosquitoes blood fed on the same bird before treatment (time zero). Two PQ derivatives (**5o**, **5c**) significantly inhibited the mosquito infection, and fewer than 15% mosquitoes presented oocysts whereas more than 90% in the control group were infected; the number of oocysts in the infected midguts was also reduced by approximately 90 %. Three PQ derivatives inhibited the mosquito infections (45%) and reduced the oocyst number; eight compounds found were inactive. For yet unknown reasons none of the gametocidal PQ-derivatives were active against the parasite blood stages, whereas PQ totally inhibited both parasite cycles. Further assays are needed to investigate the ability of **5c** and **5o** to kill the hypnozoite using experimental models. One recently described *P. cynomolgi* sporozoites in simian malaria model. Although ideal for such investigation, is not available in Latin America, in addition of being expensive and complex. Financial Support: CNPq and FAPEMIG. **E-mail:** akrettli@cpqrr.fiocruz.br

Mal165- Methemoglobinemia and therapeutic response in Brazilian patients with *P. vivax* malaria

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Methemoglobin (MetHb) is an oxidative product of hemoglobin formed when the ferrous iron in the haem moiety of hemoglobin (HB) is converted into the ferric state. Within the normal red blood cell, a small amount of MetHb is continuously formed and then reduced by an efficient methemoglobin reductase system composed by various enzymatic and non-enzymatic mechanisms which normally keeps the MetHb level < 1%. High levels of MetHb are associated with the exposure to oxidant agents, as Primaquine, which is used in the treatment of *P. vivax* malaria. The metabolism of HB has been closely related to the pathophysiology of malaria in severe infection, but no date associated the levels of MetHb with the parasitaemia and the parasitological or clinical outcomes in subjects with *P. vivax* malaria without complication living in endemic areas of Brazilian Amazon. Therefore, we determine the levels of MetHb in healthy volunteers and subjects with *P. vivax* malaria before and in the course of treatment. A total of 37 subjects with *P. vivax* malaria were studied. Among then, 9 subjects presented relapse during the follow-up period. The mean time for parasite clearance and the geometric mean of parasitaemia were similar between cured and relapse cases. The distributions of gender and ages were comparable in both groups. The levels of MetHb in healthy volunteers ranged from 0.56 % to 1.69%. In subjects with malaria the levels of MetHb on D0 in cured cases ranged from 0.35% to 3.56% and on D2 from 0.31 to 8.84% ($p=0.0083$). In cases with relapse, the levels of MetHb ranged from 0.33% to 0.95% on D0 and from 0.44 to 2.82 on D2 ($p=0.0257$). The levels of MetHb were similar between healthy volunteers and cured patients on D0, but were high as healthy volunteers ($p=0.0014$) as in cured cases on D0 ($p=0.0234$), when compared with relapse cases. The levels of MetHb in healthy volunteers were consistent with previous reports and resulting of the continuous formation and reduction of MetHb in erythrocytes. The low levels of MetHb in subjects with relapse compared to healthy volunteers and cured subjects, point out the role of oxidative stress in the pathophysiology of malaria. **E-mail:** amandagncmello@yahoo.com.br, jvieira@ufpa.br

Mal166- Is the efficacy of dihydroartemisinin-piperaquine in uncomplicated *P.vivax* malaria related to the follow-up period?

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Introduction: Multidrug-resistant *Plasmodium vivax* is emerging elsewhere across countries. Clinical studies assessing efficacy of a new fixed-dose co-formulated dihydroartemisinin-piperaquine (DP) in treating uncomplicated *falciparum* infection in endemic countries revealed the excellent efficacy. However, clinical studies assessing efficacy of DP in treating uncomplicated vivax infection in endemic countries is limited, and there is the heterogeneity of efficacy level among studies. **Objective:** To assess whether the different follow-up period of DP reveal an equal efficacy in treating uncomplicated *P. vivax* infection. **Materials and Methods:** This is a documentary review of published randomized controlled trials (RCTs) assessing the relative efficacy and safety of DP for treatment of uncomplicated *P. vivax* malaria. We assessed the risk of bias in the included trials using the guideline of the Cochrane handbook of systematic reviews of interventions. The domains applied for the risk of bias assessment for all included studies were (i) random sequence generation (ii) allocation concealment and (iii) blinding of outcome assessment. To identify the association between the reported efficacy of DP and the follow-up period, we calculated correlation coefficient. We also investigated the relationship between the risk of bias and the efficacy reported. P value at 0.05 is set for significance. **Results:** We identified 6 RCTs for the present analysis. The efficacy of DP varies from 54.9% to 100% and the follow-up period ranged from 28 to 63 days. Half of the studies identified are in low risk of bias. Overall, DP has a better efficacy than that of other comparators. The results show that there is a significant relationship between the reported efficacy of DP and the follow-up period ($r = 0.89$; $P = 0.003$). The efficacy level of DP at day 28 is better than that of the longer follow-up period (i.e. 42-63 days). **Main conclusion:** DP has no known therapeutic efficacy on the hyponozoite stage of *P. vivax*. The better efficacy level at day 28 is likely to be a consequence of the terminal elimination half life of piperquine (~ 28-35 days), which can suppress the first relapse, but cannot prevent subsequent relapse. Thus, the longer duration of follow up (i.e 42- 63 days) has potential to yield a lower efficacy. **E-mail:** cho_naing@imu.edu.my

Mal167- Characterization of phenotypes of *Plasmodium vivax*, Brazilian Amazon

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Introduction: The assessment of drug sensitivity of *Plasmodium vivax* to antimalarial is of vital interest for malaria endemic regions. Monitoring of the frequencies of drug-resistant and -sensitive in vitro can provide critical information of drug resistance, and such information can be used to predict, anticipate and even contain the spread of resistance in clinical practice. **Material and Methods:** Using a Colorimetric Double-Site Plasmodium Lactate Dehydrogenase Antigen Capture Enzyme-Linked Immunosorbent Assay (DELI) we investigated the *in vitro* chloroquine, mefloquine and dihydroartemisinin susceptibility profile of *P. vivax* isolates collected in different periods, at the time of diagnosis of uncomplicated vivax malaria patients living in two Brazilian Amazon endemic areas: Manaus, Amazonas state (2010-2011; n = 198) and Coari, Amazonas state (2011; n = 54). Pregnant women, indigenous people, prisoners and individuals less than 18 years of age were excluded. **Results:** Of the 252 samples studied, the geometric mean chloroquine IC50 for *P. vivax* isolates from Manaus (198) was 39 nM [95%CI: 37–48 nM] compared to 21 nM [95%CI: 14.7– 29.1 nM] from Coari (54). The geometric mean mefloquine IC50 from Manaus (108) was 13, 9 nM [95%CI: 11– 18 nM] to 10, 2 nM [95%CI: 9.7– 19.1 nM] from Coari (54). The geometric mean dihydroartemisinin IC50 from Manaus (108) was 2, 7 nM [95%CI: 2, 2– 3, 2 nM] to 2, 2 nM [95%CI: 1.7– 3.1 nM] from Coari (54). Correlating with a cut off for chloroquine resistance was defined as 100nM, a level exceeded in 11.6% (23/198) of Manaus isolates and 5, 5% (3/54) of Coari isolates; a cut off for mefloquine resistance was defined as 30nM, a level exceeded in 7.4% (8/108) of Manaus isolates and 5, 5% (3/54) of Coari isolates. All the isolates were sensitive to dihydroartemisinin. **Conclusions:** In vitro testing discriminates between populations with differing levels of sensibility the

drugs antimalarials. The present study showed, *Plasmodium vivax* parasites with chloroquine resistance are already circulating in the Brazilian Amazon. **Financial support:** FAPEAM, PRONEX- Rede Malária. **E-mail:** yfchehuan@gmail.com

Mal168- Whole blood levels of primaquine in therapeutic failure cases of *P. vivax* malaria

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Malaria vivax is a major public health problem in Brazilian Amazon. The treatment is based on the association between chloroquine with primaquine. A high variability in primaquine concentrations in biological media has been showed, which may be responsible by the therapeutic failure and the uncertainty about the optimum concentration range of primaquine in whole blood. In this study, we compare the levels of primaquine in whole blood of subjects with parasitological diagnosis of *P. vivax* malaria with therapeutic failure. The subjects were treated with the standard recommendation of Brazilian Official Health (n=4; group 01) and according *Center for Disease and Control* guidelines (n=4; group 02). Clinical, parasitological evaluations and serial venous blood samples were collected before (D0), and on D3, D7, D14, D21 and D28 after start the treatment. Primaquine was determined by high performance liquid chromatography after liquid extraction. Primaquine was not detected on D0. On D3 the levels of primaquine were 504±325 ng/mL and 532±325 ng/mL (p>0.05); on D7 were 196±50 ng/mL and 291±225 ng/mL (p<0.05); on D14 were 245±62 ng/mL and 939±374 ng/mL (p<0.05); on D21 were 65±56 ng/mL and 265±380ng/mL (p<0.05), in groups 01 and 02 respectively. On D28 only the patients of group 2 presented detectable levels of primaquine (209±261 ng/mL). The therapeutic recommendations of *Center for Disease and Control* guidelines for the use of primaquine promote significantly high levels of primaquine on D7, D14, D21 and D28. Further studies should compare the levels of primaquine in patients with clinical and parasitological cure. **Supported by:** CNPQ/ Rede Interdisciplinar de Pesquisa Clínica em Malária por *Plasmodium vivax* na Amazônia Brasileira: Farmacovigilância e Abordagem Clínico-Diagnóstica nos Estados do Amazonas, Pará, Amapá e Mato Grosso. **E-mail:** mmdemello@gmail.com

Mal169- Risk of drug resistance in *Plasmodium vivax* malaria's treatment due to breakdose

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Introduction: In South-western Amazonia, in practice, the less severe cases of malaria tend to be diagnosed by technicians and receive age-based prescription, in diagnostic laboratories; the more severe malaria cases are generally accompanied by physicians in hospitals and their dosage is calculated using various parameters, including age and weight among them. On top of this reality, although the Brazilian Health Ministry has included (from 2010) the patient's weight parameter in malaria treatment schemes, this was not the case with the 2011 launched template for the .Investigation of Malaria Cases Record., favouring the perpetuation of age as the standard parameter for malaria treatment dosage calculation. On the other hand, there is vast scientific literature showing that under-dosage strongly favours microbial drug-resistance. **Material and Methods:** descriptive study with convenience sample of suspected malaria cases, formed by people who came Spontaneously, from July 2011 to February 2012, to the main malaria diagnose facilities of three municipalities of the Brazilian State of Rondonia: Alto Paraíso, Buritis and Campo Novo. A weight scale was made available at each malaria diagnose facility; the weight of each suspected patient was recorded; and the treatment (whenever necessary) was prescribed considering the patient's weight for the dosage calculation, according to the weights bands found at Table 1 of the Brazilian Health Ministrys Guide for Malaria Treatment. **Results:** During the eight months studied, there were 3,007 exams, and after the exclusion of 507, the remaining 2,500 were included in this study. They

showed 136 (5.4%) underweight people, 1,653 (66.2%) normo weight people and 711 (28.4%) overweight people. The age-bands with more overweight people were, respectively 6-11 y.o. (14 cases; 35.7%) and 12-14 y.o. (90 cases; 35.5%). Despite the fact that this study was not aimed at evaluating obesity, the percentage of overweight found was consistent with the literatures obesity prevalence for the Brazilian population. **Main conclusions:** Applying the 28.4% of the overweight people found on the number of actual malaria cases reported by the above three municipalities during the year 2011, 510 treatments would be at under-dosage level if their doses were based on the age parameter alone, as opposed to their prescriptions being made based on the patient's weight for the dosage calculation. Therefore, the Brazilian Health Ministrys Malaria Coordination team must not only include the weight parameter in both the Investigation of Malaria Cases Record and in the Epidemiological Surveillance Information System. (SIVEP Malaria), but also promote the use of the patient's weight parameter in the calculation of *Plasmodium vivax* malaria treatments. Dosage by the Brazilian local malaria control health personnel, if drug-resistance is to be avoided. **E-mail:** manuel.cesario@uol.com.br

Mal170- Rate of relapse in *Plasmodium vivax* malaria using different primaquine regimens according to bod mass index

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Introduction: In Brazil, patients with *vivax* malaria are treated with the association of chloroquine to promote the disappearance of clinical signs and symptoms of the disease and primaquine to avoid relapses. The patient weight has influence on the therapeutic response to primaquine: 30 mg/day for 14 days is more effective than 30 mg/day for 7 days. Also, it has been observed a negative correlation among Body Mass Index (BMI) and the abdominal circumference of the patient with time of relapse. This study aims to evaluate the risk of relapse in patients with *vivax* malaria using two different primaquine regimens, one proposed by the Ministry of Health of Brazil and the other according to patients' BMI. **Material and Method:** No blinded, randomized clinical and therapeutic essay developed at Clinical Trial Program of Evandro Chagas Institute, Belém/Pará with the support of Toxicology Laboratory of University of Pará (UFPA), with approval of the Ethical Committee. Sixty two patients were enrolled and divided into three groups: group A - 30 mg/day of primaquine, for 7 up to 12 days according to the regimen proposed by the Ministry of Health of Brazil (2010); group B - 30 mg/day of primaquine for 14 days in patients with BMI > 25kg/m²; group C - 30 mg/day of primaquine for 7 days in patients with BMI < 25kg/m². **Results:** Relapse was observed in 36.8% (7/19) of patient of group A. and in 23.1% (6/26) of group C. In group B, that received 30 mg of primaquine/day for 14 days, relapse occurred only in 11.7% (2/17) of patients. The relative risk to relapse among patients of group A and B was 0,32 (p=0.088; CI 95% - 0.08 to 1.33). The mean abdominal circumference of the body reflecting the corporal composition of the patients was 85.8cm ± 6,9 to group A, 98.9cm ± 11,0 to group B and 75.9cm ± 10,6.to group C. The mean of fat mass index/kg, other parameter used to evaluate the corporal composition, was 18.6 kg ± 6,7 in group A, 20.6 kg ± 5.5 in group B and 15.0/kg ± 7.6 in group C. **Conclusions:** Patients that followed the primaquine regimen according to BMI (group B and C) had inferior rates of relapses when compared to patients that received primaquine according to the regimen proposed by the Ministry of Health of Brazil (2010) (Group A), and therefore schemes B and C were considered of better efficacy, despite the increased abdominal circumference and the fat mass index/kg values observed in Group B and the decreased values of the same indices in Group C when they were compared to Group A. **E-mail:** rosanalibonati@terra.com.br

Mal171- Steroidal alkaloids of *Solanum nudum* Dunal (Solanaceae) with activity against *Plasmodium vivax*

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Introduction: According to the traditional medicine of Tumaco (Nariño, Colombia), the plant *Solanum nudum* is used to treat fever. Malaria Group, university of Antioquia has shown that steroidal compounds obtained in this plant, have activity against *P. falciparum* strains sensitive or resistant to chloroquine. Has not been demonstrated activity of these compounds in *P. vivax*. In this study we determined the activity of steroidal alkaloids SN₁, SN₂ and SN₄ in Colombian isolates of *P. vivax*. **Materials and Methods: Study area and sample collection:** Between February 2011 and March 2012, 31 patients with monoinfection *P. vivax* >2000 parasites/ul of blood and without history of ingestion of antimalarials during last month, were admitted in various health institutions in Turbo and Apartado, Uraba, Colombia. Previous informed consent; we took 10 ml of blood with heparin to these patients and ruled out the presence of *P. falciparum* by rapid test (SD Bioline). **In vitro drug susceptibility assay:** Microtest (WHO) was used with modifications described (Russell et al., 2008). The 96 wells plates were dosed with 7 serial dilutions, to a maximum concentration of 300 µM for SN₁, 1200 µM for SN₂ and 500 µM for SN₄. We determined the activity of the compounds in the *P. falciparum* strain NF54 (CQ sensitive) by the radioisotope method. Parasites were obtained by venipuncture from patients with *P. vivax* and leukocytes removed through filtration by 2 rounds of cellulose (Sigma Aldrich S6288). The susceptibility testing was performed at a hematocrit 2%, with initial parasitaemia average of each isolate of 8848 parasites/µl of blood, in McCoy's medium with 25% human serum AB+, incubated at 37°C in a gas chamber (90% N₂, 5% O₂ and 5% CO₂), until >40% of the initial parasites in ring stage matured to schizonts. According to free drug controls the inhibition percentage of schizonts-treated wells was calculated and 50% inhibitory concentrations (IC₅₀) of each compound were determined by nonlinear regression analysis. **Results:** Ten of 28 assays (35.7%) reached a maturity >40% schizonts. Thirty percent (3/10) of the isolates came from Turbo, 40% (4/10) of Apartado and 30% (3/10) of other regions. The average IC₅₀ were 35.7 µM for SN₁, 31.2 µM for SN₂ and 79.4 µM for SN₄, respectively. In the same order, the maximum IC₅₀ was 153.2, 103.2 and 190.3 µM and the minimum was 6.9, 6.8 and 23.3 µM, respectively. The IC₅₀ of SN₁ in *P. falciparum* strain (NF54) was 38.7 µM, for SN₂ was 47 µM and for SN₄ was 61.6 µM. **Main conclusions:** 1We report the activity of steroidal alkaloids from *Solanum nudum* against *P. vivax*. 2The three steroidal compounds show antiplasmodial activity against blood stages circulating isolates of *P. vivax* similar to the activity found in *P. falciparum* strain CQ-sensitive. 3 The IC₅₀ of SN₂ is significantly lower in both *P. falciparum* and *P. vivax* isolates than reported in *P. falciparum* by Arango et al. in 2008. 4The activity of these compounds may provide an alternative therapy for vivax malaria in the future. **Financing:** Colciencias código 1115-493-26137, Sostenibilidad Universidad de Antioquia 2012. **E-mail:** dianifernandez@gmail.com

Mal172- Allergic reaction to chloroquine, in the treatment of patients with malaria by *P. vivax*: case report

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Introduction: Malaria has typical symptoms, manifested by episodes of high fever followed by chills and usually accompanied by profuse sweating, profound malaise and headache. After the crisis, the patient resumes normal activities. In 48 hours, on average, another crisis happens until the patient untreated evolves or die for healing in the midst of renal complications, lung or brain. As regards the time, is rarely fatal malaria. The situation becomes more delicate when there is hypersensitivity to any drug treatment, which is very little reported. **Case Report:** Wife of nineteen years, weighing 123,46 lbs, residing in a tributary of the River Pacajá, was seen in the emergency room of a hospital from the city of Portel (Pará) about the appearance of skin rashes, itchy, characterized by elevated plaques corresponding generalized urticaria, thirty minutes after the first dose of four tablets of chloroquine (150 milligrams), and despite being oriented to administer simultaneously two tablets of primaquine (15 milligrams), did not. Asked if

she had done some other medication at home, the patient denied. She was diagnosed with *Plasmodium vivax* malaria by blood smear test ten hours after the onset of symptoms of malaria. The patient had a history of hypersensitivity to Aspirin and Dipyrone Sodium. Opposite the sign of allergic reaction, was performed intravenous administration of Promethazine (50 milligrams) and Hydrocortisone (500 milligrams). The patient recovered with the disappearance of wheals, confirming the finding introductory, after an hour of observation and was released with the withdrawal of chloroquine and primaquine as a stay of treatment for malaria. The monitoring of the patient became unfeasible because this lie away from the town center location. **Main Conclusion:** The search for alternative treatment was a failure because there is no other option than in the literature of the treatment regimen recommended by the Ministry of Health of Brazil for the treatment of infection by *Plasmodium vivax*. **Email:** roberta.naves2@gmail.com

Mal173- *In vitro* susceptibility profile of Colombian *Plasmodium vivax* isolates to conventional antimalarials.

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Introduction: The Resistance of *P. vivax* to chloroquine (CQ) has been reported since 1989 in different regions of Asia and South America (Rieckman, 1989; Fryauff et al., 1998; Alecrim et al., 1999; Ruebush et al., 2003; Soho et al., 2008). This phenomenon has been poorly documented around the world because of difficulties of growing up parasite in an *in vitro* culture. Therapeutic regimen of CQ to treatment of *P. vivax* in Colombia has an efficacy of 100%, but there are no *in vitro* susceptibility studies. Here we reports the *in vitro* susceptibility of *P. vivax* to antimalarial drugs CQ, quinine (QN), mephloquine (MQ), amodiaquine (AQ), and artesunate (AS) in 10 isolates from 31 patients of the Urabá region. **Materials and Methods: Study area and sample collection:** Between February 2011 and March 2012, 31 patients who consulted in different health institutions in Turbo and Apartado, Colombia, were admitted. They had microscopic diagnosis of *P. vivax*, mono infection was confirmed by rapid test SD Bioline. Following up an informed consent; we took 10 ml of heparinized blood from patients with a microscopy count of $\geq 66.5\%$ of rings and amoeboid trophozoites and without history of ingestion of antimalarials during last month. ***In vitro* drug susceptibility assay:** *In vitro* susceptibility of *P. vivax* to antimalarials was measure by microtest according to WHO, with modifications previously described by Russell et al., 2008. The 96 wells plates were dosed with 7 serial dilutions, the maximum concentration tested was 1200 nM of CQ, 800 nM of MQ, 150 nM of AQ, 2700 nM of QN and 400 nM of AS. Quality control was performed with *P. falciparum* (strain NF54, CQ-sensitive) to verify the activity of drugs. Leukocytes from the blood obtained from each patient were removed by 2 rounds of cellulose filtration (Sigma Aldrich S6288). Parasites were re suspended in McCoy's medium with 25% human serum AB+ to 2% hematocrit, then were add to predosed plates. The assay was incubated in a gas chamber (90% N₂, 5% O₂ and 5% CO₂) at 37°C, until >40% of the initial parasites in ring stage matured to schizonts. The inhibition percentage of schizont was calculated according to free drug controls and 50% inhibitory concentrations (IC₅₀) were obtained by a nonlinear regression analysis. **Results:** Parasites in ten of 28 assays reached a maturity >40% of schizonts from initial rings stages. Thirty percent (3/10) of the isolates came from Turbo, 40% (4/10) of Apartadó and 30% (3/10) of other regions of Urabá. Only 6 isolates with CQ were valid for analysis according to the control performed in *P. falciparum*, 4 for MQ, 7 for AQ, 4 for QN and 10 for AS. In the same order, the average IC₅₀ were: 87.0, 141.9, 33.9, 271.0 and 1.5 nM, respectively, the maximum IC₅₀ was 893.8, 187.7, 109.3, 1836.0 and 7.4 nM and a low IC₅₀ was 6.1, 75.4, 14.5, 47.6 and 0.1 nM respectively. **Main conclusions:** This is the first report of *in vitro* susceptibility of *P. vivax* to different antimalarials in parasites from Colombian patients; the IC₅₀ found to antimalarial drugs tested is correlated with reports from previous studies (Russell et al. 2003, Baird et al. 2004, Russell et al. 2008). However, 50% (3/6) of the isolates tested needed a high concentration of CQ (>600 nM) to kill 50% of parasite population, this may be due to the presence of mature trophozoites at beginning of the assay, because there are reports of resistance to CQ in this parasite stage (Russell et al. 2008; Sharrock et al. 2009). **Supported by:** Colciencias código 1115-493-26137 , Sostenibilidad Universidad de Antioquia 2012. **E-mail:** dianifernandez@gmail.com

Mal174- Antimalarial activity of a plant extract (RM 109) and constituents

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Malaria remains the parasitic disease that causes the highest number of deaths worldwide (about 800,000 each year). The resistance of the parasite to available antimalarial drugs is one of the main concerns. So the search for new antimalarial drugs is an important scope for research and the investigation of plants used traditionally to treat malaria is a valid strategy that our group is exploring. In this study, we have evaluated the antimalarial activity of a plant extract with the code RM109, fractions and substances obtained from it. The plant species under investigation belongs to the Caryophyllales order and is used to treat various infectious diseases. The antimalarial tests were performed against *P. falciparum* W2 strain, by incorporation of tritiated hypoxanthine. A series of extracts in different organic solvents (hexane, dichloromethane, ethyl acetate and ethanol) was prepared and assayed. The ethanol extract was the most active one ($IC_{50} = 24.9 \mu\text{g/ml}$) among those assayed. A hydromethanol solution of this extract was submitted to extraction with ethyl acetate what has led to an increasing activity ($IC_{50} = 5.35 \mu\text{g/ml}$) for the ethylacetate soluble fraction. Bioguided fractionation of this fraction afforded two substances, RM109/4 and RM109/5, with intense antimalarial activity ($IC_{50} = 147 \text{ ng/ml}$ and 229 ng/ml , respectively) whose cytotoxicity was determined in human hepatoma cells (HepG2). No cytotoxicity was disclosed as indicated by CC_{50} values $>1000 \mu\text{g/ml}$ (selectivity index > 1000). These results show that RM109 is a source of potent antimalarial natural products and the investigation is on progress aiming to testing in *P. berghei* for evaluation of the *in vivo* antimalarial activity and to determine the mechanism of action of these isolated substances. **Acknowledgements:** To CNPq and FAPEMIG for financial support (PRONEX CNPq Process 555655/2009-1 and FAPEMIG Process CDS APQ 01129-10), to CNPq for fellowships (GCB/PNPD, RCP/Doctorate). **E-mail:** alaidebraga@terra.com.br

Mal175- Synthesis and Evaluation of Antimalarial Activity of Viscosaline and Theonelladin C Analogues

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Introduction: About one million deaths are caused by malaria every year. Without an effective vaccine, programmes to control malaria have relied on chemotherapy for malaria treatment. The development of new effective drugs is urgently needed. The relatively simple structure of viscosaline and theonelladin C, the strong antimicrobial activity of viscosaline, and our longstanding interest in the chemistry of sponge alkaloids prompted our investigation of the synthesis of oxygenated analogues of these marine alkaloids.

Material and Methods: A series of new oxygenated analogues of viscosaline and theonelladin C, 3-alkylpyridine alkaloids, were prepared from 3-pyridinepropanol in few steps and in good yields. The antimalarial effect of the oxygenated 3-alkylpyridine marine alkaloids analogues and of the control compound chloroquine was measured with the $[3\text{H}]$ -hypoxanthine incorporation assay. The inhibition of parasite growth was evaluated from the levels of $[3\text{H}]$ -hypoxanthine incorporation, IC_{50} (the drug concentration that reduced parasitaemia to 50%) values were evaluated by comparing the incorporation in drug-free control cultures. The cytotoxicity assay was performed by MTT colorimetric, to determine LC_{50} (the lethal drug concentrations), method using normal human cell line WI-26 VA4 (Lung fibroblast). **Results:** IC_{50} and LC_{50} values, determined for seven marine alkaloids analogues (5b, 6a-b, 7a, 8a, 9a and, 10b) showed that all compounds studied are active against *Plasmodium falciparum* chloroquine-resistant clone W2. However 9a stood out due to its higher activity and selectivity for *P. falciparum* ($IC_{50} < 3.38 \mu\text{M}$, $LC_{50} = 52,67 \mu\text{M}$). **Main Conclusions:** This preliminary investigation showed that the antimalarial activity and cytotoxicity of 3-alkylpyridine marine alkaloids analogues can be modified

significantly by the alterations in alkyl chain length. Among the synthesized compounds, 9a was the most active and selective. Therefore, such compounds would represent a promising matrix for developing a new class of antimalarial agents and deserves further investigation of derivative scaffolds. **Financial support:** FAPEMIG e CNPq **E-mail:** albrelima@yahoo.com.br

Mal176- Evaluation of antimalarial activity of new synthetic chalcones and flavones

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Introduction: Malaria, a disease caused by protozoan of the genus *Plasmodium*, has been a major problem in the global human health with about 86 affected countries and approximately 3 billion people exposed. The most dangerous species of malaria parasites, *Plasmodium falciparum* has emerged resistant to almost all drugs currently used. The search for new compounds that may be used in the development of new drugs for malaria treatment is a recommendation from the World Health Organization. Chalcones and flavones are associated with antitumoral and antimalarial activity. The aim of this study was to describe the antimalarial activity of new synthetic chalcones and flavones. **Materials and Methods:** The compounds were tested by the traditional method. Twelve synthetic chalcones and eight synthetic flavones were tested in vitro against *P.falciparum* chloroquine-resistant strain (W2) in parallel with chloroquine, a control antimalarial drug. Concentrations that inhibit 50% of the parasite growth (IC₅₀) were determined for each compound. **Results:** 9 synthetic chalcones (75%) were considered active, with IC₅₀ <50 µg/ml. Among the tested samples, the chalcones CTOTCPM, CETP and CR1OHPM showed an IC₅₀ <7.0 µg/ml, suggesting an action range very close to chloroquine. Seven flavones were considered active, with IC₅₀ ranging from 6.3 to 11.2 µg/ml. **Main conclusions:** This work showed the antimalarial activity of synthetic chalcones and flavones, molecules with low structural complexity that favours modifications and enables structure–activity relationship studies. **Financial support:** CNPq, FAPEMIG. **E-mail:** sarah_capelupe@hotmail.com

Mal177- Activity of chloroquine analogues complexed with Platinum (II) against *P. falciparum* blood parasites

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Introduction: Resistance of *P. falciparum* to most available drugs and the low sensitivity of *P. vivax* to chloroquine hamper malaria control. Although 4-aminoquinolines are still largely used as antimalarials combined with artemisinin derivatives, it is urgent to develop new treatment alternatives. Biological activity of antiparasitic compounds can be enhanced by metal complexation representing a possible strategy to overcome drug resistance. Indeed, three chloroquine analogues complexed with platinum (II) exhibited up to 76% suppression of parasitemia in *P. berghei*-infected mice (de Souza *et al.*, BiomedPharm2011). **Material and Methods:** the analogues aforementioned referred to as CQPt31, CQPt42 and CQPt40, having none, one or two triple-bonds in the side chain were evaluated for the *in vitro* activity against chloroquine-resistant *P. falciparum* blood cultured forms using monoclonal antibodies anti-histidin-rich protein II (HRPII assay). **Results and Conclusions:** the compounds exhibited high activities (IC₅₀ <4.5 µg/mL) and were non toxic (MDL₅₀ >1000 µg/mL) to hepatoma cells (HepG2). The data suggests that the triple-bond enhanced drug activity since CQPt40 (having two triple-bonds) was about 10 fold more active than CQPt31 (no triple bond). The selectivity indexes (MDL₅₀/IC₅₀) ranged from 236 to 1923 and such suggest that this is an interesting class of compounds to undergo further evaluation. **Work supported by** CNPq- FAPEMIG (Rede Malaria PRONEX). **E-mail:** nicolli@cpqrr.fiocruz.br

Mal178- *In vitro* and *in vivo* evaluation of antimalarial trioxalane LC50 in susceptible and resistant strains to artesunate

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Introduction: Malaria remains one of the most important diseases of the developing world, killing 1-3 million people and causing disease in 300-500 million people annually. Face of worldwide spread *P. falciparum* of resistance to the majority of available antimalarial drugs, WHO recommends since 2006, artemisinin based combination therapies (ACTs) as first-line treatment for uncomplicated malaria caused by *P. falciparum*. However, in 2008, reports from the Thai-Cambodian border revealed emergence of resistance to artemisinins (ARTs). Should ACTs fail; no suitable alternatives exist for chemotherapy of *P. falciparum* malaria. Artemisinin provided a completely new antimalarial structural prototype; that is, a molecule with peroxide bond in a unique 1,2,4-trioxane heterocyclic. The accessibility, relatively inexpensive preparation and the stability of 1,2,4-trioxane and 1,2,4,5-tetraoxane function enables the syntheses of derivatives with diverse structures, broadening the possibility of developing even more effective drugs. **Goal:** Aiming to overcome ART limitations, synthetic trioxolanes (peroxides) were evaluated for antimalarial activity *in vitro* and *in vivo*. **Material and method:** **1.** *In vitro* screening followed the MARKIII (WHO micro-test), with a positive control (chloroquine) and negative control (drug free). Screened *in vitro* for antimalarial activity with *P. falciparum* culture (Dd2 and 3D7). **2.** Cytotoxicity of the compounds was tested using HepG2 human hepatoma cells and the MTT assay. **3.** Efficacy against artesunate-resistant, AS-ATN *P. chabaudi* murine parasites was as follows: female Balb/C mice were infected (Day 0). On Day1, 2 and 3 after infection mice were treated subcutaneously with two different concentrations (mg range) of the compound, controls groups will receive artesunate, and negative controls receive solvent. After Day4, Giemsa stained blood smears were collected every other day, and parasitaemia determined. **Results:** *In vitro* tests, the IC₅₀ 3D7 = 0.848 ng/mL, IC₅₀ Dd2 1,868 ng/mL and IC₅₀ cytotoxicity = 189743 ng/mL. Animals infected with the resistant strain AS-ATN, presented dose response behaviour in the presence of the compound, being 0% at 50mg/Kg. **Conclusions:** LC50 demonstrated good activity against *P. falciparum* *in vitro* as well as low toxicity. Most importantly it was very efficient against resistant parasites, indicating that LC50 has a different mechanism of action from artesunate. Consequently, it represents a very good candidate molecule for an antimalarial. **E-mail:** liscoelho5@hotmail.com

Mal179- Inorganic Complex that Inhibits *Plasmodium* Growth as a Prototype of a New Class of Chemotherapeutic Agents to Treat Malaria

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Introduction: The human malaria parasite, *Plasmodium falciparum*, synthesizes fatty acids employing the Type II fatty acid biosynthesis system (FAS II), like bacteria and plants, unlike humans that rely on the Type I (FAS I) pathway. In *M. tuberculosis* the NADH-dependent enoyl-ACP (acyl carrier protein) reductase enzyme (ENR) has been shown to be a major target for isoniazid (INH), the most prescribed drug for active TB infection and prophylaxis. In trying to find better alternatives to INH, has been investigated INH analogs that contain a cyanoferrate moiety. An example of this class of chemical compound is the pentacyano (isoniazid) ferrate (II) complex [Fe^{II}(CN)₅(INH)]³⁻, referred to as IQG607, that inhibits enzyme activity of InhA (ENR of *M. tuberculosis*). This work investigates the efficacy *in vitro* and *in vivo* of IQG607 against malarial parasites, and his activity against PfENR *in vitro*. **Material and Methods:** *In vitro* tests: suspension of ring stage-synchronized cultures at 0,5 parasitaemia were distributed in 96-well plates in the presence of different concentrations of IQG607. The plates were incubated at 37°C, with a low oxygen gas mixture, for about 20 hr. [³H]Hypoxanthine in culture medium (5–25 µCi/ml) then was

added to each well and after a further 28-hr incubation, the cells were harvested and the cell-associated radioactivity was measured by scintillation counting. *In vivo tests*: BALB/c mice were challenged intraperitoneally with 10^7 parasitized erythrocytes with a recombinant *P. berghei* (PbGFP_{CON}) and were dosed intraperitoneally with 80 mg/kg body weight with IQG607 in PBS solution, with chloroquine (4 mg/kg) for reference treatment, daily, for 4 days. The relative GFP-fluorescence intensity of *Plasmodium* was analyzed by cytometry. The results were expressed as the inhibition percentage of parasitaemia in treated mice in comparison to the control. *In vitro* activity against PfENR assays were carried out in a UV-Visible Spectrophotometer. The enzyme was preincubated with the inhibitor for 15min, and the activity was monitored by adding enzyme-inhibitor mixture aliquots, with different times of exposure, to the reaction mixtures which contained crotonoyl-CoA and NADH concentrations in 100mM sodium phosphate buffer pH 7.5. The reactions were monitored by the decrease in absorbance at 340 nm due to the conversion of NADH to NAD⁺. **Results**: IQG607 inhibits *P. falciparum* growth *in vitro* with IC₅₀ value of 1,52 µM ($R^2 = 0,999$) on a chloroquino-sensitive strain. Preliminary results of *in vivo* tests have indicated that IQG607 in a daily dose of 80 mg/kg for 4 days exhibited a significant inhibition of parasitaemia in mice, with there being no obvious acute toxic effects from the tested dose. Kinetics data revealed that PfENR inhibition by IQG607 was time dependent. IQG607 is a slow-onset inhibitor of PfENR. **Main Conclusion**: INH analogs that contain a cyanoferrate moiety, such as IQG607, represent one class of chemical compounds that are potential inhibitors of an unused metabolic pathway by conventional antimalarial drugs. **E-mail**: hildebrando@fiocruz.br

Mal180- Antimalarial activity evaluation and cytotoxicity of derivatives from sulphonamides 4- metoxichalcones and sulphonamides quinolines

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Malaria is one of the most prevalent parasitic infections in the world and certainly the most detrimental. Caused by a parasite of the genus *Plasmodium*, and without an effective vaccine, the challenge to obtain new compounds from natural or synthetic products for the treatment of malaria remains current. This study aimed to evaluate the antimalarial activity and cytotoxicity *in vitro* of 19 synthetic compounds derived from sulfonamides 4-metoxichalcones, which are defined as aromatic ketones alpha, beta-unsaturated, consisting of two aromatic rings (substituted or not) interconnected by a chain enol and derived sulfonamides quinolines (heterocyclic compounds), assigned by the Institute of Health Sciences from UFMT. **Methods**: a) *In vitro* culture of intraerythrocytic stages of *P. falciparum*, W2 chloroquine-resistant strains were grown in human erythrocytes under conditions established by Trager and Jensen (1976). b) *In vitro* test of the samples against the W2 chloroquine-resistant strain of *P. falciparum*, using the traditional method (Carvalho et al., 1991), and determinate the reductions in percentages of parasitemia.3) Cytotoxic Assay: The cytotoxicity of the synthetic molecules was performed using the MTT (3 - (4,5 dimethylthiazol-2yl) -2,5 diphenyltetrazolium bromide) assay against human HeLa cells line. 4) Statistical analysis: The reduction of parasitaemia was calculated in percentages and cytotoxicity was determined by curves of IC₅₀ (concentration that inhibit 50% of the cells growth) using the program originPro 8.0. **Results**: 9 compounds, sulfonamides derived from 4-metoxichalcones, showed significant reduction in parasitaemia at a concentration of 25µg/mL (CR57 97,36%; CR52 95,51%; CR51 94,13%; CR53 93,89%; CR33 93,47%; CR50 93,35%; CR54 92,04%; CR34 91,80%; CR55 91,26%) . Among the 10 sulfonamides derived from quinolines, only three were able to reduce parasitaemia (CR114 87,13%; CR109 77,31%; CR98 73,11%). All the samples presented no cytotoxic activity, with IC₅₀ values >1000µg/mL. **Conclusions**: These synthetic compounds showed significant reductions in parasitaemia and low cytotoxicity, representing a promising class of candidates for the future development of drugs to treat malaria. Financial support: CNPq, FAPEMIG, UFMT, UFSC, UFSJ. **E-mail**: gicenzi@yahoo.com.br

Mal181- Studies on AP2 adaptor μ -chain, a new candidate molecular marker for artemisinin resistance in *Plasmodium falciparum*

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Introduction: There is evidence of reduced susceptibility of the malaria parasite *Plasmodium falciparum* to artemisinin derivatives, expressed by delayed parasite clearance times *in vivo*. If artemisinin resistance spreads, it would threaten global malaria control. We lack validated molecular markers for monitoring these phenotypes. Using whole genome sequencing in the rodent malaria parasite *Plasmodium chabaudi*, we identified a mutation in the μ chain of the AP2 adaptor protein complex (*pcap2-mu*) that arose along with the experimental evolution of artemisinin resistance. **Material and Methods:** We screened several field isolates of *P. falciparum* from an ACT clinical trial in Burkina Faso, that were tested *in vitro* for their response to artemisinin derivatives and other drugs, and in pre- and post- treatment samples from an *in vivo* ACT trial carried out in Kenya, for genetic polymorphisms in the *pfap2-mu* orthologue. Genetic polymorphisms in *pfap2-mu* were analysed for association with several endpoints in both trials that might indicate a drug resistant parasite phenotype. **Results & Main Conclusions:** Preliminary results indicate that polymorphisms in this adaptor protein subunit may be associated with *in vitro* and *in vivo* responses to artemisinin derivatives, quinine and lumefantrine. Further evaluation of *pfap2-mu* as a potential molecular marker of artemisinin resistance is now needed. **E-mail:** gisela.henriques@lshtm.ac.uk

Mal182- Comparative study of *in vitro* bioactivity of *Bertholletia excelsa* in strains of *Plasmodium falciparum* with the technique of microscopy and radiolabeling

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Amazon is known for its biodiversity. In the Amazon there are several species of flora which are used in folk medicine. The study sought to prove the bio-activity of the Brazilian nut (*B. excelsa*) and so on the comparison of microscopic and radiolabelling techniques. First, the study knows the two best known compounds against malaria have been isolated from plants, quinine and artemisinin. Second, because of quinine and artemisinin and others compounds, another compounds were synthesized. Finally, we have to regard the necessity for studies on bioactive principles in malaria control. In other words, the drug therapy has become unsatisfactory due to genomic plasticity of the parasite. **Methodology:** In the first place, after empirical survey, the selected parts, bark and hedgehog, were dried, crushed and subjected to ethanol PA for a specified time, and then the solvent were concentrated to obtain crude extract. The next step was the obtention of the extract, which was performed in solvents of increasing polarity in which resulted in six fractions (petroleum ether, chloroform, ethyl ether, ethyl acetate and hydroalcoholic). Next, all six fractions were solubilized at 10 $\mu\text{g} \cdot \text{mL}^{-1}$ ethanol PA and diluted with RPMI 5x, getting a stock split 2 $\mu\text{g} \cdot \text{mL}^{-1}$ followed by serial dilutions. Later on, these solutions were subjected to tests *in vitro* standard strains of *P. falciparum* - 3D7 and W2. In turn, the strains were cultivated in accordance adaptations of Trager and Jansen (1990). In conclusion, the IC_{50} were determined by microscopic examination and radiolabeling. **Results:** The preliminary cytotoxicity test revealed no toxicity to the erythrocytes. The tests revealed, also the aqueous and ethanol extracts of the bark and the hedgehog, showed less activity in the diluted concentration (100 $\mu\text{g} \cdot \text{mL}^{-1}$) in 48 hours (in the readings of microscopy and radiolabeling). In 72 hours (microscopic) the extracts reduced the hedgehog significantly in almost 100% parasitaemia. Next, fractions chloroform, ethyl ether and ethyl acetate, bark, had the highest activity with IC_{50} of 5 $\mu\text{g} \cdot \text{mL}^{-1}$, 5,5 $\mu\text{g} \cdot \text{mL}^{-1}$ which were not defined by the dilutions more concentrated (100 $\mu\text{g} \cdot \text{mL}^{-1}$.) For the time being, the fractions with ethyl ether, ethyl acetate and hydroalcoholic hedgehog showed, respectively in 72

hours, an IC_{50} of $12\mu\text{g}\cdot\text{mL}^{-1}$, $12.5\mu\text{g}\cdot\text{mL}^{-1}$ and $6.2\mu\text{g}\cdot\text{mL}^{-1}$, and also, in the dilutions more concentrated ($100\mu\text{g}\cdot\text{mL}^{-1}$) they were. Briefly, the tests with specific identification and isolation of substances are under review in LABFITO-UNIR. **E-mail:** carol_iolanda@hotmail.com

Mal183- Blood shizontiicid and gametocidal activities of primas, a new hybrid salt derived from primaquine and artesunate

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Primaquine (PQ) is the only available antimalarial to eliminate the late developing sporozoites in the hepatocytes, named hypnozoites, and promotes radical cure of *P. vivax*. PQ is an 8-aminoquinoline used in combination with chloroquine, or other antimalarials in areas where chloroquine resistance occurs. PQ also inhibits gametocytes development in the mosquito vector, being useful to control malaria transmission. Due to its side effects, PQ use is restricted in humans. Artesunate (AS), another important antimalarial that rapidly clears parasitemia, is used in drug combinations as the first line treatment for drug-resistant malaria parasites. In the present work we evaluated the biological activity of PRIMAS, a new hybrid drug obtained by the reaction of PQ with AS, in parallel through assays of incorporation of hypoxanthine. The results showed that the drug association and PRIMAS had similar activities ($IC_{50}\leq 1,80\mu\text{g}/\text{ml}$). Their cytotoxicity, measured against a human hepatoma cell line (HepG2), were tested in parallel and allowed to calculate the selectivity index (SI), a ration between toxicity and drug activity. The SI of both PRIMAS and the association were respectively 50,780 and 80,840, but this difference was not statistically significant ($p> 0,005$). Tested as a blood schizonticide in mice with *P. berghei*, PRIMAS and the drug combination caused a similar suppression of parasitemia in the first two weeks of infection; from days 18 to 25 PRIMAS was more active than PQ+AS. The gametocytocidal activity of PRIMAS, measured by its ability to inhibit the oocyst formation in *Aedes fluviatilis* mosquitoes, was evaluated after a blood meal in *P. gallinaceum* infected chicks 6h after treatment; the control mosquitos were fed on the same bird before treatment. PRIMAS and PQ inhibited 100% oocyst formation at similar doses and, to clarify if PRIMAS is a promising new hybrid compound for malaria control new tests are required. **Supported by** CNPq/FAPEMIG and PDTIS/FIOCRUZ **E-mail:** akrettli@cpqrr.fiocruz.br

Mal184- *In vitro* antimalarial activity of indole alkaloids ellipticine, olivacine and cryptolepine

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Introduction: Among the natural products, indole alkaloids represent an interesting class of compounds with significant antimalarial activity. Screening carried out to date has revealed several substances with good *in vitro* activity and selectivity index. Cryptolepine (**1**) ellipticine (**2**) and olivacine (**3**) are alkaloids isolated from antimalarial plants, however, **1** and **2** have also been synthesized. **Objective:** The aim of this work was to evaluate the *in vitro* antimalarial activity of **2** and **3** and compare it to that of structurally analogous **1**. **Material and Method:** Chloroquine resistant K1 and chloroquine-sensitive 3D7 strains of *P. falciparum* were used. Parasites were maintained in continuous culture as described by Trager & Jensen (1976) and tested *in vitro* as described by Andrade-Neto et al. (2007). Stock solutions of **1** (synthetic), **2** (Sigma-Aldrich) and **3** (isolated from *Aspidosperma olivaceum*) were prepared in DMSO. Test samples ($50\text{--}3.2\times 10^{-3}\mu\text{g}/\text{mL}$) were prepared and tested in triplicate in microplates containing red blood cells parasitized at 1-2% initial parasitaemia and 3% hematocrit by incubating for 24h at 37°C and then evaluating parasitaemia of thin smears of each test well using a microscope. **Results:** Median inhibition

concentration (IC₅₀) values for **1**, **2** and **3** were 0.81, 1.42 and 0.80 µM, respectively, against K1 strain and 0.34, 1.19 and 0.91 µM against 3D7 strain. Differences in the activities of these substances were not statistically significant against these two strains of *P. falciparum* although the lowest IC₅₀ value was assigned to ellipticine against 3D7 strain. All three substances were considered to be active. **1** has *in vitro* and *in vivo* antimalarial activity and **2** has *in vitro* antimalarial activity according to literature sources. Antimalarial activity for **3** has not been previously reported. **Conclusions:** **1**, **2** and **3** have comparable *in vitro* antimalarial activities and deserve attention as representatives of a potentially new class of antimalarials. Studies on the *in vivo* activity of **2** and **3** and studies on mechanism of action are necessary for better comprehension of how these compounds act. **Acknowledgements:** CNPq; PRONEX/FAPEAM/CNPq, DTI/INPA/CNPq, FAPEAM/PPP, Brazilian Malaria Network/CNPq. **E-mail:** ampohlit@gmail.com

Mal185- Antiplasmodial activity of *Aspidosperma ramiflorum*

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The oldest remedies used against malaria are still potent antimalarials currently used as first-line treatments of malaria, quinine and artemisinin. Both originated are from medicinal plants, Cinchona sp and *Artemisia annua*, respectively, from the Peruvian Amazon, and from ancient Chinese medicine. Over 1,000 plant species are used in folk medicine against fevers and malaria in the world. These facts reinforce the importance of searching new chemotherapeutic agents from ethnopharmacology. Surveys in the Amazon region shows that plants of the gender *Aspidosperma* are largely used to treat fever and/or malaria, especially *A. nitidum*. Another species *A. ramiflorum*, known as guatambu-yellow and yellow "peroba", has indications to treat leishmaniasis and malaria, our present subject of study. Plant barks and leaves were collected in Maringa (SC), used to prepare extracts and bio fractionated for biological tests against malaria, performed at FIOCRUZ-Minas. The *in vitro* activity against blood forms of *Plasmodium falciparum* (W2 clone, chloroquine resistant) was measured through incorporation of tritiated hypoxanthine, and by a colorimetric assay with monoclonal antibodies to protein rich in histidine (HRP-II). The cytotoxicity tests were performed against a human hepatoma (HepG2) and a monkey kidney (BGM) cell lines, using MTT. The selectivity indexes (SI) was calculated, as a ratio between MLD₅₀ and IC₅₀. Among nine tested extracts seven were active (IC₅₀ of 0,5 to 3,8 µg/mL) and high SI (>39). The extracts precipitated and filtered were inactive (IC₅₀ >50 µg/mL). The fraction non soluble was active with IC₅₀= 3,1 µg/mL, and IS= 56. The purified substances **1** was the only active and showed the highest SI (of 113); the others were toxic (IS <10). The extracts and fractions active *in vitro* should be also evaluated in mice with *P. berghei* to determine their activity. **Work supported by** CNPQ (multidisciplinary project 575746/2008) and FIOCRUZ-PAPES/MS. **E-mail:** akrettli@cpqrr.fiocruz.br

Mal186- Analysis of the *Plasmodium falciparum* ATPase6 gene in isolates from patients treated with artemisinin derivatives reveals the wild-type allele at residue 769 in all samples.

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Introduction: Since 2006, the World Health Organization recommends artemisinin-based combination therapy as first line treatment for *Plasmodium falciparum*, in order to restrain drug resistance. However, therapeutic failure with artemisinin (ART) derivatives has already been reported in South-east Asia. The SERCA-type PfATPase6 has been proposed as the primary target of ART. A specific single nucleotide polymorphism (SNP) in the PfATPase6 gene, resulting in a serine to asparagine change in residue 769, has been proposed to moderate resistance *in vitro*. **Material and Methods:** One hundred thirty blood samples diagnosed as *P. falciparum* by thick blood film and confirmed by nested PCR were assessed.

The harvest period comprised 1984 to 2011. Samples were obtained from individuals treated with ART derivatives prior to treatment and from recrudescence cases. Study sites included seven states of the Brazilian Amazon basin, seven countries in Africa, three in South America, one in Asia and one in Central America. Blood was plotted in filter paper Whatman 3[®] and DNA was extracted with Chelex[®] 100. PCR-RFLP was carried out with primers *Pf*ATP6-769F and *Pf*ATP6-769R followed by digestion with *Bsp*TI in order to detect the S769N mutation. Products were submitted to electrophoresis in 2.5% agarosis gel and stained with GelRed[™]. **Results:** With respect to the *in vivo* response, from the total 130 samples, 57 patients were treated with ART derivatives, of which nine presented recrudescence. In addition, all samples displayed the wild-type serine allele at codon 769. **Main Conclusions:** There was no correlation between the *in vivo* treatment outcome with ART derivatives and the *Pf*ATPase6 769 genotype in samples collected before and after the introduction of ACTs as first line treatment in Brazil. The entire *Pf*ATPase6 and other novel candidate genes are being sequenced in order to search for SNPs that may correlate with ART treatment outcome. **E-mail:** julianainoue@usp.br

Mal187- Evaluation of curcumin and artemisinin derivatives with potential and action antiplasmodial

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Introduction and objective: Malaria remains one of the major infectious diseases in the world, with 254 million cases annually and over one million deaths year (WHO, 2011). The therapeutic gamma against malaria is restricted due to parasitic resistance and the high cost of drugs. The search for novel compounds that can be used to develop new drugs for treatment of malaria remains current. The aim of this study was to evaluate the antimalarial activity of curcumin and artemisinin derivatives against a *Plasmodium falciparum* chloroquine resistant strain (W2). **Methodology:** Twenty samples were tested in vitro against chloroquine-resistant strain W2 of *P. falciparum* (PfCR) using the traditional method (Carvalho et al., 1991) and analyzed the levels of cytotoxicity according to the MTT assay (Mossman, 1983). **Results:** The twenty (20) samples tested, all showed significant reductions in in parasitaemia compared to control, PRG34 89,84%, PRG31 87,62%, GUT1 95,87%, WIL004 68,87%, GUT3 99,37%, COJ5 97,14%, LLF0009 96,19%, LLF001 100%, PR4 80%, LLF011 94,60%, BAL011 70,48%, LLF025 97,14%, PR14 94,60%, LLF026 63,17%, BAL010 95,97%, LLF018B 79,05%, LLF10 93,33%, PR1 88,57%, SG31 98,10%, LLF015 76,19%. **Discussion:** Preliminary results showed that among the 20 samples tested, most have selective antiplasmodial activity. **E-mail:** michaelufsj@gmail.com

Mal188- New Pterocarpanquinones derivatives as potential inhibitors of the Topoisomerase I from *Plasmodium falciparum*

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Introduction: The DNA topoisomerases have attracted considerable interest for the development of new chemotherapies against malaria based on their importance during replication, transcription, recombination, and repair of DNA. The pterocarpanquinones of type 1 comprise a new group of antineoplastic and antiparasite prototypes believed to inhibit topoisomerase. **Material and Methods:** Based on this assumption, we have performed a flexible docking of six new synthetic pterocarpanquinones in the 3D structure of Topo I from *Plasmodium falciparum* (PfTopoI). To evaluate

the *in silico* studies, we also performed the docking of camptothecin, a cytotoxic quinoline alkaloid which inhibits the human Topo I (*HssTopol*) and the *PfTopol*. These compounds fit well in the active site of *PfTopol* and, for this reason, they were tested *in vitro* against W2 (chloroquine-resistant) and 3D7 (chloroquine-sensitive) *P. falciparum* blood parasites using the immunoenzymatic assay anti-HRP II (histidine rich protein II). Their cytotoxicity was also determined against hepatoma cells (HepG2), a data used to calculate the selectivity index (SI = the ratio between the toxic and active doses). **Results:** In preliminary data, four compounds were shown to be active: three with $IC_{50} < 0.23 \mu\text{g/mL}$ and one with $IC_{50} = 4.3 \mu\text{g/mL}$; one compound showed partial activity ($IC_{50} = 12 \mu\text{g/mL}$) and one was toxic. The results of cytotoxicity test showed that all the compounds have a low MDL_{50} value, as expected since they are prototypes of antineoplastic drugs. Thus, these results should be further confirmed by evaluation of cytotoxicity to normal cells. **Main Conclusions:** These preliminary results demand further tests, to establish whether any of the compounds have a specific anti-parasite activity. Since the *HssTopol* presents 42% of similarity compared to *PfTopol*, new studies on molecular modeling will be performed to understand the differences between the active sites and to provide insights for the development of new selective *PfTopol* inhibitors. Structural modifications in the molecules will be possible in order to reduce toxicity and increase the selectivity for the plasmodial enzyme. **Supported by:** CNPq/FAPEMIG and FIOCRUZ. **E-mail:** akrettli@cpr.fiocruz.br

Mal189- Evaluation of the efficacy, safety, and pharmacokinetics of artemether-lumefantrine dispersible tablet in the treatment of acute uncomplicated *Plasmodium falciparum* malaria in infants weighing <5 kg

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Introduction: WHO recommends artemisinin-based combination therapy (ACT) as first-line therapy for infants with uncomplicated *P. falciparum* malaria. However, no ACTs are indicated in infants with BW <4.5 kg. Poor safety profile of quinine, the current standard of care, limits its use. To date, Coartem® (20 mg artemether-120 mg lumefantrine, AL), with an available pediatric formulation, has the largest clinical trial and post marketing safety experience in infants ≥ 5 kg. **Methods:** In this open-label, single-arm, multicenter study in Sub-Saharan Africa, inpatient neonates and infants of <5 kg BW with a confirmed diagnosis of uncomplicated *P. falciparum* malaria will be enrolled in two sequential age cohorts: first, age >28 days (cohort 1) and second, age ≤ 28 days (cohort 2). Enrollment will be stopped after 15 evaluable subjects are available in each cohort. A joint data monitoring committee will review efficacy, safety, and pharmacokinetic (PK) data from cohort 1 and recommend whether to proceed to cohort 2, with or without dose adjustment. The primary objectives are to evaluate the efficacy and safety of AL dispersible tablet administered as 1 tablet bid over 3 days (to adjust if required), and to determine plasma levels of artemether, its active metabolite dihydroartemisinin, and lumefantrine. Exclusion criteria include severe malaria, signs and symptoms of a critical condition, hepatic or renal abnormality, and major neurological malformation. Patients will be followed up to Day 42, and their neurodevelopmental status will be assessed at 12 months of age. Primary endpoint is PCR-corrected parasitological cure at Day 28. Secondary endpoints include reduction in parasite density at 24 hours; PCR-corrected and uncorrected parasitological cure at Days 14, and 42; time to parasite, fever and gametocyte clearance; PK assessments and safety and tolerability assessments. Use of quinine as a rescue medication will be permitted. **Results:** Study results are expected in 2014. **E-mail:** kamal.hamed@novartis.com

Mal190- Activity of *Aspidosperma pyrifolium* biofractionated extracts against *Plasmodium falciparum* blood forms *in vitro*.

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Malaria is endemic in the Brazilian Amazon where traditional healers use plants to treat the disease, especially infusions of species from *Aspidosperma* genus. Plant bark, wood and root from *A. pyrifolium*, popularly known as “pau-pereiro”, were collected and used to prepare extracts. The *in vitro* activity of compounds was evaluated on chloroquine-resistant W2 clone *P. falciparum* blood stages by tritiated hypoxanthine incorporation and immunoenzymatic anti-HRP-II assays. The ethanolic crude extract was the most active, and then fractionated by liquid-liquid partition. The resulting fractions were tested again and among seven three were active ($IC_{50} < 10.0 \mu\text{g/mL}$; concentration inhibiting 50% parasite growth), two extracted with ethyl acetate and one hydromethanolic with methanol. The activity was concentrated in components of polar fractions of the extract. Each fraction was tested against a human hepatoma cell line (HepG2) and was not cytotoxic ($MDL_{50} > 413$); the resulting therapeutic indexes (MDL_{50}/IC_{50}) were higher than 85. Taken together, the data reflect a good antimalarial profile of *A. pyrifolium* extracts that, however, need further chemical characterization, in order to elucidate which molecule(s) is(are) responsible for the anti-*P. falciparum* activity herein described. **Work supported by** Pronex Rede Malária (MCT/ CNPq/ MS/ SCTIE/ DECIT/ FAPEAM/ FAPEMA/ FAPEMAT/ FAPEMIG/ FAPESPA/ FAPERJ/ FAPESP), CPqRR, PAPES V and FIOCRUZ. **E-mail:** ceravolo@cpqrr.fiocruz.br

Mal191- Pharmacotherapy follow-up in patients with malaria treated in a non-endemic area, Rio de Janeiro

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Introduction: Malaria is a potentially severe disease, widely distributed in the tropical and subtropical regions of the world. Despite drug resistance, other factors are directly related to infection's response to anti-malarial treatment, such as adherence to the treatment, drug interactions and adverse drug events. Monitoring these factors by pharmacotherapy follow-up is important to guarantee the effectiveness of treatment preventing drug related problems and ensuring patient safety. The aim of this study was to describe the effect of pharmacotherapy follow-up (PF) in patients with malaria seen at a referral centre located in the city of Rio de Janeiro, an area without active transmission of malaria. **Methods:** Descriptive study conducted from January 2005 to February 2012 at IPEC. Data collections were carried out before and after the implementation of PF. Data were collected from medical records and follow-up interviews. The variables included were age, gender, co-morbidities, anti-malarials and concomitant medications used, adverse drug events and adherence to anti-malarial treatment. Groups from different periods were compared using chi-square test. **Results:** A hundred and fourteen patients were included. Seventeen of them were treated at least twice resulting in 138 malaria treatments. The majority was male (76.3%) with ages between 13 and 66 years. We observed 104 adverse events to anti-malarials considered as 79 adverse drug reactions, three medication errors and 22 therapeutic failures. The majority of patients (90.4%) had a high adherence behavior according to Morisky's questionnaire. Since 2009, when PF started, information related to adverse event increased in 46.0%, and to clinical and parasitological cures increased in 29.8% and 49.4%, respectively. Besides, loss to follow-up after malaria treatment reduced in 24.2% ($p < 0.05$). **Main conclusions:** Before PF, we had no accurate information about patient safety. Furthermore, treatment effectiveness was not known because patients were not followed. We concluded that an improvement in malaria patient's care was achieved by surveillance activities, including pharmacotherapy follow-up. **E-mail:** lusiele.guaraldo@ipec.fiocruz.br

Mal192- *In silico* screen for target similarity identifies several candidate drugs against proteins of the *Plasmodium falciparum* apicoplast

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Introduction: Most drugs in use against *Plasmodium falciparum* share similar modes of action. Thus, the paradigm for identifying new drugs is shifting towards finding alternative targets. One such strategy has focused on the apicoplast, a plastid-like organelle of prokaryotic source which evolved through secondary endosymbiosis. Several antibiotics have been shown to be effective at disrupting apicoplast biology. Here, we undertook a comprehensive *in silico* target identification-based approach for detecting further drugs that may be able to interfere with the *P. falciparum* apicoplast. **Materials and Methods:** The *P. falciparum* genome databases PlasmoDB and GeneDB were used to compile a list of 500+ proteins containing apicoplast signal peptides. Each of these proteins was treated as a potential drug target and its predicted sequence was used to interrogate three different freely available databases (Therapeutic Target Database, DrugBank and STITCH 3) that provide synoptic data on drugs and their primary or putative drug targets. All drugs with an output expectation value lower than 1e-5, were listed. The compiled data was subsequently compared with the drug-target data of *P. falciparum* proteins contained in the TDR Targets database. **Results:** We were able to identify several drugs that have the potential to interact with about 15% of all proteins predicted to be involved in the biology of the *P. falciparum* apicoplast. These putative targets are distributed among the processes of replication, and transcription, translation, isoprenoid biosynthesis, fatty acid metabolism, transport, antioxidation, protein folding, Fe-S cluster production, porphyrin biosynthesis, PTM modifications and very few proteins of unknown function. Drugs identified spanned many different classes, such as antivirals, antibiotics, anti-cancer drugs, anti-fungals, anti-parasitics and even anti-obesity. In addition, a significant amount of the compounds identified were not present in the TDR Targets database. **Main conclusions:** We have taken a computer-aided approach that allowed identifying a number of drugs that have the potential to disrupt the biology of the *P. falciparum* apicoplast. Many of these drugs are already approved but have never been experimentally tested against malaria parasites. The possibility of using some of these chemicals in combination with existing antimalarials will be discussed. **E-mail:** pedrovcravo@gmail.com

Mal193- S-nitrosoglutathione Prevents Experimental Cerebral Malaria

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Administration of the exogenous nitric oxide (NO) donor dipropyleneetriamine-NONOate (DPTA-NO) to mice during *Plasmodium berghei* ANKA (PbA) infection largely prevents development of experimental cerebral malaria (ECM). However, a high dose (1 mg/mouse twice a day) is necessary and causes potent side effects such as marked hypotension. In the present study we evaluated whether an alternative, physiologically relevant NO donor, S-nitrosoglutathione (GSNO), was able to prevent ECM at lower doses with minimal side effects. Prophylactic treatment with high (3.5 mg), intermediate (0.35 mg) or low (0.035 mg) doses of GSNO decreased incidence of ECM in PbA-infected mice, decreasing also edema, leukocyte accumulation and hemorrhage incidence in the brain. The high dose inhibited parasite growth and also induced transient hypotension. Low and intermediate doses had no or only mild effects on parasitaemia, blood pressure, and heart rate compared to saline-treated mice. PbA infection decreased brain total and reduced (GSH) glutathione levels. Brain levels of oxidized (GSSG) glutathione and the GSH/GSSG ratio were positively correlated with temperature and motor behavior. Low and intermediate doses of GSNO failed to restore the depleted brain total glutathione and GSH levels, suggesting that ECM prevention by GSNO was probably related to other effects such as inhibition of inflammation and

vascular protection. These results indicate that ECM is associated with depletion of the brain glutathione pool and that GSNO is able to prevent ECM development in a wide range of doses, decreasing brain inflammation and inducing milder cardiovascular side effects. **E-mail:** graziela.zanini@ipec.fiocruz.br

Mal194- Screening of vegetal products from Brazilian flora for investigation of new antimalarial agents

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Malaria is a disease occurring all over the world, having protists of the genus *Plasmodium* as pathological agents. The pathogenesis of the disease is well understood for the infection with *P. falciparum* and can lead the patient to death. Between the various clinical symptoms, the most severe is the development of Cerebral Malaria, occurring mainly in children under five years. The most commonly used drugs in malaria therapy are chloroquine and mefloquine, both derived from quinine, a natural product obtained from barks of *Cinchona spp.* These were highly effective against different species of the parasite, including *P. falciparum*. However, the resistance to multiple drugs is known, making necessary the search for alternative drugs. The pharmaceutical industries have obtained success in the research of natural products as source of new drugs. Since Brazil has the higher biodiversity of plants in the world, the aim of this study was to evaluate the effect of fractions and purified products extracted from the Brazilian Amazonian flora with anti-malaria potential. To assess a probable anti-malaria effect, male Swiss Webster mice were infected with *P. berghei* ANKA (10^7 parasitized red blood cells/mice) and four hours after infection the oral treatment was started with the products in various doses (0.2-100 mg/kg). Chloroquine was the standard drug used (25 mg/kg). The oral treatment was repeated for the next three days at the same time and in the 5th day after infection the parasitaemia was assessed by blood smears colored with Panotoc method. This test is called the Four Days Test. Around 25 samples were received by codes to preserve the identity of the samples tested and the most promising antimalarial effects were observed for the fraction coded as FCL (0.2-25 mg/kg) reducing the parasitaemia of 28-68% ($p < 0.05$). The reduction was significant for all doses tested and was not different from the standard drug chloroquine ($p > 0.05$). Our results suggest that FCL presents a potential antimalarial effect. However, complementary tests have to be done, as in Cerebral Malaria and toxicity assessment, to purpose this sample for clinical trials. **E-mail:** monicafp@ioc.fiocruz.br

Mal195- Optimal Artemisinin Combination therapies in a *Plasmodium berghei* in vivo model

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Introduction: One of the reasons why it is difficult to eradicate malaria is the widespread drug resistance of the two main human parasites *Plasmodium falciparum* and *Plasmodium vivax*. Currently, the most successful drug is artemisinin, extracted from the Chinese wormwood *Artemisia annua* and its derivatives; it cures faster than any other drug and is extremely potent against CQ- or SP-resistant *P. falciparum* *in vitro* and *in vivo*. Nevertheless, artemisinin has a very short life and a high rate of recrudescence; consequently, it needs to be used in combination with a long lasting drug. Currently, there are five artemisinin combination therapies (ACTs) that are clinically used: artemether-lumefantrine (ATM-LMF), artesunate-mefloquine (AS-MFQ), artesunate-amodiaquine (AS-ADQ), dihydroartemisinin-piperaquine (DHA-PIP), and artesunate-pyronaridine (AS-PND). Of these, ATM-LMF, AS-MFQ and AS-ADQ are already first-line therapies in many endemic countries. Nevertheless, not much is known for the PK/PD

data of these combinations. **Materials and Methods:** In order to study PK/PD properties of the existing ACTs and their individual components we have used a modified Thompson test with a drug sensitive *P. berghei* NK65 strain mouse model. **Results:** We identified minimal curative drug concentrations that rapidly clear parasite infections, prevent parasite recrudescence, and are not antagonistic. The doses required to cure varied between the different ACTs. In particular, AS-PND and ATM-LM produced cures at the lowest doses of the partner drug in combination as compared to the other ACTs tested. **Main conclusions:** Our results demonstrate that the *P. berghei* mouse model is amenable for the study of pharmacokinetic-pharmacodynamic properties of ACT drugs. Future studies will compare the ability of the ACTs to cure drug resistant infections, including resistance to both artemisinin and partner drugs. **E-mail:** fsaenz213@puce.edu.ec

Mal196- Genomewide mutation detection in rodent malaria parasites resistant to Artemisinin Combination Treatment

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Introduction: Lacking alternative effective treatments, the possible evolution of resistance to Artemisinin Combination Treatments (ACTs) by malaria parasites, will severely undermine our ability to control this devastating disease. **Materials and Methods:** Here, we have used Illumina whole genome re-sequencing to identify the mutations in *Plasmodium chabaudi* AS-ATNMF1 parasites in which resistance to ACT (artesunate + mefloquine) was experimentally evolved. **Results:** Seven point mutations, a small deletion (3bp), one large duplication (~80kbp) and one CNV (80 kbp duplication) were detected in AS-ATNMF1 relative to the artemisinin and mefloquine sensitive progenitor clone AS-3CQ. The large 80kb duplication contained an estimated twenty-two genes, including *mdr1*, encoding a multidrug resistance transporter. One point mutation (V2697F ubp1) and one 3bp deletion (I102del, PCHAS_031370) have been previously implicated in artemisinin and chloroquine resistance respectively. **Main conclusions:** *Mdr1* duplication is proposed to confer mefloquine resistance and possibly increased artesunate resistance. The other mutations may variously contribute to these phenotypes but are more likely to be deleterious, neutral or compensatory events. **E-mail:** pedrovcravo@gmail.com

Mal197- Malaria prevention in HIV-infected pregnant women: PACOME trial testing cotrimoxazole prophylaxis versus intermittent preventive treatment with mefloquine

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Introduction: The consequences of malaria in pregnancy are increased by HIV infection. Cotrimoxazole (CTX) prophylaxis given to HIV-infected pregnant women (PW) for the prevention of opportunistic infections is assumed to be protective against malaria. Alternatively, an intermittent preventive treatment (IPTp) is recommended. The PACOME randomized controlled non inferiority trial aimed to evaluate the efficacy of CTX in all HIV-infected PW. The comparator was mefloquine (MQ) IPTp, already evaluated in HIV-negative PW. **Material and methods:** HIV-infected PW were enrolled in Benin between 2009 and 2011. Two sub-trials were designed: (1) PW with low CD4 cell count or advanced HIV disease, for whom CTX prophylaxis was mandatory for the prevention of opportunistic infections, were randomized to

receive either daily CTX, or daily CTX associated with MQ IPTp (15 mg/Kg MQ given three times); (2) PW for whom CTX was not mandatory were randomized to receive either daily CTX, or MQ IPTp alone. The primary endpoint was the prevalence of placental malaria, determined by microscopy (hypothesis: 2% in each treatment group). **Results:** A total of 240 women were enrolled in the “CTX mandatory” sub trial, 190 in the “CTX not mandatory” sub trial. Malaria prevalence was 5% at enrolment. Placental blood smears were available from 305 of the 346 PW who had given birth until March 2012: In the “CTX mandatory” sub trial, no placental smear was positive in the CTX+MQ IPTp treatment group (0/103) versus 1/107 (0.9%) in the CTX group. In the “CTX not mandatory” sub trial, there was no positive placental smear (0/45) in the MQ IPTp group versus 1/50 (2%) in the CTX group. Minor side effects following MQ intake were frequently reported (2/3 of PW), mostly dizziness, vomiting and nausea. **Main conclusions:** The low prevalence of placental malaria suggests good efficacy of all prevention strategies, though null prevalence was obtained only with MQ IPTp. The final results testing non inferiority between treatment groups will soon be available, when follow-up is completed (by May 2012). This trial is the first ever evaluating the efficacy of CTX, as well as MQ, in HIV-infected PW **E-mail:** lisedenoeud@yahoo.fr

Mal198- Pharmacotherapy follow-up in patients with malaria treated in a non-endemic area, Rio de Janeiro

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Introduction: Malaria is a potentially severe disease, widely distributed in the tropical and subtropical regions of the world. Despite drug resistance, other factors are directly related to infection's response to anti-malarial treatment, such as adherence to the treatment, drug interactions and adverse drug events. Monitoring these factors by pharmacotherapy follow-up is important to guarantee the effectiveness of treatment preventing drug related problems and ensuring patient safety. The aim of this study was to describe the effect of pharmacotherapy follow-up (PF) in patients with malaria seen at a referral centre located in the city of Rio de Janeiro, an area without active transmission of malaria. **Methods:** Descriptive study conducted from January 2005 to February 2012 at IPEC. Data collections were carried out before and after the implementation of PF. Data were collected from medical records and follow-up interviews. The variables included were age, gender, co-morbidities, anti-malarials and concomitant medications used, adverse drugs events and adherence to anti-malarial treatment. Groups from different periods were compared using chi-square test. **Results:** A hundred and fourteen patients were included. Seventeen of them were treated at least twice resulting in 138 malaria treatments. The majority was male (76.3%) with ages between 13 and 66 years. We observed 104 adverse events to anti-malarials considered as 79 adverse drug reactions, three medication errors and 22 therapeutic failures. The majority of patients (90.4%) had a high adherence behavior according to Morisky's questionnaire. Since 2009, when PF started, information related to adverse event increased in 46.0%, and to clinical and parasitological cures increased in 29.8% and 49.4%, respectively. Besides, loss to follow-up after malaria treatment reduced in 24.2% ($p<0.05$). **Main conclusions:** Before PF, we had no accurate information about patient safety. Furthermore, treatment effectiveness was not known because patients were not followed. We concluded that an improvement in malaria patient's care was achieved by surveillance activities, including pharmacotherapy follow-up. **E-mail:** lusiele.guaraldo@ipec.fiocruz.br

Mal199- Antimalarial activity of quinolines derivatives

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Introduction: Malaria, along with HIV / AIDS and tuberculosis, remains to date as of the greatest public health challenges worldwide, representing major negative impact on countries where it is endemic, contributing to a cycle of poverty and limiting economic development. Joint actions, such as combating the insect vector, rapid and accurate diagnosis, and appropriate therapy, development of new therapeutics, vaccines and optimization of the action of drugs used today are of fundamental importance in the fight against the disease. One interesting approach is the use of derivatives of quinolines with different functional groups. In this scenario, quinolines have been widely used to treat malaria, until the development of resistance. The precise mechanism of action of quinolines is still not fully understood, and the main seems to be related to the accumulation of these weak bases in acidic lysosome of the parasite and the connection to Fe(II)-protoporphyrin IX, preventing the polymerization of this substance, interrupting the mechanism of detoxification, by which it converts the Fe(II)-protoporphyrin IX in an inert polymer, insoluble, non-toxic called hemozoin. The consequent accumulation of Fe(II)-protoporphyrin IX leads the parasite to death. **Material and Methods:** To evaluate the antimalarial activity, two derivatives were tested in vivo in 10mg/Kg each in a murine model using the 4-day suppressive test. They are: 8-(prop-2-ynyloxy)quinoline **(1)** and 3-(quinolin-8-yloxy)butan-2-one **(2)**. Giemsa stained blood smears were made on days 5, 7, 9 and 12 after inoculation. The antimalarial activity was expressed as percentage of inhibition of parasite multiplication. **Results:** The compound **(1)** showed increased activity over the days examined, from 0 to 5 days, 7 on 7, 52 on 9 and 47 in 12 days post-infection. In contrast, the activity of the compound **(2)** remained around 40% in the period. **Conclusion:** Given its suppression values, these compounds are promising antimalarials and therefore may be objects of future investigations. **Supported by:** CNPq, FAPEMIG and UFJF. **E-mail:** bbtareis@hotmail.com

Mal200- Consequences of Gestational Malaria on Birth Weight: Finding the Best Timeframe for Intermittent Preventive Treatment Administration

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Introduction: To prevent the consequences of malaria in pregnancy, intermittent preventive treatment (IPTp) strategy has been adopted by most African countries since the early 2000's. It consists in the administration of 2 curative doses of sulfadoxine pyrimethamine (SP) at least one month apart from the second trimester of pregnancy. The currently implemented schedule appears to leave the beginning of pregnancy unprotected, and the coverage of late pregnancy largely depends on the time of administration of the 2 doses of SP. Therefore, WHO has stressed the need to find an optimal timing for IPTp administration. **Material and methods:** We pooled data from two studies conducted in South Benin, 20 km apart from each other between 2005 and 2010: a prospective cohort of 1037 pregnant women and a randomised trial comparing SP to mefloquine in 1601 women. To assess the consequences of IPTp timing on the outcomes of pregnancy, we performed a logistic or a linear regression depending on the outcome analysed (binary or continuous). Covariates were included in the initial models on the basis of the literature and on hypothesized underlying causal relationships (directed acyclic graphs (DAGs)). **Results:** A total of 1439 women (752 in the cohort and 687 in the SP arm of the randomised trial) who had given birth to live singletons were analysed. An early intake of the first SP dose (4 months of gestation) was associated with a lower risk of LBW compared to a late intake (6-7 months of gestation) (aOR=0.5 p=0.01). There was also a borderline increased risk of placental infection when the first SP dose was administered early in pregnancy (aOR=1.7 p=0.1). **Conclusion:** This study is the first to investigate the timing of SP administration during pregnancy. We clearly demonstrated that women who had an early intake of the first SP dose were less at risk of LBW (a reduction by half) compared to those who had a late intake. Pregnant women should be encouraged to attend antenatal visits early to get their first SP dose and a third dose of SP could be recommended to cover the whole duration of pregnancy, avoiding late infections of the placenta. In the context of increasing resistance to SP, research is focusing on the finding of new candidates to replace SP for IPTp. Our results show that, whatever the drugs used, it is of the utmost importance to ensure that future trials take into account the timing of their administration, and particularly to ensure an early intake and the full coverage of the pregnancy period. **E-mail:** bichtrambe@hotmail.com

Mal201- Nutrition and malaria treatment: the challenge of incorporating weight data when dispensing chemotherapy in South-western Amazonia

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Introductio: From 2010 the Brazilian Health Ministry included patient's weight variable in malaria treatment schemes; nevertheless, this improvement was not maintained when it launched the 2011 template for the .Investigation of Malaria Cases Record., favouring the perpetuation of age as the standard variable for malaria treatment dosage calculation. Scientific literature has been documenting that under-dosage is strongly related to malaria recidivating and recrudescence. Considering recent obesity epidemics being reported in developing countries, including Brazil, we considered justifiable to bring some light into this often forgotten relationship. **Material and Methods:** descriptive study with convenience sample of suspected malaria cases, formed by people who came spontaneously , from July 2011 to February 2012, to the main malaria diagnose facilities of three municipalities of the Brazilian State of Rondonia: Alto Paraíso, Buritis e Campo Novo. A weight scale was made available at each malaria diagnose facility; the weight of each suspected patient was recorded; and the treatment (whenever necessary) was prescribed considering the patient's weight for the dosage calculation, according to the weight's bands found at Table 1 of the Brazilian Health Ministry's Guide for Malaria Treatment. **Results:** During the eight months studied, there were 3,007 exams, and after the exclusion of 507, the remaining 2,500 were included in this study. They showed 136 (5.4%) underweight people, 1,653 (66.2%) normoweight people and 711 (28.4%) overweight people. The age-bands with more overweight people were, respectively 6-11 y.o. (14 cases; 35.7%) and 12-14 y.o. (90 cases; 35.5%). The evaluation of recidivating and/or recrudescence before and after the intervention of the dosage adjustment according to the patient's weight is still ongoing. **Main conclusions:** Despite the fact that this study was not aimed at evaluating obesity, the percentage of overweight found was consistent with the literature's obesity prevalence for the Brazilian population. Applying the 28.4% of found overweight people on the number of actual malaria cases reported by the above three municipalities during the same period, 510 treated patients would risk malaria recidivating and/or recrudescence if their treatment had not been prescribed considering the patient's weight for the dosage calculation. Therefore, it shall be mandatory the inclusion of the weight data in both the .Investigation of Malaria Cases Record and the calculation of malaria treatment dosage by the Brazilian local malaria control health personnel. Similarly, there is no excuse for the Brazilian Malaria Coordination team not to include patient's weight variable in the Epidemiological Surveillance Information System (SIVEP Malaria). **E-mail:** raquelrangelcesario@gmail.com

Vaccines

Mal202- Evaluation in animal models of a malaria vaccine using the Yellow Fever virus 17D as an expression vector

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The development of a vaccine for malaria is currently considered a priority in public health due to the socioeconomic impact and morbidity of the disease with around 250 million cases registered every year. The yellow fever vaccine is considered one of the most successful vaccines for its long-lasting immunogenicity obtained after a single dose. As a result of the elucidation of the polyvalent responses

directed to YF17D vaccine virus, the use of this virus as an expression vector of heterologous antigens has been encouraged. Considering that yellow fever and malaria share the major endemic areas in American and African continents, the construction of a vaccine for malaria based on the yellow fever 17D vector became an interesting approach. Two recombinant viruses containing the heterologous protein MSP-1₁₉ from *P. falciparum* (YF17D/MSP-1₁₉fal) and *P. vivax* (YF17D/MSP-1₁₉vivax) inserted between the E/NS1 genes have been constructed. This protein consists of a 19 kDa fragment obtained after proteolytic processing of the merozoite surface protein 1 (MSP-1) during invasion of erythrocytes and is described as a target for protective antibodies in animals and immune people. Recombinant viruses were characterized *in vitro* for their proliferative capacity in Vero cells and genetic stability and expression of heterologous protein were assessed by confocal immunofluorescence and Western blotting. Immunization of BALB/c mice and the non-human primate *Saimiri sciureus* allowed the evaluation of the constructions in terms of immunogenicity. The recombinant viruses were able to proliferate *in vitro* reaching titers liable of scaling. The recombinant protein was detected in the perinuclear region, possibly associated with the endoplasmic reticulum. Both viruses were capable of inducing neutralizing antibodies to YF, but in lower titers than those induced by the vaccine virus 17DD. The induction of specific antibodies for the heterologous protein by the different recombinant viruses was also demonstrated by low levels of IgG in both models. The antibodies induced in the monkey model bound to the native protein in parasite-infected red blood cells detected by immunofluorescence. The challenge carried out in after immunization of *Saimiri* monkeys with FA17D/MSP-1₁₉fal did not generate conclusive results. *Saimiri* monkeys proved to be a good model for evaluating malaria vaccine candidates based on the yellow fever vector, with viremia and immunogenicity similar to rhesus, the model classically described. These initial data suggest the need to improve the platform of expression towards higher viral immunogenicity. **E-mail:** mbonaldo@ioc.fiocruz.br

Mal203- Mapping Epitopes on *P. vivax* Invasion-Related Antigens

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Introduction: Malaria control is still based on prophylaxis and treatment with antimalarial drugs. No vaccine is available and not much is known about host-parasite interactions and immune escape mechanisms in malaria infections, especially in those caused by *Plasmodium vivax*. This study aims to immunologically characterize a set of surface proteins of *Plasmodium vivax* merozoite by Spot-Synthesis technique. **Material and Methods:** Linear B cell epitopes were mapped on three of major proteins involved in invasion: Duffy Binding Protein, Merozoite Surface Antigen 1 and MAEBL Antigen. Sequences were obtained from UniprotKB database. A library of 929 overlapping peptides was synthesized directly on cellulose membrane covering the entire proteins sequences. Membranes were tested for antibody recognition from serum samples of Malaria-exposed individuals from Amazon. **Results:** A total of 34 linear epitopes of various lengths (8-14 residues) were identified. Different recognition patterns were observed like the absence of epitopes at hydrophobic pockets, strong recognition at repetitive low complexity regions and in the case of MSP-1 antigen, a strong recognition at N-terminal region contrasting with a poor recognition at C-terminal MSP-1₄₂ and MSP-1₁₉ fragments. **Conclusions:** The knowledge generated in this study involves innovative information about naturally acquired immunity and has potential applications in differential diagnostic approaches as well in development of subunit vaccines against Malaria. **E-mail:** brunac@ioc.fiocruz.br

Mal204- Immunogenicity of vaccine formulations containing a combination of recombinant *Plasmodium vivax* antigens AMA-1, MSP119 and MSP3β

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Introduction and Objectives: *Plasmodium vivax* is the most world-widely distributed and the most prevalent specie in America. Vaccine development to *P. vivax* is considered a priority on the global program for the eradication of malaria. In contrast to *P. falciparum* malaria, currently, there is no successful human vaccine trial against *P. vivax* infection. We consider that an effective vaccine formulation should be composed by immunodominant regions of two or more antigens of the parasite. Based on that, we studied the immune responses induced by experimental mouse immunizations with a combination of three important merozoite antigens: Apical Membrane Antigen 1 (AMA-1), 19 kDa C-terminal regions of Merozoite Surface Protein 1 (MSP1₁₉) and Merozoite Surface Protein 3 β (MSP3 β). **Material and Methods:** The recombinant proteins were expressed and purified by affinity and ion exchange chromatography. Subsequently, RP-HPLC analyses were performed to confirm the high purity of these samples. The immunogenicity of these formulations was evaluated in BALB/c and C57BL/6 mice, emulsified in two different adjuvants, Quil A or Poly(I:C). Mice were immunized with the antigens administered together or individually. The humoral immune response against recombinant proteins representing each one of them was measured by IgG antibody titers using ELISA. **Results:** C57BL/6 mice immunized with the recombinant antigen combination in the presence of Quil A or Poly(I:C) adjuvants showed high antibody titers ($\log_{10}>4$) similar to mice inject with a single antigen only. In contrast, BALB/c mice immunized with the recombinant antigen combination had lower antibody titers to MSP1₁₉ or to MSP1₁₉ and AMA-1 when administered in the presence of Quil A or Poly(I:C) adjuvants, respectively. Interestingly, we observed a significant increased in the antibody titers of the C57BL/6 mice receiving the combination of antigens against MSP3 β protein in Poly(I:C). The ratio between the IgG subclasses profile showed a mixed Th1/Th2 response, in all groups tested. Specific antibodies were still high six months after the last immunizing dose indicating a long lived immune response. **Conclusion:** Overall, the combination of these three antigens is an effective strategy for the development of a vaccine formulation when administered with Quil A or Poly(I:C). However, certain heterogeneity of the different host genotypes might be expected when the antigens are provided in combination. Whether this heterogeneity is biologically relevant is currently being investigated. **E-mail:** mayneop@usp.br

Mal205- Looking for potential vaccine candidates in *Plasmodium vivax* using bioinformatics tools

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Malaria is the major parasitic disease in the world, causing nearly one million deaths, mostly in African children. Among the species that cause human disease, *Plasmodium vivax* has the widest distribution with 40% of world population living at risk of infection. In Brazil, 85% of around 300,000 cases annually reported are caused by this species. An effective vaccine against malaria is a priority and many efforts have been made to identify promising candidates. Despite some *P. falciparum* antigens are in phase III of clinical trials in Africa, only few antigens of *P. vivax* has been tested but none in a clinical trial yet. To be a good candidate for a vaccine, a protein must be accessible to the host's immune system and it should be able to induce a lasting immunity. Therefore, the protein must be exported/secreted, integral of membrane in extracellular stages of the parasite or anchored in the membrane of the host cell. Thus, the aim of this study was search for proteins that had these characteristics at the predicted proteome of *P. vivax* and *P. falciparum*. For this, we used different programs to predict: signal peptide (SignalP); transmembrane domains (TMHMM); a sequence motif typical of exported proteins across the parasitophorous membrane, named PEXEL (in house perl script); secreted proteins by nonclassical pathways (secretomeP), and exported proteins (ExportPred). For the 5435 annotated proteins of *P. vivax*, 822 (15%) were predicted to have signal peptide and 1057 out of 5252 (20%) proteins of *P. falciparum* have this feature. The PEXEL motif in the first 80 aa of the sequence was found in 15% proteins of *P. vivax*, and in 20% of *P. falciparum*. However, only 3% of *P. vivax* proteins and 6% of *P. falciparum* proteins showed both characteristics (signal peptide and PEXEL). The ExportPred algorithm, which is also based on SignalP and PEXEL search, predicted that *P. vivax* has <1% and *P. falciparum* has 4% of proteins exported. The differences might be because of parameter settings. We found 24% of *P. vivax* proteins and 28% of *P. falciparum* proteins with at least one transmembrane domain. Combining the predictions, *P. vivax* has 4% proteins with signal peptide and transmembrane domain, whereas *P. falciparum* has 8% proteins. The proteins

with signal peptide, PEXEL and transmembrane domain are 35 (<1%) for *P. vivax* and 197 (4%) for *P. falciparum*. Only few proteins were predicted to be exported /secreted by non-classical pathways 1% of *P. vivax* and 0,5% of *P. falciparum* proteins. Among these proteins, it was possible to find some protein families that are known to be exported/secrete, such as PfEMP1, Stevor and Rifin, corroborating our approach. The differences found in the number of proteins present in each species, may reflect the particularities in the parasite biology. *P. falciparum* for example has several routes of invasion described, while *P. vivax* has only one major. The high susceptibility of *P. falciparum* to be sequestered in the capillaries could also suggests a larger number of proteins anchored in the parasite, since some of these proteins bind to adhesion molecules in the endothelium. We are selecting the programs to build a pipeline which will predict automatically the distinct forms of putative vaccine candidates. **Supported by:** Fiocruz, CNPq, Fapemig. **E-mail:** ricardosouza@cpqrr.fiocruz.br

Mal206- Erythrocyte binding antigen 175 (EBA-175) of *Plasmodium falciparum*: genotypic determination isolation of individuals naturally exposed in Brazilian endemic area

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Introduction: *P. falciparum* Erythrocyte Binding Antigen 175 (EBA-175) plays a central role in erythrocytes invasion being considered, therefore, a target for malaria vaccine development. This antigen present to well characterized regions: *the region II* - conserved and immunogenic, that contains two cysteine-rich segments (F1 and F2), which are involved in binding to glycophorin-A on the surface of erythrocytes and, *the region III* – that contains mutually exclusive C (strain CAMP) and F (strain FCR3) fragments, which defines the two allelic families of EBA-175. Several studies performed in high endemicity malaria area in Africa have shown the influence of this dimorphism on clinical disease and outcome. The differences observed between different endemic areas in relation to exposed individuals and the circulating parasites are important factors in terms of vaccine strategies since the efficacy of a potential vaccine may vary in different epidemiological scenarios. **Objective:** The goal of this study was to evaluate the genetic diversity of regions II and III of EBA- 175 in *P. falciparum* isolates from Porto Velho (RO), a region of malaria unstable transmission, and the influence of this diversity on malaria morbidity and exposure variables. **Material and Methods:** The blood samples were collected in three time points between 1994, 2002 and 2007 (PV94 group, *n*=101; PV02 group, *n*=57; PV07 group, *n*=30, respectively). The genetic polymorphism was analyzed by PCR and sequencing. **Results:** We observed in the region II only one type of fragment with 926 bp. In the region III we observed the classic dimorphism with a higher frequency of the Cfragment (84.3%). The mixed infection was observed in 1.6% of isolates. There were no differences in the frequency of fragments C and F among the 3 groups. Sequencing of region II revealed 5 nucleotide changes in 3 of 15 isolates, leading to 2 amino acids replacements. Sequencing of region III revealed that: in the C-fragment there were 8 nucleotide changes in 3 of 45 samples, leading to 7 amino acids replacements; in the fragment-F there were 2 nucleotide changes, in 2 of 11 samples, leading to 2 amino acids replacements. **Conclusion:** Our results showed: reduced genetic diversity in *P. falciparum* EBA-175 isolates circulating in Porto Velho; predominance of C-fragment and temporal stability of allelic dimorphism of the EBA-175. Moreover, no association was observed between the EBA-175 dimorphism and malaria morbidity and exposure variables. **E-mail:** banic@ioc.fiocruz.br

Mal207- Improved immunogenicity of nanoparticle-coated PyMSP-1 C-terminus DNA vaccine using different routes of administration

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Background: Several approaches have been developed and targeted towards developing an effective malaria vaccine over the years. DNA vaccination, which has the advantage of eliciting both humoral and cellular immune responses, and flexible is one of the many approaches. Efforts to identify methods of enhancing immune response of plasmid DNA vaccination have been tried, and among them is the development of delivery systems; which include delivery route and nanoparticle formulation. To enhance the immunogenicity of MSP-1; two approaches (use of NP formulation and different routes of administration) were used to evaluate immunogenicity of C-terminus fragment of MSP-1. **Materials and methods:** Plasmid encoding Plasmodium yoelii MSP-1 C-terminal was constructed and formulated with newly designed nanoparticle—a ternary complex of polyethylenimine and α -polyglutamic acid (pVR1020-MSP-1/PEI/ α -PGA) and pVR1020-MSP-1 designated as coated and naked, respectively. Groups of C57BL/6 mice were immunized either with 100 mg of NP-coated MSP-1, naked or NP-coated blank plasmids, by three different routes of administration; intravenous (i.v.), intraperitoneal (i.p.) and subcutaneous (s.c). Mice were primed and boosted twice at 3-week intervals, then challenged 2 weeks after. Sera were collected for antibody response and cytokines analysis. In addition, spleens were removed and splenocytes prepared for FACS and cytokines analyses. **Results:** Measurement of IgG and its subclass antibody titer by ELISA showed higher titer in coated group than the naked group. Flow cytometric analysis of splenic cells after immunization with coated DNA showed an increased proportion of both CD4+ and CD8+ subpopulation of T cells. Cytokines levels in the culture supernatant of merozoite antigen-stimulated splenocytes and sera were observed to be significantly higher in the coated as compared to naked or control group. High levels of Th1 and Th2 types of cytokines were observed in vaccinated mice by i.p. followed by i.v. than s.c. vaccinated mice. INF- γ ELISPOT producing cell number of splenocytes, indicated some stimulatory effect of this novel nanoparticle on coating MSP1 DNA vaccine and might have enhanced the protective immunity against blood stage malaria. **Conclusion:** In all the three different routes of administration, nanoparticle coating substantially enhanced IgG response, CD4+ and CD8+ T cell populations, cytokine induction and protection. Better protection by route of administration was observed to be in the following order i.p. > i.v. > s.c. **E-mail:** nshuaibu@yahoo.com; nasir@nagasaki-u.ac.jp

Mal208- Differential activation of dendritic cells by nanoparticle-coated PyMSP-1 DNA vaccine using different route of delivery

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Background: In malaria DNA vaccine development, there exists a critical need for additional delivery vehicles which may facilitate targeting and/or controlled release of antigen to antigen presenting cells such as dendritic cells. We have previously shown the immuno-stimulatory and protective effect of nanoparticle (NP)-coated Plasmodium yoelii merozoite surface protein 1 (PyMSP-1) plasmid with high level of IL-12 production. It has also been reported that γ -PGA NPs were preferentially internalized by DCs and induced the production of IL-12. Here we attempted to investigate the in vivo stimulatory effect

of NP-coated plasmid on dendritic cells by analyzing the expression of antigen presenting molecule MHC class II, co-stimulatory molecules and cytokines production in group of mice immunized with NP-coated and naked MSP-1 plasmids. **Methods:** Groups of six week old female C57BL/6 mice were immunized either intraperitoneally (i.p.) or subcutaneously (s.c.), with 100 µg/mouse of either NP-coated-plasmid DNA (pVR1020-MSP-1/PEI/γ-PGA) or naked plasmid DNA (pVR1020-MSP-1). Mice were prime-immunized at day 0 and two subsequent boosters at three weeks intervals. Two weeks after the last boost, IgG and its subtype antibody responses were assessed by ELISA from the individual sera. The mice were then sacrificed, and freshly isolated lymph node and splenic cells were stained to analyze the proportion of T cells and various activated DC markers by flow cytometry. Cytokine (IL-12 and IFN-γ) levels were measured in the supernatants of antigen stimulated lymph node and spleen cells and sera from immunized mice. **Results:** We observed a significant increased proportion of activated DCs and expression of CD40 in the group of mice immunized with NP-coated MSP-1 as compared to naked plasmid. CD80 and CD86 co-stimulatory molecules were significantly increased in the coated group immunized by s.c. and i.p., respectively. Higher levels of IL-12 and INF-γ production were induced in splenocyte and lymph node cells cultured supernatants from NP-coated MSP-1 vaccinated mice across the two routes of administration. It is apparent here that DC activation, CD40 expression and IL-12 production following rMSP-1 stimulation, were significantly induced in NP-coated group across the two route of delivery. **Conclusion:** These data indicate that nanoparticle-coated PyMSP-1 DNA vaccine activated DC and also, significantly influenced CD40 molecule expression on the activated DC. **E-mail:** msamafr@yahoo.fr / nshuaibu@yahoo.com

Vectors

Mal209- Effects of temperature on the survival and development of *Anopheles darlingi* (Diptera: Culicidae)

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Introduction: *Anopheles darlingi* is the main malaria vector in the north of Brazil, and comprises up to 95% of the anophelines captured in periurban regions of Porto Velho, RO. Despite of that, basic, yet fundamental, aspects of *A. darlingi* biology, such as lower, optimum and upper temperature limits for development and survival are not known. Due to the importance of temperature on mosquito biology and thus on malaria epidemiology, we investigated the effects of three constant temperatures, i.e., 20, 30 and 35°C, on the survival and development of immature and adults of *A. darlingi*. **Material and Methods:** Mosquito eggs were obtained from field mosquitoes captured using modified BG Sentinel traps in periurban areas of Porto Velho, RO, and transferred individually to ice-cube trays. Hatched larvae were fed with grounded fish food and adults fed with 20% sucrose. Insects were kept inside an insect rearing chamber on constant temperatures, 12 h photo phase and 75% RU and inspected daily for data records. **Results:** Eclosion rates did not differ significantly under the temperatures tested and ranged from 0.6 to 0.7. Despite of that, time to eclosion decreased 50% under 35°C. Larval development time also decreased in higher temperatures, ranging from 44 days at 20°C to 15 days at 35°C. Pupae metamorphosis to adult took more than 6 days at 20°C. In general, larval and adult survival were very low in all temperatures evaluated, i.e., 1,2 to 10% for larvae and 1 to 4% for adult. Interestingly, up to 80% of adult survivors on the upper and lower temperatures tested were males, but the female: male ratio remained 1:1 at 30°C. Adult longevity ranged from 20 days at 20°C to 4 days at 35°C for females. **Main conclusions:** Present data indicate that the temperatures tested significantly affected important biological parameters related to the vector capacity of *A. darlingi*. Moreover, the lower and upper temperatures tested were close to thermal limits of this species and severely affected female production. **E-mail:** eferreirademelo@hotmail.com; alealsil_bio@yahoo.com.br

Mal210- Is insecticide vector resistance a slowing up factor for malaria elimination? Impact of LLINs on malaria transmission in relation to the presence of insecticide resistance in *Anopheles gambiae*

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Background: The main trouble with ITNs implementation today in Africa is the appearance of pyrethroid-resistance in *Anopheles* (Chandre et al., 2002). In addition, N'Guessan *et al.* (2007) have demonstrated a decrease of the efficacy of lambda-cyhalothrin-treated nets on *Anopheles* in areas of strong resistance in southern Benin. If these results seem worrying, they need to be placed in a context as was done in the discussion of the published paper. In fact, the study was done at the experimental hut level and it is difficult to extrapolate from these results what will happen at community level. This study was designed to assess the impact of mass campaign of ITNs on the dynamics of resistant *Anopheles* vector populations and malaria transmission. **Methods:** The study was conducted in eight clusters selected in the southern Benin. Four clusters were characterized by a high vector resistance (mortality rate of *An. gambiae* after WHO susceptibility test <60% and Kdr frequency >80%) and four others of low resistance (mortality rate of *An. gambiae* >80% and Kdr frequency <70%). In each cluster, 2 mosquito sampling points were randomly selected and 2 houses chosen per sampling point for mosquito collections to monitor malaria transmission. Adult mosquitoes were collected twice a month. Vector species were dissected to determine the age grading and the heads/thoraxes parts analysed by ELISA method to look for CSP antigens. Abdomens of females were used for PCR analyses to identify mosquito species and molecular forms of both *An. gambiae*. Sampling of mosquitoes using morning pyrethrum spray catches (PSC) and window exit trap was done to determine eventual changes in mosquito behaviour. **Results:** The human biting rate is not lower in the low resistance clusters compared to high resistance clusters. The influence exerted by the MILDs on the 2 categories of mosquitoes (low and high resistant *An. gambiae*) doesn't differ. By other hand, 555 thoraxes of *An. gambiae* from the low resistance area collected using human landing catch, pyrethrum spray catch and exit window traps were analyzed using ELISA/CSP techniques; 55 of these thoraxes were found positive for circumsporozoite antigen: CS+ = 11.11%. In the high resistance area, CS+ = 10.74% (39/363). The difference is not significant ($p > 0.05$). Using only mosquitoes collected by human landing catch, the 2 sporozoite indices were the same, respectively 11.37% (39/343) and 11.63% (31/266). The parity rate (physiological age grading) is also the same, 84.22% (747/887) and 80% (220/275). Contrary to what was expected, the exophily rate is higher in the low resistance area (93.3%) compared to high resistance area (73.65%). **Conclusion:** These results indicate the low and high resistance of *An. gambiae* to pyrethroids have the same impact on the effectiveness of LLINs. The susceptible *versus* resistant mosquitoes will be the best material to compare. **E-mail:** akogbetom@yahoo.fr

Mal211- Stimulation of *Anopheles gambiae* immune response to *Plasmodium berghei* infection

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Introduction: Malaria parasites must go through complex developmental transitions within the mosquito vector before transmission to humans occurs. The knowledge gain so far on mosquito immune response clearly underlies its importance to control parasite transmission and the role of basal immunity as a key player to limit the infection. In the present study, we have investigated the effect of immunostimulatory molecules, known to activate mammals' innate immune system, on the outcome of *Plasmodium berghei* infection of the mosquito *Anopheles gambiae*. **Material and Methods:** In order to evaluate the effect of immunostimulatory molecules, they were injected 24h prior infection into female mosquitoes at different concentrations. The following molecules were screened: M-TriDAP, Pam2CSK4, zymosan, CL097, Rec-Fla-ST and sodium alginate. Treated mosquitoes and their controls were directly fed on *P. berghei* infected mice and the outcome of mosquito infection was monitored on the 10th day

post-infection, when infection rate and infection intensity were determined. The molecules that showed a significant affect on the infection were further analyzed by functional genomic assay. **Results:** Treatment with different concentrations of zymosan, flagellin and sodium alginate showed a reduction in infection, while treatment with Pam2CSK4, M-TriDAP and CL097 did have no effect on the outcome of the infection. A microarray analysis, currently running, shows that transcription changes are primarily associated with immune related genes. **Main Conclusions:** Mosquito immunity can be stimulated leading to significant reductions on the outcome of mosquito Plasmodium infection. **E-mail:** hsilveira@ihmt.unl.pt

Mal212- Bacterial toxicity of *Bacillus sphaericus* 2362 Brazilian larvicide regarding the aquatic (non-target) entomofauna in laboratory conditions

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Introduction: Malaria vector *Anopheles* Meigen, 1818, develops its immature forms in aquatic environments, sharing them with other beneficial insects, called non target fauna. Some aquatic insects exert regulatory pressure on the vectors by feeding on them. Moreover, the non target insects play a major role in nutrient- cycling and, some of them are used as bio-indicators of environmental impact. World Health Organization (WHO) considers the non target entomofauna safety condition as important data for underscoring the application of any insecticide, since it will reflect on the existing ecological balance. Several fish, amphibians, aquatic birds and other animals feed mainly on immature aquatic insects, which are essential to their survival. By taking the non-target insects safety into account, this study aims at to evaluate the effect of Spaherus SC on two different families of aquatic insects in the Amazon region. **Methods:** Aquatic insects were collected with aquatic entomological net at an urban anthropically altered creek for family Chironomidae and an urban preserved creek for family Euthyplociidae. Laboratory tests on families Chironomidae and Euthyplociidae have been carried out in 20x20 aquaria containing 1L of water and 20 µL of the Sphaerus Sc larvicide made up of 2362 *Bacillus sphaericus* and strain S242. Mortality was observed every 24h, 48, 72 and 96 hours, accepting mortality data up to 30% for the control. **Results:** Forty-six immatures of Euthyplociidae, and 700 Chironomidae were collected and subjected to 40 tests with larvicida and 16 controls. The mortality observed on Euthyplociidae immatures showed to be 100% following 24h and for Chironomidae immature the highest percentage was 12% in 24h. **Conclusion:** The Euthyplociidae family showed to be highly susceptible to Sphaerus SC formulation, yet further studies will be required so as to ascertain and confirm this finding. **Financial Support:** CNPq/FAPEAM Rede Malária, CTPETRO. **E-mail:** nataliellimaia@gmail.com

Mal213- Anopheline Abundance, Biting Behavior and Entomological Inoculation Rates in Two Malaria Endemic Regions of Colombia

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Introduction: The Uraba-bajo Cauca and Alto Sinu (UCS) and Pacific (PAC) regions have historically had the highest reports of malaria cases in Colombia. The characterization of entomological parameters allows for a better understanding of malaria transmission dynamics. From collections at six localities of UCS and PAC, we evaluated entomological parameters, including anopheline abundance, biting activity and human biting rate (HBR) as well as transmission parameters, including infection rate (IR) and entomological inoculation rate (EIR). **Materials and Methods:** Anophelines were collected every three months during four visits to each locality, November 2009-June 2010. Sites in UCS included El Loro - LOR, Juan Jose-JUJ and La Capilla-CAP, whereas sites in PAC included San Antonio de Padua-SAP, Zacarias-ZAC and Pindales-PIN. Collections were performed using human landing catches for six days from 18:00-24:00 h, indoors and outdoors. **Results:** 9,344 anophelines of 10 species were collected. *Anopheles nuneztovari* s.l. (45.4%) and *Anopheles darlingi* (42%) were the most abundant, followed by

Anopheles calderoni (6%), *Anopheles pseudopunctipennis* (3%) and *Anopheles albimanus* (2%). Other species including *Anopheles triannulatus* s.l., at $\leq 1\%$. *Anopheles nuneztovari* s.l. and *An. darlingi* exhibited biting activity throughout the night with the highest peak between 21:00-23:00 and 20:00-23:00 h, respectively. *Anopheles nuneztovari* s.l. exhibited endophagy in LOR, CAP and ZAC. In JUJ-UCS, *An. nuneztovari* s.l. and in SAP-PAC, *An. darlingi* showed the highest HBRs. *An. nuneztovari* s.l. was infected with *Plasmodium vivax* VK247 in UCS and with VK247 and VK 210 in PAC. *An. darlingi* was infected with *Plasmodium falciparum* in PAC and *P. vivax* VK 210 in UCS. IRs for these species was $< 0.1\%$. *An. triannulatus* s.l. was infected with *P. vivax* VK 247 in UCS (IR: 1.2%). The cumulative EIRs revealed the highest malaria transmission intensity in CAP followed by ZAC, SAP and JUJ. **Conclusions:** The major Colombian vectors *Anopheles nuneztovari* s.l. and *An. darlingi* are also primary vectors in our study sites in UCS and PAC. Nevertheless, it is important to continue to evaluate species that are of local importance in malaria transmission in other Latin American countries, such as *An. triannulatus* s.l. and *An. calderoni*, to help determine the relative importance of these species as vectors in Colombia. **E-mail:** mcorrea@quimbaya.udea.edu.co

Mal214- Anopheline dynamics involved in malaria transmission in the District of Coração, State of Amapá, Brazil

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For better understanding the dynamics of malaria transmission in an area is necessary to know the behavioral patterns of *Anopheles* species, such as hourly biting activity, and exophilic, endophilic, anthropophilic and zoophilic parameters, and attraction index for mosquitoes per man/hour. In this study, the aim was to analyze the entomological factors involved in the dynamics of malaria transmission in the District of Coração, State of Amapá, Brazil. The collections of specimens were carried out from December 2010 to November 2011, covering the whole the District of Coração. Samples were collected monthly, with three days of uninterrupted catch with protected human bait. Also, there were four collections of twelve hours and four collections in two distinct environments: anthropophilic and zoophilic. After captures, the mosquitoes were placed in plastic cups properly labeled containing the site name, date, time and mode of capture. A total of nine species were sampled (1.689 mosquitoes), which *Anopheles darlingi*, *Anopheles braziliensis* and *Anopheles marajoara* were the most frequent species. The three species showed a multimodal behavior of biting activity with the highest levels of exophilic and anthropophilic. The index of attraction for mosquitoes per man/hour estimated at collections of four hours revealed a pattern of activity later for *A. darlingi* compared to two others species, whereas in the collections of twelve hours, the results revealed that *A. darlingi* was the species more active throughout the night, with a complex multimodal pattern. Overall, *A. darlingi* was the most anthropophilic species, followed by *A. marajoara*, whereas *Anopheles nuneztovari* s.l. was the most zoophilic. There was not significant correlation between the index of attraction per man/hour and climatic variables, such as air temperature, relative humidity and rainfall. The lack of correlation may be due to the low densities and variability in the abundance of specimens collected in the area. Since the three the most frequent species obtained in this study, the District of Coração can be considered an area highly vulnerable to the development of outbreaks of malaria; therefore, we suggested that vector control measures will be need urgently in this District in order to prevent outbreaks and/or malaria epidemics. **Key words:** malaria vectors, behavioral patterns, Amazonian Brazil. **Supported by** CNPq, CAPES, UNIFAP and MCTI/INPA. **E-mail:** barbosalmc@unifap.br

Mal215- Diversity of the genus Anopheles (Culicidae) in the rural area of Cuiabá, Mato Grosso / Brazil.

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Introduction: Affecting about 500 million people each year and killing more than 3 million, malaria is one of the diseases that make more victims in the world. This is caused by protozoa of the genus *Plasmodium* which is transmitted by something about 40 species of mosquitoes of the *Anopheles* genus. In Brazil, one of the main transmitters of malaria is *Anopheles darlingi*. This study aimed to observe the fauna and the diversity of *Anophelinae* mosquitos in the rural area of Cuiabá, capital of Mato Grosso / Brazil. **Materials and Methods:** Samples were collected at the location of Rio dos Peixes, 30 km distance from Cuiabá-MT, Brazil. We used three different methods of capture: Shannon trap, a device called the Mosquito Magnet Defender and protected human bait. The insects were stored in appropriate recipients according to the methodology previously described. Samples were collected monthly from May to November of 2011 two consecutively days from (5:00 PM to 21 PM) rates of diversity and dominance were obtained using Past (v2.14) software. **Results and discussion:** A total of 896 individuals were captured and distributed in: 882 *An. darlingi* (98%), 1 *An. argyritarsis* (0.11%), 6 *An. triannulatus* (0.67%), 2 *An. minor* (0.22%) and 5 *An. sp* (0.55%). The first one is the main vector and the second and third are secondary vectors of malaria. The data show a very low diversity, 0.167 on the index Menhinick (95% CI 0.1002 to 0.1670) and dominance index Berger-Parker at *An. darlingi* was 0.9844 (95% CI 0.9754 to 0.9922 .) The research site is often visited by people for leisure purposes, so the values of dominance and abundance for *An. darlingi* probably occur due to the anthropophilic habits of this species, which does not occur with others who are generally more zoophilic. **Conclusion:** The low species diversity and high abundance and dominance rates for *An. darlingi* showed the adaptability of this transmitter to the human modified environment. Therefore, in areas used for leisure proposes can become an important epidemiological site, because of the presence of vectors that can transmit malaria, although the community of Rio dos Peixes does not have any case registered in the past years. But an entomological monitoring of these insects is recommended for the purpose of surveillance. **E-mail:** sydneyvianna2@hotmail.com

Mal216- Ceara Anophelines (Diptera Culicidae), Brazil

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Introduction: According to World Organization of Health Malaria is recognized as serious problem of public health in the world reaching about 40% of population belonging to more 100 countries, it is a sharp infectious disease of feverish character, caused by protozoa of the gender *Plasmodium* and transmitted by vectorial insects of the gender *Anopheles*. In Brazil, it is endemic in the Amazon area responsible for about 99,8% of the cases. The other cases happen in the Amazon extra area whose registrations are sporadic and isolated. About 380 anophelines species are described; however, 60 are only capable to transmit the disease. For the country of Brazil five species have larger involvement in human transmission of malaria: *Anopheles darlingi*, *Anopheles aquasalis*, *Anopheles albitarsis*, *Anopheles (Kerteszia) cruzii*, *Anopheles (Kerteszia) bellator*. Ceará was already considered as endemic area for malaria in 1930's and 1940's. Ceará territory eliminated the transmission of malaria in 1940, since then happened some few isolated autochthonous cases and occasional ones. The other cases happen in the Amazon extra area whose registrations are sporadic and isolated. **Objective:** The study had an objective to consolidate and to update the information about *Anopheles* species in order to complete the entomological letter in Ceará State. **Materials and Methods:** It was made entomological inquiry in 137 municipal districts in the period from 2004 to 2008. Each municipal district was divided in nine quadrants in which five were chosen to be worked (extreme quadrants) as well as the central one. In these quadrants were chosen a place that contained own nurseries for anophelines. During 4 consecutive nights were made collections to get adults specimens in shelters of animals and immature ones during day. All collected material was properly registered in specific form and transported for identification. The analysis of the data was done in Microsoft Office Excel 2007 program. **Results:** 137 municipal districts were investigated (74,5%) of the State. Ten identified species and their frequencies for municipal district were: *A. albitarsis* (72,8%); *A. aquasalis* (16,8%); *A. argyritarsis* (31%); *A. Brasiliensis* (12%); *A. darlingi* (18,5%); *A. evansae* (38,6%); *A. noroestensis* (23,9%); *A. nunestovari* (0,5%); *A. oswaldoi* (19%); *A. triannulatus* (38,6%). **Conclusion:** Considering the researched municipal districts, it was identified the presence of important vectors as *Anopheles darlingi* and *Anopheles albitarsis* and there is infected people's circulation coming from

endemic areas, it can be considered that the same ones are receptive and vulnerable areas to the malaria. However the knowledge of anophelines entomological fauna from Ceará and its distribution constitutes a strategy for prevention and surveillance, mainly in the municipal districts that registered presence of vectors considered competent. **E-mail:** insect.berg@gmail.com

CHAGAS DISEASE

Epidemiology and Control

Chagas001- **A ten-year prospective study of Chagas disease in the State of Ceará, northeastern Brazil**

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Introduction: Chagas disease (CD) is endemic in northeastern Brazil due to an eco-epidemiological scenario in which the native insect-vectors and man coexist in mud huts in rural areas. In this region, insecticide control of triatomines has been challenged by the constant re-invasion of houses by sylvatic vector species. **Objective:** in order to compare the prevalence rates of CD, the same four rural localities were investigated in 2001 and ten years later, in 2011, evaluating seroconversion rates during this ten year period. **Materials and Methods:** In the four localities of the rural zone of Jaguaruana municipality, Ceará state, northeastern Brazil, the two cross sectional seroprevalence surveys were carried out examining 385 people, both adults and children, in 2001 and 341 in 2011, all of which consented to participate. A cohort of 190 subjects who were determined CD seronegative in 2001 were re-investigated in 2011 with the aim to estimate the seroconversion rate and active disease transmission in the communities. Additional subjects, i.e. newborns and migrants were also tested. As in 2001, two finger prick blood samples were collected from each individual, on filter paper for an initial analysis to evaluate the CD seroprevalence. In order to detect anti- *T. cruzi* IgG, an indirect immunofluorescence assay (IIFA) (Biomanguinhos®, Rio de Janeiro, Brazil) was performed. Additional intravenous blood samples were collected in glass tubes from positive or inconclusive individuals in accordance with the IIFA, for confirmatory serology (IIFA plus ELISA [Biomanguinhos®]). **Results:** *T. cruzi* seroprevalence was 4.1% (16/385) in 2001, decreasing to 2% (7/341) in 2011. Concerning the 190 subjects who were negative in 2001 and reassessed in 2011, five (2.6%) became seropositive according to the two distinct assays. **Conclusions:** Restraining the continuous introduction of *T. cruzi* into the domestic and peridomestic environments seems to be extremely difficult due to the characteristics of the potential *T. cruzi* vectors and precarious and sometimes wretched housing in the region, therefore posing a risk of CD transmission. **E-mail:** mmlima@ioc.fiocruz.br

Chagas002 **High natural infection of *Triatoma brasiliensis* by *Trypanosoma cruzi* was detected in Caicó, Rio Grande do Norte, Brazil.**

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Introduction: Chagas disease is one of the main tropical illnesses in Latin America, caused by the etiological agent, *Trypanosoma cruzi*. The disease has many transmission routes, but the vector-borne

infection is still the most important. In nature, many triatomine species are found infected by *T. cruzi*. In the northeastern region of Brazil *Triatoma brasiliensis* deserves attention in light of its capacity to colonize different ecotopes and also because it is known for harboring *T. cruzi*. In this context, the circulation of *T. cruzi* in natural habitats of Caicó, a municipality located Rio Grande do Norte state of Brazil, was studied. **Material and Methods:** The collection of bugs was carried in the wet and dry seasons (April and November 2011, respectively), within a period of 10 days. The insects were placed in glass beakers containing filter paper. For the analysis of *T. cruzi* infection, feces were collected from adult bugs by pressure on the abdomen and subsequent analysis by optical microscopy. **Results:** In total 625 *T. brasiliensis* at different developmental stages were collected in five different localities in Caicó. From 287 examined insects, 197 were positive, resulting in a *T. cruzi* infection rate of 68,6%. It is important to stress that in some of the localities the percentage of natural infection was higher than 80%. **Main conclusions:** Caicó is a semi-arid region with caatinga vegetation under strong environmental pressure due to population growth and agricultural activities. The wild environment in this region supports the development and establishment of different triatomine species, mainly *T. brasiliensis*, because of its behavior of colonizing rock piles, being adapted to dry regions. Based on the high infection percentages and the observed presence of feces of small mammals living in the same rock outcrops of insects, a well-established sylvatic cycle of *T. cruzi* was evidenced. Since anthropization might provide a path to get closer sylvatic and human transition cycles, this area needs to be under continuous epidemiologic surveillance. **E-mail:** acbastos@ioc.fiocruz.br

Chagas003- Are dogs important Chagas' disease reservoirs in the semiarid Brazilian northeast region?

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Introduction: Chagas' disease is an anthroponosis of chronic clinical course caused by *Trypanosoma cruzi* (*T. cruzi*) and transmitted by triatomines. Dogs may play a role in the maintenance of the disease domestic cycle, but reports on natural canine infection are scarce for the Brazilian northeastern area. The aim of this study was to identify the presence of *Trypanosoma cruzi* in naturally infected dogs in this area as a first step towards understanding the role of dogs as reservoirs in Chagas' disease. **Material and Methods:** Blood samples were collected from 170 dogs in the countryside nearby the municipalities of Patos and Teixeira, in the state of Paraíba, and Caicó in the Rio Grande do Norte State. The diagnosis of *T. cruzi* was performed by direct microscopy of blood smears, blood culture, indirect fluorescent antibody reaction test (IFAT) and polymerase chain reaction (PCR). The sera were also tested to identify cross-reactions and / or co-infection with *Leishmania chagasi* by IFAT. All blood smears were negatives. Serology was positive by IFAT in 10,6% (18/170) samples. Of these, 13 dogs were tested by PCR assay and blood culture. A fragment of 330 base pairs from *T. cruzi* minicircle was amplified in 84.6% (11/13) samples and the parasite in two blood cultures. Among the Chagas' disease positive dogs, 11.1% (2/18) were positive for visceral leishmaniasis by IFAT (titers of 1:1280 and 1:40), characterizing co-infections. **Main Conclusions:** The common presence of infected animals in the urban peripheries suggests dogs can play a role as a domestic reservoir for Chagas' disease. However, the triatomines found in the same houses and in the neighborhood were typically peri-domestic. Moreover, the infection route was not investigated, as well as the *T. cruzi* strains infecting the animals. Therefore, in spite of the high incidence of infected animals, it is still precipitated to state that dogs have a definite role in the maintenance of a *T. cruzi* domestic cycle in the semidry Brazilian area. **E-mail:** almir@cstr.ufcg.edu.br

Chagas004- The 2005 outbreak of Chagas disease in Santa Catarina: six years later

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Introduction: In March 2005, 24 cases of acute human Chagas disease due to ingestion of sugar cane juice contaminated by *Trypanosoma cruzi* were identified in Santa Catarina, marking the first and only known outbreak of this neglected tropical disease in the southern Brazilian state to date. **Methods:** *T. cruzi* infection was confirmed by fresh blood examination, hemoculture, serology and PCR, and all patients were treated with 5mg per kg of body weight of benznidazole, brand name Rochagan, for 60 days according the Brazilian Health Ministry guideline. Patients were evaluated clinically by electrocardiogram and chest x-ray and by the laboratorial methods of hemoculture, serology and PCR at six month intervals during the first year and annually thereafter according to the established protocol for patient follow-up. **Results:** Of the 24 confirmed cases, three cases, including two children and one adult, died before diagnosis was established and three cases were diagnosed and treated out of state. During the acute phase of the 18 patients attended in state, symptoms suggestive of cardiac impairment were observed with increased frequency, including dyspnea (n=10, 55.6%), dry cough (n=10, 55.6%), edema (n=8, 44.4%) and palpitations (n=9, 50%). Interestingly, the presence of gastrointestinal bleeding, which are not symptoms typically reported for Chagas disease, were observed in 72.2% (n=13) of cases. In one case, histopathological analysis of a gastric biopsy demonstrated the presence of *T. cruzi*. Of these patients, only 10 patients (55.6%) completed the established protocol after five years and only 6 patients (33.3%) returned for follow-up in the 6th year. Three patients in the second year after treatment and one patient in the third year after treatment relapsed, as confirmed by positive hemoculture, increase in IgG serological titer and positive PCR. After five-year follow-up, all 10 patients who completed the protocol presented negative hemoculture, nine patients remained with positive IFI tests and six patients had positive PCR. Only one patient presented all negative exam results and was considered cured. Currently, no patients under observation have presented with severe outcomes related to the clinical manifestations of the disease or the disease itself. **Main Conclusions:** High rates of loss to follow-up have greatly hindered investigation of treatment outcomes of Chagas disease cases in Santa Catarina. The majority of these losses to follow-up resulted from a lack of uniform and persistent case management. More efficient, thorough, and unified case follow-up should be emphasized in future outbreak investigations. Specifically, cases should be carefully monitored overtime in a reference center for a 10 year follow-up to ensure completion of treatment and to allow for conclusive research. Additionally, Chagas disease is not currently endemic in Santa Catarina and the occurrence of an outbreak resulting from oral transmission, which is the less common form of transmission, could potentially signify its emergence in the region. **Supported by** CNPq, PPSUS and FAPESC **E-mail:** mario.steindel@ufsc.br

Chagas005- Acute Chagas Disease Outbreak Associated with Sugarcane Juice Consumption in a Brazilian Small Village, Maranhão State, October 2011

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Introduction: In October 2011, three individuals of a small village were hospitalized with a persistent fever. They were malaria suspect however *T. cruzi* was found in the direct parasitological test. After investigation more nine cases were confirmed for Chaga's disease (CD), performing twelve CD acute cases. Objectives of this study were: identify the transmission mode, source of infection, and the *T. cruzi* presences in triatomines and propose recommendations. **Material and Methods:** Descriptive and case-

control studies were conducted and a CD confirmed case was defined as a resident or individuals who visited the small village at September 06 or 08 and presented positive test for *T. cruzi* and controls had negative test for CD. In entomological search the vector's feed source and trypanosomatids presence were conducted. **Results:** Four (25%) of 12 CD acute cases were asymptomatic. All symptomatic reported fever and prostration, 62,5% distended abdomen and epigastric pain and 50% abdominal pain. None of the cases reported inoculation Chagoma or Romaña's sign. Also no blood transfusion or organ transplantation was done. None occurred deaths. The main food consumed were rice, beans, fish and sugarcane juice. The exposures associated with the illness were: consumption of sugarcane juice in September 06th (OR: 99; 95%CI: 9-1,058) and 08th (OR: 4.6; 95%CI: 1.1-19.5). There were found seven triatomine *Rhodnius robustus* species in a palm tree near the household, which main feed source was an opossum and trypanosomatids were negative. **Conclusion:** The vector or transfusion transmissions were discarded and gastrointestinal signs detected are common in oral CD acute cases. Sugarcane juice was an associated exposure in this outbreak. We recommended the Municipal Secretariat of Health to use the malaria surveillance to detect CD acute cases earlier in patients with fever. We also recommend to guide the small village population on sugarcane hygiene with water and detergents before use it, and store it in a covered place. **E-mail:** marcelamuhana@yahoo.com.br

Chagas006- Chagas disease in Guarani, a rural village within São Sebastião do Alto municipality in the state of Rio de Janeiro, Brazil: An eco-epidemiological and serological survey

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Introduction: Rio de Janeiro (RJ) state has never been considered a Chagas disease (CD) endemic area. However, some out clinic patients treated in the Clinical Research Institute Evandro Chagas, Oswaldo Cruz Foundation, were born and raised in rural areas of the hilly region of northern Rio de Janeiro state. None of them had received either blood transfusion or organ transplantation, and their mothers were native of the same region. In 2009, we received a 15 year-old girl from São Sebastião do Alto municipality, refused by the bone marrow bank, as she was detected CD positive through serology. The patient's mother was also diagnosed CD positive. In 2010, we conducted a field study involving an epidemiological and serological survey in Guarani, a rural area within São Sebastião do Alto, also a hilly region of Rio de Janeiro state. **Materials and methods:** We visited 106 domiciles in Guarani, collecting 234 human blood samples on filter paper for serology by ELISA and IIFA methods and afterwards applying a questionnaire for epidemiological investigation. Traps were set at various sites in the village for small mammal collection in hopes of disclosing potential *Trypanosoma cruzi* reservoirs. Infection diagnostics were confirmed by both IIFA and hemoculture. Fifty percent of the houses were investigated, searching for triatomines in both intra and peridomestic environments. Parasites isolated from reservoirs were characterized by multiplex PCR of the mini-exon gene. **Results:** The age of volunteers ranged from 2 to 88 years old. Of the 234 blood samples, only two proved serologically *T. cruzi* positive (ELISA and IIFA), both from the two patients (mother and daughter) already identified with the disease (prevalence 0.85%). Sixty-five residents (27.7%) reported some knowledge concerning the bugs. The consumption of game meat (opossum, armadillo, Brazilian guinea pig, paca) was reported by 65% of the inhabitants and fresh sugar cane juice by 77%. Seven triatomines, all adult *Triatoma vitticeps*, were captured in five houses, three in the positive patients' domicile. All bugs were *T. cruzi* negative by direct examination of feces. We captured 34 small mammals, two species of marsupials and four rodents, of which 3 (8.8%) were *T. cruzi* positive. The most prevalent species was *Akodon cursor* (24 animals), one *T. cruzi* positive through blood cultures, identified as genotype I (Tcl). One *Rattus rattus* and one opossum (*Didelphis aurita*) were positive by IIFA. The two positive rodents were captured in the peridomicile of the house where the mother and daughter were diagnosed with CD. **Main conclusions:** The findings suggest a CD transmission cycle in Guarani, where the home invasion of native triatomines, as well as the handling and consumption of under-cooked game meat from infected wild animal reservoirs may be factors justifying the autochthonous cases identified. **E-mail:** lhcsangenis@gmail.com

Chagas007- Chagas disease mortality in state of São Paulo: epidemiological aspects.

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In the period 2001 to 2007 deaths in 8261 were registered residents of the state of São Paulo, whose underlying cause was declared Chagas disease, with an average of 1180 cases / year. A reduction of the mortality of the disease over the period which is higher in the elderly. The aim of this study is to describe the profile of Chagas disease mortality in São Paulo in 2007 and relate to the death of triatomine species identified in the municipality of residence. We conducted a retrospective, descriptive. The selection of the deaths of residents in the state code was performed using the International Classification of Diseases Tenth Revision (ICD 10) (code B57), in the "Infectious Diseases" classified in Chapter I and provided by the State System of Data Analysis the State of São Paulo (Seade). The variables analyzed were sex, age (age groups 00-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70 and over 71 years) and naturalness. The presence of triatomine was sought for the decades 1950, 1960 and 1970 in the archives of Endemic Disease Control Superintendence for individuals in counties with ease. In the year 2007, there were 1,096 deaths due to Chagas disease in the state. These individuals were mostly male (55.4%), belonging to the age group 70 years or more (34.4%). The mortality rates are increasing with age, with 0.7% of deaths in individuals under the age of 30 years. The naturalness of these individuals stood out the states of São Paulo (39.0%), Minas Gerais (27.7%) and Bahia (14.8%). For individuals whose naturalness was São Paulo (427 people), 52.5% were men and the age group 70 years or more (61.8%). When we analyzed the species detected in 174 municipalities of residence of the deceased, can be observed that *Triatoma infestans* was present in 76 municipalities in the 1950s and 09 others in the 1960s. In 10 municipalities the species *Triatoma sordida* was detected in the 1950's and 07 municipalities the species was *Panstrongylus megistus* in the 1960s. In 06 municipalities the species *T. infestans*, *P. megistus* and *T. sordida* were found together in the 1950s. In 31 municipalities there was the presence of *T. infestans* and *T. sordida* together. In 13 municipalities there was no information of the detected species of triatomine decades evaluated. The average age of the observed deaths for the year of the study was 70 years, that is, individuals born in the 1930s. Chagas disease takes shape in the 1920s with its shares only systematized in the 1950s where the control program established in São Paulo preached the chemical control of insects on a routine basis. It is suggested to have been the way the main vector responsible for transmitting the diseases bygone. This information contributes to the determination of the problem and provides subsidies for the Chagasic patient care planning should not be thought of only in relation to the subgroup who died. **E-mail:** rubensantoniosilva@gmail.com

Chagas008- Epidemiological Analysis of Acute Chagas Disease in Pará State - 2006 to 2011

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Inoduction: Chagas Disease (CD), the human infection by *Trypanossoma cruzi*, is considered by WHO a neglected disease and configures an important public health domain, since causes about 14 thousand deaths/year in Latin America. In Brazil, chronic cases usually were caused by vector contact; nowadays, acute cases (isolated or outbreaks) are reported in several Brazilian States, notably in Amazon region. This study reviews the epidemiological status of Acute Chagas Disease (ACD) in Pará State, between 2006-2011. **Materials and Methods:** retrospective Study from database cases of State Chagas Coordination and National System of Information. **Results:** 761 ACD cases were reported between, with mean of 127 cases/year (Δ 77–238 cases). Highest incidence occurred in 2009 (IC=3.23 cases/100,000). Every year, cases increase from Jul and decrease in Dec (peaks between Aug-Oct); eventually there are no cases in Feb and Mar. Municipalities with higher number of cases: Belém (22,4%; n=171), Abaetetuba (16,2%; n=123) and Breves (9,1%; n=69). Most cases occurred in adolescents and adults older than 11; 15.2% of cases occurred in children younger than 12 years old (n=116), 9.2% in elderly higher than 65

years old (n=70); less than 1% in children 1 year old (n=6). Were reported 27 deaths, with annual lethality ranging from 1.25% to 11.39% with an average of 4.62%. Cases were distributed into outbreaks (68.6%; n=522), isolated cases (30.7%; n=234), another type of grouping (less than 1%; n=6). The transmission way was suggested as oral in 61.2% of cases (n=469); vectorial in 1.8% (n=14); accidental, transfusion and pregnancy related in 0.13% each (n=1 respectively); transmission way of infection was not identified in 36.7% of cases (n=256). Amazonian fruit juices were identified as food source of *T. cruzi*, such as Açaí (13.1%; n=100) and bacaba (2.6%; n=20). The direct parasitological examination was diagnostic method in 60% of cases (n=457); serology was confirmatory in 33.1% (n=252); epidemiological methods in 6.9% (n=53). Median of interval between symptoms begun and diagnosis was 19 days. **Conclusion:** Establishment of DC surveillance in Pará improved epidemiological information for analysis and control of disease. Oral form is currently the main route of contamination with *T. cruzi*. The sustained annual occurrence of cases suggests the disease became endemics in the region. It is necessary to strengthen strategies for investigating cases and outbreaks, with emphasis on the timely diagnosis and treatment, and identification of ways and means of transmission. **E-mail:** Elenild@gmail.com

Chagas009- Occurrence of Chagas infection in peri-urban area population of rural and Manaus, Amazon, Brazil

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Introduction: Chagas disease is an emerging disease in the Brazilian Amazon region, where *Trypanosoma cruzi* I predominates among the acute cases of the disease and *T. cruzi* III/Z3, a population cluster from wild areas of the Amazon basin, is rarely associated with human infections. In the Amazon region, deforestation, substandard housing conditions in rural areas, and harvesting of forest products have increased contact between peri-domiciliary vectors, wild reservoirs, and humans, which also increases the number of cases of ChD that apparently originate from wild transmission in these locales.

Objective: The purpose of this work was to estimate the infection rate of Chagas disease in its chronic form among inhabitants of periurban and rural areas in the city of Manaus, Amazonas.

Material and Methods: This was a sectional and descriptive study of the local population, which willingly agreed to participate, allowing visits to their dwellings. A questionnaire about epidemiological, socioeconomic, and sanitary information, as well as other clinical data, was applied to gather epidemiological variables. A 10ml blood sample was collected by venous puncture; its serum portion was submitted to immunoenzymatic assay (ELISA) for qualitative analysis of IgG anti-*T. cruzi* antibodies in human serum. After this stage, the samples classified as reactive, were submitted to indirect immunofluorescence antibody test (IFI) at 1:40 and 1:80 dilutions and were analyzed by Western blot (WB).

Results: Of the 1,850 subjects interviewed 1,611 (87%) were from rural area and 239 (13%) periurban area. 162/1,850 (8,7%) serum samples were reactive, by ELISA, 50/162 (31%) IFI were reactive and 10/162 (6,2%) were reactive by Western blot (WB). Ten patients had serologically confirmed *T. cruzi* infection; six of them were autochthonous to the state of Amazonas and the other four were from endemic areas. Seven of the 10 cases were males, and the average age was 35-57 years old; most were farmers with low education.

Conclusion: The detection of occasional clinical CD cases in this area provides data that allow the implementation of actions against its dissemination in the area. **E-mail:** MGVB: gvbarbosa@fmt.am.gov.br

Chagas010- The effect of deforestation in the transmission of Chagas disease in two municipalities in the Pará State, Brazil

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Introduction: Chagas disease, caused by *Trypanosoma cruzi* is endemic in 21 American countries. Estimates from serological surveys indicate that about 7.5 million individuals are infected in the Americas.

In Brazil, it is estimated that two million people are infected and another 22 million are at risk of infection. *T. cruzi* can be transmitted to humans by different mechanisms, but the vector is the most frequent. The vectors, belonging to the subfamily Triatominae, are hematophagous bugs, which inhabit different ecological niches within the forest. Currently, the main problem of health surveillance of Chagas disease is increasing records of infested triatomines within housings in endemic areas. Deforestation is supposed to be the main factor for this increase, leading to withdrawal of the primary vegetation, extinction of wild animals and to an increase in synanthropic animal populations, e.g. rodents and marsupials, which are known to be competent host reservoirs of *T. cruzi*. The vector species can infest peridomestic ecotopes (mainly palm trees) are in close contact to humans, raising the possibility of human-vector contact. In the present work, we have tested the hypothesis of deforestation as being the major factor for increasing numbers of acute Chagas disease cases in two municipalities in the State of Pará. **Material and methods:** We have used data from the increment of deforestation (ID), obtained from PRODES/INPE, and related it to data on acute Chagas disease (ACD) in the municipalities of Abaetetuba and Barcarena/Pará, obtained in the public database SINAN/MS. In these municipalities, increasing numbers of cases have been reported during the past nine years (2002 to 2010) [Abaetetuba n=110, mean=9.6 p/year; Barcarena n=48, mean=9.6 p/year]. We have tested a linear model of correlation between deforestation and notified human cases. **Results:** Although the number of reported cases increased in some years proportionally to ID, the linear correlation index was low for both localities: Abaetetuba ($r^2=0.0111$) and Barcarena ($r^2=0.0293$). **Main conclusions:** These results suggest, (1) that deforestation has no direct influence on the number of acute Chagas disease cases in both localities, (2) these areas reached a level of rebalance after ecological disturbance, where the vectors are already re-adapted to peridomestic ecotopes; (3) because of this state of rebalance we suggest that the increase of cases is due to other mechanisms, probably due to oral transmission of *T. cruzi* via contaminated food or even to synanthropic animals, which cohabit the residences with humans; (4) large peridomestic palms should be regarded as major potential objects of entomological surveillance. **E-mail:** andrea20_livia@hotmail.com

Chagas011- Epidemiological and clinical profile of Chagasic patients followed in a reference service

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Introduction: Chagas disease was first described in 1909 and it is caused by the protozoan *Trypanosoma cruzi*. The incidence of Chagasic infection in Brazil has decreased over the past years with the vectorial and transfusion transmission control. This new situation leads to important changes regarding the infected population which has become older. **Objective:** The aim of this study was to evaluate the epidemiological and the clinical profile of elderly patients with Chagas disease followed in a reference service. **Material and Methods:** To participate of this study, 85 Chagasic patients aged 60 years old and above were enrolled. All of them were assisted in the Outpatient Unit of the Group for Studies into Chagas Disease (GEDoCh) at the Clinical Hospital of the University of Campinas between Jul 2010 to December 2011. The following data were collected through an interview questionnaire: age, gender, epidemiological antecedents to Chagasic infection (previous transfusion, Chagas disease among relatives and in the neighborhood), human-vector contact and residence in endemic rural or urban areas. Medical records were reviewed to determine the current clinical form of the disease. **Results:** Out of the 85 patients, 44 (51.8%) were women and 41 (48.2%) were men. The mean ages were 64.95 and 66.09 years old, respectively. Considering epidemiological antecedents, 24 (28%) patients reported at least one blood transfusion in the past, 56 (66%) patients had chagasi relatives with 12 (14%) with infected mother. Infected people in their neighborhood were reported by 31 (36.5%) patients. Only one patient did not report living in rural area during its life and four patients did not migrate to urban areas. The mean time of habitation in endemic areas was 19 years. The vector insect was unknown for only two (2.3%) patients and 26 (30.5%) patients reported that they had been bitten by the insect. Regarding clinical forms of the disease, 36 (42%) patients presented the cardiac form, 21 (25%) the digestive form, 14 (16.5%) the cardio digestive form and 14 (16.5%) presented the indeterminate form. **Conclusion:** The present study shows that the epidemiological profile of the chagasi patients is characterized by young elderly people, with important antecedents for Chagas disease and most of them lived in rural and endemic areas in the

past. The majority of the patients studied presented the cardiac form of Chagas disease, followed by the digestive form. **E-mail:** barroso@fcm.unicamp.br

Chagas012- Seroprevalence of Chagas disease in blood donors assisted by Hemominas in Triângulo Mineiro, Minas Gerais, Brazil

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Introduction: The Chagas disease, in spite of the public health campaigns and the actions the epidemiological surveillance, is still a serious health problem in Latin America, causing about 50 thousand deaths per year and having over 18 million people affected by the parasite. In Brazil there are two million infected people approximately and they are facing the chronic phase of the disease. There are not studies of epidemiological around Triângulo Mineiro area, that show blood donors who are affected by Chagas disease. **Objective:** To evaluate the prevalence of the seropositivity in blood donors registered at Hemominas, Minas Gerais. **Material and Methods:** The diagnosis of Chagas disease depends on the results of serological tests, being used tests with antigens *Trypanosoma cruzi*: indirect hemagglutination (HA), indirect immunofluorescence (IFI) and enzyme-linked immunosorbent assay (ELISA), being used 2 or 3 tests at the same time, both in the selection tests and in the confirmatory tests. **Results:** The results of the blood donor candidate, registered at Hemominas from 1991 to 2011 were analyzed. From the 53,941 donors, 159 were positive to disease. From these 159, 102 (19%) seropositives and 57 (0.10%) uncertain for the Chagas infection finding higher numbers of seropositivity in people from 40 to 60 years old. In relation to the gender, it was observed that in the confirmatory cases 68 (64.26%) men were seropositive, 44 (39.6%) uncertain and 34 (70.8%) women seropositive, 13 (27.1%) uncertain. **Conclusion:** Although the quantity of men with Chagas disease is bigger, the higher percentage of seropositivity is among the women. When it comes to the prevalence of the infection between the candidates to be a donor, it's possible to perceive that it is in agreement to the nation statistics level because, nowadays, the prevalence of Chagas disease significantly diminished in comparison to the last decade's data, verifying the severe serological selection and the interruption of the vectorial transmission. **E-mail:** patty_lopes19@yahoo.com.br

Chagas013- Epidemiology and public policy of acute Chagas disease among the years 2001 and 2006

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Introduction: There are an estimated that 16 million are infected and 80 million are at risk of contamination by *Trypanosoma cruzi* in Latin America. Disease discovery by Carlos Chagas in 1909, is closely related to environmental and sociopolitical factors. Today, after more than one hundred years of studies and attempts to control, the disease is still endemic in many parts of the world, with areas of high prevalence in Brazil. The protozoan was urbanized over the years with the invasion of wild ecotopes by man, which is included as part of the epidemiological cycle of triatomines. Currently, Brazil has no registered cases of *Triatoma infestans*, but four other species of triatomines have clinical relevance: *T. brasiliensis*, *Panstrongylus megistus*, *T. pseudomaculata* and *T. sordida*. This study, therefore, aims at an epidemiological profile of the main forms of infection by *T. cruzi*, the prevalent area of residence in new confirmed cases and education of these individuals. **Material and Methods:** Epidemiological retrospective documentary research in database of the Sistema de Informações de Agravos de Notificação (SINAN). To this end, it was used descriptors Year of the first symptom and Zone Property, on which have changed epidemiological characteristics specifics of the group, obtaining data from Brazil between the years 2001 to 2006. **Results:** Between 2001 and 2006, there were 2250 new confirmed cases of Acute Chagas Disease (ACD), 64.04% of these are carried through classical vector. For the same period, 3.95% of patients died, while 49.1% of the reported cases were not reported their evolution.

In all the years considered there was more reporting of cases in urban areas, except in 2003, where the number of cases in rural areas was slightly higher than the cases of the urban area. Taking into account the total period covered, 53.95% of the cases occurred in urban areas, compared to 40.08% of cases occurred in rural areas. Also visible is the higher prevalence in patients with three years of schooling or less, corresponding to 44.8% of notifications. It is worth noting that 34.97% of reported cases in the period were ignored about the place of origin. **Conclusion:** The urbanization of the ACD has remained relatively constant during the study period. It can be inferred, then, that although controlled epidemiological point of view, deserves constant attention and reformulation of public policy in order to accomplish the combat and control the disease, with expanded vision of the determinants and constraints that she hostel. The urbanization of the disease is a challenge that goes beyond welfare policies therefore beyond the host's immune status and characteristics of the insect vector, socioeconomic conditions and lifestyles are important in the construction of structural interventions that resignify values. It is in these aspects that the reconstruction of socio-cultural environment in the country takes part as a modulator of intersectoral action in health. **E-mail:** taynamaria@gmail.com

Chagas014- Evaluation of the Chagas Disease Control Program in the Minas Gerais State, Brazil, after the decentralization of health actions, 2001-2011

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In Minas Gerais State, Brazil, after the decentralization of the Brazilian health system to states and municipalities occurred the early 2000s, certain administrative and operational difficulties have been observed in the Chagas Disease Control Program. For this reason, an evaluation of the results achieved is necessary in order to provide analytical support for changes in current operational policies. The objective of this study was to evaluate entomological survey performed by the Chagas Disease Control Program in Minas Gerais state, Brazil, from 2001 to 2011. We analyzed the secondary data in the information system of the Chagas Disease Control Program (PCDCh): number of municipalities that conducted surveillance and control activities in the period, frequency of domiciliary units investigated, domiciliary unit infestation, insecticide application for chemical control of triatomines, number of insects captured and percentage of insects examined. From 2001 to 2011, 695 municipalities (81.5% of the state total) conducted survey and control activities in at least one year under evaluation. Of these, 486 (69.9%) municipalities had triatomines captured records. A total of 1,591,191 domiciliary units were investigated, and 130,815 were positive for the presence of triatomines, an infestation rate of the 8.2% in the period. The entomological research was not conducted in 169,157 domiciliary units. A total of 137,885 residual applications of insecticide were made, with the consumption of 321,235 loads of insecticide. However, according to the system, 0.5% (n=693) of positive home units were not treated (refusal or closed house). Approximately 348,889 specimens of triatomines were captured in the period, and a high percentage (19.3%, n=67,269) were not examined. A total of 1.4% (n=3,919) of the specimens were positive for *Trypanosoma cruzi*, being distributed in 320 municipalities. The results demonstrate the importance of maintaining the activities of the Chagas Disease Program Control in Minas Gerais. In conclusion, it is essential to adapt the existing operational policies to the current situation, as shown in the entomological survey, with the objective of ensuring the sustainability of control, and the consequent interruption of vectorial transmission of Chagas disease in Minas Gerais state. For more effective control, political commitment, multi-sector articulation and rational use of insecticide are required. **E-mail:** marcela.ferraz@saude.mg.gov.br

Chagas015- Housing Improvement for Chagas disease Control in RS State: 2001 - 2011

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Introduction: The National Health Foundation (FUNASA) in a partnership with the Rio Grande do Sul State Health Secretary (SES/RS) has implemented the housing improvement for Chagas disease (PMHCh) on the RS Northeast. The program aim is to turn the housing units refractory to the *Triatoma infestans* (blood-sucking bug), the Chagas disease transmitter. **Methods:** The program is based in epidemiological criteria nominated by SES/RS, following the guidelines of the technical and financial cooperation agreement signed with FUNASA in the election of both, town and inhabitants, to receive the benefits in the hazardous area with breeding persistence in the last 5 years. It's developed and conducted by a multidisciplinary staff and integrated with Health Learning and Social Mobilization Program (PESMS), which remains along the project development and continues after the physical reforms as an evaluation scope. Are part of the process, the municipal technicians, community and the civil construction workers training, domiciliary orientation and monitoring visits and educative workshops. The health education in this process is understood like a citizenship practice, social communion and development of critical conscience to the operating people in the transformation of the reality. **Results:** the program already took care of, approximately, 1500 families and it is having continuity through the Growth Accelerating Program – PAC, remaining the same choice criteria. In 2007, more than 30 priority municipalities, with vector persistent infestation, were indicated to receive financial resources (PAC). The indications are endorsed in epidemiological studies and meetings between SES/RS entomological monitoring team, FUNASA/RS and National Management Committee for the Chagas Disease Control Program. it can be noticed the housing improvements, as well as, the behavioral change, resulting in self-esteem and quality of life improvement to the benefited families. **Conclusion:** The decentralization on the diseases control actions to the states became pressing the partnership between SUS managers to ensure the continuity and the viability to that program, enabling the housing and culture and socio-economic environment to interact in the motions on dwelling, where the environmental sanitation isn't enough to control the endemic Chagas disease. It is perceived that the sanitation and the housing improvement, not only promotes the Chagas disease control, but, prevents various health worsening; in addition to improving the community quality of life and the environment. The vector tenacity is attributed to the habits and behavior on environment manipulation, as, for example, the inadequate wood and utensil storage and the maintenance of a variety of house annexes in poor conditions. The program is exciting the community, improving the financial resources circulation on the cities and, also, inducing the integration between SUS (Sistema Único de Saúde) managers. The epidemiological impact evaluation of the vector control is continuous and permanent, looking, always, keep healthful the habitation. **E-mail:** tania-wilhelms@saude.rs.gov.br

Chagas016- How to control Chagas disease orally transmitted in Brazilian Amazon?

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Introduction: Outbreaks and isolated cases of Acute Chagas Disease (ACD) have been reported for more than 40 years in Brazilian Amazon. The oral route is an important way of *Trypanosoma cruzi* transmission especially in Pará, Amapá and Amazonas States. The oral transmission is frequently related to the acai juice ingestion, a typical regional food. This study describes the casuistic of the ADC in Brazilian Amazon since 1969, with particular reference to Pará State and the difficulties for the local disease control. **Material and Methods:** The occurrences of ACD in the Brazilian Amazon, from 1969 to 2011, were investigated in databases files from the Instituto Evandro Chagas/Ministry of Healthy and from the National Information System of Disease Notification (SINAN). In addition, we exam the public health guidance applied by the health authorities for the Chagas' disease control in Pará State. **Results:** From 1969 to 2011, there were registered 1260 cases of ACD in the Brazilian Amazon, with 5% of deaths. The

occurrences were distributed in five States: Pará (842; 67%), Amapá 279 (279, 22%), Amazonas (81; 6%), Maranhão (40; 3%) and Acre (18; 1%). In the last five years, 700 cases of ACD were reported in Pará (5 to 7% deaths), equivalent to 56% of the occurrences in the Brazilian Amazon along the investigated period. The actions carried out in Pará State were: (i) professional capacitating for diagnosis and treatment; (ii) improvement of the notification proceedings; (iii) support for investigating outbreaks; (iv) epidemiological/entomological surveillance and (v) sanitary inspection on foods related to transmission. **Conclusions:** Pará is the Amazonian State with the higher number of ACD registered cases. The applied control measures favored the improvement of the diagnosis, however, the difficulties regard to sanitary inspection on foods related to transmission, in particular the acai juice, are persistent. This product has a strong economical meaning since moves more than 250 million dollars and provides more than 150 thousand jobs. Nevertheless, the productive chain is highly vulnerable. The artisanal making of the acai juice, under precarious hygiene proceedings, represents a conditioning risk factor for oral transmission. In the capital of Pará (Belém city) and metropolitan region, the number of small shops dealing in acai juice varies as of 3000 (from January to July) to 7000 (from July to December), since this number increases in the harvest time, what make difficult the inspection by the health authorities. The control of the orally transmitted Chagas' disease depends on the economical support to small undertakers for providing adequate environment and hygiene good practices for handling the acai. By the other hand, public health teams are insufficient and need to be trained. **E-mail:** aldovalente@iec.pa.gov.br

Chagas017- Importance of the Chagas' disease in Corrientes, Argentina

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Introduction: Chagas' disease is still a major epidemiologic problem in many Latin American countries, including Argentina. The migration of people from the country into towns has resulted, in recent decades, in the urbanization of this rural disease and the arrival in non-endemic areas of numerous seropositive individuals. Up to the mid-20th century, the epidemiology of the disease was closely linked to the extreme poverty of the peasant population and to their housing, the rancho, which offers a suitable habitat for the vectors *Triatoma infestans* and encourages their proliferation. Systematized studies aiming to determine the prevalence in the chagasic population is scarce, especially in the province of Corrientes. The aim of this work is to characterize the distribution of the Chagas' infection in patients of different ages and localities of the province of Corrientes that were diagnosed in the Cenpetrop. **Material and Methods:** This province is located in the humid subtropical region of Argentina northeast (27°50' N and 52°50' W). We studied 571 patients. Patients age ranged from 2 to 82 years and 307 were male (52.3%). Serological study by Immunofluorescence test was performed. The parasitological condition was determined by xenodiagnosis (XD) and parasitological study by direct examination (Strout). **Results:** Among the 586 participants a total number of 343 positives (58.5%) were detected by TIF (58.3% were males and 58.7% females). From total patients, 47.0% were younger than 4 y-old. In 53% (116) patients *T. cruzi* was demonstrated by XD. Heart failure was the most severe clinical manifestation of chronic phase of infection. They were natives from 20 (80.0 %) of 25 Departments of the province (Capital, San Luis del Palmar, Bella Vista, Empedrado, Santo Tomé, San Cosme, San Roque, General Paz, Saladas, Paso de los Libres, Concepción, Curuzú Cuatiá, Mburucuyá, Itatí, Goya, Mercedes, Sauce, Lavalle, San Martín and San Miguel). From total patients positives, 36 were pregnant women from 17 and 46 y-old and 26 neonates were examined. In one child was demonstrated the parasite. Treatment with Benznidazol 5-7.5 mg /Kg/day was carried out within 28% (69) of people in the chronic phase, and in the case of congenital Chagas transmission. **Conclusion:** On the decade of seventy, a coordinate effort to eradicate vectorial transmission of the disease was established. Despite all of this, in the province of Corrientes persists the vectorial transmission by domestic vectors. Also protocols must be established to control of congenital Chagas transmission, in order to apply early treatment to infected neonates. **E-mail:** cenpetrop@hotmail.com

Chagas018- Lessons, challenges and a roadmap for action on implementation research for Chagas Disease in Colombia

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Introduction: Colombia is one of the top endemic countries for Chagas Disease (ChD). Over the last two decades a National Control Program, mainly executed by research centers, identified priority risk areas for intervention (domiciliary transmission and transfusion control). Despite significant progress, there were both political and economic barriers limiting the application of specific actions. In addition, the emergence of newly identified problems, such as outbreaks by suspected oral transmission, the high prevalence in pregnant women, and cases of congenital transmission brought new challenges. In turn, migration from rural to urban areas has increased awareness for the care and needs of chronic patients with an increasing demand for health services. Therefore, we aim at developing a Research Integrated Program that identifies major gaps in understanding and contributes to reduce the burden of ChD in Colombia.

Materials and Methods: We describe the program and strategies planned for the Red Colombiana de Investigación en enfermedad de Chagas (RedChagasCol). We hope to generate basic and applied research on prevention, control and rehabilitation including several areas (Epidemiology, Entomology, Molecular Biology, Cardiology, Social Science and Health Economics). Financial support comes mainly from the Departamento Nacional de Ciencia y Tecnología e Innovación (COLCIENCIAS) and internal resources from participating institutions. **Results:** A network of 35 public and private institutions (universities, research centers, patients associations and nongovernmental organizations) will conduct 19 inter-related projects, with support from a Data Coordinator Center (DCC). Expected products will include advances in: 1) Classic and molecular epidemiology, social determination and economy: studying distribution of vectors, cases, parasites, using risk predictive models, researching the role of social and cultural factors as well as coverage, opportunity and obstacles to health care, and performing economic evaluations; 2) Transmission prevention or control (in vectorial, trasfusional, congenital and oral routes) researching strategies to decrease the incidence 3) early diagnosis and treatment (etiologic and supportive) in both acute and chronic phases, and 4) rehabilitation and prognosis for chagasic cardiomyopathy, researching about prognosis biomarkers, electric devices and cardiologic evaluations. The program will also enhance research capacities and train new human resources at a top level. **Conclusion:** By developing an articulate research program Colombia has a new opportunity to achieve integration of scientific knowledge, with application in public health, establishing research priorities regarding prevention, control and rehabilitation of patients, for reducing Chagas burden of disease. **E-mail:** zcucunuba@gmail.com

Chagas019- Trypanosoma cruzi antibody assay for blood donor candidates at epidemiological risk of infection Blood Bank of Hospital General de Mexico

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Introduction: When a blood donor candidate declare to know the insect vector, have been bitten or inhabiting dwellings at risk of Chagas disease, he is automatically dismissed as donor without performing any tests to confirm or refute infection. The present study aims to identify rejected blood donor, candidates using two serological tests for *T. cruzi*. The study was performed at the Blood Bank of Hospital General de Mexico which is a national concentration hospital and receives people from all the States. **Material and methods:** All applicants who attended Blood Bank between April 2005 and February 2009 were interrogated using a brief questionnaire to find out risk factors such as birthplace, residence and journeys made, dwelling characteristics and coexistence with animals, history of blood

transfusions and donations, meet or have been bitten by the Reduviid bugs. Individuals detected with risk factors were serologically tested using ELISA and IFA. **Results:** Seventy nine otherwise eligible donors, 68 male and 11 female, were disqualified because of their answers on the questionnaire. Of this group, 70 % came from endemic areas (Veracruz, Guerrero and Oaxaca), 95% met the vector bug, 40% have seen it inside his home, 27% out or close to their houses and 21% in their work place and 17% declared have been bitten by it. Only 6% (5/79) of the individuals were confirmed positive to ELISA and IFA tests and were referred for medical care. **Conclusions:** Due to the low percentage of serologically confirmed individuals we conclude that presenting some risk factors for Chagas disease is not reason enough to disqualify blood donor candidates, even when more than 85% of the blood banks in Mexico perform screening for Chagas disease. It is important to highlight the necessity to perform serological screening and confirmatory test for Chagas disease. Currently a project is been carried out at blood banks in the state of Oaxaca where we found a seropositive blood donor candidate. **Acknowledgement:** DGAPA/PAPIIT-IN204710 **E-mail:** alruiz@unma.mx

Chagas020- Chagas disease in Mexico

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Introduction: Studies in Mexico on Chagas disease are insufficient to assess their situation and define its importance in public health. The work carried out show objectives, methodologies and different criteria, making it difficult to compare the results. In relation to the transmitters, the country has diverse geography and climate that has allowed the distribution of a wide variety of genera and species of triatomines in and around dwellings. **Material and Methods:** In order to add contributions to the knowledge of this disease, is a review with the results of investigations of their own and other groups working in Mexico. **Results:** During the period between 1998 and 2010, there is seroprevalence in rural areas from 0.9% to 44%. In blood banks seroprevalence between 1978 and 2010 ranges from 0.01% to 17%, now more than 85% of blood banks perform screening tests for Chagas. The states of Veracruz, Oaxaca and Morelos have submitted the largest number of cases and disease between 2003 and 2011. In the period from 1998 to 2007 and from 25 years of age, have increased deaths from this disease. The highest number of deaths in the age group over 65 years. Eight genera and 32 species of which have been reported involved in the transmission of the parasite have been identified, eleven species of triatomines peridomiciliados (over 50% of Mexico) and 2 species of triatomines intradomiciliados. 4 genera and nineteen species have been reported in wilderness, with predominance of the genus *Triatoma*. The distribution of the phyllosoma complex occupies a strip from northern Mexico to the south on the Pacific coast. **Conclusions:** Information sources for this work are UNAM, CNTS and the Ministry of Health of Mexico. The results show that studies with varying methodologies and criteria, does not provide an objective comparison of these, with such varied information it is difficult to determine objectively the extent of the problem of this disease in the country. On the other hand, control of the transmitters is difficult due to the existence of a large number of genera and species of triatomines peridomiciliados; with respect to the transmitters' greater interest in controlling *Triatoma barberi* and *Triatoma dimidiata* should be placed intradomiciliados. Mexico has a diverse geography, climatic and socio-cultural in rural and suburban areas to be considered for studies of Chagas disease in the country. **Acknowledgement:** DGAPA/PAPIIT-IN204710. **E-mail:** pazmar@unam.mx

Chagas021- Epidemiology and perspectives of Chagas disease Control in Mexico

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Introduction: As part of an Epidemiological Intelligence practice, we here present an analysis of the revised literature on Chagas Disease in Mexico which includes epidemiological aspects of the last 15 years. We show officially notified data on morbidity and mortality, aiming to describe the actual state of the disease from an epidemiological point of view, including observations made by groups of scientists

and experts that have analyzed the problem. The goal of this exercise is to discuss the data with groups of experts and authorities responsible of making decisions. **Materials and Methods:** The national and international literature published on epidemiological aspects of Chagas Disease in Mexico during the last 15 years (01-1997 to 01-2012) was analyzed using databases from Chagmex, Pubmed and Lilacs. Additionally, 5 groups of researchers, including the head of the National Programme, were interviewed with the goal of obtaining their opinion regarding the challenge and perspectives of disease control. **Results:** Of the 119 publications existing on the topic, 33 dealing with epidemiological aspects and disease control were chosen and are summarized as follows: 30 states of the Mexico have notified the disease, although 64% of the cases are concentrated in 5 states (Veracruz 22.5%, Morelos 17%, Oaxaca 11.4%, Yucatán 6.65% and Chiapas 6.44%). In these states the accumulated incidence is more than twice the value of national levels (0.295 for 100,000 habitants) showing a tendency to increase. This has been associated with a closer attention paid to the notification of the disease and the results are in accordance with national seroepemiological surveys that show the most affected age group ranges from 25-44 years and corresponds to 44% of the cases. The mortality data show that 87.5% of the deaths are concentrated in 5 states including Oaxaca 53.2%, Guerrero 18.6%, Chiapas 6.8%, Distrito Federal 5.3% and Veracruz 3.7%. **Conclusions:** The most conspicuous observations obtained from these publications include the lack of a National Programme with adequate financing that leads to reduced attention to the disease. Additionally, scientists are dissociated from Federal and State authorities responsible for making decisions, which leads to an inadequate epidemiological surveillance and disease control. This is associated with an insufficient budget approved by the National Programme of Vector Born Diseases for the disease control. There is important national scientific evidence on all aspects of the disease generated by a vast number of scientists, yet the overall impact on public health decisions towards this neglected disease, that primarily affects the poor population, is absent. It is clearly stated that alliances must be made between governments and scientists to control this disease that causes a great disease burden for disability and premature death; however is not official recognition of this health problem, research groups continue to develop methods to implement a surveillance and control efficient program. **E-mail:** vacmarin@hotmail.com

Chagas022- Synanthropic reservoirs of *Trypanosoma cruzi* cluster domestic vectors in geographic co-existence networks

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Introduction: The role of interspecific interactions in structuring communities at fine spatial scales is well documented, but the signature of species interactions at coarser spatial scales is unclear. Network analysis offers an important tool for understanding and visualizing biotic interactions given the difficulty to track a large number of ecological interactions, and hence the need to use alternate data sources. Triatomine species are obligate blood feeders on terrestrial/arboreal mammals, providing the opportunity for *Trypanosoma cruzi* transmission. Since vector-host adaptations are the fundamental route for Chagas disease risk, describing and understanding bug blood-feeding patterns across vertebrate hosts is important for *T. cruzi* epidemiology, and monitoring its circulation. **Materials and Methods:** We developed interaction networks from distributional datasets between triatomines and mammals in Mexico. Our database comprises 2967 georeferenced data points of 38 triatomine species and 62629 of 499 mammal species. Potential interactions were estimated using a data mining approach and their results and visualizations were analyzed. Network topology was constructed using the kamada-kawai algorithm and connectivity metrics were calculated using one script for R v2.14.0 statistical computing software developed by LVB. **Results:** When ranked by interaction importance, mammals confirmed as *Trypanosoma cruzi* reservoirs, (reported from independent studies) are located in the first quartile of vector hosts, thereby validating our interaction models. The most prevalent top ranked mammals are frugivorous bats, agricultural and domestic rodent pests, and other medium size synanthropic mammals. There is a regional cluster arrangement of the triatomine species in the networks, showing that the most important hosts for groups of Chagas vectors are more connected between them than expected at random. Node degree differed in vector species' complex. The *protracta* complex presented stronger but

few links, while *phyllosoma* and *dimidiata* complexes had the highest number of significant links, albeit the edge value was low. **Main conclusions:** Synanthropic mammals and domestic vectors play an important role in structuring the network topology for *T. cruzi* flow. We found a biogeographic association between Triatominae-mammal interactions, which creates distinct vector clusters connected by synanthropic species. The analysis of network topology will provide information regarding selection and evolutionary patterns of *T. cruzi* lineage dispersion, and regarding potential intervention strategies. **E-mail:** lbarra.cerdena@gmail.google.com

Chagas023- *Trypanosoma cruzi* in domesticated and wild mammals in a natural park landscape from the Yucatan Peninsula

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Introduction: The differential role of domestic, livestock and wild mammals as reservoirs of *Trypanosoma cruzi* and for the transmission cycle of Chagas disease (CD) to humans in Mexico is unknown. The present study in Zoh-Laguna, Calakmul, Campeche, analyzes the role of wild and domesticated mammals for parasite flow within a composite landscape which includes *Triatoma dimidiata* and a human community with reported cases of CD, modified crop/livestock ecotone areas and conserved sylvatic fragments. **Material and Methods:** Wildlife (Rodentia, Marsupialia, and Chiroptera), livestock (cattle, equines, goats, sheep, pigs) and domestic pets (dogs and cats) were collected or sampled from domestic, ecotone and conserved sylvatic habitats in/around Zoh Laguna, over two seasons. Wildlife was taxonomically identified, and tissues preserved in ethanol, while blood samples from domesticated animals were preserved in guanidine. Samples were extracted and presence of *T. cruzi* analyzed using mtDNA markers and all samples analyzed for DTU using mini-exon markers. Parallel bug collections were conducted for blood meal and *T. cruzi* infection analysis. **Results:** A total of 303 wildlife specimens were collected from 19 species (9 bat, 3 marsupial, 7 rodent) in the rainy season, and 114 specimens from 18 species in the dry season (2 bat, 1 marsupial and 1 rodent additional/unique). Five bat, *Artibeus jamaicensis* (Aj), *A. lituratus*, *Sturnira lilium* (Sl), *S. ludovici*, *Dermanura phaeotis*, and one rodent species (*Heteromys gaumeri*) were collected across all 3 habitats, although only Aj, Sl, and Hg were infected, with infection prevalence significantly higher in the sylvatic habitat for Aj, while in the ecotone, for Sl. *Sigmodon hispidus*, only collected from ecotone and domestic, was also infected. Infection in 389 livestock and pets representing 60.7% of all domesticated animals was variable according to species, ranging 1.6% in sheep to 13.3% dogs. Despite high human (4.1%), dog, and bug (36.3%) infection prevalence in the domestic habitat, none of the 4 marsupial species were infected. **Conclusions:** The Zoh Laguna landscape demonstrated high bat, rodent and marsupial diversity, although few rodent and no marsupial species across habitats were infected. The same two bat species previously reported infected from sites in Chiapas, Aj and Sl, were also infected in Zoh Laguna, albeit with lower prevalence. The relatively high mammal host diversity may be playing a role in the dilution of *T. cruzi* species' prevalences, which is an additional important consideration for species diversity conservation. **E-mail:** jramsey@insp.mx

Chagas024- American Trypanosomiasis in the Venezuelan Amazon, the last frontier

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Introduction: In spite that Chagas disease was discovered 100 years ago, recently in Venezuela it begins to know its details in the Amazon region. The project "Eco-epidemiologic studies on Chagas disease at the south of the Orinoco" managed to demonstrate the presence of *Trypanosoma cruzi* in triatomines, animals and humans. **Material and Methods:** A cross-sectional study on Yanomami in the Venezuelan Amazon state communities and creole and indigenous Panare individuals in Bolívar State was conducted in Venezuela. Blood samples were collected to determine the presence of Ig-G anti-*Trypanosoma cruzi* in serum using ELISA, HAI and IFI and extraction of DNA with Chelex resin of blood for *Trypanosoma cruzi* DNA amplification to achieve k-PCR and PCR-sat protocols. In these same communities, wild, domestic and synanthropic mammals were surveyed through xenodiagnóstico and applied PCR DNA-sat and DNA-k for molecular diagnosis of *T. cruzi*. Triatomines were searched in palm trees, houses, collected with community participation. They were identified taxonomically and the presence of *T. cruzi* or DNA in feces by optical microscope observation and PCR was determined. Fresh material was inoculated in mice and parasites were isolated in cultures for molecular characterization. **Results:** A total of 425 individuals were assessed. Seroreactivity to *T. cruzi* by two different serological tests was found in 20 individuals (4.7%), all from Hasüpiwei community. Two of them were positive by PCR. All xenodiagnosis (n=58) were negative. In this community, it was determined the presence of *T. cruzi* DNA by PCR-sat in a dog. 15 triatomines were collected in the Amazon state from which 3 *Panstrongylus geniculatus* were positivity at the fresh exam. In the Upper Orinoco basin one *P. geniculatus* was positive by PCR. In Bolívar State 4 *Triatoma maculata* were collected, being two positive. The latter were characterized as TcI. **Conclusions:** This is the first study showing *T. cruzi* infected human population in the Venezuelan Amazon with impact on the Yanomami indigenous ethnic group. It was also demonstrated triatomines and reservoirs infected with *T. cruzi* as well as the presence of the genotype TcI in the Venezuelan Amazon. These findings stimulated the creation of the regional program on prevention and control of Chagas disease in the Amazon state. **E-mail:** ozonoya6@yahoo.com

Chagas025- Eco social conditions and infection by *Trypanosoma cruzi*, etiological agent of Chagas disease in human populations of José Gregorio Monagas municipality, Anzoátegui-Venezuela

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Introduction: Chagas disease in Venezuela is found in 18 entities, with 34% of 28 million of the national population at risk. We proposed to determine environmental and social conditions in conjunction with the presence of the etiological agent of Chagas disease, in humans of the José Gregorio Monagas municipality in Anzoátegui State- Venezuela, during 2010-2011. **Materials and methods:** Socio-ecological index were studied by socio demographical and semi open questionnaire to residents. *T. cruzi* infection was studied by antibodies detection using Enzyme-linked immunosorbent assay (ELISA) and Kinetoplasto minicircle *T. cruzi* DNA identification in human blood samples collected in Whatman 1 filter paper. Each county was geo referenced and eco-social, parasitological and geographical parameters were performed by Arc Gis software. **Results:** A total of 13 dwellings/town, in 9 counties, were visited with informed consent, covering 34 % of dwellings/community, in 4 sampling events of 500 hours/person for 5 persons. Counties were identified in savannah and *morichal* biomes, with 80 to 100% of bahareque houses. No significant differences in poverty rates and/or overcrowded were observed in *bahareque* houses with respects to semi-built houses. A total of 120 sampled human were studied with 17.5 % sero positives and 15% with evidence of *T. cruzi* DNA in their blood. The sero prevalence and presence of *T. cruzi* DNA in children under ten years old were 13 %, indicating active transmission. **Conclusions:** The regions revealed a major poverty index in relation to Anzoátegui State, with use of natural resources as element of the zoonoses inserted in dwellings building. *T. cruzi* infection is present in a significant percentage of the human population, with active transmission and forced migration by cause of economic activity replacement towards the oil resource. The exposed elements lead to consider the existence of elements of risk for Chagas disease transmission of in the counties. **Keywords:** *Trypanosoma cruzi*,

Ecology, social conditions. **Financial support:** Project Red Misión Ciencia N° 2008000911-6 and Estratégico FONACIT. No 2011000470. **E-mail:** herrerleidi@yahoo.com

Chagas026- Evidence of recent transmission of Chagas disease in humans from rural areas of Cojedes State, Venezuela.

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Introduction: Chagas Disease is produced by the parasite *Trypanosoma cruzi* and transmitted by blood-sucking insects (Reduviidae. Triatominae). This disease may present an acute phase, followed by recovery or the establishment of the chronic phase with an unpredictable clinical course with cardiovascular compromise that can result in death. Immunological methods for detection of anti-*T. cruzi* antibodies are suitable for the diagnosis of Chagas disease. The detection of anti-*T. cruzi* antibodies in children under ten years old may be indicative of recent transmission. The purpose of this study was to determine the seroprevalence of Chagas disease in rural communities of Cojedes state, especially in children as evidence of possible recent transmission of the disease in the studied areas. **Material and methods:** 1107 samples were taken from individuals of 6 rural communities (Nuevo Mundo, Las Rosas, Solano, Valle Hondo, Hacienda Vieja, and Tierra Caliente) of Cojedes State. With each serum sample was performed the ELISA, the indirect hemagglutination (IHA) and the indirect immunofluorescence (IFI) for the determination of anti-*T. cruzi* antibodies. Were considered positive those with 2 or more positive tests. **Results:** We found an overall seroprevalence of 14.7% (163/1107), being the community with the lowest seroprevalence Hacienda Vieja with 9.5% and the highest Las Rosas with 23.1%. There were no significant differences in seropositivity regarding to sex of the individuals studied. In terms of age, although more than 70% of the positives were older than 40 years, 6.2% of positives detected were children under 10 years, especially in Hacienda Vieja and Tierra Caliente communities where the seroprevalence in children under ten years old were 13.2% and 14.3% respectively. **Conclusion:** The detection of anti-*T. cruzi* antibodies in children under ten years old indicate recent transmission in the study areas. **Financial support:** Project: Proyecto en Red Misión Ciencia N° 2007001442 and N° 2008000911-6, Proyecto FONACIT N° G-2005000827 and Ayudas Menores CDCH-UC-0440-10 y 0450-10 Universidad de Carabobo. **Email:** elizabeth.ferrer@gmail.com

Chagas027- Prevalence of Chagas Disease in the Bolivian community in Palma de Mallorca, Spain

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Objectives: To estimate the seroprevalence of Chagas disease in Bolivian residents in Mallorca, Spain, and compare sociodemographic and risk factors. **Material and Methods:** A descriptive cross-sectional study in the Mallorca Health Area. Included Bolivian patients > 18 years old, assigned to 2 basic health areas were selected by systematic random sampling health care database and were recruited by telephone. **Measurements:** In consultation with interview of sociodemographic variables, risk factors and symptoms for Chagas. Case was confirmed after 2 positive ELISA test. If results were positive or inconclusive, the sample was sent to the National Microbiology Centre for confirmation. **Results:** 251 subjects were included, 57.8% were men, originally from rural areas. Mean age 34.6 years (SD = 9.3). 48 cases were positive (19.1%). All positive cases were confirmed by two different techniques (no false positives). There were no statistically significant differences for any of the heart or digestive symptoms, in subjects with positive results in relation with the negative. We observed a higher prevalence in those who had lived in rural areas, in houses of adobe, and those who had a family history of Chagas. No significant differences were found by age or in those who had received blood transfusion. **Conclusions:** We found a

high prevalence in the Bolivian population and especially in those with the usual risk factors for this disease. Should be consider to make screening for Chagas disease on this population to prevent progression and to include it on the protocols of our health system. **Supported by:** Doctors of the World Balears and the Mallorcan Family Medicine Trainee Unit **E-mail:** p.favila@gmail.com

Chagas028- Colonization of *Panstrongylus geniculatus* (Latreille, 1811) in an endemic area of Chagas disease in Colombia

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Introduction: Colombia has about 10% of its population in risk of contracting Chagas disease in endemic areas, with up to 436,000 infected in 2005. However it has considered that because of the prevalence of the disease and entomological index some states, they enter are the processes of interrupting transmission of *T. cruzi* domestic by vector *R. prolixus*. **Materials and methods:** The municipality of the state of Casanare Tamara, located at 5°49'58.77"N and 72°09'42.05"O, is one of the prioritized in that process and it's need a baseline to define indicators to evaluate during the pre- and post-intervention. During the month of November 2011 to February 2012 has given the following entomological index: Index of Dispersion (ID), Index of Infestation Domestic (IID), Peridomestic Infestation Index (PII), Natural Infestation Index (NII), Colonization Index (CI), based on WHO. The taxonomic identification of adult Triatominae was made by the keys of Lent and Wygodzinsky (1979), nymphs differed among species by the insertion of antennae face and reared to adult. The NII was performed by analysis of gut contents of specimens at the Medical Entomology Laboratory of Casanare (LEM) and was confirmed with the species by the INS. **Results:** Our survey found three species: *R. prolixus*, *P. geniculatus* and *R. pictipe* (only two adults found) with the entomological indices for the first two species: ID=44.65 and 29.79%, IID=2.52 and 2.19%, PII=0.34 and 0.0%, NII=1.8 and 0.0% and CI=19,64 and 31.82%. **Conclusions:** We confirm the domiciliation of *R. prolixus* and its natural infection with *Trypanosoma* spp., presence of exuviae and nymphs in artificial habitats. Besides the confirmation of the species *R. pictipe* found recently in the state of Casanare and new register for Tamara, attention must be paid to the presence of nymphs in domestic (dorms and beds) indicating colonization by *P. geniculatus*, the latter information of extreme importance because in Ecuador, French Guiana, Venezuela and the Amazon Brazil it has been found in peridomestic attracted by light and blood from pigs and humans at night. The results are related to the findings in the city of Amalfi in Antioquia - Colombia where *P. geniculatus* was found into the domicile. However, this species have not found naturally infected it's necessary to be considered the surveillance and control, evaluate the food habits, the ability to produce metacyclic parasites, variability genetic, reproductive biology, synanthropic, time of defection, in order to define its epidemiological importance for the transmission the transmission of disease and possibility of domiciliation. **E-mail:** diegocam2003@yahoo.com

Clinical and Pathogenesis

Chagas029- The Indeterminate Form of Chagas Disease: Impact of Inadequate Lifestyle as Risk for Cardiovascular Disease

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Chagas disease affects 8 to 10 million people worldwide, and it is estimated that 40 thousand new cases occur every year. In the General Infectious Diseases Outpatient Unit of the Botucatu School of Medicine, more than 400 patients are followed up, most of whom have the indeterminate form of the disease. This study aimed at evaluating the impact of inadequate lifestyle as a risk factor for the development of

cardiovascular disease in this population. Clinical, laboratory and anthropometric (bio impedance) data were collected. Sixty-one individuals participated, of whom 37 were females (56 ± 8.2 years), and 24 were males (55 ± 6.5 years). The participants' family history showed high incidence of cardiovascular diseases (78%), *diabetes mellitus* (41%) and arterial hypertension (60.6%). Although the participants were given nutritional orientation, only a small number followed the dietitian's prescription, and 50.8% reported to perform regular physical activity. Obesity (BMI > 30) was observed in 46% of females and in 41.7% of males. The percentage of fat mass was higher than 25% in 90% of the participants. Increased waist circumference was found in more than 50% of male and female individuals, and dyslipidemia in 81% of females and in 67% of males. Reduced HDL-cholesterol levels and hypertriglyceridemia were more prevalent in women, respectively 40.5% and 51.3%. Females also showed a higher incidence of arterial hypertension (48.6%), and males showed a higher incidence of hyperglycemia (33%). The metabolic syndrome was diagnosed in 24 individuals (22.9% females 16.4% males), and of these, 6% showed the five factors in the syndrome. The use of the logistic regression model, considering the metabolic syndrome as a response variable, found a positive correlation between HDL reduction and increased triglycerides in the studied population. It is noteworthy that the obtained data indicate high risk for cardiovascular disease, particularly increased waist circumference, HDL-cholesterol reduction and increased triglycerides. The alterations observed can be modified by lifestyle changes, which include a balanced diet and daily physical exercise practice that would lead to fat mass reduction, especially of that located in the abdominal region. Considering that the metabolic syndrome has a strong relation to cardiovascular disease development, it was concluded that, in addition to clinical evaluation, it is important to develop the awareness of patients with the indeterminate form of Chagas Disease to the need for a healthy lifestyle in order to prevent the onset of such pathologies. **Keywords:** Chagas Disease, Metabolic Syndrome, Cardiovascular Disease **Financial Support:** FAPESP **E-mail:** navarro@fc.unesp.br

Chagas030- Trypanocidal Agents for Chronic Asymptomatic *T. cruzi* Infection: An Updated Systematic Review and Meta-analysis

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Introduction: Prevention of Chronic Chagasic Cardiomyopathy (CCC) by treating infected populations with trypanocidal therapy (TT) remains a challenge. Despite the enthusiasm for TT, uncertainty on its efficacy, concerns about its safety, and limited availability are barriers for a wider use of conventional agents. **Methods:** We systematically reviewed studies comparing the outcome of cohorts of seropositive individuals exposed to TT versus placebo or no treatment. We sought eligible studies in electronic databases (CENTRAL, MEDLINE, EMBASE and LILACS) up to April-May 2010. The search also included a Google search, hand search for references in review or selected articles, and expert files. Other than randomized controlled trials (RCTs), studies deemed inclusion when providing data on either mortality or progression of CCC, after at least 4 years of follow up. Groups of two reviewers independently selected eligible studies, extracted data and assessed quality, with a referee resolving discrepancies. We defined outcome data as parasite-related (positive serology, xenodiagnosis or PCR after TT) and patient-related (including efficacy outcomes such as progression of CCC and all-cause mortality, and side effects of TT). We report pooled outcome data as Mantel-Haenszel Odds ratios (OR) along with their 95% confidence intervals (CI), following the Random-Models approach. The I^2 statistics provided an estimate of heterogeneity across studies. **Results:** We included 13 studies involving 4229 participants (6 RCTs, n=1096, five of intermediate quality, one of low quality; 10 testing Nitroderivative agents Nifurtimox or Benznidazole, and 10 conducted in Brazil or Argentina). TT was associated with substantial, but heterogeneous reductions on parasite-related outcomes (positive serology, 9 studies, OR=0.19, 95%CI 0.09-0.40, $I^2=76\%$; positive PCR, 2 studies, OR=0.50 95%CI 0.27-0.92, $I^2=0\%$; positive xenodiagnosis 6 studies, OR=0.35, 95%CI 0.14-0.86, $I^2=79\%$). Efficacy data on patient-related outcomes comes largely from non-RCTs. Treatment with Nitroderivatives was associated with potentially important, but imprecise and inconsistent reductions in progression of CCC (4 studies, 106 events, OR=0.74, 95%CI 0.32-1.73,

I²=66%) and mortality (6 studies, 99 events, OR=0.55, 95%CI 0.26-1.14, I²=48%). The overall median incidence of any severe side effects among individuals receiving TT was 2.7%, and the overall discontinuation of this 2-month therapy in RCTs (5 studies, 134 events) was 20.5% (versus 4.3% among controls) and 10.4% in other five studies (125 events). **Conclusions:** The notorious efficacy of TT on parasite-related outcomes still requires confirmation in terms of patient-related outcomes. More geographically diverse RCTs testing newer forms of TT should help to a) estimate efficacy more precisely, b) explore the heterogeneity of results and c) allow a better efficacy/tolerance balance of conventional TT. **E-mail:** jvillar@unab.edu.co

Chagas031- Towards the understanding of Chagas disease pathogenesis based on cytokine profiles in Chronic and Indeterminate patients

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Chagas disease is caused by *Trypanosoma cruzi*, is an important problem in Latin America. The immunological mechanisms involved in Chagas disease pathogenesis are still not unraveled. The aim of this study was to find possible incrimination of cytokines associated to the susceptibility of Chagas' disease. This study included 50 *T. cruzi* positive serology patients from Bolivia and Colombia (categorized as cardiomyopathic, N=16; asymptomatic, N=33). We performed flow cytometry assays for 13 cytokines on human sera; we found a switch between anti-inflammatory and pro-inflammatory cytokines profiles. The asymptomatic patients showed higher frequency of anti-inflammatory cytokines and lower frequency of pro-inflammatory cytokines while the cardiomyopathic displayed higher pro-inflammatory and lower anti-inflammatory cytokines. The cytokines involved for the anti-inflammatory profile were IL-13, IL-5 and IL-10 and pro-inflammatory IL-2, IL-6, IL-9 and IL12. We suggest that regulation of these cytokines have an important role in the chagasic cardiomyopathy outcome. **E-mail:** c.poveda80@uniandes.edu.co

Chagas032- Lisophosphatidylcholine: an enhancer of Chagas disease transmission and pathogenesis.

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Introduction: Chagas disease is caused by the trypanosomatid protozoan *Trypanosoma cruzi*. Unfortunately, almost one hundred years later, specific and efficient methods to block transmission of this parasite or treat this disease still remain controversial. The current number of infected patients is 11 million, with 200,000 new cases and at least 21,000 associated deaths each year. Lisophosphatidylcholine (LPC) is a lysophospholipid released upon the action of secretory phospholipases A₂ (sPLA₂) on phosphatidylcholine associated to LDL particle. We have previously shown that LPC acts as an enhancer of *T. cruzi* infection, especially by inhibiting nitric oxide production (Mesquita et al. 2008). Here, we show that such bioactive lipid may play a role during chronic phase of the disease. **Material and Methods:** LPC levels were estimated by AZWELL LPC Assay Kit, using 5 µL of plasma from mice or human. **Results:** the levels of LPC during acute infection in mice are usually kept at 400 µM but at the day 14 after infection suddenly decrease to 200 µM. The evaluation of LPC levels in plasma of 46 human patients chronically suffering from Chagas disease shows that very different levels according Los Andes International Classification (gradual stages of disease severity: IA, IB, II and III) are present in tested individuals. This suggests a correlation not yet demonstrated with the severity of the disease. Curiously, 10 patients also suffering from different heart diseases with the same severity of the

stage III of Los Andes Classification presented a lower level of LPC in their plasma. Main conclusions: LPC may be an immunomodulatory of Chagas disease in both the acute and chronic phase of such disease. Such molecule may be either an indicator of disease progress or a modulator of its severity. These points must be clarified by future investigation. **E-mail:** iaciura@bioqmed.ufrj.br, maneto@bioqmed.ufrj.br.

Chagas033- Low levels of serotonin in Chagasi patients with megacolon is related to high concentration of inflammatory cells

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Abstract: Chagas' disease, (caused by *Trypanosoma cruzi*) affects 8-10 million people in the Americas, with an additional 40 million people at risk. It presents mainly as two clinical phases: acute and chronic. The chronic phase, in principle, can last for the patient's entire lifetime, beginning with the decline of parasitemia. It is defined by an initial absence of symptoms; however, several years after acute phase ends, some organs and systems may be injured. One of the chronic forms affects the gastrointestinal tract the main syndrome is represented by megacolon, leading to severe constipation and fecal retention. Recent reports about chagasic megacolon indicated that disturbance of immune system and enteric nervous system (ENS) has also been associated with this form development. Some substances act in both systems and fulfill a link between the nervous and immune systems. One of these substances is the serotonin. Serotonin or 5-Hydroxytryptamine (5-HT) makes up the group of biogenic amines (neurotransmitters) that also include the catecholamines (epinephrine, norepinephrine and dopamine). About 90% of serotonin present in the human body is produced in the intestine. We believe that the intestinal levels of serotonin may provide a balance between ENS and immune system. To evaluate whether serotonin intestinal levels are related with regulation of immune system, we investigated the relation among 5-HT expression and intensity of inflammatory process in colon samples from chagasic patients with megacolon. To perform this, we used, with confocal microscope, specific antibodies linked with immunofluorescent markers to measure the presence of serotonin, CD3, CD4, CD8 lymphocytes and CD68 macrophages. Our results indicated that chagasic patients with megacolon presents low levels of serotonin compared with non-infected individuals, and, the inflammatory process is intense in chagasic patients that showed the lower serotonin levels. We believe that serotonin act in the intestine as a regulator of intestinal inflammation, and some chagasic patients may be benefited by the use of antidepressive drugs that can replace the intestinal serotonin. **E-mail:**mfreitas@icbim.ufu.br

Chagas034- Investigation of total Timp-1 and TGF-β1 levels in serum from patients showing different clinical forms of the chagas disease

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Introduction: Endogenous tissue inhibitors of metalloproteinase (TIMPs) regulate degradation of EMC by inhibiting matrix metalloproteinase proteolytic activities. The cytokine TGF-β1 stimulates production of extracellular matrix proteins and induces cardiac fibrosis. Both animal and clinical studies show increased extracellular matrix components expression during acute and chronic phase in *Trypanosoma cruzi* infection leading to cardiac dysfunction and progressive fibrosis, a hallmark of Chagas disease.

Objectives: Here in, we investigated the total TIMP-1 and TGF-β1 levels in serum from Chagasic patients showing different clinical forms of the disease. **Methods:** Sera were obtained from Chagasic chronic patients whom had confirmed serology positivity by ELISA and IFI. The groups were divided into cardiac form (CARD) (n=16); indeterminate form (IND) (n=13) and controls (CONT) (n=11) according to clinical examination. Measurement of total TIMP-1 and TGF-β1 was performed using an ELISA kit (R&D

Systems) following the instruction's guide and the values are representing in pg/mL. **Results:** Our results shows that groups of patients in the CARD (5056.4 ± 2875.9) and IND (4850.7 ± 2566.9) had significantly higher circulating total TIMP-1 levels than the CONT (2611.8 ± 1020.1) ($p < 0.02$). Similar correlation was found for TGF- $\beta 1$; CARD (4219.6 ± 1124.1), IND (3920.9 ± 1552.0) and CONT (3374.8 ± 994.9) ($p < 0.03$). **Conclusion:** We demonstrated that Chagasic patients show higher circulating total TIMP-1 in the serum, for the first time, and corroborate, partially, with others colleagues finds that high levels of TGF- $\beta 1$ correlates with progressive fibrotic lesions in the heart. The overlap patterns of TIMP-1 and TGF- $\beta 1$ raise the possibility that they could be useful prognostic markers of disease progression. A prospective study on a large population of patients in the indeterminate form of disease would indicate whether the increase in TIMP-1 and TGF- $\beta 1$ levels could predict which patient in the indeterminate form of disease will progress to the cardiac form of disease. Financial support: CNPq. **E-mail:** alineeagp@hotmail.com

Chagas035- Indeterminate Form of Chagas Disease: Lipid profile and relationship with overweight and obesity

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Introduction: Chagas Disease is one of the main social and medical problems in Brazil as it presently affects approximately four to six million people. It is estimated that approximately 50% of the infected individuals are in the indeterminate stage or indeterminate chronic form of the disease. Other forms in the chronic phase are the cardiac and digestive forms. Dyslipidemia and obesity have been associated with Chagas Disease, and they have been regarded as a risk factor for the development of cardiovascular diseases in addition to hypertension and diabetes mellitus, among others. Lifestyle alterations, such as sedentariness and unhealthy eating habits resulting in overweight and obesity, may be factors that contribute to increase in these variables. Data published by WHO show that there are approximately 250 million obese adults, and at least 500 million overweight individuals, thus indicating that obesity is a world epidemic. Studies suggest that obesity significantly increases morbi-mortality. Therefore, this study aimed at evaluating the lipid profile and its relationship with overweight and obesity in patients with the indeterminate form of Chagas Disease. **Material and Methods:** Forty-three patients diagnosed with the indeterminate form of Chagas Disease and attended to at the Infectious and Parasitic Diseases Outpatient Unit participated in this study. Serum lipid profile tests (total cholesterol, LDL, HDL and triglycerides) were performed in the routine of the Botucatu School of Medicine (UNESP) laboratory, and anthropometric evaluation was conducted by a dietitian by measuring height using a stadiometer and weight using an electronic scale. From these indicators, the Body Mass Index (BMI) was calculated, and the patients were classified as overweight or obese. Statistical analysis was performed by the t test with $p < 0.05$. **Results:** Forty-three patients were studied, of whom 27 were females, and 16 were males. Such participants were classified as follows: 53.49% were obese, and 46.51% were overweight. The overweight and obese patients showed the following mean serum levels, respectively: total cholesterol $212.5\text{mg/dl} \pm 35.5$ and $189.8\text{mg/dl} \pm 25.9$; HDL $47.6\text{ mg/dl} \pm 11.6$ and $48.9\text{ mg/dl} \pm 23.8$; LDL $132.5\text{ mg/dl} \pm 33.7$ and $110.5\text{ mg/dl} \pm 22.38$ and triglycerides $178.0\text{mg/dl} \pm 54.1$ and $181.0\text{mg/dl} \pm 79.2$. In both groups, the relationship with serum levels of LDL were considered to be significant ($p < 0.0157$). **Conclusion:** Patients with the indeterminate form of Chagas Disease showed a high rate of overweight and obesity and increased levels for their lipid profile. These factors contribute to the onset of chronic and cardiovascular diseases. Further studies must be conducted in order to investigate these involved factors and prevent these diseases. **Keywords:** Chagas Disease, Body Mass Index, Lipid Profile. **E-mail:** canutri_@hotmail.com

Chagas036- IgG subclasses response against GPI associated proteins in Cardiopathy and asymptomatic Chagasic subjects

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The humoral immune response such as IgG antibodies are detected generally after a month post infection. Diagnosis of *Trypanosoma cruzi* infection after the acute phase is fundamentally done in serological means. It is known that 70-80 % of infected subject do not develop symptoms whereas the rest progress to cardiopathy. The objective of this work was to describe the IgG response against to epimastigote superficial antigens in cardiopathy (CCC) and asymptomatic (Asy) infected subjects. We studied 60 chagasic samples (30 asymptomatic and 30 with cardiopathy). The detection of seric IgG was done by ELISA (crude antigen) and IIF (epimastigotes). To obtain GPI- anchored proteins we used TX-114 2% protocol and verified by SDS-PAGE and WB. There were obtained S2, S3 and GPI fractions to be used in ELISA. The total IgG response against the three fractions was similar in both groups (CCC and Asy) with no statistical differences. However, the level of IgG1 against S2 and S3 fractions was higher in Asy than CCC group ($p<0.05$), but IgG4 against S2 fraction was negative in the majority of Asy group ($p<0.05$). The Avidity of IgG response against the three fraction in the presence of Urea in both groups showed no differences between CCC and Asy subjects. Conclusion: The used of parasites superficial antigens coupled to IgG1 and IgG4 subclasses may differentiate between CCC and Asy subjects. **E-mail:** victormonteon@yahoo.com.mx

Chagas037- Follow-up of patients in the indeterminate form of Chagas disease and progression to heart disease

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Introduction: Recent epidemiological changes promoted urbanization and increasing age of patients with Chagas disease. The rate of progression to chronic chagasic cardiomyopathy in these patients is not known. The aim of this study was determine rate of progression to chronic chagasic cardiomyopathy in Chagas disease patients without apparent cardiopathy. **Material and Methods:** a prospective observational follow-up study, comprising 581 patients with Chagas disease, without apparent cardiopathy, was followed from march/1990 to December/2005. All patients underwent clinical examination, ECG, chest radiography and echocardiography (ECHO) at admission (baseline) and followed-up with annual ECG and ECHO where indicated. The ECG analysis and definition of chronic chagasic cardiomyopathy were performed as recommended by Brazilian consensus Chagas disease (2005). Statistical analysis estimated incidence-density progression. In the comparison between progressors and nonprogressors were used chi-square tests, Fisher exact test, Student, t test and Mann-Whitney as indicated. Kaplan-Meier curves were constructed and compared by log-rank test. **Results:** The cohort average age was 44 ± 11 years, and 49% were male. After a mean follow up of 61 ± 42 months, 16 cases of progression at ECG were observed, resulting in cumulative incidence of 2.75% and an incidence-density of 0.55×100 patients-year. Patients with progression outcome had a higher follow-up time median (mean) (106 ± 39 vs 61 ± 42 months, $p<0.0001$). There were no differences between progressors and non-progressors when compared to age, sex, presence of diabetes and use of benznidazole. Progression was more frequent in hypertensive patients (4.7% vs 2%), approaching statistical significance ($p = 0.065$). ECHO was performed in 15 progressors, showing development of contractile dysfunction in 1 patient, preceded by electrocardiographic progression. **Main Conclusions:** In this urban cohort of patients with Chagas disease, without apparent cardiopathy, the rate of progression to chronic chagasic cardiomyopathy was low and less than previous studies conducted in rural and endemic areas. However, as compared to studies in urban and off-endemic area, our results are similar. The absence of re-exposure to the disease could explain this lower incidence of progression. **E-mail:** alejandro.hasslocher@ipecc.fiocruz.br

Chagas038- Ficolin-1 promoter polymorphisms are associated with susceptibility to Chagas Disease

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Introduction: Chagas disease (CD) is caused by intracellular *Trypanosoma cruzi* infection and is transmitted principally by blood-sucking triatomine insects. Circa 120 million people are currently at risk of infection and 300,000 new cases are estimated to occur every year, the majority of which in less favored social strata. Asymptomatic patients are serologically identified and referred to as 'indeterminate' (CDI). Although some individuals stay indefinitely in this phase, 27% of those infected develop the cardiac form of the disease (CDC), further 6% develop digestive damage (CDD), and a small percentage present the associated cardiac digestive form (CDA). In a previous study, we found high mannose-binding lectin (MBL) levels associated with the severity of CD cardiomyopathy. MBL and ficolins (FCN) both initiate the lectin pathway of complement upon recognition of pathogen-associated molecular patterns and activation of the MBL-associated serine protease 2 (MASP-2). Ficolin 1 (FCN-1, M-ficolin) presents high interindividual variability in serum levels. **Material and Methods:** In order to identify *FCN1* promoter single nucleotide polymorphisms (SNPs) that could be responsible for the susceptibility to Chagas disease, we genotyped rs2989727 (-1981G>A), rs10120023 (-542G>A) and rs10117466 (-144C>A) using PCR with sequence-specific primers (SSPs) in 206 CD patients (mean age 56.5 years [range 34-90]; 56% female, 44% male; 79% Euro-Brazilian, 18% Afro-Brazilian, 2.4% Amerindian, 0.5% Asian-Brazilian) from Southern Brazil, being 78 CDI, 70 CDC, 19 CDD and 27 CDA (12 were undefined). Informed written consent was obtained from all individuals and the study was approved by the local medical ethics committee. Interpretation was based on the electrophoretic pattern of the amplified fragments. Statistics was done using the Arlequin v.3.1 software package and Fisher's exact test. **Results:** Genotype distributions were in Hardy-Weinberg equilibrium. Four haplotypes were found: AAA (-1981A-542A-144A), AAC (-1981A-542A-144C), AGC (-1981A-542G-144A) and GGC (-1981G-542G-144C). Genotype AAA/AGC was more prevalent in CDI, than in CDC ($P=0.006$, $OR=0.133$ [$CI_{95\%}=0.07-0.72$]). The -542 and -144 polymorphisms were further predicted to modify transcription factor binding sites in silico which may reduce FCN-1 concentration at the membrane and in serum, discouraging *T. cruzi* infection. **Conclusions:** these points to the importance of *FCN1* promoter polymorphisms in modulating susceptibility to Chagas disease. The functional relevance of the *FCN1**AAA/AGC genotype was inferred and should be addressed in further functional studies. **Financial support:** CAPES/CNPq.

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Chagas039- *FCN2* genotypes in chronic Chagas disease patients from Brazil

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Introduction: Chagas disease (CD), caused by *Trypanosoma cruzi*, affects ten million people in Latin America where 90 million people are at risk of the infection. The pathogenesis of chronic CD is still poorly understood although there is a general consensus that the host immune response plays a determinant role in the development of the clinical forms and prognosis of CD. The complement system has been shown to be particularly important in the development and control of *T. cruzi* infection. It is known that *T. cruzi* activates the three pathways of the complement (classic, alternative and the lectin pathways) and that variants of the alternative proteins are associated with the disease evolution. Ficolins are pattern-recognition proteins which bind to specific pathogen-associated molecular patterns on microorganism surfaces, triggering the innate immune response through the lectin pathway activation. A fast binding of ficolin-2 on the surface of *T. cruzi* was demonstrated and it was observed that depletion of these molecules from the serum leads to failure in the parasite elimination, indicating that the lectin pathway clearly plays an important role in host defense. Three single nucleotide polymorphisms (SNPs) in the promoter region of the *FCN2* gene (-986, -602 and -4) have been associated with changes in the ficolin-2

serum concentrations. Given the important participation of ficolin-2 in the host defense, besides of the lack of studies on *FCN2* in chagasic individuals, we evaluate the influence of ficolin-2 levels and *FCN2* genotypes on the modulation of clinical expression of CD. **Material and Methods:** A total of 220 patients with the different clinical forms of CD and 196 healthy controls were genotyped using the Fluorescence Resonance Energy Transfer (FRET) based Real Time PCR assay. Three *FCN2* polymorphisms located in the promoter region (-986, -602 and -4) were determined. The quantification of serum ficolin-2 was performed in 153 patients and 71 controls by ELISA using commercial kits (Hycult® Biotech, Uden, Netherlands). Statistics was done using the statistical package for social sciences (SPSS) version 10.0. Ficolin-2 levels in the different genotypes were compared between groups using nonparametric Kruskal-Wallis and Wilcoxon signed rank test. Twotailed P-values less than 5% were considered significant and Bonferroni correction was applied when appropriate. **Results:** Genotype distribution was in Hardy and Weinberg equilibrium. Control subjects had higher ficolin-2 plasma concentrations than patients (medians: 4252 ng/ml vs. 2546 ng/ml, $p < 0.0001$), but there was no significant difference between genotype and haplotype distribution in the investigated groups. **Conclusions:** Decreased levels of ficolin-2 in CD patients may be due to the disease process itself, since ficolin-2 can bind to *T. cruzi* antigens and to the C-Reactive protein, which might affect circulating values. Otherwise, it may be due to *FCN2* variants not identified in the present study. Ficolin-2 levels and consumption perhaps could be used as markers of disease activity, but further experiments should be performed to prove this hypothesis. **Financial support:** CAPES and CNPq. **E-mail:** paola.imuno@ufpr.br

Chagas040- DARC genotypes and Chronic Chagasic Cardiomyopathy

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Introduction: Chagas disease remains relevant social and economic problem in many Latin American, about 100 years after its description. Chemokines may play an important role in cardiac damage observed in Chronic Chagasic Cardiomyopathy (CCC), which is most prevalent form of Chagas disease. DARC (Duffy Antigen/Receptor for Chemokine) can remove the excess of chemokine from sites of inflammation and is associated with various diseases. Thus, the objectives were to determine the DARC genotypes between patients and controls, comparing the patients' genotypes with the degrees of the disease severity and to investigate the relationship between gender and age of individuals. **Material and Methods:** 95 patients were evaluated (CCC, $n=74$; asymptomatic controls: $n=21$). The antibodies anti-*T. cruzi* was identified by ELISA. DARC Genotyping was performed by PCR-RFLP. The multiple logistic regression analysis, Fisher's exact test and Odds Ratio were used to compare the results. **Results:** The degrees of severity of moderate and severe CCC were more frequent in patients aged less than or equal to 60 years ($p=0.0098$). The male gender was a predictor factor for CCC (OR=3.28, 95% CI: 1.14-9.43; $p=0.0421$). The DARC genotypic differences between patients and controls were not statistically significant ($p>0,05$). **Main Conclusions:** Patients aged less than or equal to 60 years are at increased risk of developing the most severe forms of the cardiomyopathy. The male gender is a predictor for the CCC. Further studies are needed to determine the real importance of DARC in the pathogenesis of Chagas disease and the CCC. **Financial support:** FAPESP (Grant 2011/08075-4; 2011/19439-7); CAPES (DS). **E-mail:** apriolii@yahoo.com.br

Chagas041- ABO system and digestive Chagas disease: a FUT2 gene dependent association

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Introduction: One third of the individuals infected by *Trypanosoma cruzi* show distinct clinical manifestations of Chagas Disease (CD) (cardiomyopathy; gastro-intestinal). Despite of the comprehension of CD, there are no genetic markers indicating what form of the disease will occur. Previous reports evaluated the ABO system in CD but did not explore the influence of *FUT2* gene which controls the expression of ABO antigens in non hematopoietic tissues. The aim of this study was to verify the combined effect of *FUT2* gene and ABO system in CD. **Material and methods:** 240 patients were enrolled (cardiomyopathy: n = 120; gastro-intestinal: n = 120). The *FUT2* genotyping and the ABO phenotyping were identified by PCR-RFLP and hemagglutination methods, respectively. The qui-square test, the exact Fisher's test, and the Odds Ratio were used to compare the proportions. **Results:** The differences between cardiomyopathy and gastro-intestinal CD were not statistically significant in relation to *FUT2* gene (χ^2 : 1.141; DF: 1; p=0.2854) and ABO phenotypes (χ^2 : 1.855; DF: 3; p=0.6031). However, when these two markers were analyzed in combination, the differences were statistically significant for gastro-intestinal CD (χ^2 : 9.961; DF: 3; p=0.0189) and associated with the B and AB phenotypes (OR: 10.969; CI 95%: 1.415-85.022; p=0.0114). **Main Conclusions:** The role of the *FUT2* gene and the ABO system in CD remains unclear and there are no evidences that ABO antigens act as receptors for *T. cruzi*. The *FUT2* gene controls the expression of ABO antigens in the gastro-intestinal tract which are structurally distinct from those expressed in the heart tissues. Maybe these differences increase the risk for CD in the gastro-intestinal tract, especially among those from B and AB blood groups. In conclusion the expression of ABO antigens under the control of the *FUT2* gene in the gastro-intestinal tract influences the clinical manifestation of CD. **Financial support:** FAPESP Grant 2011/08075-4; CRB and APO are supported by Brazilian Ministry of Education - CAPES; AVSC is supported by FAPESP Grant 2011/19439-7 and Biotechnology Research Institute, Auckland University of Technology - AUT. **E-mail:** cassiarubia.b@gmail.com

Chagas042- Attempt to associate clinical manifestations of Chagas disease with *Trypanosoma cruzi* minicircle kDNA mutations at specific loci

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Introduction: Outbreaks of acute Chagas disease has been reported in the Pará State, BR, and these cases have received medical assistance at the Gaspar Vianna Hospital. In this study we documented clinical findings, and attempted to associate those physical manifestations to the kDNA mutations present in parental and progeny. **Study population and methods:** 109 individuals belonging to four families from Counties of Barcarena and Breves, showing acute *T. cruzi* infections, comprise the study group. Family members were subject to ECG and 24 h Holter recording, echocardiograph, and ergometric exams. The DNA extracted from those cases was subject to PCR with specific nDNA and kDNA primer sets. All the cases showing kDNA amplification products were subject to *tp*TAIL-PCR and the amplicons were cloned and sequenced. Then, results of clinical exams were analyzed aside the genetical markers, aiming at statistical association of the clinical findings to the kDNA mutations in specific loci in the human genome. **Results:** Among 109 study cases, 82 yielded nDNA and kDNA amplicons, but 19 cases yielded kDNA only. Interestingly, among 101 cases with positive kDNA marker, 41 had normal ECG, and the remaining showed abnormalities such as sinus bradycardia, left ventricle repolarization disturbance, ventricular premature contractions, and branch blocks. 27 ecodopplercardiograms were normal but 11 cases had mitral and tricuspid valves alterations, ectasia of the aortic arc, insufficiency of pulmonary and tricuspid valves, and pericardial effusion. Additionally, average maximal VO₂ and MET recorded among the kDNA positive cases were, respectively, 38,87(±14,51) ml/Kg/min, and 10,95 (±08). These results are slightly different from those obtained in the control group (p> 0.05), but there was a significative difference in four cases showing positive kDNA markers. The data showing association between the clinical findings with kDNA mutation loci will be presented in the Poster. **Main conclusions:** Regardless of 29 cases in the study population had presented symptoms of acute Chagas disease, the clinical exams in 101 family members yielded similar results as those obtained in family members showing kDNA positive marker. The result suggests that with exception of four cases showing complete right bundle branch block and mild

cardiomyopathy, all the remaining cases had the intermediate chronic infections and no disease. **E-mail:** adrijbag@gmail.com

Chagas043- Dual chamber pacemaker implantation in a patient treated by the project Pharmaceutical Care to the Chagasic patient of the Ceará state:A case report.

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Introduction: One of the main manifestations of Chagas disease (CD) is cardiac involvement. In the chronic phase may occur Brady arrhythmias, heart failure as well as develop the substrate for ventricular arrhythmias, this happens as a result of degeneration and myocardial fibrosis. Sudden death and heart failure are major causes of death in these patients. **Methods:** Female patient, 38 years old, suffering from Chagas disease. Since 2005, shows RBBB (right bundle branch block) evidenced by ECG (electrocardiogram conventional). The treatment was started with Benznidazole in 2006 (the Laboratory of Research in Chagas disease) and continued with clinical follow-up at the Cardiology Ambulatory of the Hospital Universitário Walter Cantídio (HUWC). **Results:** The electrocardiographic results in the following years were: RBBB, VES (ventricular extrasystole), sinus bradychardia (2006), RBBB, sinus bradychardia (2007), RBBB (2008). A Holter Performed (2008) showed breaks (≥ 2.0 seconds) and isolated ventricular arrhythmias. Given these results and clinical evaluation of the patient, the attending physician asked the patient to the hospital to perform a pacemaker implantation. The dual chamber pacemaker implantation was successfully performed in May 2008. The patient continued to be monitored by the cardiology ambulatory of the HUWC. **Conclusions:** According to these results, we can say that although the patient had presented a right bundle branch block before the start of treatment, the Benznidazole was unable to prevent the development of Chagas heart disease to more severe forms. **E-mail:** erlanefreitas3@hotmail.com

Chagas044- Series of cases of chronic Chagas disease, autochthonous in the Brazilian Amazon state of Pará

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Introduction: chronic Chagas disease (CHD) in the Brazilian Amazon has few records of autochthonous cases. By the year 2010 were reported only five cases with chronic infection. **Material and Methods:** We present the case report of three patients with CHD in Pará, one with fatal outcome. **Case 01:** A female patient, aged 36, born and raised in Cameta-PA, diagnosed by ELISA serological tests in 2008: Reagent, indirect immunofluorescence 1:80, Rx Thoracic enlarged cardiac silhouette with pulmonary congestion, electrocardiogram AVB first degree, bundle branch block right, anteroseptal inactive area; echocardiogram (ECHO) with severe ventricular dysfunction (EYF 22%), mitral valve regurgitation, aortic and tricuspid regurgitation, mild pericardial effusion, and apical thrombus. Holter monitoring was performed with episodes of NSVT. She evolved with refractory heart failure and death due to ventricular arrhythmia. **Case 02:** Patient 33, male accompanied the cardiology clinic, Serology: ELISA reagent, IFI 1:60; chest X-ray, an enlarged cardiac silhouette due to increased right ventricular echocardiography with left ventricular dysfunction (55% EYF) dilatation and hypokinesia right ventricle, with episodes of continuous Holter 2:1 AVB, which subsequently reverted to sinus rhythm. Evolved with recurrent heart failure, sudden worsening of presenting episode and CHB requiring implantation of a pacemaker. **Case 03:** IHB 23 years male, born and raised in Para Waterfall Pirie, with positive serology for Chagas in

November 2008, Elisa Reagent, HA reagent IFA IgM 1/40 and IgG 1/320. Presented with fever, fatigue developed with signs of heart failure and hospitalized for compensation and treatment with benznidazole. Evolved to functional class I. ECG showed a first degree AV block and right bundle branch block. X-ray revealed an enlarged cardiac silhouette. When ECO depressed ventricular function (EYF 53%), moderate increase in left ventricular dysfunction (EYF 41%), Holter with episodes of atrial tachycardia, serology: ELISA reagent, indirect immunofluorescence 1:60. **Conclusion:** We draw attention to cases of dilated cardiomyopathy in the State of Pará, research is needed to investigate the etiology of these cardiomyopathies to the actual size of the DCC Brazilian Amazon. **E-mail:** dilmasouza@ufpa.br

Chagas045- Chagasi cardiopathy associated to active transmission at Sucre state, Venezuela

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Chagas disease is a serious Latin American health problem caused by the parasite *Trypanosoma cruzi*. Chagasic cardiopathy in the chronic phase is the only clinical manifestation present in Venezuela, although a lot of multi disciplinary studies carried out in order to eradicate the disease there is still no cure for this. Additionally Sucre state of Venezuela constitutes an interesting area of study because effective anti-Chagasic campaigns were eliminated in 1982 driven to alarming resurgence. In this sense the present work consist of an epidemiologic and cardiologic evaluation at San Pedro and Yaguaracual villages of Sucre state, Venezuela, located at 10°12'48'' NL, 64°25'00'' WL and 10°18'40'' NL, 64°21'15''WL, respectively. Immunodiagnostic by IgG ELISA, Direct Agglutination and Immunofluorescence followed by ECG, Echocardiogram, X Rays, Dynamic Holter and Stress Test were realized on serum positive individuals. New York Heart Association classification for clinical manifestations and risk factors was applied to define functional group. San Pedro seropositivity was 25.% (50/194) which 35% of them less than 20 year old whilst Yaguaracual seropositivity was 49% (22/45) which 18% of them less than 20 year old too, indicated an active transmission. Between San Pedro Chagasic cardiopathy patients 88% of them corresponded to Group I (Asymptomatic) and 12% belong to group II (with evidences of myocardial damage), comparable to Yaguaracual with 73% in the group I and 27% in the group II. These findings pointed out a strong active transmission of Chagas disease at Sucre villages studied associated with asymptomatic condition in the majority of affected individuals but in high risk to development myocardial damage in the future. **Financial support by:** FONACIT Project G-2005000827. **E-mail:** emarchanmarcano@yahoo.es

Chagas046- Chagas heart disease in Latin American immigrants in Alicante (Spain): a multicentre experience.

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Introduction: There has been a recent increase in the number of patients with Chagas' disease outside areas classically considered endemic for the diseases. The aim of this investigation is to describe the clinical profile of a series of heart Chagas' disease attended in (Spain). **Material and Methods:** This study was performed in four general hospitals in the Alicante province (Spain) between January 2002 and May 2011. Theses centres were not a referral institution for imported diseases, tropical medicine or international health. Laboratory diagnosis of *Trypanosoma cruzi* infection was made by two serological tests: ELISA or immunochromatographic and the second was a indirect immunofluorescence. The cardiac

involvement of Chagas disease was based on the presence of any of the ECG criteria and/or echocardiogram criteria. **Results:** A total 128 patients from 7 countries were *T. cruzi* infected. The main country of origin was Bolivia (n=101; 78.9%). The median of age was 35 years (range: 0-72); 63.3% were female. Clinical data were available for 108 patients, 27 (24.5%) had cardiac involvement. Twenty five (23.1%) patients showed electrocardiogram disorders: right bundle branch block (12.0%), supero-anterior hemiblock (6.5%), sinus bradychardia (4.6%), complex ventricular extrasistolia (3.7%), auriculo-ventricular block (2.8%), left bundle branch block (1.9%), and sustained ventricular tachycardia (1.9%). Echocardiography was performed on 67 patients, 14.9% was abnormal: 13.3% had wall movement abnormalities, 10.4% had a decreases ejection fraction, and 3% had apical aneurysm. From 27 patients with cardiac disorder, five illnesses had pacemakers, one patient was heart transplantation and one was waiting cardiac transplantation. There were no significant differences in cardiac involvement by sex, however the patients with cardiac disorder were discretely older (median: 39; range: 21-52) than illness without cardiomyopathy (median: 33,4; range: 0-72) (p=0.09). The patients with cardiac involvement referred more commonly syncope and dyspnea than illness without cardiomyopathy (32.0% versus 11.7%, p<0.001; and 28.0% versus 5.2%; p=0.029); after using multivariate logistic regression analysis, only the syncope was associated with cardiomyopathy (OR: 6.5; 95% CI: 1.5-27.1). **Conclusions:** The rates of cardiac involvement (25.4%) were slightly higher than observed in the Spanish literature (18.6% and 19%). **E-mail:** torrus_die@gva.es

Chagas047- Renal Involvement in Chagas Disease: A Retrospective COHORT of 41 Patients in Northeast Brazil

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Introduction: Chagas disease is an endemic zoonosis in South America caused by the protozoan parasite *Trypanosoma cruzi*. There are an estimated 300,000 new cases per year in the American continent. About 30% of infected people develop the disease and 23,000 die due to Chagas disease. The aim of this study was to investigate the correlation between Chagas disease and renal functional.

Material and Methods: This is a retrospective cohort with 41 patients with Chagas disease followed in a tertiary hospital in Fortaleza city, Northeast Brazil. The patients were identified using the registers of the reference laboratory for Chagas disease in the Faculty of Pharmacy, Federal University of Ceará. The sample was divided into two groups: patients with serum creatinine (Scr) <1.3mg/dL and Scr ≥ 1.3mg/dL and two subgroups, men and women. **Results:** Only two men patients had Scr ≥ 1.3mg/dL (4.8%). Of all patients, 51% were male and 66.6% were older than 40 years, while 80% of woman over 40 years. The mean serum creatinine value was 0.8±0.02mg/dL for men and 0.6±0.02mg/dL for women. The mean median serum creatinine value was 0.9±0.02mg/dL for men and 0.7±0.02mg/dL for women. There was statistically significant difference within groups. When comparing the subgroups there was statistically significant difference with p<0.0001. **Main Conclusions:** Renal dysfunction is an important feature of tropical diseases such as Chagas disease. According to the results found correlation between Chagas disease and kidney function. Although many studies have already established the development of the disease, there are no reports of renal involvement in Chagas disease. **Financial Support:** CNPq (Brazilian Research Council). **E-mail:** joyceblimaa@hotmail.com

Diagnosis and Treatment

Chagas048- The problem of cross-reactions in serologic tests used for diagnosis Chagas' disease and Leishmaniasis in endemic regions for both diseases. A systematic review.

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Introduction: The interpretation of the results of the diagnostic tests for Chagas' disease and Leishmaniasis in endemic areas for both diseases, as currently occurs in the Brazilian Amazon region, should be approached with great caution. The objective of this study was to do a systematic review of epidemiologic data on cross-reactions in serologic tests used for diagnosis of Chagas disease and visceral Leishmaniasis in endemic regions for both diseases. **Methods:** Electronic databases from Medline, LILACS, and Scholar Google were searched for the terms: "cross-reactions" and "diagnostic tests" and "Chagas disease" and "visceral Leishmaniasis". Seventy articles have been retrieved. From these, six articles included data from sero-epidemiologic cross sectional studies. **Results: Main results included:** Malchiodi (1994), using data from patients of Salta, Argentina, already drew attention to the cautious interpretation of serologic tests in endemic areas for Chagas' disease and Leishmaniasis. In their study, using immunoblot, the authors identified specific bands for each agent, and criteria for establishing the diagnosis of co-infection. Caballero *et al* (2007) makes an analysis of the main tests currently used for the diagnosis of Chagas disease, using as a gold standard Western blot for *T. cruzi*. The authors found that the sensitivity of ELISA tests showed 100% but the specificity ranged between 82.84% and 100% when cases of leishmaniasis were included and between 100% when 95.57% and cases of leishmaniasis were excluded. Another aspect highlighted by the authors is that the tests that use recombinant antigens or synthetic peptides are more specific than those using crude extracts of epimastigotes forms of *T. cruzi*. From a serologic investigation with dogs, in São Paulo, Troncarelli *et al* (2008), using indirect Immunofluorescence method, observed that 17% of the dogs presented both serology for *T. cruzi* and Leishmania, of which 1.8% with identical titles, 13.7% with titles for Leishmania above those of Chagas, and 0.85% titles were superior for Chagas; in 2.5% to positive serology for Leishmania and negative was for Chagas, and positive for Chagas and 1.7% negative for Leishmania. and conclude that in cases of possible cross reactions, other techniques such as PCR, should be used in diagnostic confirmation. **Discussion and Conclusion:** Epidemiologic and clinical correlation is fundamental for a proper interpretation of diagnostic tests for Chagas disease and Visceral Leishmaniasis, especially in endemic areas for both diseases. Efforts should also be made on use of more specific methods for distinguishing between the two diseases or confirm co-infection. **E-mail:** haroldodematos@gmail.com

Chagas049- New DPP® rapid point-of-care immuno-assay for detection of Chagas disease

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Introduction: Chagas Disease, a tropical disease caused by the parasite *Trypanosoma cruzi*, is transmitted by insect vectors. It can also be transmitted by blood transfusion or from mother to baby during pregnancy. Improved point of care test for Chagas is urgently needed, especially for countries with limited resources. DPP® Chagas Test is a joint developed product between Chembio USA and Bio-Manguinhos Fiocruz Brazil. **Materials and Methods:** The DPP® Chagas strip employs three novel recombinant antigens, immobilized as a mixture onto nitrocellulose membrane. The assay uses 10 µl of blood or serum sample to produce results within 15-20 minutes. The reading test is performed visually or

by a handheld reader. A collection of 275 known Chagas positive Brazil, 348 negative (148 Brazil and 200 USA) and 60 reactive with other disease (20 HIV, 10 Syphilis, 15 HCV, 15 HTLV) samples from Brazil and two commercial panels (PMT201, PMT202) from BBI Diagnostics, USA, were used for the DPP assay evaluation. Studies conducted in Brazil and USA. **Results:** DPP® Chagas test showed a 98.5% (271/275) Sensitivity on Brazil Chagas Positive Samples, 100% (27/27) Sensitivity on BBI Panel 202 and BBI Panel 203, 100% (148/148) Specificity on Brazil Blood Bank Specimen and 98% (196/200) Specificity on USA Specimen. DPP® Chagas test showed no cross-reactivity with 20 HIV, 10 Syphilis, 15 HCV and 15 HTLV positive specimens. **Conclusions:** The DPP® Chagas assay demonstrated an excellent diagnostic accuracy. This novel test can provide a convenient and reliable point-of-care test for use in primary health care clinics or in resource-poor settings. **E-mail:** edmilson@bio.fiocruz.br

Chagas050- Evaluation of lytic antibodies levels induced by *Trypanosoma cruzi* Complement Regulatory Protein (Tc-CRP) from different *T. cruzi* strains after experimental infection

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The *Trypanosoma cruzi* Complement Regulatory Protein (Tc-CRP) is a major epitope that elicits lytic antibodies during *T. cruzi* infection. In recent decades, biochemical and molecular studies classified Tc-CRP as a member of the *trans*-sialidase family and evaluated its application as a molecular marker in serological tests for diagnosis before and after treatment. However, so far, all studies were performed using the Y strain, prompting the necessity to assess the presence and expression of this protein in other strains, as well as its ability to induce lytic antibodies in different hosts. So, this study aimed to evaluate lytic antibody levels induced after experimental infection of BALB/c and C57Bl/6 mice using different parasite strains isolated from patients (Colombian, Hel and Y) and vectors (AQ-4 and CLBrener). Infections carried out using AQ-4 and CLBrener strains resulted in a subpatent parasitaemia on both mice lineages, whereas the other groups of infection, performed with Colombian, Hel and Y strains resulted in patent parasitaemia, but at varying levels. The evaluation of lytic antibody levels induced after parasites inoculation was carried out using the Tc-rCRP ELISA and LMCo tests. In general, there was an increasing trend in lytic antibody levels, since late phases of infection showed the highest levels, mainly in sera from BALB/c mice. Comparing the two techniques applied, direct correlation was detected only in samples collected during the chronic phase of infection when the lytic antibody titers resulted in greater reactivity of Tc-rCRP ELISA test. There was a low reactivity observed in sera samples obtained after infection with AQ-4 and CLBrener strains and these results are probably due to the low capacity of inducing lytic antibodies from proteins present on the surface of parasites inoculated or to the absence of patent parasitaemia. Sera related to groups infected by parasites from the Colombian, Hel and Y strains presented higher lytic antibody titles when compared to the previous two groups. Among the evaluated strains, Hel strain presented the highest capacity of inducing lytic antibodies during the chronic phase. In conclusion, this study showed that parasites isolated from vectors are less capable of inducing lytic antibodies than those isolated from patients in the evaluated mouse models. **Keywords:** Tc-rCRP ELISA, lytic antibodies, BALB/c mice, C57Bl/6 mice. **E-mail:** tathymarques@yahoo.com.br

Chagas051- Serological diagnosis of Chagas disease in IPB-LACEN/FEPPS/RS/BRASIL - Ten years of history

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The prevalence and distribution of Chagas Disease and its association with mortality are constantly changing as a result of the impact of control programs, rural migration and changes in socioeconomic

conditions of high-risk communities. Currently, the prevalence of Chagas Disease is estimated at about 13 million infected individuals, with three million of symptomatic cases and an annual incidence of approximately 200,000 new cases in 15 countries in Central and South America. The goal of this work is assess the prevalence of chronic Chagas infection in patients over ten years of serodiagnosis in IPB-LACEN/FEPPS-RS. We analyzed data from 19,077 patients who underwent serological diagnosis (ELISA and immunofluorescence) for Chagas Disease, from January 2001 to December 2010, of whom 7,683 were positive (40.27%). Excluding the year 2005, due to the outbreak of oral transmission occurred in Santa Catarina, with 7,258 cases and 1,119 positives (15.42%). During these ten years the total number was 11,819 patients, with 6,564 positives (55.54%). Between 2001 and 2006 there was a gradual increase in the number of patients of 1,171 patients in 2001 reached 1,828 in 2006, an increase of 657 (56.11%). From 2006 to 2010, there was an inversion: from 1,828 patients to 882, a decrease of 946 (51.75%). The positivity, irrespective of the year ranged from 49.73% to 59.82%. Patients who performed serological diagnosis of Chagas Disease have been declining year by year from 2007 (20.86%, 15.75% and 7.53%). These patients were from blood banks, medical clinics, and public hospitals of the state and private medical offices. We can conclude that regardless of the number of patients, the change in prevalence was not significant. **E-mail:** cloefernandes@terra.com.br

Chagas052- Serologic evolution of patients followed by the program of pharmaceutical care for Chagas patients from State of Ceará

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Introduction: Chagas disease (AD), endemic in South and Central America, is a chronic disease caused by *Trypanosoma cruzi*. There are no doubts as to the benefit brought by the use of benznidazole during the acute phase of the CD, on the other hand, treatment on the chronic phase remains controversial. There are no convincing studies in the medical literature with sufficiently large samples and appropriate control groups that may indicate whether specific treatment is effective in preventing the development of chronic AD (except BENEFIT, in progress) (COURA JR & J BORGES-PEREIRA, 2011). **Methodology:** This is a longitudinal study, involving 15 chronic chagasic patients treated with benznidazole. we considerate serological analysis, initial serum samples (before the treatment, 2006) and 2011. The qualitative and semi-quantitative IgG anti-*T.cruzi* was performed by conventional ELISA, samples of serum in dilution 1/80. Was used as a evolution criterion, the change in optical density (OD). **Results:** For each serological test we classify the evolution in the following types: Unchanged = Variation OD <1.5 times; Progressive Increase in OD ≥ 1.5 times; Regressive = downfall OD ≥ 1.5 times (Zauza et al., 2001). Of the 15 patients followed up, 10 had serologic evolution unchanged, 2 progressive and 3 regressive. **Conclusions:** From these results, we can say that although benznidazole have limited effectiveness in the chronic phase, it is worth treating the patient, 15 subjects followed up, 13 had a positive serological response (unchanged or regressive) over the years. **E-mail:** monicacoelhoandrade@yahoo.com.br

Chagas053- Monitoring the evolution of parasite load, IgG levels and cardiac manifestations in patients with chronic Chagas disease from Mato Grosso do Sul, Brazil, eleven years after benznidazole treatment

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Trypanosoma cruzi is the etiological agent of Chagas disease that affects about 8 million people in Latin America. This disease is still endemic in some areas of Brazil, including the west-central region of the country. In order to analyze the impact of benznidazole (BZ) treatment in chronic Chagas patients, we

carried out a longitudinal study to compare parasite detection in blood, serology and clinical manifestations outcome in a group of 34 chronic infected patients, taking samples before and eleven years after BZ treatment (5mg/kg/day - 60 days). The sampling was constituted by individuals inhabitant in Rio Verde sanitary district, Mato Grosso do Sul state, west-central region of Brazil. From the 34 Chagas disease patients, 19 were treated and 15 did not receive the drug. Clinical examination, resting electrocardiogram, indirect immunofluorescence (IFI), enzyme linked immunosorbent assay (recombinant ELISA) and conventional polymerase chain reaction (PCR) were performed before and eleven years after treatment. From the 19 treated patients, 13 (68.4%) were PCR-positive and 6 (31.6%) PCR-negative before drug administration. Eleven years after treatment, the number of PCR-positive patients decreased to 4 (21.1%) and PCR-negative increased to 15 (78.9%). On the other hand, in the group of 15 untreated patients, 9 (60.0%) were PCR-positive and 6 were (40.0%) PCR-negative. Eleven years later, the number of PCR-positive patients decreased to 1 (6.7%) and PCR-negative increased to 14 (93.3%). Relative to the serological results, the recombinant ELISA showed an increase in the optical density values, which were more evident in the treated group. However, for the same group, we observed a significant decrease in the levels of IgG anti-*T.cruzi* detected with IFI. To assess clinical manifestation outcome, the electrocardiograms are still in progress. In addition, real-time quantitative PCR assays are being performed to estimate the parasite load in both patient groups, before and after treatment. Taking together, it is expected that these results can contribute to better understand Chagas disease progression and the efficacy of benznidazole treatment in chronic infected patients. **Supported by:** CNPq-473430/2009-6 **E-mail:** clara.oliveira@ioc.fiocruz.br

Chagas054- Evaluation of aminotransferase levels in chronic chagasi patients treated with Benznidazole in Ceará, Brazil.

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Introduction: In Brazil, the only drug currently available for the treatment of Chagas disease is Benznidazole (Bz), which acts through the formation of free radicals and/or nucleophilic metabolites. These compounds have low specificity of action for the biochemical pathways of the parasite, which contributes to the cytotoxic effects observed for the treatment in about 30% of patients. This work aims to determine the levels of aspartate aminotransferase, AST, and alanine aminotransferase, ALT, as markers of liver injury, since drug metabolism is mainly hepatic and the action of free radicals is nonspecific. **Methods:** We analyzed the levels of aminotransferases (ALT and AST) of 27 chronic chagasi patients of both sexes, above 25 years old, attended by the Pharmaceutical Care for the Chagasic Patients of the Ceará State being treated with Benznidazole (5mg/kg / day) in 2011. The tests were conducted before treatment begins, during (30 days) and at the end of treatment (60 days) in all patients. Graph Pad Prism 5.0 was used for statistical analysis of the data. **Results:** The Wilcoxon test showed no statistical significance when assessing the levels of aminotransferases in the samples analyzed in the three moments of pharmacotherapy. The initial levels average of AST was 24.07 (standard deviation - 2.126), with 30 days of treatment showed a mean of 23.80 ± 2.501 and at the end of treatment an average of 25.20 ± 1.987 . The initial levels average of ALT was 27.22 ± 4.289 , with 30 days of treatment showed a mean of 26.72 ± 5.200 and at the end of treatment an average of 28.44 ± 3.666 . **Conclusion:** According to the results presented, we can see that the averages of samples from all patients showed no change in liver enzymes during treatment, although it is reported in literature that the metabolism of Bz can lead to liver damage. **E-mail:** Jdsf_junior@hotmail.com

Chagas055- Autoantibodies in chronic Chagas disease: association with clinical and laboratory aspects

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Introduction: Chagas disease (CHD) is caused by the protozoan *Trypanosoma cruzi* and transmitted by hematophagous *Triatoma* insects, affecting approximately 12 million people in Latin America. Various autoimmune diseases (AID) including multiple sclerosis and diabetes mellitus have been associated with persistent infections, suggesting that chronic infections are related to autoimmune processes. The contribution of autoantibodies to the pathogenesis of CHD is not fully understood, but different studies have suggested the importance of autoantibodies in chagasic cardiomyopathy. **Material and Methods:** To investigate the presence of following autoantibodies in sera from chronic chagasic patients: smooth anti-muscle (SMA), anti-gastric parietal cells (APAC) and anti-endomysial (IgA-EmA) using indirect immunofluorescence assay, in serum samples from 50 patients previously diagnosed with Chagas disease in the Chagasic Patient Ambulatory Care Unit of the Hospital de Clínicas da Universidade Federal do Paraná (HC/UFPR) and in the sera of 100 ethnically healthy volunteers. **Results:** There was a trend for higher SMA and APAC frequencies in chagasic patients. When compared to controls (3/50, 6% vs 1/100, 1% for SMA, OR=6.3, 95%CI=0.6-62.4); While 4% at the patients were positive for APAC (2/50) and none for the controls. All individuals were negative for IgA-EmA. **Conclusions:** These preliminary results suggest that chronic Chagas disease may be associated with the development of autoimmunity. Nevertheless in order to confirm this hypothesis a larger number of patients will be further investigated. **Financial support:** CAPES. **E-mail:** matiascosta.angel@gmail.com

Chagas056- Clinical decisions when disagreements between PCR and serology among patients in diagnostic investigation for chronic Chagas disease occur.

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Introduction: Since 2008, Ipec – Fiocruz is conducting a project to develop and turn available a PCR test to be used in clinical practice, mainly for the diagnosis of chronic Chagas disease. The main motivation was to have an alternative to serology when this is persistently inconclusive, following Brazilian guidelines. This work aims to briefly discuss clinical course of action when serology are negative and PCR is positive for chronic Chagas disease. **Methods:** This was a case series, from a sequential selection of patients suspected of chronic Chagas disease. From March 2008 to March 2012 patients looking for Chagas disease diagnosis at Ipec were screened to be a volunteer to a project aiming to validate a PCR test for Chagas disease. All volunteer with PCR for Chagas disease available up to January 2012 were included. Two commercial serological tests, one EIE and one IIF, were conducted. The protocol was to collect three blood samples for each patient to conduct PCR test. *PCR was conducted in each sample with primers designed to two target regions: nuclear satellite and kinetoplast.* **Results:** 151 patients had data available for both PCR and serological tests. These patients did seek chronic Chagas disease diagnosis due to a variety of reasons, including: blood bank positive screening (20.63%); referred from other health units due to heart disease (30.95%); referred from other health units due to digestive disease (13.49%); due to Chagas disease among relatives (25.40%). The proportion of males was 42.28%, the mean age was 47.53, 73.60% reported ever lived at rural areas and 65.87% reported ever lived in mud houses and 13.49% ever received blood transfusion. Ninety three had initial diagnosis as without Chagas disease and 10 had inconclusive diagnosis. From these 93, 48 (53.30%) had at least one positive PCR. From the 10 patients initially with inconclusive diagnosis, 6 had positive PCR. Three patients had 2 (2.64%) consecutive samples with inconclusive serology results. Patients were called back and 5 of them were further tested so far. All of these 5 remained with at least one positive PCR, one did seroconvert from negative to positive and one had two inconclusive Serologies. **Conclusions:** Similar results were observed before; however the appropriate medical course of action is seldom discussed. Three main points come from this results: as currently stated in Brazilian guideline, PCR will probably add very little information on decision making; although further confirmation is required it seems that PCR is able to identify a considerable proportions of patients with Chagas disease that serology cannot; as PCR becomes technically less laboriously and expensive, it will be relevant to discuss its roll as first line test. Patients' follow-up and investigations about clinical characteristics contributions to decision making are required to better understand this phenomena. **E-mail:** pedro.brasil@ippec.fiocruz.br

Chagas057- Development of a real time PCR assay to determine parasitaemia in chronic patients with Chagas Disease: comparison between TaqMan and SYBR GREEN systems

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Chagas disease (CD) is a neglected endemic disease in Latin America. It is caused by the protozoan *Trypanosoma cruzi* and affects about 8 million individuals of whom 30-40% either have or will develop cardiomyopathy, digestive megasyndromes or both. Although a treatment exists, it is known that the available drugs are more effective in the initial stage of the disease. Thus, the search for new markers for cure criteria is extremely important to investigate the therapeutic efficacy in CD. Quantitative real-time PCR (qPCR) is an accurate method to determine parasite load in clinical samples and could be useful as an indicator of the therapeutic response during the prolonged natural history of CD. In this study, we intend to compare TaqMan and SYBR Green systems in order to improve a methodology for CD molecular diagnosis, using primers targeted to *T. cruzi* nuclear satellite DNA (Piron *et al.*, 2007). The SYBR Green based real time PCR, besides being used to estimate parasitaemia in chronic infected individuals also shows its potential in molecular typing the parasite through melting curve analysis of the amplified products. DNA was extracted from human blood spiked with *T. cruzi* epimastigotes, using the QIAmp DNA mini kit (QIAGEN). Standard curves for the absolute quantification were performed in triplicate with 10-fold serial dilutions, ranging from 10^6 to 10^{-3} parasite equivalents/mL. In the SYBR Green assays, the optimal tested concentration for primers targeting the parasite satellite DNA was 300nM. In order to check for DNA integrity and possible inhibitors present in blood samples, the human β -globin gene (200nM primers) was used as control. TaqMan system assays were carried-out in multiplex and directed for both *T. cruzi* satellite DNA and human RNase P. In this case, the best primers and probe concentrations for the *T. cruzi* target were 300nM and 100nM, respectively. The pre-developed reagent for the RNase P human gene was included as an internal control of amplification (0,5X). The analytical sensitivity (<0.01 parasite equivalents/mL) as well as the standard curves parameters were similar in both systems ($E>90\%$; $R^2>0.98$). A group of 50 chronic cardiomyopathy Chagas (CCC) disease patients from the BENEFIT trial was evaluated for parasite load estimative with the SYBR Green protocol. As expected, a scarce parasitaemia was observed, varying from 0.01 ± 0.01 to 4.76 ± 2.84 parasite equivalents/mL. In summary, qPCR showed high ability to diagnose and quantify very low parasitaemia burden in CCC patients, revealing its potential as a complementary diagnostic tool to serology, for monitoring circulating parasites in patients submitted to etiological treatment. **E-mail:** carolina.lima@hotmail.com

Chagas058- Evaluation of multiplex PCR test for detection of *Trypanosoma Cruzi* IN in peripheral blood of patients with chronic Chagas disease and dried feces of triatomine bugs used on xenodiagnosis

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Introduction: The intracellular parasite *T. cruzi* is the causative agent of Chagas disease, which affects 17 million people in Latin America. The chronic phase is characterized by low parasitaemia and high level of Anti-*T. cruzi* antibodies and the diagnosis is done preferably using serological methods, including indirect immunofluorescence (IIF), indirect hemagglutination (IHA) and ELISA. These tests show high sensitivity, and low specificity, and a variable number of individuals present inconclusive serological tests. Nowadays, many studies have used the technology of Polymerase Chain Reaction (PCR) to detect DNA sequences of *T. cruzi* in blood of chronic chagasic patients. The high specificity of the PCR has pointed to its use as a confirmatory method for diagnosing patients with inconclusive serology. This study aimed to comparative analysis between PCR and conventional methods commonly used for diagnosis - serology and xenodiagnosis in the detection of infection by *T. cruzi* in individuals with chronic Chagas' disease.

Material and Methods: Blood of 67 chronic chagasic patients, both sex, from the Chagas' disease clinic at the Hospital Universitário Walter Cantídio, of Universidade Federal do Ceará, were tested for *T. cruzi*,

using serological tests (IFI, IHA and ELISA), M-PCR and xenodiagnosis. Here in we evaluated of Multiplex - Polymerase Chain Reaction (M-PCR) for detection of DNA of *T. cruzi*. M-PCR was used to detect the fragment of 330bp kDNA and other fragment of 195bp nDNA of *T. cruzi* in peripheral blood and dried feces of triatomine bugs from the xenodiagnosis of these same patients. **Results:** According to the results of serological tests, patients were classified into 3 groups: 1. Positive test results (when 2 or 3 results from the three serological tests were positive), 2. Inconclusive or indeterminate serology (when 2 results were negative), and 3. Negative serology (when 3 results were negative). Among the 59 seropositive samples that obtained 18 (30,5%) positive results of M-PCR for peripheral blood and 41 (69,5%) negative results. In M-PCR for waste of triatomine bugs were obtained 20 (33,9%) positive and 39 (66,1%) negative results. The two samples with inconclusive serology were negative in the M-PCR for peripheral blood and for the waste of triatomine bugs. Samples with negative serology (n=6) had 2 (33,3%) positive and 4 (66,7%) negative results of M-PCR for blood. For the waste of triatomine bugs, the 6 seronegative samples were negative by M-PCR. **Main conclusions:** These results showed that M-PCR assay in peripheral blood samples and in waste of triatomine bugs, using primers for different regions, in this case, nuclear DNA and kinetoplast DNA, could be applied to the diagnosis of chronic Chagas' disease, even with different methods of extraction of total DNA. Data also showed that M-PCR in waste of triatomine bugs is more sensitive than the test of xenodiagnosis. **Keywords:** *Trypanosoma cruzi*, Chagas disease, PCR-Multiplex, peripheral blood, stool of triatomine bugs. **E-mail:** mjteixeira601@gmail.com

Chagas059- Evaluation of parasitaemia by PCR in individuals chronically infected by *Trypanosoma cruzi* treated with benzonidazole

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Introduction: Etiologic treatment of Chagas disease is focused on the elimination of the *T. cruzi* in infected tissues, which should arrest the evolution of the disease and avert its irreversible long-term consequences. The drug available in Brazil is the benzonidazole, but therapeutic efficacy varies according to different geographical areas and disease stages. In addition to the lower efficacy of etiological treatment, the lack of safe methods for cure evaluation is a great challenge of Chagas disease. In chronic stage, the persistence of positive results of immunological assays is frequent and the negative results, when it happens, can take years to appear, requiring much time of following. **Objective:** This study aimed to evaluate the serological and parasitological status of patients with chronic Chagas disease after chemotherapy with benzonidazole. **Material and Methods:** A retrospective study was carried out with patients treated with benzonidazole (5mg/Kg/day for 60 days) between 1980 and 2010. Twenty-nine patients who had Chagas disease confirmed by two reagent immunological tests and/or one positive xenodiagnosis before treatment were included. Conventional serology (ELISA and IIF) and parasitological tests (hemoculture and *Nested-PCR*) were performed after chemotherapy. **Results:** At the time of treatment, the mean age of patients was 36 ± 7.24 years old (20-39 years) and the time post-treatment varied from 1-29 years. After chemotherapy, 100.0% (29/29) of the cases were reagent to ELISA. According to IIF, two individuals (6.9%) showed inconclusive results with titers under the 1:40 cut-off. The rest of the group (27/29) had positive results with titers between 1:40 and 1:1280. Comparing the IIF titers, 61.5% (16/26) of them decreased after treatment, 11.6% (3/26) remained with the same titration and 26.9% (7/26) increased. *T. cruzi* DNA was detected by N-PCR in 48.3% (14/29) of the cases. Negative and inconclusive results were observed in 51.7% (15/29) of the samples. Hemoculture was negative for all individuals. **Conclusions:** Our results suggest that the conventional immunological assays remain with positive results after years post-treatment and the antibody titers decreasing do not represent the cure itself. However, N-PCR may be useful in early identification of therapeutic failure of Chagas disease. Although it is difficult to determine parasitological cure in negative N-PCR cases, we can infer that this condition represents a declination of parasitaemia as a favorable consequence of etiological treatment. **E-mail:** camilasmil@yahoo.com.br

Chagas060- International workshop on standardization and validation of qPCR methods for quantification of *Trypanosoma cruzi* DNA in blood samples from Chagas disease patients

Ramírez JC and Cura C, Moreira OC, Aznar C, Velázquez E, Ramírez JD, Alberti A, Pavia P, Lages E, Flores MD, Muñoz A, Pérez D, Santalla J, Guedes P, Marcet P, Peneau J, Padilla C, Robles DC, Valencia E, Crisante GE, Greif G, Zulantay I, Costales J, Álvarez M, Martínez NE, Villarroel R, Villarroel S, Sánchez Z, Juiz N, Bisio M, Parrado R, Britto C, Yadón Z, Schijman A.

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An international workshop was launched by 26 expert PCR laboratories from 14 countries to assess the performance of Multiplex qPCR strategies based on TaqMan probes to monitor the parasite load of Chagas disease patients. Methodology included DNA extraction from 300 µL of blood previously treated with Guanidine Hydrochloride – EDTA buffer (GEB) using the High Pure PCR Template Preparation Kit from Roche. Two qPCR methods were assayed: 1) Satellite DNA qPCR and 2) kDNA qPCR. Both methods included an internal amplification control. Reportable range, analytical sensitivity and precision were estimated following international guidelines using human blood spiked with parasite cells. Inclusivity and selectivity were also estimated using purified DNA from stocks representing the different *T. cruzi* discrete typing units (DTUs) and *Trypanosoma rangeli* and *Leishmania* sp, respectively. DNA integrity was tested in stored samples using TaqMan RNase P Control Reagents Kit from Applied Biosystems. Both methods were challenged against 210 clinical samples provided by the participant labs, including patients with different epidemiological and clinical settings: congenital and oral cases, chronic asymptomatic and symptomatic patients. kDNA qPCR showed a better analytical sensitivity than Satellite DNA qPCR with detection limits of 0.25 and 0.5 parasites/mL, respectively. Both methods showed higher precision at high concentration levels. A high concordance was observed between Satellite DNA and kDNA qPCRs results for the same clinical samples. kDNA qPCR results should be taken with caution in regions where infections with *T. rangeli* are suspected. Good conservation of DNA integrity in stored GEB samples was observed. The use of the internal amplification control allowed detecting cases of low DNA extraction efficiency or presence of PCR inhibitors in the samples. This effort is a major goal towards international harmonization of qPCR methods for the quantification of *T. cruzi* DNA in human blood samples, leading to provide a reliable surrogate biomarker of therapeutic response in Chagas disease patients. **E-mail:** aleschijman@gmail.com

Chagas061- Molecular diagnosis and characterization of acute *Trypanosoma cruzi* infection due to organ transplantation

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Introduction: Chagas disease, caused by *T. cruzi*, is transmitted mainly by triatomine insect vectors, blood transfusion or by infected women to offspring. The infectious cycle includes intermittent subclinical parasitemia for prolonged periods of time, which in the long term may cause chronic organ damage. Amastigotes have been isolated from various organs, thus transplantation (Tx) plus immunosuppressive therapy is a novel way of disease transmission. Herein, we report the incidence and molecular characterization of *T. cruzi* acute infection in previously uninfected organ Tx recipients that received organs from seropositive donors. **Materials and Methods:** Case 1: Infected deceased donor (IDD) and 3 recipients (1 lung, 1 liver, 1 kidney). Case 2: IDD and 1 liver recipient. Case 3: IDD and 2 kidney recipients. Peripheral blood or cerebrospinal fluid (CSF) samples were collected for detection of *T. cruzi* by means of kDNA-PCR. Positive samples were subjected to a PCR algorithm for identification of *T. cruzi* Discrete Typing Units (DTU) and to a real-time PCR strategy to quantify *T. cruzi* DNA in blood samples. Minicircle signatures of *T. cruzi* infecting populations were analyzed using RFLP-PCR. **Results:** Case 1:

blood samples from two organ recipients were kDNA-PCR positive after 72 and 98 days post-Tx, respectively, and both were infected by DTU Tc V (or Tc V + Tc VI). The comparison between their minicircle signatures revealed nearly identical RFLP profiles, suggesting a common source of infection. Case 2: the recipient exhibited positive kDNA-PCR 36 days post-Tx and was also infected by Tc V (or Tc V + Tc VI). Case 3: One of the recipients showed kDNA-PCR positive results 93 days after Tx and central nervous system involvement. *T. cruzi* infecting populations were characterized as Tc V in blood and CSF samples. It is worth noting that there are two other kidney recipients from cases 1 and 3 that have not had a positive kDNA-PCR result until the present moment. **Main Conclusions:** Molecular tools allowed for diagnosis of acute *T. cruzi* infection. The routes of transmission could be inferred by fingerprinting of the detected *T. cruzi* populations, directly in peripheral blood and CSF samples from transplant recipients. Furthermore, this report reveals the relevance of systematic monitoring of recipients by PCR strategies in order to provide prompt diagnosis and subsequent anti-trypanosomal treatment. **E-mail:** cura.carolina@gmail.com

Chagas062- PCR as an alternative diagnosis tool for megaesophagus associated with cardiomyopathy in patients with inconclusive or negative serology for Chagas disease

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Introduction: Infection by *Trypanosoma cruzi* is the main etiology of megaesophagus in Brazil and the prevalence of chronic cardiopathy associated with digestive forms of Chagas disease is estimated around 5-8%. Although diagnosis in chronic phase relies on conventional serology, some authors have reported false-negative results. The polymerase chain reaction (PCR) has been used as an alternative diagnostic test when patient presents inconclusive serology. **Objectives:** The aims of this study were to investigate the seroprevalence for Chagas disease in a population presenting esophageal motility disorders and to detect *T. cruzi* satellite DNA by molecular methods in blood samples of patients with inconclusive or negative serology. **Material and Methods:** A retrospective study was conducted in order to assess the seroprevalence for Chagas disease in patients with megaesophagus assisted in a reference service. Epidemiologic profile of patients included in this study was evaluated according to the age, place of birth, serological status and association with cardiac involvement. **Results:** The seroprevalence for Chagas disease determined by conventional serology (IIF and ELISA) was 79% (409/518) in persons with megaesophagus. We investigated 109 cases when serological tests were inconclusive or negative [31/518 (6%) and 78/518 (15%), respectively]. Out of the 109 patients (female= 65; male=44), 21 (20%) also presented cardiomyopathy compatible to chagasic infection. Mean age was 49.18 ± 18.66 years. From 21 patients with digestive and cardiac involvement, 20 (95%) were born in areas where Chagas disease was considered endemic in the past. PCR was performed in blood samples of 13/21 patients and positive results were observed in 10/13 (77%) cases, clarifying the megaesophagus etiology. **Conclusions:** The high seropositivity rate for Chagas disease among patients with megaesophagus found in this study corroborates other Brazilian reports. Since most of the patients presented epidemiologic antecedents for Chagas disease, *T. cruzi* infection must be considered regardless the serological status. Our findings highlight the possibility of misdiagnosis and also raise the importance of clinical and epidemiologic investigation to determine megaesophagus etiology. PCR can be useful to clarify some of those specific cases. **E-mail:** gelikks@fcm.unicamp.br

Chagas063- Post-therapeutic cure criteria in Chagas disease: conventional serology screening followed by PCR and supplementary serological tests

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Introduction: A critical review of conventional serology, parasitological and supplementary serological tests for assessing the efficacy of treatment of Chagas disease was undertaken. **Material and Methods:** For this study, 94 Chagas disease patients treated with benznidazole >10 years before, were evaluated by laboratorial (ELISA, IIF, IHA, Hemoculture, PCR, rec-ELISA and TESA-cruzi) and clinical (anamnesis, physical examination, conventional electrocardiogram, thorax X-ray, esophagus and colon barium-contrasted X-ray) analysis. **Results:** Percentages of 10.6% and 8.5% of patients were considered cured by the classic (any two tests) and the more rigorous cure criteria (three tests) of conventional serology (ELISA, IIF and IHA), respectively. Following, patients were evaluated by parasitological (hemoculture and PCR) and supplementary tests (rec-ELISA and TESA-cruzi). Hemoculture and PCR were negative in all treated cured (TC) patients, regardless the criterion used. The results of rec-ELISA were similar to three tests criterion. TESA-cruzi showed high percentage (21.3%) of negative results when compared with both cure criteria. Rec-ELISA and TESA-cruzi test revealed negative results in 70% and 87.5% of the patients categorized as TC by the classic and three tests criteria, respectively. In patients with discordant conventional serology the parasitological (PCR) and supplementary serological tests were decisive to verify the therapeutic failure. Analysis of clinical features showed that 62.5% of TC patients presented the indeterminate form of the disease. **Conclusions:** Global data demonstrated that the cure control of Chagas disease must be done carefully using conventional serology, parasitological and supplementary tests and that treated patients negative in TESA-cruzi must be evaluated later to verify if this test is able for early assessment of parasitological cure in Chagas disease. **E-mail:** girleyfrancisco@nupeb.ufop.br

Chagas064- *Trypanosoma cruzi* and *Leishmania* spp. detection in dogs from Triângulo Mineiro and Alto Paranaíba regions for PCR

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Introduction: Visceral Leishmaniasis and American Trypanosomiasis are parasitosis in which the dog is the main domestic reservoir, mainly in urban areas due to its proximity to man. Domestic dog has great epidemiological importance, due to the high canine prevalence in endemic areas, as well as precede the occurrence of human cases. Most infected dogs are asymptomatic, but infective for vectors sandflies and triatomines. **Material and methods:** Were collected blood from 240 dogs resident in Triângulo Mineiro and Alto Paranaíba's regions (Uberaba, Perdizes, Delta, Água Comprida, Nova Ponte) and, those with symptoms suggestive of leishmaniasis, were euthanized for organ collection (liver, spleen, lymph nodes, skin and bone marrow). Parasitological diagnosis was performed for direct (imprint) and indirect (blood and bone marrow culture) techniques. During samples collection, imprints and bone marrow smears were performed and stained with Panotic kit or Leishman staining. Strains isolation were performed in LIT, LIT + NNN (Novy-Nicolle-MacNeil) and Schneider media. For *Leishmania* spp. molecular diagnosis, we applied a Nested PCR technique (Cruz *et al.*, 2002), using the specific set of primers R221 (GGTTCCTTTCCTGATTACG) and R332 (GGCCGGTAAAGGCCGAATAG) in the first amplification, and R223 (TCCCATGCAACCTCGGTT), and R333 (AAAGCGGGCGCGGTGCTG) in the second amplification. Positives samples amplified a specific fragment of 358 bp. For *Trypanosoma cruzi* molecular diagnosis, PCR was performed with the set of primers 121 (TAAAAATGTACGCATATGGGGGAGAG) and 122 (GGTATTGCTGGGGTGGTGAATATTA) (Wincker *et al.*, 1994), which amplifies a specific fragment of 330 bp. **Results:** In Uberaba city, it was possible to identify amastigotes of *Leishmania* spp. in two (8%) out of 25 euthanized dogs in spleen, liver, lymph node and bone marrow smears by direct parasitological methods (smears and imprint). Blood and bone marrow cultures from these dogs were also positive in Schneider's medium. *Trypanosome cruzi* parasitic forms were not identified in culture, however, the PCR technique permitted the amplification of specific fragments in 18 (7,5%) blood samples, in which further one amplified *T. cruzi* DNA in liver sample and five dogs were positive in tissue fragments, total of 23 (9,6%) infected animals out of 240 analyzed dogs. The two dogs that were positive for *Leishmania* spp. in parasitological and molecular methods applied were

also positive for *T. cruzi* in PCR technique. **Conclusion:** Dogs of the surveyed area are roosting *Leishmania* spp. and *T. cruzi*, being an important reservoir for these protozoosis, enabling the domestic cycle of these parasites. Once canine infection precede or have a significant relationship to human infection, it should be emphasized the possibility of spreading the disease among domestic animals in the evaluated regions, as well as starting a new focus for human disease. **E-mail:** larallyn@hotmail.com

Chagas065- Long-term evaluation of etiologic treatment with benznidazole in patients with indeterminate chronic Chagas' disease

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Introduction: The aim of this study was to assess the effect of benznidazole treatment on the electrocardiographic, serological and parasitological evolution of patients with indeterminate form of Chagas disease. **Material and Methods:** We retrospectively analyzed a group of 62 patients that were treated with benznidazole and compared their clinical findings with the findings of an untreated group (n=62) of age-matched patients with indeterminate form of Chagas disease. **Results:** The frequency of electrocardiographic alterations in the treated group was followed for 118.3 ± 61.7 months (7333.7 patients-months) and in the untreated group was followed for 144.51 ± 49 months (8959.9 patients-months). The rate of disease progression from indeterminate to the cardiac form of Chagas disease based on electrocardiographic findings was 12.9% (8/62) among treated patients and 16.1% (10/62) among untreated ($p = 0.4$). The incidence densities of this outcome was 1.09/1000 patients-months and 1.11/1000 patients-months in treated and untreated groups, respectively (relative risk = 0.98). There was no correlation between the progression to cardiac form and age, sex or place of birth. The serological titers of benznidazole treated and untreated patients were followed for 88.7 ± 49.6 months and 140.9 ± 47.8 months, respectively. The titers were converted to a linear scale, where 1 represented a 1:40 dilution and 6 a 1:1280 dilution. The serological titer reduced significantly after benznidazole treatment (4.07 before treatment vs. 2.85 post-treatment, $p < 0.001$). Such reduction was not observed among untreated persons (4.22 at the beginning of the follow-up vs. 4.11 at the end, $p = 0.503$). Despite the observed reduction of the serological titers after benznidazole treatment, the post-treatment titers tended to increase throughout the follow-up period, mainly after 100-150 months. All treated patients were submitted to xenodiagnosis, before treatment, and 32 proved positive. After treatment, xenodiagnosis remained positive in only one patient. **Main Conclusions:** We concluded that: **i)** among studied patients there was no relationship between etiologic treatment and electrocardiographic progression, **ii)** treatment with benznidazole is associated with a significant reduction in serological titers, and **iii)** parasitaemia seems to be suppressed soon after specific treatment. **E-mail:** alejandro.hasslocher@ipecc.fiocruz.br

Chagas066- Benznidazole evaluation in the treatment of the experimental Chagas' disease

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Introduction: Chagas' disease is caused by the flagellate parasite *Trypanosoma cruzi*. Although parasite transmission by its natural vector has mostly been controlled, Chagas' disease still deserves attention from researchers since many people remain infected and new infection routes have been described. New drugs must be tested and different diagnostic methods must be used to determinate the parasitological cure. The total parasite elimination is crucial, since its presence can trigger a new acute phase, mainly in the immunosuppressed patient. The present study proposes to evaluate the Benznidazole effectiveness and the efficacy of the diagnostic methodologies in the experimental Chagas' disease treatment. **Material and methods:** Swiss mice were infected intraperitoneally with 10^4 *T. cruzi* trypomastigotes forms and in the early acute phase the treatment was initiated with Benznidazole at 100mg/kg body weight for 90 consecutive days, by oral route. Every other day, blood samples were collected from the tail vein for

different diagnostic methods, such as ELISA, indirect immunofluorescence, PCR and fresh blood examination. At the end of the protocol, a set of mice were euthanized and the heart, kidney, adrenal gland, liver, esophagus and spleen were removed for histopathological analysis. **Results:** The non-treated group presented high mortality rate, high parasitaemia and positive results for all diagnostic methodologies used. In the other hand, we have not seen any mortality in the treated group and parasitaemia fell as soon as treatment began. Furthermore, all diagnostic tests were negative and there were no significant alterations in the histopathological analysis. The PCR was able to detect parasite DNA in the non-treated animal tissues, whereas it has not amplify DNA fragments in the samples derived from treated mice. **Conclusion:** Our results corroborate the literature data stating that the Benznidazole promote the parasitological cure for Chaga's disease, and set the PCR as an excellent methodology for diagnostic, demonstrating consistency with other methodologies and presenting greater sensibility. **E-mail:** anacd07@yahoo.com.br

Chagas067- Benznidazole decreases cardiac inflammation and cytokine expression in dogs treated in acute phase of experimental Chagas disease

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The Benznidazole (Bz) is the only drug available for Chagas disease treatment in Brazil. Despite the side effects, it is still indicated in the treatment of Chagasic patients, especially children, due its effectiveness during the acute phase of infection. In this study, we evaluated during the chronic phase the inflammatory process and cytokines expressed by inflammatory cells in the heart of dogs infected with strains susceptible or partially resistant to treatment with Bz. To this, 14 dogs were infected with 2000 blood trypomastigotes per kg of body weight of Be-78 or Y strains, and treated with 7 mg of Bz/kg, divided into two daily doses for 45 days. The oral treatment was started immediately after the appearance of parasitaemia, detected by fresh blood examination. The cure was evaluated by PCR, hemoculture and serological tests, the negativation of these tests was observed in 12% and 100% of animals infected with Be-78 or Y strain, respectively. Six months after treatment, in the chronic phase, the groups untreated, treated cured or not cured were euthanized and the right atrium and interventricular septum were collected. After this, these fragments were routinely processed for obtaining serial sections that were stained with H&E and immunohistochemical staining for cytokines (IFN-gamma, TNF-alpha, IL-10 and IL-4) for quantification of inflammation and inflammatory cells expressing each of these cytokines, respectively. We observed that the dogs infected with Be-78 strain treated/cured exhibited a significant reduction of inflammation and the expression of IFN-g, TNF-a, IL-4 and IL-10 in relation to the groups infected/ untreated and infected/not-cured, otherwise the animals treated/not-cured showed a profile similar to untreated group. Dogs infected with the Y strain treated/cured exhibited a significant reduction of inflammation and IL-10 expression in relation to the infected/untreated. Therefore, when the Bz treatment promotes healing, the cardiac damage caused by inflammation is minimized. **Supported by:** CNPq, CAPES and FAPEMIG. **E-mail:** cmcarneiro@gmail.com

Chagas068- Side effects of benznidazol treatment and stage of Chagas disease in Fortaleza city, Northeast Brazil

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Introduction and Aim: Chagas heart disease (CHD), caused by the protozoan *Trypanosoma cruzi*, is a significant cause of morbidity and mortality in South and Central America. Benznidazole is the most

commonly used drug for the etiological treatment of the disease although its effectiveness varies according to the phase of the same and toxic side adverse effects are frequent. The aim of this study was to investigate the correlation between side effects associated with benznidazole and stage of the disease. **Methodology:** This is a retrospective cohort with 99 patients with Chagas disease followed in a tertiary hospital in Fortaleza city, Northeast Brazil. The patients were identified using the registries of the reference laboratory for Chagas disease in the Faculty of Pharmacy, Federal University of Ceará. The sample was divided into three groups: patients with indeterminate, cardiac and digestive forms. Some patients have not been classified. Results: Of the 99 patients that have received treatment with benznidazole, 58 patients (58.5%) presented side reactions. The most frequent reactions were headache (50%) and nausea (42%) followed by dermatological reactions (35.2%). Addition to the reactions described patients had vomiting and tingling in the body. A total of 15 patients (15%) had to withdrawn the treatment due to side effects. No deaths occurred. In 58.1% of the women was a developed side effects while 48.2% of men were affected. There was no statistically significant difference between the forms indeterminate (43.3%) and digestive (33.3%) in relation to side effects, and patients with the cardiac form (75%) were most affected. **Conclusions:** The effects of benznidazole use in patients with the cardiac form of Chagas disease may be due to greater physical impairment of these individuals when compared to patients included in other forms. Despite side effects caused by treatment, benznidazole has the best safety against record and is better tolerated than most, making it the first choice for treatment. **E-mail:** nataliamorais6@yahoo.com.br

Chagas069- Adverse reactions in elderly patients with Chagas disease treated with Benznidazole in the State of Ceará

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Introduction: As result of the effectiveness of control measures of the vector transmission and blood transfusion in many countries of Latin America, Chagas disease (CD) has showed a strong tendency to become most prevalent in elderly patients. The only drug available in Brazil for the etiological treatment of this disease is the Benznidazole (BZN), however it's presents as drawbacks the partial effectiveness and toxicity. Elderly patients are more susceptible to the development of adverse reactions (AR) probably as result of high prevalence of co-morbidities associated with hepatic and renal disease and the large number of medicines that they use. This justified the necessity of studies directed to this population. Objective: Describe AR in elderly patients undergoing the treatment of DC with BZN. Materials and methods: This was a retrospective study with survey of the data filed in the Laboratory of research in CD located in Department of Clinical and Toxicological Analyses of the Universidade Federal do Ceará. The study included all patients aged 60 years or more attended in the Laboratory between the period of January/2008 to December/2011, who had the data sheet of Monitoring side effects during the treatment filed. The patients without the file or with incomplete data were excluded of the study. The collected data were tabulated in worksheets in Excel for statistics. Results: Of 43 patients included in the study, 53.49% (23) were males and 46.51% (20) were female. Sixteen patients (37.21%) reported not having shown any AR during treatment with BZN, while 62.79% (27) reported to present at least one type of AR. The 27 patients reported a total of 93 AR, with an average of 3.44 ± 2.47 reactions per patient. The reactions most frequently reported were muscle weakness and dizziness, both with percentage of 11.83% (11), followed by pruritus (10.75%), red spots on the skin (9.68%), headache (9.68%) and nausea (9.68%). Nine patients (20.93%) didn't completed treatment with BZN, one for lack of medicine available through the Secretary of health of the State and the others were because of the AR caused by the medicine. Conclusion: It was noted the occurrence of AR in more than half of elderly patients undergoing treatment with BZN, which shows the necessity of a pharmacotherapeutic follow-up more thorough of this population in order to prevent and detect AR earlier and increasing patient adherence to therapy. **E-mail:** laisesantospereira@hotmail.com

Chagas070- Arthritis during treatment with Benznidazole

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Introduction: American trypanosomiasis or Chagas disease is caused by the parasite *Trypanosoma cruzi*, endemic in Latin America and an emerging disease in non-endemic countries due to the recent trends in migration. Benznidazole is the most commonly used drug for the etiological treatment. Patients treated with Benznidazole suffer frequent side effects, and whereas arthromyalgias is common, arthritis has been reported as a very rare symptom. The objective of this study is to describe the importance and characteristics of arthritis as a side effect of Benznidazole. **Materials and Methods:** We performed a retrospective cohort study of 178 patients initiating treatment with Benznidazole from June 2009 to June 2011, attending to the Tropical Medicine Unit in the Hospital Clínic of Barcelona. *T. cruzi* infection diagnosis was confirmed by two serological enzyme-linked immunosorbent assay (ELISA) tests (BIOELISA®-Biokit and ELISA-Ortho®), and an electrocardiogram and chest X-ray were performed. All patients received Benznidazole 5 mg/kg/day for 60 days (maximum dose of 400 mg/day) and were visited at least every fortnight for clinical evaluation and blood test. **Results:** 178 patients in chronic phase and indeterminate or early cardiac form were treated with Benznidazole during the study period. Most patients were from Bolivia, age ranged from 25 to 43 years, and 135 (75.8%) were women. Treatment was interrupted in 45 patients (25.3%), in 5 of them (11.1%) due to arthritis. Another case of arthritis happened 2 days after the treatment was abandoned for dermatological adverse event. Arthritis developed in the 6 patients (3.4% of all treated) between day 25 and 42 after starting treatment. One case had additive arthritis, 3 had oligoarthritis and 2 had polyarthritis. In 5 of them was asymmetric. Shoulders, elbows, wrists, knees and metatarsophalangeal joints were the most affected. Autoimmunity test were performed, and only 1 case had significant results with positive antinuclear antibodies 1/226, positive antiRo antibodies and strong positive CCP. All affected patients were treated with nonsteroidal antiinflammatory drugs (NSAID) and steroids and the symptoms were solved in 7-62 days, with no relapse or signs of chronic autoimmune disease (follow-up between 9 and 24 months). **Main Conclusions:** Arthritis seems to be more frequent than previously described as an adverse reaction caused by Benznidazole. Although arthromyalgias is well known to be common, we have detected that arthritis is not unusual and can be the reason to stop treatment for Chagas disease. In our series we did not find an established pattern of joint involvement, and no patient developed chronic symptoms afterwards. It appears that Benznidazole could cause arthritis, but there is no evidence to think that it could trigger a chronic autoimmune disease. Guidelines for the management of these adverse events are still lacking but clinicians should be aware of the possibility of *T. cruzi* reactivation when using corticosteroids in the context of arthritis during treatment with Benznidazole. **E-mail:** jgascon@clinic.ub.es

Chagas071- Adverse events associated to use of Nifurtimox in children with Chagas disease in Colombia

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Introduction: The etiological treatment for Chagas disease is usually associated with a high incidence of adverse drug reactions (ADRs). Historically, the use of Benznidazole in Colombia has been more extended than any other Trypanocidal drug. In this sense, ADRs associated to the use of Nifurtimox in children have not been sufficiently characterized in this population. We aimed to describe ADRs observed in children treated with Nifurtimox in an endemic area of Casanare, Colombia. **Materials and Methods:** We conducted a prospective cohort study of children with Chagas disease treated with Nifurtimox (dose from 10 to 12 mg/kg in two divided doses for 60 days). Laboratory tests for hepatic, renal and hematological functions were carried out at days: 0, 20, 40 and 60th of treatment. Interrogatory and

physical examination was carried out in the same dates. **Results:** A total of 62 children with chronic asymptomatic Chagas disease (mean age 11.5 years) diagnosed by two different serologic tests were enrolled in the study. The most important symptoms considered Nifurtimox related were: at 20th day: hyporexia (32.2%), asthenia (15.3%), headache (16.9%) and abdominal pain (6.8%). At 40th day were: hyporexia (27.9%), and headache (6.6%). At 60th day were: hyporexia (19.7%), headache (19.7%) and abdominal pain (9.8%). Weight loss, of at least 1 pound, increased with time 45.6% at 20th day, 60.0% at 40th day and 67.2% at 60th day ($p<0.05$). In the same way, significant weight loss ($>3\text{kg}$) increased with time 5.3% at 20th day, 10.0% at 40th day and 11.7% at 60th day ($p<0.05$). Significant weight loss ($>3\text{kg}$) was related to age OR: 2.25 (CI 95%: 1.2-4.1). In general the laboratory tests kept in normal ranges. A mild tendency to decrease the media of leucocytes from 0 to 40th day with a final recovery at 60th day: (9,421; 9,822; 8,309; 8,631; $p<0.05$) was observed. Hemoglobin, transaminases and creatinine were stable throughout following-up. Only one patient required temporal suspension of the treatment by 3 days. The 100% finished 60 days of treatment. **Main conclusions:** In concordance with most of authors, overall treatment with Nifurtimox was well tolerated in children. Hyporexia and weight loss were the most important ADRs. Most ADRs were mild and did not require treatment suspension. Follow-up was successful and guaranteed the satisfactory termination of etiologic treatment. **E-mail:** zcucunuba@gmail.com

Chagas072- Control of Chagas disease in the State of Minas Gerais, Brazil: the registration process of using benznidazole in the service network of the Unified Health System

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Neglected diseases are a major global cause of illness, long-term disability and death. Among these, Chagas' disease is a parasitic infection caused by *Trypanosoma cruzi*, widely distributed throughout Latin America, and that leads to high morbidity and mortality. The available drug for treatment of Chagas disease, benznidazole (BZ), has potential toxic side effects and variable efficacy, contributing to its low rate of use. However, BZ is currently the only drug available in Brazil, being distributed by the Ministry of Health for use in the states. In this work, we evaluated the registration process of BZ use in the State of Minas Gerais, Brazil, in the period of 2009 to 2011. The analysis was based on data from the registration form used by the SES/MG to request specific treatment with BZ from 2009 on, as a way to control of drug distribution in the state. The questionnaire data include the patient's personal data, city of residence, treatment indication and prescribed dosage. From 2009 to 2011, a total of 286 patients treated with BZ. Of these, 9.8% ($n=28$) were treated in 2009, 38.8% ($n=111$) in 2010 and 51.4% ($n=147$) in 2011, showing an increase in the number of requests over the years. 47.130 tablets were consumed. Patients treated in the period were from 49 different municipalities, and a total of 70.6% of the requests came from the regional health unit of Montes Claros, followed by the unit of Januária (7.6%). The distribution by sex was even, with 50% of patients being female ($n = 143$) and 50% male. The mean age was 42.8 years, ranging from 19 to 70 years. 107 (37.4%) were under 40 years of age. Of all patients who requested treatment, 96.2% were reported as having an indeterminate form of Chagas disease and 22.8% were described as having some heart disease. Only one patient was found as a carrier of the acute disease, but the analysis found this to be a chronic case. During the period, only 17.5% of prescriptions were appropriate under current recommendations, showing the need to empower the health care network. This evaluation shows the need for a better structure within the control actions of Chagas disease focusing on better qualification of health care providers and enhanced access to treatment. **E-mail:** marcela.ferraz@saude.mg.gov.br

Chagas073- In vivo Benznidazole Susceptibility to *Trypanosoma cruzi* Strains from the Western Brazilian Amazon.

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Introduction: The Amazon is currently an area of active transmission of *Trypanosoma cruzi*. However, it remains unknown the susceptibility to benznidazole (BZ) for the strains in this region. **Material e Methods:** Thirteen are TcIV and were obtained from human acute phase of the DCh (12) and *Rhodnius robustus triatominae* (1) from Amazon. Five TcI strains from the same state, obtained from *Didelphis marsupialis* (3) and *R. robustus* (2) were included as well as six other strains obtained from the State of Paraná, in chronic patients (5) and *Panstrongylus megistus* (1), TcII and TcI, respectively. For each strain, groups of 20 Swiss mice were inoculated with 10,000 TS/animal or 2 x 10⁴ TS/animal, when the parasitemia was subpatent: 10 were treated with BZ 100 mg/kg/day (TBZ), for 20 consecutive days, and 10 constituted the untreated control group (CNT). To monitor the cure were used fresh blood examination (ESF), blood culture (HC), polymerase chain reaction (PCR) and Enzyme - Linked Immunoabsorbent assay (ELISA). Using the arbitrary criteria of Toledo et al 2003, the strains were classified as resistant when the cure rate ranged from 0 to 33%, intermediate sensitivity, 34 - 66% and above 67% sensitive. **Results:** TBZ mice showed a significant reduction in the parasitological, molecular and serological parameters in relation with CNT animals, to strains belonging to the DTU TcI and TcIV from Amazon and they showed more significant reductions ($p < 0.005$) of the evaluated parameters (8/9) when compared to TcII strains from Paraná (4/9). The specific treatment promoted suppression of parasitemia in all mice inoculated with strains of *T. cruzi* studied. Moreover, we observed a significant reduction ($p = 0.000$) in the time passed after the initiation of treatment and the negative FBE to the TBZ animals. The general rate of cure obtained was about 60% and ranged from 27.3% to 100%, including strains resistant, intermediate sensitive and sensitivity to BZ, being independent from DTU, geographical origin and host. The resistance profile was observed, even among natural populations of the parasite without prior drug exposure. It wasn't possible to establish a predominant profile of susceptibility to the studied strains according to the DTU for which they belong, since this is justified by the presence of distinct haplotypes within the strains tested, and also by use of PCR, a technique proven to be more sensitive, allowing a greater detection of treatment failure. **Main Conclusions:** The greatest number of significant reductions to the strains of Amazon TcI and TcIV as well as the suppression of parasitaemia, negative FBE and cure rate of around 60% indicate the one hand, the use of BZ in patients of this region, and in other hand, show the need for continuous search for new more effective treatments for Chagas disease. **Keywords:** *Trypanosoma cruzi*; mice; benznidazole; Amazon. **Supported by:** Fundação Araucária / CNPq. **E-mail:** anapeteson@hotmail.com

Chagas074- Quality of life and barriers in access to health care and treatment for Chagasic mothers in Colombia

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Introduction: In Colombia, Chagas disease is estimated to affect near 140,000 women in fertile age and near 1,000 annual congenital cases occurs. As a neglected disease, Chagas is coupled with some social conditions. Gender has been low explored in terms to access to health service and quality of life. Our objective is to describe some health care access conditions and quality of life in a group of mothers with Chagas disease in Colombia. **Material and methods:** As part of the first Pilot Program for Surveillance of Congenital Chagas Disease of Colombia, trypanocidal treatment with Nifurtimox was administered for diagnosed mothers included in the screening phase who ended their breastfeeding period. Both telephonic and personal medical supervision of their treatment were provided, following with laboratory ordered at 20, 40 and 60 days, being performed at the closest health care center from their location. A medical consultation after the ending of the 60 day period was given. Perceptions about the disease, social network, family support, economic conditions and access barriers to health services were characterized by a structured questionnaire. In turn, quality of life was measured with the WHOQOL-BREF standardized questionnaire. **Results:** Overall, 55 mothers from three departments (14 from Boyacá, 39 from Casanare and from Meta) with a mean age of 29.3 years (SD 7.9) were included. Of all, 57.7% reside in rural area. Mean of children was 2.4 (range: 0 to 10). Civil status was: common law-marriage (65.5%), married (29.1%) and single (6.7%); health insurance: contributed (6.8%), subsidized

(94.1%) and no insurance (5.5%). Socioeconomic stratum was I (86.8%), II (11.3%) and III (1.9%). Level of education was any (1.8%), incomplete primary school (32.7%) complete primary school (23.6%), high school (3.4%), technician/university education (10.9%). Main occupation was house: (89.1%). Of all, 22.6% reported Chagas in relatives. Regarding perceptions of the disease most women perceived the disease as a mortal threat, taking religious beliefs as a tool for confronting health troubles. In different domains of the WHOQOL-BREF scale, women with Chagas having lower school level, lower income level and perceiving less support from their friends, family or religion, were more prone to obtain lower scores. **Main conclusions:** Most mothers with Chagas disease are inhabitants of rural communities, have subsidized insurance and low school and income levels. When these characteristics are conjugated with no support from their social network and low personal/religious beliefs, measured quality of life tends to be lower. **E-mail:** elgrancombo@gmail.com

Chagas075- Evaluation of anti-*Trypanosoma cruzi* activities of nitrosyl/nitro ruthenium complexes *in vitro* and *in vivo*

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Benznidazole has been used to treat Chagas disease since the decade of 70ies. Both drugs are effective in acute phase or recent chronic phase of the disease, but they can induce side effects for the patients. Therefore, more efficient drugs with lower toxicity are need for the treatment of this disease. Synthesis of transition metal compounds, especially ruthenium, has increased over the years and data have shown many biological applications of these compounds. The discovery of pharmacological functions of nitric oxide (NO), mainly on infection by protozoa, has led the development of NO donor compounds as therapeutic agents. This study aimed to evaluate the anti-*T. cruzi* activity of the nitrosyl/nitro ruthenium complexes, *cis*-[RuCl(NO₂)(dppb)(5-mebipy)], *cis*-[Ru(NO₂)₂(dppb)(5-mebipy)], *ct*-[RuCl(NO)(dppb)(5-mebipy)](PF₆)₂ and *cc*-[RuCl(NO)(dppb)(5-mebipy)](PF₆)₂; (dppb=1,4-bis(diphenylphosphino)butane; 5-mebipy=5,5'-dimethyl-2,2'-bipyridine). The cytotoxicity assay was performed using splenocytes from BALB/c mice in the presence of the compounds at different concentrations. Trypanocidal effects were evaluated *in vitro* and *in vivo*. The evaluation of anti-*T. cruzi* activity *in vitro* was performed by incubating trypomastigotes and epimastigotes forms in the presence of the complexes. *In vivo* experiment was performed using female BALB/c mice infected with 10⁴ parasites per mouse and orally treated with 25 mmol/kg/day of each compound, daily, for 5 consecutive days. Scanning electron microscopy was performed to evaluate ultrastructural effects of the most active compound. The LC₅₀ values of *cis*-[RuCl(NO₂)(dppb)(5-mebipy)], *cis*-[Ru(NO₂)₂(dppb)(5-mebipy)], *ct*-[RuCl(NO)(dppb)(5-mebipy)](PF₆)₂ and *cc*-[RuCl(NO)(dppb)(5-mebipy)](PF₆)₂ were 34.43 μM, 16.34 μM, 34.05 μM and 27.96 μM, and the IC₅₀ values for trypomastigotes were 8.38 μM, 2.87 μM, 2.08 μM and 5.85 μM, respectively. The first compound was not active against epimastigote form and the IC₅₀ values of the other complexes were 16.64 μM, 5.69 μM, 26.66 μM, respectively. IC₅₀ values of benznidazole, under the same conditions, were 11.41 μM and 10.68 μM. *In vivo* treatment with the compounds reduced parasitaemia and the groups treated with the compounds *cis*-[RuCl(NO₂)(dppb)(5-mebipy)] and *ct*-[RuCl(NO)(dppb)(5-mebipy)](PF₆)₂ increased the survival of BALB/c mice in acute phase of the Chagas disease. The complex *ct*-[RuCl(NO)(dppb)(5-mebipy)](PF₆)₂ presented the best results *in vitro* and *in vivo*. Scanning electron microscopy demonstrated that the treatment of trypomastigotes with this compound caused membrane fragmentation, surface discontinuities and shrinkage of the parasites. In conclusion, we observed that ruthenium complexes were effective against *T. cruzi* using *in vitro* and *in vivo* experimental models. **Supported by:** CNPq, CAPES, PRONEX and FAPESP. **E-mail:** taniramaturino@aluno.bahia.fiocruz.br

Chagas076- *In vitro* and *in vivo* evaluation of anti-*Trypanosoma cruzi* activity of derivatives of vitamin K

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About 10 million people worldwide are infected by *Trypanosoma cruzi*, etiologic agent of Chagas disease. The absence of effective trypanocidal chemotherapy reflects the need for constant research in this area. Some compounds such as naphthoquinones can reduce trypanothione reductase (TR), specific enzyme of trypanosomatids, which controls cellular oxidative stress. Inhibition of TR favors an oxidative process and death of the parasite. In this work, we investigate the trypanocidal potential of phytylmenadione (K1) and menadione (K3), both vitamins K derived from naphthoquinones. The trypanocidal activity of K1 and K3 was evaluated by *in vitro* assays with replicative form epimastigote, the bloodstream trypomastigotes and the intracellular form amastigote of Y and Colombian strains. Cytotoxicity was determined by mode of incorporation of [³H]-thymidine. Scanning and transmission electron microscopy were performed to analyze the effect of the treatment with K1 and K3 on the ultrastructural of trypomastigotes. *In vivo*, trypanocidal activity was evaluated by observing the levels of parasitemia. Our data demonstrated the high trypanocidal activity of K1 and K3. Both compounds were able to inhibit the proliferation of epimastigotes and amastigotes and reduce viability of trypomastigotes on *in vitro* assays. With emphasis of vitamin K3 which showed lower IC₅₀ values, for all the forms, when compared with the reference drug, benznidazole. For example, K3 had an IC₅₀ against trypomastigotes (Y strain) of 2.19 ± 0.02 µM and the positive control benznidazole presented an IC₅₀ of 12.43 ± 0.52 µM. And for amastigotes (Y strain) the IC₅₀ of K3 was 4.90 ± 0.29 µM and the positive control benznidazole was 13.99 ± 0.39 µM. K1 and K3 presented less cytotoxicity on mammalian cells compared to the parasites, demonstrating selective character. Transmission electron microscopy revealed that the treatment with vitamin K1 and K3, with their respectively IC₅₀ values, resulted in kinetoplast and mitochondria disorganization and the appearance of vacuoles. The scanning electron microscopy revealed that the treatment with vitamin K1 and K3 cause the appearance of membrane protusions, shrinkage of the parasites and discontinuities on the surface of the trypomastigotes after treatment. *In vivo*, the treatment using the dose of 25 mg/kg/day with K3 was able to reduce significantly parasitemia (*p* < 0.05). New research may propose molecular structural improvements to enhance the activity of K1 and K3, offering thus an effective alternative for chemotherapy against Chagas disease. **E-mail:** taniramaturino@aluno.bahia.fiocruz.br

Chagas077- The association of heart and spleen highly diluted medication decreases parasitaemia but not mortality in mice infected by *Trypanosoma cruzi*

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Chagas disease is spread all over American affecting millions of people in Central and South America. Murine experimental infection by *Trypanosoma cruzi* is widely used by researchers seeking for a more effective treatment. This study aimed at evaluating the effect of spleen and heart highly diluted medication over infection by *T. cruzi* in mice. In a blind trial, 40 male Swiss mice with 8 weeks of age were randomly allocated in groups: CG – control group (n=10) treated with the preparation vehicle (7% hydro alcoholic solution) of the medication; SHG – group treated with spleen highly diluted medication 1:10²⁶ (n=10), HHG – group treated with heart highly diluted medication 1:10²⁶ (n=10) and SHG+HHG – group treated with spleen and heart medications associated 1:10²⁶ (n=10). Animals were intraperitoneally inoculated with 1,400 blood trypomastigotes of Y strain of *T. cruzi*. Treatment was carried out 96 hours after inoculation, with medication diluted in water (1mL/100mL) *ad libitum*, from amber recipient during 16 hours. Parasitological parameters assessed: total parasitaemia, parasitaemia peak, pre-patent period. Parasitaemia was evaluated by Brener technique, through daily counting, from the first day of infection. Clinical parameters assessed: weight, temperature, water and food intake and excreta were measured 5

days before infection and daily after inoculation until animals' death or until checking negative parasitaemia for 3 consecutive days. Mortality was registered for a period of 64 days after infection. State University of Maringá's Ethics Committee for Experiments in Animals reg. 054/11. Parasitaemia was compared between control and treated groups with Kruskal-Wallis test, with 5% significance. The association of heart and spleen highly diluted medications lowered parasitaemia significantly which was expressed by lowest maximum peak of parasites ($4,6 \times 10^7 \pm 1,8 \times 10^7$; $4,7 \times 10^7 \pm 3,9 \times 10^7$; $3,9 \times 10^7 \pm 2,6 \times 10^7$; $3,8 \times 10^7 \pm 1,7 \times 10^7$) ($p < 0,00$), smaller total parasitaemia ($8,6 \times 10^7 \pm 3,4 \times 10^7$; $1,0 \times 10^8 \pm 6,1 \times 10^7$; $7,8 \times 10^7 \pm 4,2 \times 10^7$; $8,0 \times 10^7 \pm 4,4 \times 10^7$) ($p < 0,00$) measured in trypomastigotes/mL, considering CG, SHG, HHG and SHG+HHG, respectively. Besides, SHG+HHG association increased in the pre-patente period ($6,56 \pm 0,82$) in relation to CG ($5,25 \pm 1,48$), SHG ($4,70 \pm 1,62$) and HHG ($5,68 \pm 1,41$) ($p < 0,00$). Mortality did not present any difference among CG, SHG, HHG and SHG+HHG ($p = 0,373$; $p = 0,287$; $p = 0,287$). No significant clinical difference was noticed, although SHG+HHG association has showed the worst clinical results among the groups. Such results – lowest parasitaemia peak, longer pre-patent period and worst clinical performance – suggest that other factors linked to *T. cruzi* infection, apart from the parasite number itself, and are determining the illness and mortality levels in murine model. It shows that such medications influence murine infection by *T. cruzi* and that it is necessary to adjust the medication treatment dose/scheme to find better results. **Keywords:** *Trypanosoma cruzi*, highly diluted medication, parasitaemia, mortality, mice **E-mail:** gisajanaina@hotmail.com

Chagas078- Therapies of restriction on Angiotensin II actions in experimental acute *Trypanosoma cruzi* infection

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Introduction: Chagas heart disease (CHD) is the most important clinical manifestation of *Trypanosoma cruzi* infection and presents variable clinical course from asymptomatic to severe form of heart failure. Drugs previously used to improve functional capacity or mitigate cardiac remodeling in CHD (eg. inhibitors of angiotensin converting enzyme – ACE) have also presented actions on inflammatory mechanisms, a *sine qua non* condition for the pathogenesis of CHD. In this study, we evaluated the single and combined action of Enalapril (ACE inhibitor) and Losartan (angiotensin II receptor blocker) during the acute inflammatory phase of experimental Chagas disease. **Material and Methods:** C57BL/6 mice were infected with tripomastigotes forms of “Colombian” strain of *T. cruzi* and treated for 20 days with Enalapril (25mg/Kg), Losartan (15mg/Kg), a combination of both (15mg/Kg each), benznidazole (100 mg/Kg) and vehicle (untreated control). These doses were previously standardized in our laboratory. Parasitaemia and mortality were evaluated daily and after 22 days of infection, animals were euthanized to collect biological samples. The heart was used to perform histological analysis (inflammation and parasitism) and serum for immunoassays. **Results:** It was observed a reduction of blood and tissue parasites load in animals treated with Losartan or Enalapril, but not with the combination of both. Serum levels of inflammatory mediators TNF-alpha, CCL2 and CCL5 were reduced to those Losartan-treated animals which also showed an increase of IL-17 and IL-10 levels. Treatment with Enalapril leads to a reduction of TNF-alpha, IL-17 and CCL5, but maintained IL-10 and CCL2 serum levels. For the immunoassays, treatment with the combination showed similar results to those observed for Enalapril. All treatments induced a reduction in cardiac inflammation and tissue parasitism. **Conclusion:** Our data showed pleiotropic effects of treatments with the monotherapies of drugs which restrict the actions of Angio II through the interference of parasite replication and immune response modulation, culminating in the reduction of the inflammatory infiltrate in cardiac muscle tissue. Together, these findings suggest that these treatments can lead to a protection of heart damage mediated by immune response during acute experimental *T. cruzi* infection. **Supported by:** FAPEMIG, CNPq, UFOP **E-mail:** gpcosta@nupeb.ufop.br

Chagas079- *In vitro* and *in vivo* evaluation anti-*Trypanosoma cruzi* activity of *N*-acylhydrazones oxadiazoles

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Chagas disease, or American trypanosomiasis, is caused by the flagellate protozoan *Trypanosoma cruzi*. One century after its discovery, Chagas disease still remains a major health problem in Latin America. Treatment is based on benznidazole that have toxic effects and limited efficacy. Therefore, new chemotherapeutic agents are urgently needed. In this context, the effects of sixteen *N*-acylhydrazone oxadiazoles were evaluated as potential candidates for anti-*T. cruzi* agents. The cytotoxicity of compounds was determined by incorporation of [³H]-thymidine in splenocyte cultures obtained from normal mice. The trypanocidal effects of the compounds were evaluated *in vitro* with the three forms of the parasite (epimastigotes, trypomastigotes and amastigotes of the Y strain *T. cruzi*). Scanning and transmission electron microscopy were performed to analyze the effect of the most active compounds in the ultrastructural of trypomastigotes. *In vivo*, trypanocidal activity was evaluated by observing the levels of parasitaemia. All *N*-acylhydrazone oxadiazoles tested showed no toxicity to mammalian cells. The *N*-acylhydrazones 6c, 6d, and 6e showed antiproliferative activity for the replicative form epimastigote, as well as cytotoxicity for the infective form trypomastigote and the intracellular form amastigotes of *T. cruzi*. Oxadiazoles 6c and 6d had an IC₅₀ against trypomastigotes of 3.5 ± 3.1 µM and 11.2 ± 3.1 µM, respectively, and the positive control benznidazole presented an IC₅₀ of 11.3 ± 1.88 µM. Transmission electron microscopy (TEM) revealed that the treatment with hydrazones 6c and 6d, with their respectively IC₅₀ values, resulted in deep ultrastructural changes on the mitochondria, kinetoplast, Golgi apparatus and endoplasmatic reticulum of trypomastigotes. The scanning electron microscopy (MEV) revealed that the treatment with hydrazones 6c and 6d cause the appearance of membrane protusions and descotinuities on the surface of the trypomastigotes after treatment. *In vivo*, the compounds 6c and 6d were able to reduce significantly the parasitaemia (*p* < 0.001), as observed in benznidazole-treated controls. Our results showed that the hydrazones were active in vitro and in vivo assays. **E-mail:** calcio0303@hotmail.com

Chagas080- *In vitro* evaluation of anti-*Trypanosoma cruzi* activity of physalins purified from *Physalis angulate*

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Physalis angulata L., Solanaceae, is an annual herb commonly used in popular medicine in many tropical and subtropical countries, and its extracts contain a variety of substances, including a class of seco-steroids (physalins). We have previously demonstrated the immunomodulatory, antimalarial and antileishmanial activity of physalins purified from *P. angulata*. Here we investigated the trypanocidal activity of physalins B, D, F and G. The cytotoxicity of compounds was determined by incorporation of [³H]-thymidine, in cultures of splenocytes obtained from normal mice. The trypanocidal effect was first evaluated by light microscopy through the determination of IC₅₀ values for epimastigote and trypomastigote forms of *T. cruzi* (Y strain). We also evaluated the effects of the compounds in intracellular forms in cultures of macrophages infected with *T. cruzi* trypomastigotes. Transmission electron microscopy was performed to analyze the effects of physalin B in the ultrastructure of trypomastigotes. Our data demonstrated the high trypanocidal activity of the physalins against bloodstream trypomastigote and epimastigote forms. Physalins B and F had an IC₅₀ for trypomastigotes of 0.68 ± 0.007 µM and 0.84 ± 0.04 µM, respectively, whereas the positive control benznidazole presented an IC₅₀ of 11.3 ± 1.88 µM. Physalin B is approximately fifty times more cytotoxic for trypomastigotes than to mammalian cells. In the

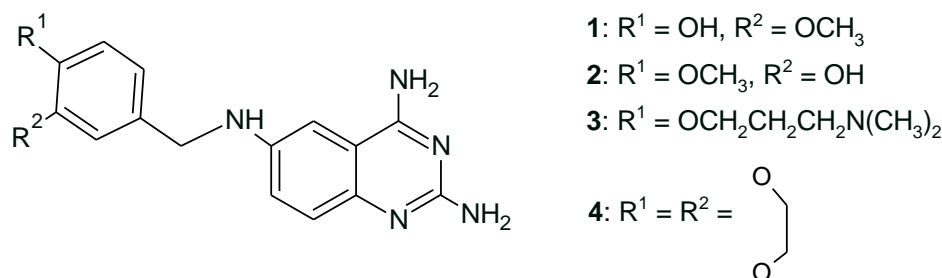
model of macrophage infection, all four physalins were able to reduce the percentage of infected cells and the intracellular parasite number at concentrations non-cytotoxic to macrophages. Transmission electron microscopy revealed that the treatment of trypomastigotes with physalin B at 0.68 μM induced kinetoplast disorganization, alterations in the Golgi apparatus cisternae and endoplasmatic reticulum, a light extraction of the cytoplasm of the parasite and the appearance of myelin figure that may indicate autophagy. Our results showed that the physalins tested were very active *in vitro* against both extracellular and intracellular forms of *Trypanosoma cruzi* and suggest their potential use in the development of new antichagasic chemotherapy. **E-mail:** calcio0303@hotmail.com

Chagas081- Synthesis and biological activity of *N*⁶-benzylquinazoline-2,4,6-triamine derivatives as anti-*Trypanosoma* agents

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Introduction: Chagas' disease (American Trypanosomiasis) is an endemic disease caused by the protozoan *Trypanosoma cruzi*.¹ currently; Nifurtimox (**Nfx**) and benznidazole (**Bnz**) are the only available drugs for the treatment of this illness. However, they induce significant side-effects and their efficacy varies among different *T. cruzi* strains according to the geographical area.² consequently, the development of safe and affordable compounds with anti-*T. cruzi* activity is urgently needed. In this context lies the present work where a series of quinazolin-2,4,6-triamine (**1–4**) were synthesized and initially they were in vitro screened against bloodstream trypomastigotes of two *T. cruzi* strains (NINOA, INC5). Subsequently, the most active compound was submitted to in vivo evaluation.



Material and methods: The synthesis of compounds (**1–4**) began with the cyclocondensation of 5-nitroanthranilonitrile with guanidine hydrochloride led to 6-nitroquinazoline-2,4-diamine; which was treated with acetic anhydride followed by hydrogenation to furnish *N,N*-(6-nitroquinazoline-2,4-diyl) diacetamide. Condensation of amide with substituted benzaldehydes gave intermediates imines, respectively. The imine group was reduced and then hydrolyzed to yield **1–4**. Target compounds were tested in vitro against bloodstream trypomastigotes of two *T. cruzi* strains (NINOA, INC5). The percentage of lysis for each compound was determined after 24 h of parasite exposition. **Nfx** and **Bnz** were used as reference drugs. As regards in vivo evaluation, mice were infected with *T. cruzi* NINOA strain. Compound **4** was applied as oral single dosage of 200 mg/Kg at 14 days post-infection. Furthermore, the parasitaemia was determinate at 0, 2, 4 and 6 h. The positive control drugs were **Bzn** and **Nfx**. **Results:** Target compounds (**1–4**) were obtained in fair yields and purity as solids with sharp melting points. All the spectrometric and spectroscopic data for these compounds are in agreement with the expected structures. Of the four compounds tested, only **4** showed a better profile of lytic activity with respect to reference drugs against two trypomastigotes strains. The same compound also showed antiprotozoal activity in infected mice; although in this case, it was not better to reference drugs. **Main conclusion:** Our results indicate that despite its significant activity against the bloodstream trypomastigotes of *T. cruzi*, compound **4** needs to be further modified in order to improve their in vivo activity. **Financial Support:** PAPIIT-UNAM IT216411 **E-mail:** franher@unam.mx

Chagas082- Lack of effect of simvastatin on structural remodeling in animal model of Chagas cardiomyopathy

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Introduction: Chagas cardiomyopathy (CM) is characterized by a large amount of fibrosis and inflammation. As simvastatin (simva) has anti-inflammatory effects, we hypothesized that it could be an important drug in the treatment of patients with CM. The purpose was to evaluate simva in the myocardium remodeling and inflammation in an animal model of CM. **Methods:** 123 hamsters were divided: C-controls(25), CSimva-controls with simva 10mg/Kg/day(25), Simva1-infected treated from the beginning with the same dose of simva (25), Simva2-infected treated after 4 months(24); Infect-untreated(24). Follow-up of 10 months. Interstitial collagen volume fraction (ICVF) RV and LV measured using videomorphometry and Picrosirius red stained heart. Metalloproteinase9 (MMP9) was obtained by zymography. Gene expression of TNFalpha, IFNgamma, IL10 by real time PCR and ΔCt . Survival by Kaplan-Meier and log rank. Comparison between groups by Kruskal-Wallis; $p \leq 0.05$. **Results:** infected animals (Simva1 = 189 ± 133 days Simva2 = 150 ± 124 ; Infect = 138 ± 123) lived less than controls(C = 257 ± 80 ; CSimva = 283 ± 58) ($p \leq 0.05$) with no difference among infected. ICVF-RV (%) was greater in infected groups (Simva1 = 3.88 ± 1.14 , Simva2 = 2.22 ± 0.64 ; Infect = 4.38 ± 0.83) than in controls(C = 1.12 ± 0.31 ; CSimva = 2.18 ± 0.73) ($p \leq 0.05$) with no difference among infected groups. ICVF-LV(%) was greater in infected animals (Simva1 = 1.83 ± 1.01 , Simva2 = 1.52 ± 0.93 ; Infect = 3.01 ± 0.66) than in controls(C = 0.68 ± 0.31 ; CSimva = 0.81 ± 0.28) ($p \leq 0.05$) with no difference among infected. MMP9 was higher in infected groups(Simva1 = 2394 ± 2441 , Simva2 = 5673 ± 4091 ; Infect = 2392 ± 2042) compared to controls(C = 954 ± 2332 ; CSimva = 454 ± 1123) ($p \leq 0.05$) with no difference among infected. TNFalpha did not have difference among infected groups (Simva1 = 5.33 ± 3.66 , Simva2 = 4.44 ± 2.17 ; Infect = 6.13 ± 3.24). IFNgamma in infected groups(Simva1 = 5.47 ± 3.56 , Simva2 = 4.46 ± 2.08 ; Infect = 4.21 ± 2.09) was higher than in controls(C = 8.50 ± 2.59 ; CSimva = 6.84 ± 2.53) ($p \leq 0.05$) with no difference among infected. IL10 in infected animals(Simva1 = 9.07 ± 4.62 , Simva2 = 7.76 ± 4.77 ; Infect = 8.11 ± 4.48) did not have difference and the values were greater than controls(C = 14.11 ± 4.40 ; CSimva = 12.55 ± 3.90) ($p \leq 0.05$). **Conclusions:** simva did not attenuate deposition of interstitial collagen, did not change dynamics of collagen degradation, did not decrease inflammation, and did not reduce mortality. **E-mail:** barbara.ianni@incor.usp.br

Chagas083- Effects of a combined treatment with Itraconazole/Pravastatin and Allendronate for asymptomatic *T. cruzi*-infected individuals: A randomized controlled trial in the CHICAMocha Study

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Introduction: Prevention of Chronic Chagasic Cardiomyopathy by giving trypanocidal therapy (TT) to asymptomatic infected populations remains a challenge. Uncertainty on its efficacy, unfavorable tolerance and limited availability prevent wider use of conventional agents (Nifurtimox or Benznidazole). Commonly used imidazolic agents in combination with statins or biphosphonates have all been suggested as potential trypanocidal agents. **Materials and Methods:** We designed a randomized, controlled, blinded, parallel group, phase II trial testing the hypothesis that an 8-week combined therapy (CT) with Itraconazole/Pravastatin (200/40 mg/day) and Allendronate (A: 70 mg/week) modified parasitic load of exposed individuals. Eligible individuals (18-50 years) needed to have positive serostatus, negligible probability of re-infection, and to be apparently healthy. We conducted two study protocols comparing first CT with all agents, A as a single treatment, and no treatment (1:1:1), and then CT with A (for 8 versus 16 weeks) and matching placebos (1:1:1:1). Our main outcome measure for both protocols was having a positive PCR at least one year after randomization. To test reproducibility and increase sensitivity, we ran

repeated PCR at baseline, 1st, and 3rd year for the first protocol, and 3 PCR with 10-day intervals over the 12th month for the second protocol. Additional efficacy outcomes were changes in F29 serology and pro-BNP. Safety outcomes included a composite of severe clinical reactions (SCR) or significant raise in blood markers throughout the study, and incidence of mild symptoms (in the placebo-controlled protocol). **Results:** We randomized 242 consenting individuals to CT (n=71), A (n=103), and no treatment or placebo (n=70), with 218 having PCR (636 tests overall, mean=2.9 tests/individual). Forty-five participants (71.4%) had at least one positive PCR (primer 121/121-122) in the CT group, compared to 62 (69.7%) and 46 (68.7%) in the A and untreated/placebo groups, respectively (p=0.941). Participants with positive PCR for primer S35/S35-S36 were 25 (39.7%), 46 (51.7%) and 30 (44.8%), for CT, A and untreated/placebo groups, respectively (p=0.331). We did not observe differences in the change of F29 serology titers or pro-BNP levels across treatment groups over follow-up. Logistic regression showed that neither CT nor A reduced the likelihood of positive PCR compared to the reference group. There were no SCRs reported, nor differences in the blood work or mild symptoms between groups up to 1 year after randomization. **Main conclusions:** Although safe, treatment with a combination of Itraconazole/Pravastatin or Allendronate did not modify parasitic load, antibody titers or the neurohormonal response in our study. This protocol proved to be a feasible and valid strategy to rule in/out candidates for TT in the future. **E-mail:** jvillar@unab.edu.co

Chagas084- Activity of essential oils components obtained from Colombian plantas against *Trypanosoma cruzi* and *Leishmania spp*

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Introduction: Chagas disease and leishmaniasis are infections that affect million people and constitute a major public health problem in endemic countries. The treatments available are reduced and associate to inadequate schemes of administration. The search of new options is a priority. The essential oils (EO) have presented widely biological activities and could be a major source of antimicrobial substances. Similarly, EO from some Colombian aromatics plants has shown activity against *Trypanosoma cruzi* and *Leishmania* species in prior studies. However EO is complex mixtures from various chemical natures, therefore some their pure components could be lead compounds in the search of antiparasitic treatment. Few studies provide anti-leishmanial or anti-tripanosomal effect of components of this EO. The aim of this work was to determine *in vitro* antiparasitic and cytotoxic activity of components of EO obtained from Colombian plants in *T. cruzi* and *Leishmania* species as a potential and interesting option of treatment of these important parasitic diseases. **Material and Methods:** Biological activities of 17 components of EO were tested. Initially components were selected from chemicals composition of active EO against parasites in a previous study of our groups. The toxicity in Vero and THP-1 mammalian cells lines was evaluated using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide)] reduction assay. The antiparasitic activity was tested in free and intracellular forms of *T. cruzi* and *Leishmania* species. The results were expressed in inhibitory concentrations (IC₅₀ and ₉₀) and cytotoxic (CC₅₀ and ₉₀). They were calculated by sigmoidal regression analyses (Mxlfittm, ID Business Solution, Guildford, UK). The selectivity indices were calculated (IS) and the component activity with respect to the EO that contained them too was calculated using Index of Activity (IA). Chemoinformatics Analysis by toxicity were done through software Osiris property Chemistry (<http://www.organic-chemistry.org/prog/peo/>). **Results:** In Vero cells the components α -pinene, linalool, citral, R- carvone, carvacrol and thymol showed CC₅₀ between 2.22 and 37, 22 μ g/mL. *Trans*- β -caryophyllene showed toxicity with CC₅₀: 17,77 and CC₉₀: 26,11 μ g/mL. In THP-1 cells, citral and eugenol was toxicity with CC₅₀ between 2, 94 and 49,97 μ g/mL. In *T. cruzi*, α -pinene, carvacrol, thymol and *trans*- β -cariofileno were activities with CI₅₀ between 0.34 and 2.90 μ g/mL and IS greater of 3 in epimastigotes. α -pinene, s-carvone and thymol presented partial intracellular and selective activities between 1.92 and 3.16 μ g/mL in amastigotes. *trans*- β -caryophyllene was the most active in amastigotes (CI₅₀: 25,13 and CI₉₀: 71,07 μ g/mL) nevertheless this activity was not selective. The components α -pinene, carvacrol, thymol and *trans*- β -caryophyllene were more active than the EO that contained them with values of IA between 10.79 to 94,71. In *Leishmania*, geranial, carvacrol *trans*- β -caryophyllene and eugenol showed partial activities between 12.74 to 38.5 μ g/mL in

promastigotes and *trans*- β -caryophyllene was partially active in amastigotes intracellular of *L. chagasi* and *L. panamensis* with IS de3, 44 and 4.54 respectively. Mutagenic and tumorigenic risk was established by Thymol and S-carvone through chemoinformatics analysis. None components evaluated was more active than reference drugs (nifurtimox and anfotericina B). **Conclusions:** The components α -pineno, carvacrol and *trans*- β -cariofileno showed antiparasitic activity in forms of *T. cruzi* and low cytotoxicity therefore could be lead compounds being necessary continue the related studies. Otherwise results of IA demonstrated that activity of EO not always directly was related to its majority component, it being possible minority components even showed better effect than the active EO that contained them. **E-mail:** l.vivianaherrera@gmail.com

Characterization and Pathogenicity of T. cruzi

Chagas085- Characterization of *Trypanosoma cruzi* in domiciliary triatominae from municipalities of Minas Gerais, Brazil

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The current stage of the Chagas disease Control Program is the epidemiological surveillance, nevertheless major challenges exists for its maintenance. Minas Gerais (MG) presents a rich fauna with 20 triatomine species and situations of persistence or recurrence of domiciliary foci. The objective of the present study was to confirm former diagnosis of triatomine infection performed by health service personnel and to determine the epidemiological pattern of *Trypanosoma cruzi* circulating in intra and peridomestic environments. Samples of triatomines received by the Reference Laboratory on Triatominae and Epidemiology of Chagas Disease (LATEC) in the period 2010-2011 were used in analysis. The insects were collected from households in municipalities considering: high and medium risk of triatominae reinfestation and risks of human *T. cruzi* infection occurrence, according to the criteria established by the Secretaria de Saúde de MG. All insects were examined for *T. cruzi* infection by laboratory workers of regional health departments. In LATEC, insects guts contents of 220 triatomines were stored on filter paper. The multiplex PCR was used to confirm the triatomine infection diagnosis performed by health services personnel and for molecular characterization of *T. cruzi* I (TcI) and *T. cruzi* II (TcII). DNA extraction was made by lysis of the samples. Six *T. vitticeps* specimens were analyzed (5 intra and 1 peri) from 5 municipalities, 13 samples of *P. megistus* (3 intra and 10 peri) from a single municipality and 201 *T. sordida* specimens from 32 municipalities (peridomestic). The Insects infection rate was 2.1%, and all parasites characterized as TcI. Considering the studied species, *T. vitticeps* presented the highest rate of infection, corresponding to 83.33%, whereas *T. sordida* infection rate was 1.4%. From the samples of *P. megistus* no *T. cruzi* fragments was amplified, confirming the analyzes conducted by laboratory technicians of health services. An exception on findings was the detection of a *T. sordida* specimen *T. cruzi* infection using only PCR. These results are in agreement with former studies that indicated high rates of *T. cruzi* infection on *T. vitticeps*. Registries aging 20 years demonstrated presence of TcII in triatomines from MG state, which was an indicative of active transmission. Results in the present study confirms the interruption between domestic and sylvatic cycles, thereby parasites (TcI) are exclusively of sylvatic origin. Nevertheless, the occurrence of *T. cruzi* infected insects inside houses points to the needing of continuous epidemiologic surveillance on MG, once there are native species capable of domiciliary invasion and colonization, those hosting sylvatic *T. cruzi* strains. **E-mail:** silvia@cpqrr.fiocruz.br

Chagas086- Biological behavior of different *Trypanosoma cruzi* strains in an experimental model of mixed infection in immunosuppressed Balb/c mice

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Introduction: Chagas disease can be marked for an acute phase with patent parasitaemia followed by a long asymptomatic state on which almost 40% of the patients grow deadly clinical forms of disease. Due to *T. cruzi* diversity the presence of mixed infections at the same host both in vertebrate or invertebrate particularly, in endemic areas has been observed. Additionally the interactions between different populations of the parasite, may result in changes in biological properties as a result of reinfection. In this study, we evaluate the behavior of two Tc I strains (AQ1-7 and MUTUM) and one Tc II strain (JG), in mixed infections of JG+AQ1-7 and JG+MUTUM, analyzing biological behavior in blood at 15, 45 and 70 days p.i. in Balb/c mice, before and after immunosuppression induced by Cyclophosphamide. **Material and Methods:** Animals were i.p. inoculated with 5×10^3 trypomastigotes of JG, AQ1-7 or MUTUM (single infection) or a mixture of 2.5×10^3 trypomastigotes of each JG plus AQ1-7 or JG plus MUTUM (double infection). On acute phase parasitaemia was detected by direct microscopy of fresh blood each 2 days during 40 days. During immunosuppression parasitaemia was determined by microhematocrit test every day during 30 days. **Results:** In the acute phase, only animals inoculated with JG strain and mixed infections, showed patent parasitaemia. In addition, the mixed infection group JG + AQ1-7 showed a positive association presenting in average higher parasitaemia levels and parasitaemia peak when compared to JG strain single infection, in contrast, the infection with JG + MUTUM, showed a negative association, presenting in average lower parasitaemia levels and parasitemia peak when compared to JG strain single infection. The differences between parasitaemia peak of JG+AQ1-7 and JG+MUTUM were statically significant ($p < 0.05$). The infections of Tc I strains seemed to be more pathogenic than the Tc II, due to the highest mortality rate observed between JG strain and AQ1-7 ($p < 0.01$) or MUTUM strain ($p < 0.01$). In addition, the association of JG strain with Tc I strains seems to play a protective role, due to a lower mortality rate, especially when associated with AQ1-7 strain. During immunosuppression Tc I strains showed patent parasitaemia but blood parasitism was less intense than Tc II single infection or in mixed infections. **Main conclusions:** This study showed important differences between different *T. cruzi* strains biological behaviors in blood in acute phase or during immunosuppression. The JG strain seemed to play a protective role in mixed infections while Tc I strains have shown to be more pathogenic particularly after immunosuppression. **E-mail:** tonsales@hotmail.com

Chagas087- Biological and genetic characterization of *Trypanosoma cruzi* isolates in Venezuela

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Introduction: *Trypanosoma cruzi* etiological agent of Chagas disease is comprised by heterogeneous subpopulations at biological, genetic, clinical and epidemiological level. In Venezuela, this disease is considered controlled. Nevertheless, recently have emerged some outbreaks in non endemic areas. The objective of this work was the characterization at biological and genetic level some isolates from *T. cruzi* found in vectors and reservoirs of Venezuela. **Materials and methods:** *T. cruzi* isolates (n=34) derived from *Panstrongylus geniculatus* (17), *Rhodnius prolixus* (5), *Didelphis marsupialis* (8), *Rattus rattus* (2) and *Homo sapiens* (2) were characterized in murine model and maintained in NNN medium for DNA extraction by Chelex resin, and amplification of kinetoplast DNA from *T. cruzi* (PCR-kDNA). The genotyping was performed through of PCR based on amplification of non-transcribed mini-exon spacer, and 24Sα and 18 ribosomal DNA. **Results:** Infection in murine model revealed a prepatente period of 13

days, parasitic peak of 7×10^4 flagellates per ml blood, myotropism and 80% of mortality. All strains revealed biological heterogeneity and correspondence with *T. cruzi* I subpopulation. **Conclusions:** *T. cruzi* I has been traditionally associated with wild and synanthropic environments, however in Venezuela has been found in domestic cycles. This can be indicative of gene flow from wild environments to human housing. **Financial support:** Projects: Proyecto en Red Misión Ciencia N° 2007001442 and N° 2008000911-6, Proyecto FONACIT N° G-2005000827 and Ayudas Menores CDCH-UC-0440-10 y 0450-10 Universidad de Carabobo. **E-mail:** daisy8328@hotmail.com

Chagas088- Analysis in vitro and in vivo genetic and biological behavior of different taxonomic groups of *Trypanosoma cruzi*

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Introduction: Factors such infectivity and capacity of intracellular multiplication of six taxonomic groups (DTUs) of *T. cruzi* are poorly studied. The aim was analyze the genetic and biological behavior of different DTUs of *T. cruzi* infection *in vitro* and in different cell types and *in vivo* in mice. **Material and methods:** Eight strains (TcI - Alv, AQ1-7 and Mut; TcII - 425 and Rom; TcIII - 115; TcIV - 3048 and TcV - PV) were studied and *in vitro* analysis was performed by infection with MK2 cells, VERO and macrophages. The infectivity was assessed three hours after infection and intracellular multiplication capacity after 24, 48 and 72 hours by counting of intracellular parasites. The analysis was performed *in vivo* in mice (n=10/cepa) to evaluate the parasitaemia and tissue tropism. Genetic characterization was conducted in the three cell types and genetic profiles found compared to the inoculum through LSSP-PCR technique. **Results:** MK2 infectivity of all strains was similar (73%). Two strains of the TCI (AQ1 and Alv-7) showed similar capacity of infective in all cells, however the strain Mut infected VERO cells and macrophages with low efficiency. Strains TcII (425 and Rom) showed infectivity variable (59.6% and 81% respectively) and a high efficiency of intracellular multiplication in 24 hours and a decline to 48 and 72 hours after infection in Vero cells and MK2. Macrophage proliferation was higher at 48 hours and reduced at 72 hours. The strains TcIII, TCIV and TCV showed low infectivity in macrophages and variability of results in VERO. The strain TcIII showed high intracellular capacity of multiplication in all cells presenting multiplication factors of 89, 149 and 91 in MK2, VERO and macrophages cells respectively. The hybrid populations TcIV and TcV showed the highest factor of intracellular multiplication which increased in all cells analyzed. The *in vivo* analysis showed a possible classification of the strains: Mut, PV, 115, and 425, classified in high infectivity and low pathogenicity and strains ALV, 3048 AQ1-7 classified in high infectivity and low pathogenicity. Genetic characterization showed that the strains remained grouped with similarities between 32.8% and 41.1% in two divergent arms which were stable at all times showing high similarity to the profile of the inoculum (T0). The populations of *T. cruzi* were not clustered according to their taxonomic groups. The stability of the groups showed that there was no selection of populations *in vitro*. The LSSP-PCR profiles of strains of *T. cruzi* showed high similarity to MK2, VERO and macrophages. **Conclusion:** Our results demonstrate a pattern of behavior strain-dependent and thus could not establish correlations with taxonomic groups and genetic characteristics and / or biological characteristics of parasites. **E-mail:** tiago_pereira_lima@hotmail.com

Chagas089- DNA repair enzymes are related to *T. cruzi* infection persistence

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To establish a chronic infection *T. cruzi* must resist the oxidative damage to its DNA exerted by oxygen and nitrogen free radicals (ROS/RNS) generated by their own metabolism and by the insect vector as well as the mammalian host cells. We propose that the DNA repair BER pathway is activated when *T. cruzi* is exposed to ROS/RNS, allowing its survival. The mechanisms responsible for DNA repair of the DNA damage by ROS/RNS in *T. cruzi* are unknown. Two recombinant *T. cruzi* DNA repair apurinic/apyrimidinic endonucleases (TcAP1, TcAP2) were identified in the parasite genome. Modeling of deduced amino acid sequences present structural characteristics similar though not equal than the

corresponding mammalian enzymes. Using an antibody prepared against a TcAP1 or TcAP2 peptides those enzymes were recognized in the three cellular forms of the parasite by western blot. Those enzymes do not increase its expression in epimastigotes or trypomastigotes treated with oxidative species. TcAP1 and TcAP2 were cloned in a parasite expression vector. Transfected TcAP1-GFP and TcAP2-GFP epimastigotes show that the DNA repair enzymes are localized in the nucleus but not in the kinetoplast of the parasite. Interestingly, independent overexpression of TcAP1 or TcAP2 moderately increases survival of parasites when submitted to oxidative stress. These results suggest that the BER pathway and particularly TcAP1 and TcAP2 play an important role in *T. cruzi* oxidative DNA damage resistance leading to parasite persistence in the insect vector and in the mammalian host cells. **Supported by:** FONDECYT-Chile1090124 (to NG), 1120230 (to UK) and CONICYT PIA-Act 112. **E-mail:** gcabrera@med.uchile.cl

Chagas090- Epidemiology and molecular characterization of *Trypanosoma cruzi* isolates from humans, triatomines and wild mammals from the oriental Amazonia

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Introduction: Acute Chagas disease (ACD) is mainly transmitted via oral route in the Brazilian Amazon, in outbreaks of an average of 100 cases per year and a mortality rate of 5% in the States of Pará (PA), Amapá (AP) and Maranhão (MA), Brazil. Its regional cycle involves mammals (including humans) and triatomines in distinct ecotopes infected with *Trypanosoma cruzi* which have molecular patterns of distinct lineages. We analyzed four ACD outbreaks in the cities of Barcarena, Belém and Cachoeira do Arari (PA), and in Santana (AP), whereas in the city of São Luís (MA) we studied cycles of *T. cruzi* and there were no reported cases of ACD. **Material and methods:** Parasitological (thick blood film–GE, QBC[®]; xenodiagnosis–XE; and hemoculture–HC) and serological diagnosis (indirect hemagglutination–IH; and IgG and IgM indirect immunofluorescence assays–IIF) were performed. Wild mammals and triatomines associated with the outbreaks were collected using traps in regional palm trees. The isolates of *T. cruzi* were genotyped using the mini-exon gene. **Results:** A total of 63 cases of ACD were confirmed: 41.3% (26/63) through GE; 58.7% (37/63) QBC[®]; 79.4 (50/63) XE; and 61.9% (39/63) HC. The IH test was positive in 3.05% (81/2648) of the cases, and the IIF was IgG positive in 2.49% (66/2648) and IgM in 2.37% (63/2648) of the 2648 samples. The samples collected in São Luis were all negative. We captured 24 mammals: 13 *D. marsupialis*, 1 *M. cinerea*, 5 *P. opossum*, 3 *M. nudicaudatus*, 1 *O. macconnelli*, 1 *O. bicolor* and 433 *R. rattus*. The infection rate (IR) for *T. cruzi* was 7.14% (29/404). A total of 3279 triatomines were captured and analyzed: *T. rubrofasciata* (n=3008), IR 30.46% (39/128); *R. robustus* (n=137), IR 76% (79/104); *R. pictipes* (n=94), IR 56.9% (49/86%); *E. mucronatus* (n=6) and *P. geniculatus* (n=12) with an IR of 50%; and the uninfected *R. neglectus* (n=5) and *P. lignarius* (n=6). The TCI lineage was identified in 46, 31 and 74 human, mammal and triatomines isolates, respectively. **Conclusions:** Parasitological test confirmed cases of ACD in Pará and Amapá and XE, HC and QBC[®] were more sensitive than the GE. IH and IIF assays were sensitive to detect ACD in different infection periods. Wild mammals and triatomines infected with *T. cruzi* captured near de patients' houses were associated with its transmission. Only the TCI lineage (the most frequent in the region) was identified. In São Luis, the parasite presents a domestic cycle associated with the domestic rat and with *T. rubrofasciata*, and a sylvatic cycle maintained by Didelphidae. Both circulate with the TCI. Markers with a better resolution can determine the transmission cycles, contamination routes and hosts involved in cases of ACD in the Amazon. **E-mail:** veravalente@iec.pa.gov.br

Chagas091- Genotyping of *Trypanosoma cruzi* samples isolated from chagasic patients from two municipalities in the Jequitinhonha Valley, MG, Brazil

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Introduction: Currently *T. cruzi* is classified into six discrete taxonomic units (DTUs) TcI, TcII, TcIII, TcIV, TcV and TcVI that present significant differences concerning to geographic distribution and biological properties. The objective of this study was to verify the genetic profile of parasite's samples isolated of patients from Berilo and José Gonçalves de Minas municipalities, Jequitinhonha Valley, MG, an important endemic area of Chagas disease in Brazil. **Material and Methods:** Molecular characterization was performed by five different markers in 51 *T. cruzi* samples isolated by hemoculture from patients in the chronic phase of the disease. DNA extraction was carried out using the DNA Purification KIT (Promega, USA). The first identification of the six *T. cruzi* genetic groups followed the methodology of Lewis *et al.* (2009). This methodology explores the profiles of bands of dominium D7 of 24Sα rDNA, digestion of amplified products of two genes with their respective restriction enzymes (HSP60/ECORV e GPI/HhaI) via RFLP-PCR. **Results:** In this first phase of characterization 43 samples were typed as *T. cruzi* of TcII DTU according to the new consensual classification of Zingales *et al.* (2009). However, it was not possible to define the genetic identity of 8 isolates based on this methodology. Following, the products obtained were submitted to polymorphic analysis of the subunit II of cytochrome oxidase and of the spliced leader intergenic region of mini-exon which when associated classified these isolates as TcVI. After, the RAPD technique was employed in the evaluation of intra-specific variability of the *T. cruzi* samples using ten primers. Data obtained were submitted to the UPGMA analysis and the results corroborated the originated from the other markers identifying the majority of the isolates as TcII and the other eight isolates as TcVI. Additionally, this analysis showed lower intra-group variability in TcII revealing two groups. **Conclusions:** The results of this work confirmed the preliminary data obtained of this region, showing a predominance of *T. cruzi* isolates of TcII in Berilo, similar than the verified in other studies in samples of patients of other regions in the Northeast/South axis of Brazil. Additionally a considerable isolates of TcVI, less frequent in Brazil, were identified. These data confirmed distinct profile of *T. cruzi* in Brazil if compared with other countries of South America. **Financial Support:** FAPEMIG, CNPq, SESU-MEC, UFOP. **E-mail:** maykontavares@yahoo.com.br

Chagas092- Novel approach to design selective and potent inhibitors against *Trypanosoma cruzi* dihydroorotate dehydrogenase

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Introduction: Dihydroorotate dehydrogenase (DHOD) from *Trypanosoma cruzi* (TcDHOD) is a flavoenzyme that catalyzes the oxidation of dihydroorotate to orotate and then the reduction of fumarate to succinate in the *de novo* pyrimidine biosynthesis pathway. TcDHOD is known to be essential for survival and growth of *T. cruzi* and a validated drug target. Previously, we reported the crystal structures of TcDHOD in the native form and in complexes with all physiological substrates and products, which bound to the active site in a closed state. The small volume of the closed active site pocket (178 Å³) has made difficult to design nM order inhibitors. To solve this problem it has been needed to obtain inhibitors bound to the active site in an open state that would give more wide binding volume. In this study we develop a novel approach to obtain potent and specific inhibitors for TcDHOD by obtaining the active site in open state. **Material and Methods:** To get the active site loop in open state, we designed NL-2 and MII-3-045 that were predicted to cause steric repulsion with the loop moiety of the closed form. A library of 5-substituted orotate derivatives were designed based in the open state. Compounds with high docking score were selected and synthesized. Inhibitory activity and selectivity were evaluated by assaying inhibition against *T. cruzi* and human DHODs. Co-crystal structures were obtained by soaking method and X-ray data collected at Spring-8. Inhibitory activity against *T. cruzi* amastigote was also assessed. **Result:** X-ray structure analyses of TcDHOD complexed with NL-2 and MII-3-045 revealed that they bound to the active site in the open state increasing the active site volume to 694 Å³, as expected. The best 5-substituted orotate derivative shows the IC₅₀ of 150 nM and selectivity against TcDHOD more than 11900 times compared to the human DHOD. We present here a total of 50 crystal structures of TcDHOD complexed with 5-substituted orotate derivatives, and derived interactions critical for the selectivity, as well as potential interactions with several amino acid residues around the inhibitor binding site to get more

excellent inhibitors. Some compounds showed growth inhibitory activity against intracellular amastigotes. **Conclusion:** We succeeded to obtain highly potent and selective inhibitors against TcDHOD by a new approach. The designed TcDHOD inhibitors, showed inhibitory activity against *T. cruzi* amastigote stage, chemically validating TcDHOD as drug target. **E-mail:** danielkeninaoka@yahoo.co.jp

Chagas093- Preliminary results of *Trypanosoma cruzi* molecular characterization isolated from sylvatic triatomines in Espírito Santo, Brazil.

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Trypanosoma cruzi, the agent of Chagas disease, presents complex populations that differ in their biological, genetic characteristics and behavior in vertebrate hosts. Various molecular studies are used for genotyping *T. cruzi*. Strains isolated from wild triatomines were characterized by molecular markers based on the mini-exon gene. *T. cruzi* samples were obtained from Atlantic Forest triatomines from June 2010 to May 2011, in Espírito Santo state. The samples were plated in NNN medium supplemented with BHI. DNA extraction was performed by a salting-out method and amplified by polymerase chain reaction multiplex for intergenic spacer for mini-exon gene, using the following primers: Tc1 (5'-TTG CTC GCA CAC TCG GCT GCAT-3'), Tc2 (5'-ACA CTT TCT GTG GCG CTG ATC G-3'), zymodeme 3 (CCG CGW ACA ACC CCT MAT AAA AAT G-3'), *Trypanosoma rangeli* (CCT ATT GTG ATC CCC ATC CCC ATC TTC G-3'), mini-exon gene (5'-TAC CAA TAT AGT ACAGAA ACT G-3'). The amplifications were analyzed by 2% agarose gel electrophoresis. In a total of 51 samples, 38 were characterized for mini-exon gene, as follow: *T. cruzi* I = 15 (39.47%), *T. cruzi* II = 12 (31.57%) and zymodeme 3 = 11 (28.95%). In this study, *T. cruzi* I strain showed the highest circulation among wild triatomines (*Triatoma vitticeps* and *Panstrongylus geniculatus*) in the Atlantic Forest. The strain circulation is related to the presence of animals reservoirs, like marsupials (*Didelphis aurita*) and rodents as food sources, maintaining the parasite sylvatic cycle in the environment. *T. cruzi* II was found in *T. vitticeps* in the wild environment however is linked to the domestic cycle of Chagas disease. Despite the absence of domestic triatomine, *T. cruzi* II circulating are capable to induce parasitaemia in humans if the insect become domiciled. Zymodeme 3 was found in *T. vitticeps* and *P. geniculatus*, in the Atlantic Forest region, but this strain only appear at the Amazon region. Some authors consider this region of the Atlantic Forest as an enclave of the Amazon Forest, so it is possible this strain circulation. This strain shows that the Atlantic Forest presents some similar flora and fauna to Amazon region. The results showed *T. cruzi* population diversity in the Atlantic Forest however ES is not an endemic area for Chagas disease. There is little information about the parasite population and further studies should be conducted to confirm the presence of these strains in the wild environment, providing a better understanding of the transmission in order to reduce the risk of establishment of the disease in domestic environment. **E-mail:** falqueto@ndp.ufes.br

Chagas094- The role of High density lipoprotein (HDL) on the *Trypanosoma cruzi* agglutination by digestive tube extract of *Rhodnius prolixus*

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Rhodnius prolixus is an important vector of *Trypanosoma cruzi*, which is the etiologic agent of Chagas disease. This parasite develops a part of its life cycle along the digestive tube of this invertebrate host. Some aggregated parasites can be observed on the anterior midgut lumen after vector infective feeding. In a previous study Mello et al. (1996) showed that the infection of *R. prolixus* by different *T. cruzi* strains can be positively correlated with *in vitro* agglutination activity of digestive tube extract (DTE) against these parasites strains. The agglutination activity of DTE is effective against rabbit erythrocytes and

epimastigotes of *T. cruzi*, but not against trypomastigotes from cell culture. This kind of agglutination activity against erythrocytes and parasites can also be verified using *in vitro* experiment with *R. prolixus* hemolymph (Ratcliffe et al., 1996). This hemolymph activity can be inhibited by some carbohydrates related to galactose. On the other hand, several other sugars tested in the assays were unable to inhibit the agglutination activity of DTE. However, the DTE agglutination activity was inhibited by *p*-nitrophenol and Polysorbate 20, which are classic inhibitors of hydrophobic interactions. Adsorption experiments showed that the supernatant of DTE previously incubated with erythrocytes no longer agglutinate these blood cells but maintained its agglutination activity against *T. cruzi*. Furthermore, DTE previously incubated with *T. cruzi* did not agglutinate parasites but maintained its agglutination activity against erythrocytes, indicating that these activities against *T. cruzi* or erythrocytes must involve different molecules from DTE. Experiments with various blood diets (whole blood, washed erythrocytes or plasma alone) showed that only DTE from insects fed with plasma in its diets are able to agglutinate the epimastigotes. However, the agglutination activity against erythrocytes has been detected with DTE obtained from insects only fed with washed erythrocytes, showing that this hemagglutination is not plasma dependent as observed when agglutinating against epimastigotes. Moreover, assays with complement inactivated plasma from rabbit or human blood showed that plasma alone is able to agglutinate epimastigotes *in vitro*, but did not agglutinate trypomastigotes from cell culture or washed rabbit erythrocytes. Defatted plasma from rabbit and DTE obtained from insects fed with reconstituted blood with defatted plasma plus erythrocytes didn't agglutinate the epimastigotes. In addition, *in vitro* assays showed that parasites are agglutinated with HDL and they are not agglutinated with LDL. Finally, these results suggest that *T. cruzi* epimastigotes are agglutinated by HDL, probably through hydrophobic interaction. **E-mail:** cjcmoreira@gmail.com

Chagas095- Topoisomerase I AND DUAL Inhibitors affect cell proliferation and Ultrastructure of Trypanosoma cruzi Epimastigote forms

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The protozoa *Trypanosoma cruzi* is the aetiological agent of Chaga's disease, a tropical parasitic illness endemic in Latin America. *T. cruzi* contains an unique mitochondrion with an enlarged portion, the kinetoplast, which contains a catenated arrangement of circles, the kDNA. In trypanosomatids, the nucleus presents distinct compartments, as the nucleolus, and a condensed chromatin associated with the nuclear envelope. The topological state of DNA is modulated by topoisomerases that revert supercoilings during replication, transcription, recombination and repair, thereby representing an interesting target in chemotherapeutic studies. In this work we evaluated the effects of different topo I inhibitors, as camptothecin and its derivatives (topotecan and irinotecan), as well as dual inhibitors, that target both topo I and II simultaneously (baicalein and luteolin) on proliferation and ultrastructure of *T. cruzi* epimastigotes. Cells were cultivated in culture medium containing different drug concentrations and samples were collected after each 24 hours (until 96 hours of cultivation) for counting on Neubauer's chamber or for processing to transmission electron microscopy. Cell cycle arrest was checked using flow cytometry and the cell viability was evaluated using MTS/PMS method. In order to check the generation of reactive oxygen species and the mitochondrial membrane potential, cells were incubated with the markers H₂DCFDA and JC-1, respectively. Camptothecin reduced cell viability and promoted a strong proliferation inhibition (IC₅₀ = 2, 08 µM), in a dose-dependent manner, leading to cell cycle arrest in G2 phase. On the other hand, topotecan, irinotecan, baicalein and luteolin did not cause such growth impairment. Transmission electron microscopy analysis revealed that camptothecin and topotecan promoted nuclear ultrastructural alterations, as an unpacking of the perinuclear chromatin, and mitochondrial swelling. Camptothecin induced higher levels of reactive oxygen species and loss of mitochondrial membrane potential. Taken together, our data suggest that camptothecin is the most effective compound against *T. cruzi* proliferation when compared to other topoisomerase inhibitors, including their derivatives which are known to be more potent against tumor cells. This study provides information for designing new therapeutic compounds, considering that topoisomerase I represents a promising target for antitrypanosomal chemotherapy. **Supported by** CNPq and FAPERJ **E-mail:** zuma@biof.ufrj.br

Chagas096- *Trypanosoma cruzi* Discrete Typing Units distribution among infected asymptomatic individuals from an endemic area in Colombia

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Introduction: Chagas disease, a public health problem in Colombia, is caused by the parasite *Trypanosoma cruzi*. This parasite presents a high genetic diversity evidenced in six Discrete Typing Units (DTU's) being TcI-TcVI, that are widely distributed in the American continent. The aim of this study was to observe the distributions of the DTUs in patients treated at three different moments. **Materials and methods:** This is a sub study from a phase II clinical trial which aimed to evaluate the efficacy of a combined therapy among 242 asymptomatic infected individuals (CHICAMOGA study). We selected a subset of 54 participants from the state of Santander with both positive conventional serology and PCR at baseline. We obtained three Guanidine-EDTA blood samples at three point times (PCR1, PCR2, and PCR3) of the treatment (10 days intervals, one year post-randomization). The DNA samples were submitted to molecular characterization using three seminested assays targeted to the intergenic region of the mini-exon, 24S rDNA and A10 genes. **Results:** Fifty four percent of samples in the first point time were positive to TcI, 17% to TcII, 17% to both TcI and TcII, 7% to both TcI and TcIV, 2% to TcVI, and 3% to TcIV. In PCR2 we identified 77% samples as positive to TcI, 17% to TcII, 2% to TcVI, and 4% were not determined. In PCR3 we identified 52% positive samples to TcI, 13% to TcII, 2% to TcVI, and 33% were not determined. **Conclusions:** Our results show the predominance of the TcI genotype among infected individuals from Colombia and the presence of other DTUs as TcII, TcIV, and TcVI in single and mixed infections. Whether these patterns relate to clinical manifestations of the disease or the response to therapy remains unknown. **E-mail:** gipcumng@gmail.com

Chagas097- *Trypanosoma cruzi* I haplotypes and genetic characterization of the LSSP-PCR kDNA in endemic areas of Minas Gerais and Bahia - Brazil

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Introduction: The intraspecific variability and the behavior of *T. cruzi* I (TcI) in the epidemiology of Chagas disease is poorly studied in Brazil. **Materials and Methods:** TcI populations from triatomines, *Didelphis albiventris* and humans of two major endemic areas in Brazil were genetically characterized by the variable region of kDNA by LSSP-PCR of kDNA and amplification of haplotypes of TcI based on microsatellite motif of spliced leader gene (SL-IR) proposed by Herrera et al. (2007). **Results:** High genetic variability was demonstrated in kDNA of TcI circulating in sylvatic cycles in two areas of large epidemiological importance in Brazil: Minas Gerais and Bahia, observed among different hosts, suggesting the presence of clones in each major region. In the dendrogram generated (Gel Compar II software) isolates clustered into five groups with distinct populations circulating in vectors, *D. albiventris* and humans. It was possible to observe the presence of two independent and heterogeneous populations belonging to Santa Maria da Vitória province (Bahia state) from the identical vector species: *T. sordida* (12.9% ± 9.3% of bands shared). A similar fact occurred in isolates from Minas Gerais state that also had two different populations but with different hosts. The amplification of haplotypes target in microsatellite motif to spliced leader gene (SL-IR) proposed by Herrera et al. (2007) demonstrated the presence of haplotype D in only six of the isolates tested. The remaining samples showed no amplification to other haplotypes (a, b and c). **Conclusions:** We suggest the maintenance of surveillance in relation to TcI and the application of specific measures of control for each region studied, considering the presence of principal clones and vectors in each. **E-mail:** henriquekappel@yahoo.com.br

Chagas098- Vertical transfer of *Trypanosoma Cruzi* minicircle sequences to descendents of chagasic

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Introduction: Outbreaks of acute *T. cruzi* infections have been reported all over the Amazon, for the last four decades. Humans showing acute Chagas disease were subjected to treatment with trypanocidal nitroarenes. To evaluate the effect of treatment we analyzed the germ line cells aiming at the evaluation of the effectiveness of the therapeutic regime on the prevention of transfer of *T. cruzi* nuclear DNA (nDNA) and mitochondrial DNA (kDNA). **Study Population and methods:** This study comprises 34 male adults from four families living in the Counties of Barcarena and Breves, State of Pará, Brazil. Additionally 19 males who had shown clinical manifestations of an acute infection were included. Each male who adhered voluntarily to the research protocol yielded semen for the assessment of *T. cruzi* genetic markers. Semen collected from 53 patients was used for DNA extraction. The haploid DNA was subjected to PCR with specific nDNA (Tcz1/2) and kDNA primers S35/S36. The PCR amplification products hybridized with specific probes. Additionally, each haploid DNA sample was subjected to a *targeting-primer* Thermal Asymmetric Interlaced-PCR (tp-TAIL PCR) using kDNA primer sets S34, S36, S35, S35 reverse, S67, and S67 reverse, combined with human DNA primers L1-1 to L1-6. The amplification products that hybridized to kDNA probes were cloned in pGEM *T-Easy* vector (Promega) and sequenced commercially. **Results:** Among 53 host DNA samples the PCR amplified the nDNA in 43 cases (81,1%). Also, these DNA samples showed PCR amplified kDNA in 47 cases (88,1%). The genomic DNA showing kDNA positive cases were further subjected to tpTAIL-PCR. The amplicons hybridizing with kDNA probe, which were cloned and sequenced, revealed *T. cruzi* minicircle kDNA sequences integrated at several chromosomes. The chimera sequences Blastn analyses showed the kDNA minicircles The CA-repeats micro homology present at joining regions suggest homologous recombination is the mechanism of kDNA integration in LINE-1. **Main Conclusions:** The study reveals vertical transfer of kDNA minicircle sequences to retrotransposon LINE-1 located at various chromosomes. This main finding is similar to that obtained in haploid cells from chronic Chagas disease parental and their descendents. Interestingly, these kDNA mutations were obtained from cases showing the active infection (88,1%), having the nDNA marker, but also from patients retaining the parasite kDNA only. The results show that treatment with the trypanocidal drug did not abrogate the infection and the transfer of kDNA. Actually, the positive nDNA in the semen suggest the living parasite can be transferred from a male chagasic to its mate. **E-mail:** ferbiol_1@yahoo.com.br

Chagas099- New molecular marker to differentiate *Trypanosoma cruzi* from *Trypanosoma rangeli* and their major genetic groups

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Introduction: Natural co-infection of *Trypanosoma cruzi* and *T. rangeli* occurs in triatomines, wild and domestic mammals as well as in humans and can affect the specific diagnosis of infection by *T. cruzi*, the etiological agent of Chagas disease. **Material and Methods:** In this study the restriction fragment length polymorphism of subunit 2 from Cytochrome Cxidase (RFLP-COII) was used to differentiate between all *T. cruzi* Discrete Typing Units - DTUs (Tcl-TcVI) from the two distinct *T. rangeli* groups (KP1+ and KP1-). DNA of related parasites *Leishmania braziliensis*, *L. amazonensis*, *L. chagasi*, *T. evansi* and *T. vivax* were used as controls. **Results:** The RFLP-COII was able to differentiate *T. cruzi* from *T. rangeli* genetic groups as well as *T. cruzi* from five other trypanosomatids species. Five out of six *T. cruzi* DTUs could be clearly distinguished by the presence of bands that varied from approximately 250 to 350 bp. A single *T. cruzi*-specific band of ~80 bp was also observed. The restriction profile of *T. rangeli* showed bands of

~120 and 280 bp for KP1+ and a single product of ~400 bp for KP1-. Sequencing of the amplified products confirmed the absence of the specific site for *Alu* I for uncut amplicons. The results show that RFLP-COII was able to separate *T. cruzi* DTUs from *T. rangeli* genetic groups in artificial mixtures with distinct proportions of DNA. Moreover, this method also clearly identified mixed *T. cruzi* x *T. rangeli* infection in triatomines feces infected with *T. cruzi* (Tcl or TcII) and *T. rangeli* (KP1+ or KP1-) strains. The observed specificity of this marker was confirmed by multiplex PCR and southern blot analysis. **Main Conclusions:** The results point out that RFLP-COII is a specific, sensitive and simple method to differentiate *T. cruzi* from *T. rangeli* as well as to define their genetic groups. Considering the sympatric distribution of both parasite species and the mixed infection in reservoirs and vectors, the RFLP-COII is valuable method to assess *T. cruzi* and/or *T. rangeli* infection and typing, being of major importance for Chagas disease epidemiology. **Support:** CNPq/CAPES/FINEP **E-mail:** amandadesa@brturbo.com.br

Chagas100- Relationship between the thickness of DNA polymerase chain reaction band and different amounts of *Trypanosoma cruzi*

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Introduction: The total number of people infected with *T. cruzi* is estimated at 8 to 9 million and in Brazil, the population of chronically patients is 2 to 3 million individuals. The PCR technique is considered a promising tool for monitoring the etiologic treatment of Chagas disease. Its success depends on the amount of circulating parasites in patients' blood stream. *T. cruzi* circulates in very small amounts at the chronic phase and dynamics about its circulation is not predictable. **Material e Methods:** The aim of this study was to evaluate the relationship between different amounts of *T. cruzi* from axenic culture serially diluted and the thickness of the band of parasite DNA detected by PCR. Successive dilutions from Y strain LIT culture were performed from the initial volume of 1 mL in the concentrations of culture: 10^5 , 10^4 , 10^3 , 10^2 , 10^1 , 10^0 , 10^{-1} parasites. The DNA extraction using the Gomes et al. (1998) protocol was carried out in an aliquot of 100 μ L, with amounts varying from 10.000 to 0,00001 parasites resuspended in 10 μ L. Each one of the concentrations 10^1 , 10^0 and 10^{-1} were further diluted (1/10, 1/100 and 1/1000). PCR products visualized in 4.5% polyacrylamide silver-stained gel were obtained from the amplification of 2 μ L of DNA, corresponding to the variation of 2000 to 0.000002 parasites. **Results:** We have found the following thicknesses for 330 bp bands: 9mm to amounts of parasites varying to 200 to 0,2, 7mm to 0,02 and 3.5mm to 0,002. For the 660 pb bands were found fragments of 7mm thickness to amounts of parasites varying to 200 to 0,2; to 0,02 parasites we found a band of 4mm, and to 0,002 parasites none products was observed. In the second step of dilution, the 330 bp band of dilution 1/10 from 10^{-1} (0,0002 parasites) thicknesses band was 2.5mm in three bands together, to 1/100 (0,00002 parasites) we found 0.3mm in 3 fragments, for 1/1000 (0,000002 parasites) wasn't possible to detect any band. In 1/10 from 10^0 (0,002 parasites) was observed band of 4mm, in 1/100 (0,0002 parasites) was not observed any band, 1/1000 (0,00002 parasites) a band of 1mm. In 10^1 diluted 1/10 (0,02 parasites) the band measured 7.5mm, 1/100 (0,002 parasites) = 5mm, 1/1000 (0,0002 parasites) band of 2mm in 2 fragments. **Main Conclusions:** This results suggest that the thickness of the band of DNA in the PCR are related to amount of *T. cruzi*, indicating that this protocol may help in monitoring the amount of circulating parasites in infected host, submitted or not to etiologic treatment. This study also showed that PCR is a very sensitive technique, although false negatives are possible to occur. **Keywords:** *Trypanosoma cruzi*; Chagas disease; DNA detection; PCR; Serial dilutions, Diagnosis in chronic phase. **E-mail:** fabiana_nabarro@hotmail.com

Chagas101- Molecular identification of blood and tissue distribution of different *Trypanosoma cruzi* II and I strains in an experimental model of mixed infection in immunosuppressed Balb/c mice

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Introduction: The *T. cruzi* diversity has been determined by different biological, genetic and biochemical markers and is grouped into six Discrete Typing Units (DTU) or taxonomic groups (Tc I - Tc VI). This diversity, coupled with reinfection, favors the presence of infections by different DTU in both vertebrate and invertebrate hosts. Clinically, these associations may play an important role, mainly in reactivation cases. In this study, we evaluate the behavior of two Tc I strains (AQ1-7 and MUTUM) and one Tc II strain (JG), in mixed infections of JG+AQ1-7 and JG+MUTUM, analyzing molecular behavior both in tissue and blood samples at 15, 45 and 70 days p.i. in Balb/c mice, before and after immunosuppression induced by Cyclophosphamide. **Material and Methods:** Tissue samples were collected, at 15, 45 and 70 days p.i. and DNA was extracted using alkaline lysis method. Blood samples were collected at the same time as tissue samples in 6 M guanidine HCl plus 0.2 M EDTA buffer (v/v). Then, DNA was extracted by the standard phenol-chloroform method. So tissues and blood samples were subjected to electrophoresis on 1.5% agarose gel and the bands corresponding to 330pb regions of *T. cruzi* kDNA were used as a template to a second PCR assay at low stringency conditions. The products from LSSP-PCR were analyzed by 7.5% polyacrylamide gel electrophoresis followed by data analysis using the GelComparII software (Applied Maths NV). **Results:** The relative proportions of JG, AQ1-7 and MUTUM in the positive tissue and blood samples from unique and mixed infected animals were estimated using the LSSP-PCR technique. In acute phase the JG strain showed, in mixed infections a tendency to overlap Tc I strains, both in blood and tissues. In this period the group of mixed infection JG+AQ1-7, demonstrated a slight trend of the Tc I strain to overlap the JG strain in tissues. The opposite was observed in immunosuppressed animals with a predominance of Tc II in blood. In JG + MUTUM group, there was a predominance of Tc I strain over Tc II in all tissues, both in groups that have suffered or not immunosuppression, and Tc II in blood over Tc I strains. **Conclusions:** This study showed important differences in mixed infections behavior both in blood and tissues samples. Thus, we observed a trend of Tc II strain to overlap Tc I strains in the blood at different periods. However, a predominance of Tc I strains in tissues mainly during chronic phase, when compared to Tc II strains was also observed. **E-mail:** henriekappel@yahoo.com.br

Chagas102- Pathogenicity for mice of *Trypanosoma cruzi* strains from the states of Amazonas (TcIV) and Paraná (TcII), Brazil

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Introduction: The geographical heterogeneity of Chagas disease (ChD) is mainly caused by genetic variation of the etiological agent *Trypanosoma cruzi*, whose strains are currently referred as six Discrete Typing Units (DTUs): *T. cruzi* I - VI. Our working hypothesis is that the pathogenicity of *T. cruzi* for mice varies with genetic lineage of the parasite. To test this hypothesis were conducted parasitological and histopathological evaluation of mice inoculated with strains of *T. cruzi* IV (TcIV) from the State of Amazonas and *T. cruzi* II (TcII) from Paraná State, Brazil. **Material and Methods:** We studied eight strains belonging to DTU TcIV obtained from acute cases (7) and triatomine *Rhodnius robustus* (1) from the Amazonas and three TcII strains obtained from chronic patients in Paraná. Groups of 10 Swiss mice were inoculated via IP with 1×10^4 blood trypomastigotes / animal of each strain. Parasitaemia was evaluated daily from day 3rd after inoculation (dai). The animals were sacrificed one day after the day of peak parasitaemia (Dpmax) and at 30th dai. Fragments were obtained from the heart, skeletal muscle, liver, spleen, brain, diaphragm, abdominal wall and rectum, which were stained with hematoxylin / eosin (HE) and examined under a microscope. For each strain were obtained the pre-patent period (PPP), patent period (PP), peak parasitaemia (Pmax), day of peak parasitaemia (Dpmax), inflammatory process and tissue parasitism in the acute phase. **Results:** TcIV strains of Amazonas had lower PPP, PP, earlier Dpmax, and low levels of parasitaemia, whereas TcII strains of Paraná showed higher PP and Pmax, and were considered more virulent. Discrete tissue parasitism in the heart and skeletal muscle and moderate inflammatory process in the heart, liver, intestine and brain were observed by HE only in mice inoculated with the three TcII strains studied, whereas mild inflammatory process for both DTUs. Mice inoculated with strains of both lineages had involvement of CNS and large intestine; however, the inflammatory lesions were more intense for TcII. **Conclusion:** The results confirm our working hypothesis since the TcII

strains of Paraná were considered of variable pathogenicity for mice while TcIV strains of Amazonas were considered of low pathogenicity, agreeing with the lower severity of the ChD in the Amazon region. **Keywords:** *Trypanosoma cruzi*, DTUs, Amazon; Pathogenicity; Mice. **E-mail:** aninha_gru@bol.com.br

Chagas103- Inflammatory angiogenesis in acute and chronic infection in C57BL/6 mice by different strains of *Trypanosoma cruzi*

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Introduction: Inflammation is a key immune-pathophysiological characteristic of cardiomyopathy induced by *Trypanosoma cruzi*. Different inflammatory cells can contribute to the release of pro- and anti-inflammatory mediators which can directly or indirectly promote vascular endothelial growth factor (VEGF) mediated angiogenesis. VEGF is considered as important indicator molecule acting on heart remodeling particularly during chronic phase of infection. We have recently shown that total *T. cruzi* antigen induces inflammatory angiogenesis in murine sponge implantation model. **Objectives:** In this study, we investigated acute and chronic phase of the infection for the possible formation of new vessels in the inflammatory heart infection in C57BL/6 mice infected with Colombiana and VL-10 strains of *T. cruzi*. In parallel, pharmacological therapy (ACE inhibitors, Statin and Benznidazol) were administered during acute infection to evaluate interferences on immune response modulation. **Methodology:** Animals were euthanized at end of acute phase and 100 days of post-infection. Blood and heart samples were collected to study production of inflammatory and angiogenesis mediators in acute as well as chronic phase by immunoassay (TNF-alpha, CCL-2, CCL5, VEGF and IL-17), real time-PCR (VEGF, Angiopoietin-1, 2 and Thrombospondin 1, 2), immunocytochemistry (MMP-2 and 9, a-CD31 and a-VEGF) and conventional histology (inflammation, parasites and vessels). **Results:** Our preliminary data showed that serum VEGF was elevated in acute and chronic phase in those animals infected with Colombiana strain, coincidently with elevation of TNF-alpha and CCL2, but not IL-17 which was only elevated in chronic phase. VL-10 strain infected mice had low parasitaemia and low levels of serum TNF-alpha and CCL2, but IL-17 production was significantly elevated in chronic phase. **Conclusion:** Our initial data suggest that different strains of *T. cruzi* may regulate VEGF in dependent of inflammatory response that may trigger formation of new vessels in infected heart tissue. **Supported by:** CNPq, FAPEMIG, TWAS, ISID, UFOP. **E-mail:** deenabajra@gmail.com

Chagas104- Virulence of *Trypanosoma cruzi* in açai pulp in immunocompetent mice C57BL/6/J Unib

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Introduction: Acai fruit is the main dietary supplement in the northern Brazilian state of Pará and because of its high productivity is an important source of income for the local economy. However, oral transmission through the ingestion of metacyclic trypomastigotes of *Trypanosoma cruzi* in açai pulp has contributed to the emergence of microepidemics of acute Chagas disease (ACD). Recent studies indicated that *T. cruzi* can survive for 144h in açai pulp and retain its virulence in immunodeficient mice and now it is important to demonstrate its behavior in mice with distinct genome on the level of resistance to the parasite. The aim of this study was to evaluate possible changes in virulence of *T. cruzi* incubated in açai pulp under different thermal treatments. **Material and Methods:** Aliquots *in natura* açai pulp produced in Belém city were mixed with 100.000 trypomastigotes of *T. cruzi* Y strain, obtained from CBA/J Unib. The inoculum was obtained by forced sieving from the contaminated mixture. During the experimental infections, 100 µL of inocula were administered to the C57BL/6/J Unib, intraperitoneally. The mice were

pretreated with cephalexin at 1,75mg/day. It were utilized 6 animals in each experimental group – negative control (eluate from açai pulp), positive control (plasma contaminated with *T. cruzi*) and test groups (eluate from the mixture açai pulp and *T. cruzi*), with the samples incubated at room temperature, at 4°C or at - 20°C, for 24 hours. This study was approved by the CEUA/Unicamp, protocol 2180-1, according to Brazilian law 11794/08. After the infections, animals were observed during 60 days and the parasitemia was scored as recommended by Brener. Results: In the positive control group, the mean days to onset of infection were 7,0±1,0 (samples incubated at room temperature); 11,0±4,0 (at 4°C) and 12,0±1,0 (at -20°C). In the test groups, the mean days to onset of infection were 7,0±0,0 (at room temperature); 7,0±0,0 (at 4°C) and 13,0±0,0 (at -20°C). After this, in the positive control group, the mean days of the disappearance of the parasitemia were 28,0±5,0 (at room temperature); 28,0±5,0 (at 4°C) and 33,0±0,0 (at -20°C). In the test groups, the mean days of the disappearance of the parasitemia were 19,0±5,0 (at room temperature); 25,0±9,0 (at 4°C) and 33,0±0,0 (at -20°C). There were no deaths at the experimental groups. Main conclusions: *T. cruzi* can retain its virulence in açai pulp for 24 hours at room temperature, at 4°C and -20°C and cause infection in C57BL/6/J Unib. These results can indicate interesting epidemiological data to public health and mechanisms involved in foodborne ACD in immunocompetent hosts should be investigated. **Supported by** CAPES and cooperative agreement 667/2008 between the Brazilian Ministry of Health and the Universidade Estadual de Campinas **E-mail:** rodrigo@cemib.unicamp.br

Chagas105- Congenital Chagas disease: Protective mechanisms of the placental trophoblast to *Trypanosoma cruzi* infection

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Congenital Chagas' disease is caused by the haemophagelated protozoan *Trypanosoma cruzi*. However, only a percentage of the infected mothers transmit parasites to their fetus; therefore local placental factors that impair vertical transmission may exist. During vertical transmission, the parasite reaches the fetus by crossing the placental barrier, which principal tissue is the trophoblast, composed of two cellular layers: the syncytiotrophoblast (ST) and the cytotrophoblast (CT). The CT displays high proliferative properties, whereas the differentiated ST loses its generative capacity and is not able to proliferate. The ST is a multinucleated layer that is in direct contact with maternal blood. The trophoblast, as covering epithelia, forms a physical barrier to pathogens. The epithelial turnover has been considered part of the innate immune system due to the fact that pathogens, prior to cell invasion, must attach to the surface of cells. As these cells are continuously eliminated, the attached pathogens are removed with them. The trophoblastic cell line BeWo can be maintained in a non-differentiated stage and induced to undergo differentiation and fusion with forskolin (50 µM) within 48 to 72 h. In order to determine the ability of the parasite to infect the BeWo cells, we incubated 10⁵ cells with different concentrations of *T. cruzi* trypomastigotes Y strain (0,25:1; 0,5:1 and 1:1 parasite: cell ratio) for 2 hours. 48 hours after we performed DAPI staining and the percentage of infected cells as well as number of intracellular amastigotes were determined. On the other side, the differentiation and fusion of BeWo cells were measured by a two-color fluorescence fusion assay 72 hours after incubation with the parasite in the presence or absence of forskolin. Cells were visualized in a Nikon Eclipse E400 epifluorescence microscope; the images were obtained with a Digital DS-Ri1 Nikon camera and analyzed with the ImageJ software. *T. cruzi* trypomastigotes are able to infect BeWo cells. A low concentration of parasites (0,25:1 parasite: cell ratio) infects a higher percentage of BeWo cells (23 ± 3%) but produces less number of amastigotes per cell (25,3 ± 1,5) than a 1:1 parasite cell ratio (17 ± 1,9 % infected cells and 41,1 ± 3,4 amastigotes per cell). The fusion rate of BeWo cells increases from 30 ± 5,5% to 45 ± 4,2 % in presence of the parasite. Our results suggest that *T. cruzi* increases the trophoblast turnover; this could be a protective mechanism of the placental tissue to impair the congenital transmission. **Acknowledgment:** FONDECYT Grants 1120230 (to UK), 1090124 (to NG) and 1090078 (to JM). CONICYT-PIA ACT 112, Chile. **E-mail:** ukemmerling@med.uchile.cl

Chagas106- Chagas Disease in the Chicken Model: Inhibition of Pathology by Bone-Marrow Transplantation

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Introduction: To eliminate a role persisting *T. cruzi* infection play in the outcome of Chagas heart disease we used birds' refractory to the parasitic infection. The chicken innate immunity is a tight barrier against the *T. cruzi* after the first week of embryo growth. The chick hatched from *T. cruzi*-infected egg is parasite-free, but it retains minicircle kDNA in the genome. This model system showing kDNA mutations at several chromosomes develop parasite-free Chagas-like heart disease. In this study we show involvement of the immune system effectors cells in the rejection of the kDNA-mutated chicken heart, and propose a treatment for the heart disease. **Materials and methods:** One hundred virulent *T. cruzi* trypomastigotes are inoculated in the air chamber of fertile chicken eggs from congenic birds of Prague. The kDNA+ (group A) and the kDNA- (group B) adult birds are treated with cytostatic (Myleran, (5mg/kg) and Bulsufan (14 mg/kg) to destroy bone marrow cells. Two days after treatment group A and B chickens are grafted bone marrow cells, respectively, from healthy donors, and from sick kDNA+ chicken. Two weeks after marrow transplantation, group A and B chickens are grafted one day-old chick reporter heart. Eleven days after graftings, the heart graft is removed from A and B and subjected to histopathology and phenotype analyses. Control, synchronized homograft experiments consisted in one day-old chick heart graft in healthy congenic birds of Prague. **Results:** Control homograft's in congenic chickens did not show histopathological lesions and heart rejection after 11 days. In the experiments of group A, kDNA+ chicken that had bone marrow destroyed by drugs and that received bone marrow cells from healthy recipients did not reject heart homograft, after 11 days. Contrastingly, in the experiment of group B, healthy chicken that had bone marrow cells destroyed by drugs and received injection of sick kDNA+ marrow cells did have the heart homograft fully rejected, after 11 days. The histopathology revealed lyses of the reporter heart graft by immune system effectors mononuclear cells. The phenotyping of the immune system cells revealed that CD45, TCR $\gamma\delta$, CD8 α , CD8 β , TCRVb1, and TCRVb2 cytotoxic lymphocytes carried out the homograft rejection. **Conclusion:** The transfer of the heart pathology by means of sick, kDNA+ bone marrow cells to naive recipients suggests that treatment of the human Chagas heart disease by bone marrow transplantation is feasible. However, the success of such treatment requires an effective anti-trypanocidal drug to eliminate possible recrudescence of the cryptic *T. cruzi* infection. **E-mail:** neide@unb.br

Chagas107- Functional features of *Trypanosoma cruzi* kDNA mutations in *Gallus gallus*

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Introduction: An accelerated rejection of heart cells by immune effectors lymphocytes from the chagasic animal has suggested the autoimmune theory of Chagas disease. Studies aiming at the origin of the autoimmune phenomenon have suggested that transfer of *T. cruzi* kDNA minicircles to the host genome may explain the pathogenesis of the disease. In order to avoid the argument that residual cryptic infection could masquerade transfer of the parasitic kDNA, and the importance of the genotype alterations in the pathogenesis of Chagas studies it was thought to use a transkingdom animal model. Birds are refractory to *T. cruzi* but the infection can be established in the early embryo. Chicks hatched from *T. cruzi*-infected fertile eggs are parasite free, but the kDNA minicircles are retained in the genome. The kDNA-mutated birds develop Chagas heart disease similar to that seen in humans. In this study we show vertical transfer and fixation of kDNA mutations in *Gallus gallus* genome. **Material and Methods:** The *tp*TAIL-PCR (Hecht et al, 2010) made with kDNA nested primers and specific chicken genome primers C31-1 to 6. The amplicons hybridized to a radio labeled kDNA probe and were cloned and sequenced. **Results:** The integrations of *T. cruzi* kDNA minicircle spread to several chromosomes; 58% out of 133 events were present in macrochromosomes, 1 to 4 and 9. Circa 34% of these events were in coding regions and a

majority was in retroelement. The demonstration of kDNA mutations in the cyclin M2 and at the tetraspanin 18 loci suggested the use of primers sets upstream and downstream to the kDNA insertion site. The *tpTAIL* PCR amplifications showed kDNA mutations in parental is usually different from that variable region in the progeny. **Main Conclusions:** The results showed lateral and vertical transfer of kDNA minicircle sequences in parasite-free chicken hatched from *T. cruzi*-infected eggs. The transkingdom chicken model showing the kDNA mutations at several chromosomes may develop progressive disease and die of heart failure. The lesions in the heart reveal lyses of the heart cells by cytotoxic effectors lymphocytes. The Chagas heart disease in kDNA-mutated chicken appears to be a genetically driven autoimmune disease. The kDNA-mutations reveal different minicircle variable regions in parental and progeny. These results suggest that lateral and vertical transfer of kDNA minicircles in the *Gallus gallus* genome do not follow Mendelian Law. Contrarily, it appears to carry features of semiconservative inheritance that favors an increasing genetic diversity. **E-mail:** maroldiniz@gmail.com

Chagas108- Genetic vaccination against chronic experimental *Trypanosoma cruzi* infection

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Introduction: In the last 15 years evidence supported by a number of experimental studies provided that genetic vaccination elicited protective immunity against acute infection with *T. cruzi*. The present work evaluated the prophylactic and therapeutic vaccination against experimental chronic infection. **Materials and Methods:** F1 (BALB/cXCB10) inbred mice were primed intramuscularly with plasmid DNA and subsequently boosted 21 days later with replication defective recombinant human type 5 adenovirus. Plasmids and adenoviruses contained the genes of *T. cruzi* TS or ASP-2. In addition, we used during priming a plasmid containing the gene of the murine IL12 (pIL-12). Immune responses were estimated two weeks after the last immunizing dose. The immunological analyzes were performed by ELISPOT assay and intracellular staining (ICS), using as stimulus the CD8 epitopes TS-Epi (IYNVGQVSI H2-k^d restricted) and VNHRFTLV (H2-k^b restricted). Mice were challenged with 10³ bloodstream trypomastigotes of the Brazil or Colombian strain of *T. cruzi*. We evaluated daily parasitemia, survival, ECG, and serum CKMB. **Results:** Immunization elicited strong CD8⁺ T cells mediated immune response. Most cells were multifunctional expressing surface CD107a, IFN- γ and TNF- α simultaneously. The presence of pIL-12 during priming improved the CD8 immune responses. In the case of prophylactic vaccination, following challenge with Brazil or Colombian strains, we observed a significant reduction in the peak parasitemia of immunized animals when compared to control mice (p<0.01 in all cases). The presence of pIL-12 further reduced the parasitemia (p<0.01). ECG (cardiac alteration) and serum CKMB were significantly reduced in all vaccinated animals challenged with Colombian strain when compared to control mice. We are currently evaluating the results of the prophylactic vaccination with the Brazil strain and the therapeutic vaccination with the Colombian and Brazil strains. **Conclusions:** Our preliminary results show that prophylactic genetic vaccination with *T. cruzi* genes encoding TS and ASP-2 impacts favorably the chronic experimental infection with Colombian strain of *T. cruzi*. **Supported by:** FAPESP, INCTV (CNPq) and FAPEMIG. **E-mail:** mrodrigues@unifesp.br

Chagas109- Inflammation and cytokine profiles in the esophagus of dogs experimentally infected with *Trypanosoma cruzi* is associated with the phase of infection and / or strain used

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Immunological characteristics of the host may be related to pathological manifestations in Chagas disease. Thus the evaluation of inflammatory cells and cytokines produced by them, may allow a better understanding of the genesis of chronic injuries. In this study, *Beagle* dogs were infected with Y or Berenice-78 (Be-78) strains. Subsequently, these animals were necropsied during the acute or chronic phase and esophagus fragments were collected to perform inflammation quantification, immunohistochemistry to detect T CD4+ and CD8+ lymphocytes and real-time PCR for quantification of cytokines IL-6, IL-4, IL-10, TGF- β , IFN- γ , IL-12, TNF- α and iNOS. The acute inflammatory process observed was predominantly mononuclear and significantly higher in the infected groups compared to uninfected animals. Immunohistochemistry analysis of animals infected by Y or Be-78 strains showed that the majority of inflammatory cells were positive for CD4+ or CD8+ in similar proportions. However, in the chronic phase, the inflammatory infiltrate was reduced when compared to the acute phase and it was observed only in animals infected with Be-78 strain. During acute phase was observed an increase in mRNA expression of proinflammatory cytokines TNF- α and IFN- γ in animals infected with the Y strain compared to the other groups. In the chronic phase there was an increase in mRNA expression of IL-6, IL-12, TNF- α and iNOS enzyme in animals infected with Be-78 strain in relation to the group of animals infected with the Y strain and the uninfected animals. Regarding anti-inflammatory cytokines, there was only a significant increase in mRNA expression of IL-10 in animals infected with the Y strain compared to the group of uninfected animals in the acute phase. Thus, in experimental infection of *Beagle* dogs with the Y or Be-78 strains, both infections promote an acute inflammatory response, predominantly lymphocytic with similar proportions of T cells CD4+ and CD8+, restricted, in the chronic phase, to the Be-78 strain. In addition, in animals infected with the Y strain the acute inflammation was associated with expression of proinflammatory cytokines, whereas in animals infected with Be-78 strain is not associated with expression of proinflammatory cytokines evolving to chronic focal inflammation associated with expression of these cytokines and iNOS enzyme. Supported by FAPEMIG and CNPq. **E-mail:** katia.fonseca@gmail.com

Chagas110- Modulation of lipid bodies' formation and the host immune response Mediated by phagocytosis of apoptotic cells during infection by *Trypanosoma cruzi*: involvement of the nuclear receptor PPAR γ

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Chagas' disease is a chronic systemic infection caused by the protozoan parasite *Trypanosoma cruzi*. During the infection, changes in the lipid metabolism, including increase of lipid bodies (LBs) numbers, are observed within host cells. LBs are key organelles involved in nutritional and immunoregulatory functions during intracellular pathogen infection. Induction of apoptosis is associated with infection by pathogens, and generally is performed by efficient removal of apoptotic cells by professional phagocytes, especially macrophages. Previous studies have demonstrated that the phagocytosis of apoptotic cells by macrophages favors parasite replication in part through modulation of the inflammatory response. Peroxisome proliferators-activated receptors (PPARs) are molecules functionally active in different immunoregulatory processes with ability to regulate the expression of varied genes involved in inflammation and lipid metabolism. In this work, we evaluated the effects of the phagocytosis-mediated immunosuppression of apoptotic cells on LBs formation and host response against *T. cruzi* infection. Moreover, the participation of the receptor PPAR γ in these processes was evaluated. Histological analysis of heart and spleen from infected rats demonstrated increase of apoptotic cells in these organs. The immunomodulatory effects from interaction with apoptotic cells during infection with *T. cruzi* and the role of PPAR γ in this process was evaluated using peritoneal macrophages infected *in vitro* with metacyclic trypomastigotes forms of *T. cruzi*, DM28c strain, treated or not with rosiglitazone and GW99662 (PPAR γ agonist and antagonist, respectively) and co-cultured with apoptotic splenocytes. Pretreatment with GW9662 reduced the ability of macrophages to phagocytize apoptotic cells. Both *T. cruzi* infection and co-culture with apoptotic cells induced LB formation in macrophages, however treatment with GW9662 reduced only LB formation triggered by infection, but not by apoptotic cell interaction. Cultures only infected and treated with rosiglitazone showed significant reduction in the

production of proinflammatory cytokines triggered by *T. cruzi* infection such IL-6, TNF- α and KC, but increased the amounts of PGE₂. The addition of apoptotic cells also significantly reduced the production of proinflammatory cytokines IL-6 and TNF- α , but increased levels of KC in the supernatant of infected cell cultures. PPAR γ appears to be involved in the modulation of TNF- α and KC. Together these results suggest that PPAR γ are involved on LB formation and host inflammatory response during *T. cruzi* infection, and the presence of apoptotic cells can modulates this inflammatory response by mechanisms partially dependent of PPAR γ . **Supported:** FAPERJ, CNPq, CAPES, FAPEMIG and PAPES/FIOCRUZ. **E-mail:** liviaaufj@hotmail.com

Chagas111- *Trypanosoma cruzi* stocks belonging to TcI and TcIV DTUs from Brazilian Amazon are divergent in terms of biological and medical properties in mice

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Chagas disease is an important health problem, affecting 8–9 million individuals, with approximately 50,000 new cases annually, in Central and Latin America. In the Brazilian Amazon, clinical and epidemiological frameworks of Chagas disease are very dissimilar in relation to the endemic classical areas of transmission, possibly because the diverse clinical outcome of human *T. cruzi* infection has been attributed to the genetic heterogeneity of parasite populations and to the host's genetic background. In this work, twenty five *T. cruzi* stocks from Western Amazon Region attributed to the TcI and TcIV DTUs were comparatively studied in Swiss mice, inoculate either for TS or TM by IP via, to test the hypothesis that *T. cruzi* clonal structure has a major impact on its biological and medical properties. Seventeen parameters were assayed: (1) pre-patent period (PPP); (2) patent period (PP); (3) mean daily parasitemia (MDP); (4) maximum of parasitaemia (Pmax); (5) day of maximum of parasitaemia (DPmax); (6) mortality (%MOR) in the acute phase; (7) %MOR in the chronic phase; (8) day of maximum mortality (DMM); (9) infectivity (%INF); (10) percentage of positive fresh blood examination (%+FBE); (11) percentage of positive hemoculture (%+HC); (12) percentage of positive PCR (%+PCR); (13) percentage of mice with inflammatory process in any organ; (14) percentage of mice with tissue parasitism in any organ; (15) percentage of positive ELISA (%+ELISA) in the acute phase; (16) %+ELISA in the chronic phase; and (17) in vivo susceptibility to benznidazole. Clonal theory assumes that an evolutionary divergence accumulated between different lineages possibly involves genes that govern important properties related to the parasite virulence, pathogenicity, and clinical presentation of Chagas disease. Statistical comparisons showed that 14 out 17 parameters were significantly different between the two DTUs. TcIV showed higher values of PP, MDP, Pmax, %MOR in the acute phase, %INF, %+FBE, %+ELISA in the acute phase, and %+ELISA in the chronic phase than TcI. On the other hand TcI showed higher values of PPP, DPmax, %MOR in the chronic phase, DMM, frequency of mice with inflammatory process in any organ and frequency of mice with tissue parasitism in any organ than TcIV. Results strongly support that biological differences are proportional to the evolutionary divergence among the DTUs, and highlight the need to take into account the phylogenetic diversity of *T. cruzi* natural stocks circulating in the emergent areas for Chagas disease in all applied studies dealing with clinical diversity of Chagas disease, immunology, diagnosis, prognosis, and drug and vaccine trials. This shows that evolutionary divergence and biological differences do not evolve independently. Concluding, *T. cruzi* stocks belonging to TcI and TcIV DTUs from Brazilian Amazon are divergent in terms of biological and medical properties in mice.

Keywords: *Trypanosoma cruzi* lineages TcI and TcIV; Chagas disease; Amazonia; Experimental infection; Biological properties; Mice. **Supported by:** Fundação Araucária / CNPq **E-mai:** mjtotoledo@uem.br

Chagas112- 2-Iminothiazolidin-4-ones arrest *Trypanosoma cruzi* growth and impair trypomastigote development in macrophages

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Chagas disease, caused by the flagellate protozoan *Trypanosoma cruzi*, affects about 18 million people in the Americas. Current chemotherapy of Chagas disease is based on benznidazole, which is toxic and has limited efficacy. Therefore, new chemotherapeutic agents need to be developed. In this work, we describe the identification of eight *Trypanosoma cruzi* inhibitors through a combination of thiazoles and hydrazones chemistry. The trypanocidal effect against *T. cruzi* was first evaluated by light microscopy through the determination of IC₅₀ values for the replicative form epimastigote and bloodstream trypomastigotes of Y strain. Cytotoxicity was determined by incorporation of [³H]- thymidine by splenocytes obtained from normal mice. The trypanocidal activity of the iminothiazolidin-4-ones was tested in a model of infection *in vitro* of macrophage cultures. All compounds showed activity for the replicative form epimastigote and the infective form trypomastigote, showing IC₅₀ values ranging from 0.5 to 51.5 µM and 1.2 to 48.2 µM, respectively. In terms of cytotoxicity, compounds TS-40 and TS-59 showed the best profiles. The compounds had IC₅₀ values of 9 µM and 1.2 µM, respectively, for trypomastigotes and IC₅₀ values of 5.1 and 0.5 µM, respectively, for epimastigotes. All the values smaller than the IC₅₀ of the standard drug (benznidazole IC₅₀ = 11.2 µM for trypomastigotes and 7.5 µM for epimastigotes). In splenocyte cultures, all the compounds showed no or moderate cytotoxicity, demonstrating a selective toxicity of these compounds, especially compound TS-59, which is over 180 times more cytotoxic to trypomastigote than for mammalian cells. In the model of macrophage infection with *Trypanosoma cruzi*, all the iminothiazolidin-4-ones were able to reduce the percentage of macrophages infected and the relative number of amastigotes per cell. The compounds TS-40 and TS-59 showed the best activity. The IC₅₀ of these compounds were 10.11± 0.09 µM for TS-40, and of 5.20±0.54 µM for TS-59, while benznidazole had an IC₅₀ of 13.99±0.39 µM. Our results with iminothiazolidin-4-ones TS-40 and TS-59 argue for the evaluation of these compounds in the *in vivo* infection model in mice. **E-mail:** calcio0303@hotmail.com

Chagas113- Lung tissue damage dynamics in mice with acute Trypanosomiasis in an acute phase of the experimental infection

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Introduction: Pneumonitis provoked by *T. cruzi* was described in international scientific literature initially in mice and afterwards in human beings. Due to its relatively rare occurrence in human patients this pathology has not been studied in detail. No pathologic data have been reported, other than the autopsy series reported by Köberle in 1959 and congenital pneumonitis described by Bittencourt in 1981. In 2005 our group has published an article on pathological changes in mouse lungs during experiments. Present study elucidates the dynamics of pathological changes in mouse lungs during the acute phase of an experimental *T. cruzi* infection. **Materials and methods:** Four groups of eight to ten-week-old male BALB/C mice were infected with an intraperitoneal injection of 10⁵ blood trypomastigotes. These mice were sacrificed at different periods of the acute infection phase. Lungs were taken and processed for Hematoxylin-Eosin and Masson staining's. A full digital image with 400X magnification of each sample was obtained using an Axiocam 5 HR digital camera and a motorized Axioplan light microscope. Each panoramic image was analyzed in an automatic interactive mode. Alveoli wall thickness and the number of nuclei per alveolar wall length were measured; all bronchial wall damage and parasitism data were documented. The presence of fibrotic changes was quantified at all periods of acute infection phase and

in the control group. One way ANOVA, T tests and χ^2 square methods were used for the statistical comparison using different parameters. **Results:** Gradual alveolar wall thickening and cellular hyperplasia in alveoli were observed during the rise and maximum phase of parasitaemia, decreasing both parameters during the dropping parasitaemia level phase. These parameters were stabilized at normal levels during the post-acute infection phase. Bronchial wall destruction and mononuclear infiltration were also observed during the acute infection phase, being replaced by fibrotic tissue in the final acute phase. Pulmonary parenchyma aeration has shown similar behavior as wall thickness during the acute infection phase. **Conclusions:** Acute pneumopathy is part of the clinical picture in American Trypanosomiasis developing during the acute infection phase and parasitaemia. Our work shows that this pathologic condition is limited by decreasing parasitaemia levels and reactive inflammation. The data reported by Koberle for human autopsies confirm the presence of sequelae to this pathologic condition in at least 6% of all Chagasic patients. **E-mail:** valery.melnikoff@gmail.com

Chagas114- Single nucleotide polymorphisms of cytokines genes in chronic Chagas disease: an overview

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Introduction: Chagas disease is a parasite infection that is a significant public health in Latin America. The mechanisms responsible for the susceptibility to the infection and the mechanisms involved in the development of cardiac and digestive forms of chronic Chagas disease remains poorly understood. However, there is growing evidence that differential susceptibility in endemic areas may be attributable to host genetic factors. The aim of this overview was to analyze the genetic susceptibility to human Chagas disease, particularly the single nucleotide polymorphism of cytokines genes. **Methods:** A review was conducted in the following databases: PubMed/MEDLINE and Scopus. The search strategy included the following terms: "Cytokines", "Single Nucleotide Polymorphisms" and "Chagas Disease", according to MeSH (Medical Subjects Heading Terms/PubMed). There was no restriction regarding language or publication date. The research period was covered until January 2012. The eligible studies were those which included in their analysis, the genotype or allelic frequencies of SNPs of cytokines genes probably related to Chagas disease susceptibility or development to cardiac and digestive forms of chronic Chagas disease. **Results:** After screening 25 non duplicated citations from the databases, 19 studies were selected for the overview. Of the 19 eligible articles, 11 had been conducted with chagasic patients and healthy individuals as a control group and 8 had been conducted only with chagasic patients, presenting different clinical manifestations of the chronic phase. The selected studies are presented in a table (that contains the studied populations, target genes, number of individuals), in chronological order of publication and a narrative synthesis of their main findings was conducted. All studies were published in English language and have been conducted in Latin American populations. **Conclusion:** A critical analysis of the data presented in the articles suggests that the genetic susceptibility to Chagas disease and chronic Chagas cardiomyopathy is highly influenced by the complexity of the immune response of the host. In approximately 14 years of research of SNPs of cytokines genes in Chagas disease, many loci were studied and some of them showed possible association with disease susceptibility and/or progression. Prospective studies with other populations where Chagas disease is endemic (with distinct ethnic and genetic backgrounds) and with a large sample size need to be conducted to establish the cytokines genes that are involved in disease susceptibility and/or progression. **E-mail:** meroricky@hotmail.com

Chagas115- Experimental neuropathology by triatomine isolates of *Trypanosoma cruzi* from Anzoátegui state, Venezuela.

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Introduction: American Trypanosomiasis is a progressive, chronic and debilitating entity that affects the health and human productivity constituting a public health problem in Latin America. *Trypanosoma cruzi*, the causal agent, is a paninfective hemoflagellated with wide tissue parasitism. Its transmission occurs by contact of the skin or exposed mucous from human or other mammals with feces from infected triatomines. The objective of this work was to study the histopathological behavior of *T. cruzi* isolates in murine model for detect lung invasion. **Materials and methods:** Intestine of collected triatomines from "El Eneal I" and "Valley of the Neveri" villages from Anzoátegui State, Venezuela, were dissected in isotonic saline solution. The material was microscopically observed (400X) and positive samples with flagellates were inoculated i.p. in albino mice NMRI (12 g wt) with 200 metacyclic trypomastigotes/g wt for each isolate. Tisular tropism study, lung parasite colonization and histopathology were performed in euthanized mouse with maximum parasitaemia. Tissue samples of lung and other organs were fixed with neutral 10% formaline and included in paraffin for serial microtome cuts (0.3 µm thick) and staining with hematoxiline/eosin. **Results:** In sections of lungs of infected mice of all groups showed varying degrees alveolar edema, hypertrophy of pneumocytes, increases total cellularity of the alveolar wall, as well as capillary congestion, perivascular infiltrates and destruction of the vessel wall and parasites myocytes in the lung tissue and vascular walls. **Conclusion:** Pulmonary complications should be considered as a possible clinical manifestation in the acute phase of Chagas disease in the mammalian host and lung damage, its extent and severity will depend on the virulence of the isolate. **Financial support:** Projects: Proyecto en Red Misión Ciencia N° 2007001442 and N° 2008000911-6, Proyecto FONACIT N° G-2005000827 and Ayudas Menores CDCH-UC-0440-10 y 0450-10 Universidad de Carabobo. **E-mail:** amorocoima@gmail.com

Chagas116- Identifying potential hosts of *T. cruzi* and *T. rangeli* from the analysis of Cytochrome B from the digestive tube of *Rhodnius* spp collected in the region of the middle Tapajós River, Pará, Brazil.

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Introduction: In the last few years, most severe outbreaks of Chagas disease in Brazil are concentrated in the Amazon region. Determining triatomines' food sources is a useful tool to understand the Chagas' disease eco-epidemiology, helping to identify natural hosts of triatomines and potential carriers of *T. cruzi* and/or *T. rangeli* in several environments. **Methodology:** Specimens of *Rhodnius* spp were collected from *Attalea* spp palm trees in three communities of the middle Tapajós River, Pará, Brazil. Insects had DNA extracted from their digestive tube contents as well as from the contents of their legs. In order to identify the infection with *T. cruzi* and with *T. rangeli*, specific PCRs were performed in triatomines. Intestinal contents of insects were evaluated using Cytochrome B gene, followed by sequencing and comparing with GenBank (Blast) database. **Results:** Infections with *T. cruzi* and with *T. rangeli* were verified in the 740 *Rhodnius* spp specimens collected. Out of these, 125 were infected with *T. cruzi* and 69 with *T. rangeli*. Furthermore, the presence of two parasites (mixed infection) was found in 25 triatomines. In order to study their food sources, intestinal contents of 191 triatomines were identified. Result has shown specimens of *Rhodnius* spp fed in three different classes of animals (mammals, birds and reptiles). In the Mammalian class, four orders of animals were identified – Primates (four spp), Didelphimorpha (three spp), Rodentia (seven spp) and Xenarthra (one sp), distributed in 13 different genera. In the bird class, three different genera were identified (three spp), whereas in the Reptilia class, only one genus was identified (two spp). Associating these results with the infection with *Trypanosoma* spp has shown that *Tamandua tetradactyla* is a reservoir of *T. rangeli* and, possibly, of *Oecomys Roberti*. In addition, an association was found among *T. cruzi*-infected triatomines whose intestinal contents were identified as *Didelphis marsupialis*. **Conclusions:** Molecular identification of triatomines has shown that the triatomine species associated with *Attalea* palm trees corresponded to *R. robustus*. Results: have

shown significant dynamics between triatomines and different reservoirs in the canopy of *Attalea* genus palm trees. Molecular identification of vectors' food sources, associated with the infection with parasites, is an important tool to help understand sylvatic transmission cycles. Moreover, it may be employed in order to elucidate mechanisms and potential hosts of *T. cruzi* associated with severe outbreaks which occurred in the Amazon region. **E-mail:** fbragasdias@gmail.com

LEISHMANIASIS

Epidemiology

Leish001- Epidemiological and entomological aspects of American tegumentary leishmaniasis (ATL) in the municipality of Monte Negro, state of Rondônia, Brazil

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Introduction: The municipality of Monte Negro (10° 15'92" S and 63° 17'14" W), located 250 km southwest from the city of Porto Velho, is an historical area of transmission of ATL that accounted for a prevalence of 59 cases/100.000 inhabitants in 2010. This work was carried out on the purpose of identifying the species of phlebotomine sandflies in this area that may have been transmitting the disease, and concisely describe the epidemiological aspects of ATL. **Material and Methods:** epidemiologic data was obtained from the Ministry of Health of Brazil and from the Secretary of Health of Monte Negro. The phlebotomine sandflies were captured using CDC light traps between July 2006 to July 2008 in nine different localities of the municipality. Environmental, economic and demographic indicators were obtained from government institutions, namely IPEA, IBAMA and IBGE. **Results:** There has been a significant decrease in the incidence a ATL of about 50% over the last ten years in the municipality and 25% in Rondônia. 1,935 specimens of 52 sand fly species were captured, two of the genus *Brumptomyia* genus and 50 of the genus *Lutzomyia*. The species of the genus *Lutzomyia* found belong to the subgenera *Evandromyia*, *Lutzomyia*, *Micropygomyia*, *Nyssomyia*, *Pressatia*, *Psathyromyia*, *Psychodopygus*, *Sciopemyia*, *Trichophoromyia*, *Viannamyia*; and the following groups: Aragaoi, Migonei, Oswaldoi, Saulensis, Verrucarum and an ungrouped species *Lutzomyia acanthopharynx*. Brazilian socioeconomical and environmental indicators demonstrated an increase by 18% in the government family allowances provided in this region, reduction of migration to Amazônia (minus 35.000 inhabitants), increase of employs in the south and southwest of Brazil and 50% decrease in deforestation. **Main Conclusions:** Four sandflies species were found in the state of Rondonia for the first time: *B. brumpti*, *Lu. tarapacaensis*, *Lu. melloi*, and *Lu. lenti*. Other species captured as *Lu. whitmani* and *Lu. davisii* have proved to be significant vectors of *Leishmania* in the enzootic and in the anthroponotic cycle. The detection of these vectors suggests an increase of the transmission risk in the peridomestic environment. Socioeconomical improvement of Brazilian economy in the last 15 years collaborated in the decrease of people exposed to vectors of ATL, as they fixed these people in areas of low risk of transmission (urban areas, south and southwest of Brazil). **Supported by** FINEP/FAPEX **E-mail:** spider@icbusp.org

Leish002- Epidemiological characteristics of American cutaneous leishmaniasis patients received by the Center of Research Aggeu Magalhães/ Fiocruz, between the years 2010 and 2011

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Leishmaniasis are diseases with wide spectrum of clinical manifestations, varying from skin lesions to severe involvement of the viscera, may leading the patients to death. American cutaneous leishmaniasis (ACL), the most predominant form in the Americas, is registered since the South of Argentina, being found in almost all Latin American countries, with the exception of Chile and Uruguay. In 2010, 22,472 cases were registered in Brazil, and 437 of them, were from the Pernambuco State, location that offers own eco-epidemiological characteristics for the maintenance of the disease. In this sense, the aim of this study is to describe and analyze the epidemiological profile of patients with ACL received in the Laboratory Reference for Leishmaniasis of the Center of Research Aggeu Magalhães/Fiocruz between the years 2010 and 2011. The data were obtained from records of 119 ACL suspects patients. Were analyzed the variables: sex, age, occupation, origin of residence, clinical form presented, localization and diameter of the lesions and the evolution time. Were considered as formal case patients who presented at least one positive laboratory test, resulting, thus in 82 patients. In the analyzed period, there was higher prevalence in males 65.8% (54/82), being the average of age bracket 20 to 50 years the most affected 57.3% (47/82). A total of 47.6% (39/82) of the patients develop agricultural activity and 65 of them (79.3%) leaving in the rural area of Moreno municipality. Analyzing the clinical forms of the lesions, the form ulcerated is predominant, being observed in 90.2% (74/82), as ulcer diameter varying from 1cm x 1cm to 8cm x 6cm, located mostly in the lower 68.3% (56/82) and 47.6% upper limbs (39/82). Concerning the time between the appearance of the first lesion and the start to treatment, it was observed a mean interval of 1 month 30.5% (25/82). The results reflect the profile of ACL, disease that affects mainly males in rural environment, being the productive age group most affected. The ulcerated lesions, the location of them and the evolution time observed in this study are usual to the characteristics of ACL in the most endemic areas of Brazil. **Financial support:** CNPq and FACEPE **E-mail:** julianamedeirosviana@gmail.com

Leish003- Characteristics of visceral leishmaniasis in Reference Unit in the State of Mato Grosso do Sul

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Introduction: Visceral leishmaniasis (VL) is a serious chronic disease in which mortality may reach up to 10% of cases when no appropriate treatment is instituted. It is caused by species of the genus *Leishmania*, and in Brazil, the etiologic agent is *Leishmania chagasi*, transmitted by *Lutzomyia longipalpis*. Recently, *Lutzomyia cruzi* was indicted as a vector in focus in the State of Mato Grosso do Sul (MS), which since nineties influences significantly the VL statistics in Brazil. The epidemiological study of VL is important to provide support to health professionals who deal with the parasites, so that the diagnosis and early treatment may contribute to health promotion and control of this endemic disease in MS. **Objectives:** to describe the epidemiological characteristics of VL cases treated in Reference Unit of Campo Grande, MS. **Materials and Method:** this work is a cross sectional study, carried out through analysis of VL medical records and Chips of Epidemiological Research (CER) of the Hospital Epidemiology Service, generated from visits to the hospital and to the outpatient care in 2011. **Results:** There were 142 reported cases of VL, including 15 (10.1%) were discarded by the absence of laboratory confirmation and / or clinical improvement with treatment. Of 127 confirmed cases, 88 were from Campo Grande (69.3%), 123 were new cases (96.9%) and 120 were autochthonous cases (80.3%). 75 patients were male (59.1%), with ages ranging from six months to 89 years, mean 24.8 years. The disease was

detected in 54 patients aged below 10 years (42.5%) and 17 patients aged 50 years (13.4%). Eleven (8.7%) were HIV positive and in 14 CER (11%) this data was ignored. In 30 cases, confirmation was made by clinical and epidemiological criteria (23.6%) and laboratory diagnosis in 97 (76.4%). Of all the drugs used for treatment, pentavalent antimony has been first choice in 53 cases (41.7%), changing therapy to amphotericin B or liposomal amphotericin B in 21 cases (16.5%). Of four described recurrences (3.1%), three were children under 10 years old and an adult HIV. Only one death due to VL in adult no HIV status was recorded in that year. **Conclusions:** The patient infected with *Leishmania chagasi* was often male, aged 0-10, or more than 50 years, residing in the city of Campo Grande, where the Hospital in Study is located. New autochthonous cases were diagnosed by immunological tests. The drug generally used was pentavalent antimony, and only 16.5% of cases occurred some therapeutic change. There were four recurrences and only one death was recorded in the period. **E-mail:** joslaine.nunes @ bol.com.br

Leish004- Epidemiological profile of human visceral leishmaniasis in Barcarena, a mining municipality in the state of Pará, Brazil

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Introduction: Visceral leishmaniasis (VL) is increasing in Para, where the mining municipality of Barcarena is an area of intense disease transmission. The objective of the study was to describe the recent epidemiological profile of human VL in Barcarena and the principal areas of risk in order to orientate surveillance and control methods. **Material and Methods:** The study was a descriptive observational study, of the ecological type. Analysis was performed (χ^2 , $p=0.05$) on epidemiological data about new and autochthonous cases of VL in Barcarena notified to SINAN (National Information System of Disease Notification) from 2004 to 2008. A geostatistical analysis (Kernel) identified hotspots of cases. **Results:** There were 201 new cases of VL in the study period, with a reduction in the incidence per 100,000 inhabitants each year: 76.8; 82.3; 55.2; 27.0 and 25.6 respectively ($p<0.0001$). There were no deaths recorded, except in 2007 (1 death = 4.2%). The months with the highest numbers of new cases were December (23), January (24) and May (26), compared to 12-20 cases in the other months. Male children below the age of 5 years from the rural zone were the most at risk of disease ($p<0.05$), though the proportion of new cases in the youngest and oldest ages (<5 and >60) also declined each year ($p<0.05$). Laboratory confirmation of VL was more common than simple clinical diagnosis. In 2004, the majority of cases were confirmed by direct parasitological examination (60%) and the remainder by indirect immunofluorescence (IFAT) (40%). From 2005 onwards the reverse pattern occurred with the majority of new cases confirmed by IFAT (2005: 54%; 2006: 71%; 2007: 86% and 2008: 90%). Treatment schemes for periods shorter or longer than that recommended by the Ministry of Health were registered during the 5 years (30%). Kernel analysis revealed hotspots of VL cases in the centre and north of Barcarena, spanning the localities of Santa Maria, Bacuri e Araticu, a rural zone of dense forest and roads, as well as the locality of Aipi and limited areas of the urban center. **Conclusion:** From 2004 to 2008, serology was offered and used efficiently. The gradual reduction in the incidence of disease and in the proportion of severe cases (extremes of age), together with the low death rate, may reflect the restructuring of the VL control program in Barcarena in 2005. Various problems still persist and incomplete data, particularly on the 'evolution of the cases', hindered some analyses. Most transmission was in rural areas, but with hotspots of cases at the urban-rural interface, and greater risk of transmission in the rainy season. The priority targets for disease surveillance and control in Barcarena come into three categories: 1 – Morbidity, severity and geographical distribution; 2 – Registers of information and diagnostic criteria; 3 – Treatment criteria. The intensification of these actions is a priority in the center and north of the municipality. **E-mail:** lourdesgarcez@iec.pa.gov.br

Leish005- Epidemiological Profile of Pediatric Patients Diagnosed with Visceral Leishmaniasis (VL) at the Dr. Waldemar de Alcântara General Hospital in Fortaleza, Ceará – A Retrospective Analysis of 319 cases.

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Introduction: The Visceral Leishmaniasis (VL), popularly known as Calazar disease, is a systemic infective disease, endemic in tropical and subtropical regions, including Ceará, in Brazil northeast. Aiming to contribute to the scientific community and feeling responsible for public health policy better prevention strategies development, we strongly believe that the early diagnosis and treatment are highly important in decreasing morbidity and mortality in pediatric patients with VL. **Material and Methods:** This paper attempts to give an epidemiological indicators overview of this disease in Fortaleza, Ceará, from the analysis of all cases of pediatric patients hospitalized due to VL over a period of six (06) years in a hospital unit center. The study is retrospective, descriptive and analytical, by reviewing records from 2004 to 2009, when the patients admitted had VL as the main diagnosis, comprising three hundred and nineteen (319) patients in the children's range. Data collection was performed by standardized questionnaire, including clinical, laboratory and treatment parameters. **Results:** Their ages varied from under 1 year to 16 years old, and the group under 10 years old was the most affected (95,6%). As to gender, there was a slight predominance of females (52%) over males (48%). Most of the patients (58%) were from Fortaleza. The main clinical manifestations were: fever (100%), splenomegaly (98,4%), hepatomegaly (94,3%), pale skin and mucosa (94%), asthenia (49,5%) and cough (38,9%). Other reported clinical manifestations, but with lower incidence were: diarrhea (28,5%), lower limb edema (21,9%), weight loss (19%), somnolence (8,1%), vomiting (6,3%) and gingival bleeding (3,8%). The main laboratory findings were reversed albumin-globulin concentration in plasma (72,4%), pancytopenia (70%) and elevated ESR or PCR (59%). The tests used for diagnostic confirmation were myelogram (84,3%), K39 antigen search (59%) and serology by immunofluorescence (35,4%). From the sample, 94% of the patients were discharged with clinical symptoms solved and 2,5% died. There was clinical segment loss with 3 patients, precluding the access to the outcome. **Main Conclusions:** The data indicated that VL is quite prevalent in pediatric patients under 10 years old and that this disease can be easily recognized for characteristic clinical features, facilitating the diagnostic ratification in early investigations based on the main confirmatory diagnostic methods. The early diagnosis is crucial in saving lives. **E-mail:** angelicapessoamorais@gmail.com

Leish006- Epidemiology The American Cutaneous Leishmaniasis (ACL) in Montes Claros, Minas Gerais, Brasil

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The American Cutaneous Leishmaniasis (ACL) is an infectious disease caused by different species of *Leishmania* spp and it is transmitted by insects from the Phlebotominae subfamily. Primarily, it is a zoonotic infection, where the human being acting as a secondary host in the transmission chain, is affected by polymorphic manifestations in the skin and mucous membranes. Regarding to the occurrence, the municipality of Montes Claros is considered endemic, with a no homogeneous distribution of cases in its territory. Given the importance of this disease, this study aimed to evaluate the epidemiological aspects of CL cases reported in the city from 2007 to 2011. It was conducted a retrospective descriptive study of the cases of this disease, obtained from the Information System for Notification of Grievous Diseases (SINAN). The following variables were evaluated using Excel and Tabwin software: number of cases, sex, age, region of residence, and the criterion used for confirmation,

and evolution,. During the study period was registered 281 cases of ATL in Montes Claros, with an annual average of 56.2 cases. Transmission peaks were observed in the years 2010 (n = 76) and 2011 (n = 77) with an incidence of 20.9 cases per 100,000 inhabitants. Among the cases studied, were observed that LTA affects both sexes, with male predominance (n = 181 cases: 64.4%) and in patients aged more than 10, which corresponded to 91.4% (n = 254) of the cases. In relation to the patients place of residence 75.5% (n = 210) are from the urban area. The prevalent clinical form is cutaneous, registered in 96.4% cases (n = 268). There were 88.26% (n = 248) cases confirmed through laboratory tests, and the Montenegro intradermal technique (n = 185 cases, 65.8%) was the most widely used. In terms of outcome, 247 cases (87.9%) progressed to clinical cure, 04 (1.4%) abandoned the treatment and 01 (0.35%) died of other causes. There was no further information about evolution in 22 cases (7.8%), since they are still being studied and monitored for closure. The evaluation of all these indicators refers back to the importance of improving the information system, which is an important tool for monitoring and for surveillance of the cases, as well as for the construction of monitoring indicators for the disease, establishment of the transmission pattern, and performance of operational assessment for the control activities. It is also necessary to strengthen the care network for ACL cases in Montes Claros by structuring how to perform parasitological diagnosis and give appropriate treatment for patients through health professional training. **E-mail:** rfmarilia@hotmail.com

Leish007- Epidemiological Aspects of Visceral Leishmaniasis in the city of Caxias – MA

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In Brazil, visceral leishmaniasis (VL) or kala-azar is endemic Neotropical, mainly rural, with trends towards urbanization. The etiological agent of VL is *Leishmania chagasi*, the protozoan intracellular mononuclear phagocytic system. The LV is widely distributed throughout the world. The estimated risk population of 350 million people, currently endemic in 88 countries. In Latin America, Brazil is responsible for 90% of reported cases, 56% in the Northeast. Between 1984 and 2002, 66% of cases occurred in the states of Maranhão, Piauí, Ceará and Bahia. The VL in Brazil affects mainly children, with higher prevalence in males and with the main reservoir Dog From 2006, in Brazil, every case of VL is notifiable, and the data recorded in the Information System Diseases Notification - SINAN. The VT has interrelationships geographic, climatic, social and cultural, which diversifies its clinical manifestations in different regions of the country, as well as being risk factors for the occurrence of the disease. **Material and Methods:** The study was based on retrospective analysis, cross sectional, descriptive, and quantitative nature documentary of 180 cases of patients diagnosed with visceral leishmaniasis in the period 2007 to 2011, in the city of Caxias - MA. Data were provided by the Department of Epidemiological Surveillance. Were analyzed the following variables: age, sex, race, neighborhood, co-infection with HIV, area of residence, type of entry, education, relationship with work and evolution. **Results:** We observed that the total of 180 cases (100%), 70% were aged 9 years old, 108 cases were male (60%) and 72 cases were female (40%). As for race, 80.6% were mixed. The neighborhood undertakers were most prevalent (11.25%). Only 3.33% had HIV co-infection. Regarding the area of residence, 88.4% of cases occurred in urban areas, 96.6% were new cases, 43% of cases had incomplete primary education, 3% of the cases were related to work and 92.3% were cured. **Main Conclusions:** Visceral leishmaniasis is endemic in the city of Caxias. The council has shown a reduction in the number of cases of illness due to actions of the Municipal Center for Health Education (NMES) with lectures and action of health workers in educating the population; however it still needs more government support in the implementation of most effective measures for the control of VL. **E-mail:** wilson-jardim@hotmail.com

Leish008- Prevalence of asymptomatic infection of *Leishmania chagasi* among blood donors in endemic areas for leishmaniasis in Ceará

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Introduction: Visceral leishmaniasis (VL) is expanding in Brazil, and the northeast region holds the largest number of cases. Clinical manifestations can range from asymptomatic to severe, mainly affecting the spleen and liver. VL is transmitted to humans through the bite of infected phlebotomine *Lutzomyia longipalpis* females, but other forms of transmission have been reported, like sharing needles, laboratory accidents, congenital infection, and sexual contact and by blood transfusion. The transmission by transfusion has been cited since 1948, in China, but other cases have been reported in Brazil, India, France, Sweden, Belgium and England. Hemotherapy Brazilian Legislation, RDC No. 153 of 2004, recommends, as routine in blood banks, serological tests for Chagas, hepatitis B and C, syphilis, HTLV 1 and 2, HIV 1 and 2. However, the LV serology is not recommended. Thus, the following study aims to determine the prevalence of isolation of *Leishmania* by culture and evaluate the detection of DNA of this parasite in the same buffy coat from the blood donors, by PCR. **Materials and methods:** Bags of buffy coat from blood donors from the Blood Center of Fortaleza (HEMOCE) were analyzed in this study. The culture of peripheral blood mononuclear cells was performed in duplicate in NNN medium with supplemented Schneider and maintained for four weeks with weekly evaluations using an inverted microscope. The primers used in PCR for *Leishmania* DNA are related to a conserved sequence of minicircle kinetoplast DNA (kDNA), generating an amplicon corresponding to 120 bp. The presence and integrity of the human DNA was verified by amplifying the β -globin gene generating a fragment of 252 bp. **Results:** Of the 293 blood donors analyzed, 228 (77.8%) are residents of Fortaleza, 31 (10.5%) of the metropolitan area and 34 (11.6%) resides in the interior of the state. In relation to gender, 187 (63.8%) are male and 106 (36.2%) are female, with an average age of 34.7 years. Among the donors, 282 (96.2%) tested negative in tests conducted by HEMOCE and 11 (3.7%) were positive in at least one of the tests. Cultures were performed for all donors and they were negative and can be explained by low parasite load found in patients with asymptomatic LV. The extracted DNA integrity was checked by PCR using β -globin and all were positive for the gene. All the 100 samples submitted to PCR for *Leishmania* were negative until now. **Conclusion:** Although it still hasn't being detected any cases of asymptomatic LV among the donors from HEMOCE-Fortaleza, we can not rule out the possibility of transmission by blood products, because a greater number of donors still has to be evaluated and submitted to these techniques that are still in progress. **E-mail:** mpompeu@gmail.com

Leish009- Prevalence of visceral leishmaniasis in the period 2008 to 2010 in the state of Piauí - Brazil

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Introduction: The Visceral leishmaniasis (VL) or Calazar, is a chronic serious disease and potentially fatal, being caused by species of the genus *Leishmania*, that are part of the *Leishmania donovani* complex. At Brazil, the etiologic agent is *L. chagase*. The illness is recognized by the world health organization as an important problem of public health, being endemic in more than 60 countries. Brazil is one of the responsible for more than 90% of the cases that are registered around the world. In our country, the disease is spreading both numerically and geographically, especially in the northwest, highlighting in these states: Bahia, Ceará, Piauí e Maranhão, where 90% of the notifications are noticed. Besides the high incidence and the wide distribution, another important factor related to the LV is the possibility to assume serious and lethal forms when associated with bad nutrition and concomitant infections. The following work aims to show the distribution of the human LV cases registered at the states of Maranhão and Piauí, between the years of 2008 and 2010, and also at the epidemic period of the disease and the cities with a higher prevalence. **Material and Methods:** Research with data originally from Sistema de Notificação de Agravos – SINAN in the period from 2008 to 2010. **Results:** in this period, 742 cases were diagnosed at PI: 10 at the city of Paulistana, 11 at Miguel Alves, 1 at Picos,

49 at Parnaíba and 575 at the capital Teresina, being 235 of all, registered at 2008. At MA, there were 1.429 cases between them 47 at Barra do Corda, 47 at Itapecuru Mirim, 65 at Açailândia, 152 at Caxias, 164 at Imperatriz and 409 at São Luís, being 404 of all cases registered at 2008. **Conclusion:** After a review of all cases of visceral leishmaniasis diagnosed in the States of Maranhão and Piauí, one might note that MA in the city of Caxias was most prevalent. In the PI, Teresina was deemed to be most prevalent in the state. As for the endemic period, 2008 had a higher number of reported cases in both states. **E-mail:** yasmin.vali@hotmail.com; pedro.hca@hotmail.com; luka.ferreira@hotmail.com

Leish010- "Environmental Risk Factors for American Visceral Leishmaniasis in the State of Sao Paulo - Brazil"

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Introduction: The American visceral leishmaniasis (AVL) is a serious public health problem. In 2010 the State of Sao Paulo had 26% municipalities with intense transmission. Among the actions of the transmission control the environmental stewardship has been paramount in spite of the complexity of the environment in urban centers. **Objective:** Identify environmental risk factors in the city of Florida Paulista classified as transmission intense. **Material and Method:** Florida Paulista has 13,761 population and free service for collecting organic material. Since 2006 registered 28 cases indigenous. In 2011 the prevalence of canine was 11%. To conduct the canine census survey, Zoonoses agents were previously trained for environmental assessment of the residences by a specific form. The information was analyzed in Epi Info 2006 software according to size of peridomicile, presence of green area, type of construction of housing, pets, accumulation of organic matter, implementation of environmental stewardship and protection measures of dwellers. **Results:** In 3,947 households, were visited 1820 without dogs and 1091 with dogs, was not possible to survey households in 1036 because were closed by the time of visit or refusal of the residents. Were recorded 1627 dogs in the properties surveyed, an average of one dog per household (64.4%). When comparing homes without dogs with those with presence, there are no differences between the type of construction of houses, both brick towed, peridomicile of 10 to 200 m² and 10 to 200 m² green area. In properties without dogs, 30.6% of dwellers cohabit with other types of animals: birds (66.2%) and rabbits (4.2%) the most cited. In these households, 18.0% had conditions for proliferation of the vector as the presence of organic fertilizer (43.8%) and animal feces (54.3%). The residents do environmental stewardship (81.7%) and additional measures such as use of insecticides (98.9%) and repellents (90.2%). In households with dogs the residents also live with birds (39.8%) and rabbits (3.7%), mainly. In this case, accumulation of organic matter such as leaves, fruits, stems, roots and animal feces were the most expressive. The control measures showed that residents of homes with dogs make less use of insecticides (42.4%) and repellents (67.2%), but perform animal protection measures such as application of chemical products (98.5%) and vaccine canine AVL (6.2%). **Conclusion:** In this study was observed that most residences offer conditions for breeding of the vector *Lutzomyia longipalpis* and transmission risks. The domestic dogs are in critical properties, which due to environmental factors become more susceptible to infection. The diagnosis allowed new proposals for environmental stewardship and protection measures, beyond represent an important indicator for monitoring the practices of the actions. **E-mail:** luciah@sucen.sp.gov.br

Leish011- Distribution os American tegumentary leishmaniasis (ATL) in the state of Amazonas from 2001 to 2010

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Introduction: American tegumentary leishmaniasis (ATL) represents a public health problem in the Brazilian Amazon. The Amazonas State constitutes the second Brazilian state in reported cases of ATL. The transmission has been associated with same activities as forest extractive tasks, disorganized land allocation, and deforestation tasks. In the last years these activities have been favored by the construction of roads connecting the main metropolitan areas. In order to describe the geographical distribution of the disease in the Amazonas State, we have conducted this observational retrospective study. **Methods:** We searched for all reported ATL cases in the Amazonas State through the national information system on diseases of compulsory declaration (SINAN) for the period between 2001 to 2010 and analyzed the epidemiology of the resulting data, and characterized the leishmaniasis in cutaneous and mucosal. **Results:** We identified 21,492 reported cases in this period, with an average of 2149 cases per year. Geographical distribution results are as followed: Manaus: 10879 (50.6%), Rio Preto da Eva: 2621 (12.2%), Presidente Figueiredo: 1730 (8%), Itacoatiara: 991 (4.6%), Coari: 447 (2.1%), Boca do Acre: 411 cases (1.9%). 16,372 cases were males (76.2%) were males. Almost half of the cases were related to forest extractive tasks (43.71%). The group of age most commonly affected was between 11 and 44 years, accounting for 67.17% of the cases. Regarding the clinical classification 20,733 (96.46%) was cutaneous. **Conclusion:** The most affected area in the area in the Amazonas States is Manaus, accounting for half of the cases. The disease is associated with male sex, young people, and is related with activities involving the forest. These findings are consistent with other studies, and confirm the association between forest tasks and leishmaniasis, which are mainly developed by young men. As an official database lead study, we are probably missing some data which constituted a limitation of the study. **E-mail:** jguerra291@gmail.com

Leish012- An Ecological Risk Model for Visceral Leishmaniasis in Brazil Based on Environmental and Socioeconomic Risk Factors.

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Introduction: Visceral leishmaniasis (VL) is a serious public health problem due to its resistance to modern control efforts. VL represents a disease complex with important clinical impacts and epidemiological diversity that belongs to the group of neglected tropical diseases intrinsically associated with poverty and health inequities. The circumstances of transmission of VL are continually changing in relation to environmental, demographic and behavioral factors and the use of remote sensing and computer software, such as geographic information system (GIS), has emerged as an alternative tool in the study of endemics, using climate, environmental and socioeconomic data to better understand the dynamics of endemic VL and predict future outbreaks. This study aimed to identify socioeconomic and environmental factors associated with VL cases in Brazil from 2005 to 2009, using GIS and maximum entropy method (Maxent) modeling software to predict disease risk at the municipality level. **Material and Methods:** The GIS database was constructed using records of VL cases by municipality available in the national notifiable diseases information system (SINAN) database; records from the Brazilian Institute of Geography and Statistics (IBGE) and the Pan-American Health Organization (unsatisfied basic needs for people (UBNp) and housing (UBNh)) were used to compile the socioeconomic data. The environmental database was constructed using long-term normal monthly climate data from WorldClim and MODIS remote sensing annual composite image data. Probability distribution models for VL based on environmental and socioeconomic features were executed using Maxent and maps of the spatial distribution and prediction models for VL were created. **Results:** 13,563 cases of VL were registered at SINAN, but only 2.2 % of the municipalities reported cases of the disease. The majority of the cases occurred in the North and Northeast regions (21.31 and 47.42% respectively). A linear regression model showed that population, garbage collection, literacy rate and UBNh water availability were the socioeconomic indicators that would better explain the presence of VL ($p < 0.0001$; $r^2 = 0.414$). The Maxent model for environment variables showed a higher contribution by precipitation seasonality (14.5%) and precipitation of October (11.6%) (AUC=0.948). The socioeconomic model showed that the most influencing variables were poverty incidence (35%; AUC= 0.894); UBNh - drinking water availability (49%;

AUC= 0.745) and UBNp - education (38.5%; AUC= 0.845). The model combining environmental and socioeconomic data showed precipitation of October (21.6%), literacy rate (15.4%) and UBNp sanitation (14.9%) as the most influencing variables (AUC= 0.942). **Conclusions:** The techniques used are powerful tools that can identify areas suitable for disease based on a known distribution and improve the allocation of resources to better control endemics. **E-mail:** newmeh2004@yahoo.com.br

Leish013- High canine seroprevalence as a risk factor to occurrence of human visceral leishmaniasis in Governador Valadares (Minas Gerais, Brazil)

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Introduction: In the municipality of Governador Valadares/MG were notified 86 cases of visceral leishmaniasis between the years of 2008 and 2011, with 14 deaths. The presence of a large number of infected dogs and the high density of *Lutzomyia longipalpis* has been appointed as the main risk factors to occurrence of the disease. **Objectives:** This study aimed to measure the canine seroprevalence in many neighborhoods of city. **Material and Methods:** Blood samples was collected through venous puncture of the auricular marginal vein and transferred to filter paper. For the serological process has been used the immunoenzymatic test (ELISA), with confirmation by the indirect immunofluorescence assay (IFAT) in reactive animals by the first method. Therefore, titles $\geq 1/40$ was considered positives. **Results:** Between the years of 2008 and 2011 were collected 17,516 samples, being diagnosed 4,904 seropositive dogs establishing an average prevalence of 28%. However, in some neighborhoods of city, as Lourdes, Santa Helena, Vila Mariana, Nossa Senhora das Graças and Mãe de Deus, the canine prevalence exceeds 40%. **Conclusions:** The results showed that the dog has an important role on transmission cycle of VL in the city increasing the necessity of rigid control actions through euthanasia of seropositives dogs with the residual insecticide spraying and environmental management in residences. **Support:** FAPEMIG, UFVJM, FIOCRUZ. **E-mail:** ricbarata@hotmail.com

Leish014- Identification of risk factors associated to canine visceral leishmaniasis in an endemic area of Bahia

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At urban areas, the dog is the main reservoir for *Leishmania infantum* (syn chagasi). Identify risk factors for *L. chagasi* infection is essential to plan effective actions to control visceral leishmaniasis. The present study aimed to perform a serological surveillance in dogs from an endemic area for visceral leishmaniasis. A cross-sectional study was performed including 22 neighborhoods in the municipality of Camaçari, Bahia, Brazil. The houses included in this study were chosen randomly. Five hundred dogs living in 348 households were included, evaluated clinically and an epidemiological questionnaire was done with their owners. The diagnosis of visceral leishmaniasis was determined by both ELISA and culture positivity in splenic aspirates. The chi-square test ($P < 0.05$) was used to evaluate the association between risk factors and canine visceral leishmaniasis diagnosis. *Leishmania* infection was diagnosed in 27% of animals evaluated. According to the report of the owners, dogs presenting some signals of canine visceral leishmaniasis in the last month correlated with the diagnosis of the disease. The signs included apathy 37% (OR1.7; IC1.1-2.8), weight loss 36% (OR1.7; IC1.1-2.5) and loss of appetite 35% (OR1.6; IC1.0-2.6). In addition, the owner report their dogs have been sick 63% (OR5.9; IC2.9-11.4) or convalescent 35% (OR1.8; IC1.0-3.4) in the last month. In the households, presence of vegetation 32% (OR3.4; IC1.9-6.0), other dogs 39% (OR2.1; IC1.3-3.2), and birds in the backyard 37% (OR2.0; IC1.2-3.2)

were identified as potential risk factors for canine visceral leishmaniasis. The positive diagnosis of dogs with visceral leishmaniasis was higher in houses where the animals were guard dogs 37% (OR1.9; IC1.2-2.8) and exclusively remained in the backyard 28% (OR3.2; IC1.2-8.2) Potential protective factors against Leishmania infection were also identified: dogs living in household located on paved streets 20% (OR0.5; IC0.3-0.7), as well as the use of protective methods in dogs 42% (OR0.5; IC0.3-0.8). A positive correlation was found between detection of dogs infected with Leishmania in either homes (OR4.6; IC2.6-7.9) or neighborhoods (OR3.3; IC2.0-5.6) where dogs have been previously diagnosed with leishmaniasis. These findings indicate that new cases appear more often in areas where disease in dogs has been previously diagnosed. In sum, the data presented herein open the possibility of implementation of more adequate control actions in areas where factors of greatest risk of canine visceral leishmaniasis have been identified. Furthermore, this may help targeting effective measures to control this illness in human population. **Support by** FAPESB, INCT-CNPq, PDTIS, PST Veras' grant (CNPq:306672/2008-1). **E-mail:** dmfraga@hotmail.com

Leish015- Positivity rate for canine visceral leishmaniasis in areas with strong transmission in the State of Bahia

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Introduction: In Brazil, canine visceral leishmaniasis (CVL) coexists with the human disease in all known outbreaks although it is usually more prevalent and generally precedes the occurrence of human disease. Dogs infected by *Leishmania donovani*, the Mediterranean cause of canine leishmaniasis, have a well-known spectrum of clinical features ranging from apparently healthy to severe end-stage disease. From the epidemiological point of view, the canine disease is considered more important than the human disease because it has large numbers of asymptomatic animals harboring parasites. In urban areas, thus the dog becomes the main source of infection for humans. The enzootic canine disease has preceded the occurrence of human cases and infection in dogs has been more prevalent than in men. The disease in dogs has a slow and insidious onset and epidemiological studies are a decisive factor for effective planning strategies for the control of VL. **Objectives:** This study aims to demonstrate the positive canine index (CPI) for CVL in cities with strong transmission in the state of Bahia in the period of 2008 to 2010, specifically: América Dourada, Boquira, Cafarnaum, Canarana, Feira de Santana, Guanambi, Iraquara, Irecê Jequié, Juazeiro Macaúbas, Salinas da Margarida, Salvador, Sento Sé and Uibaí, totaling 341 human cases in that period. **Methods:** Data were obtained from monthly reports submitted by the Regional Health Boards in the State (DIREs). The calculation of the CPI was based on a formula contained in the Manual of Surveillance and Control of Leishmaniasis. **Results:** In the period 2008 to 2010, the highest levels of CPI were observed in Feira de Santana (11.1%), Jequié (11.1%), Guanambi (7.9%) and Iraquara (6.6%), coinciding with the highest number of human incidents in the same period, accounting for 34% of the total cases in the 15 municipalities with strong transmission currently in the state. **Conclusion:** The surveillance of the disease includes the entomological surveillance of human cases and canine cases. The latter becomes increasingly important both for confirmation of the urbanization of VL, in this way monitoring the parasite movement that serves as an important indicator of the occurrence of human cases, and to help health professionals in decision-making in order to adopt strategies aimed at reducing morbidity and mortality in the counties of strong transmission for VL in Bahia. **E-mail:** leish.divep@saude.ba.gov.br

Leish016- A survey of canine visceral leishmaniasis in the tourist town of Embu das Artes -SP, Brazil

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Introduction: One of the biggest problems about leishmaniasis is the lack of a specific diagnosis capable to identify *Leishmania* species and control the spread of the disease. The PCR technique are being described as a rapid and reliable technique for prevention, control of the spread, and treatment support. Since 2002, several cases of autochthonous canine visceral leishmaniasis (CVL) were detected in the tourist town of Embu das Artes located around São Paulo, one of the largest cities in the world. Concerned about the spread of the disease in humans, an inquiry was created in order to investigate some aspects involved the transmission and epidemiology of CVL in this touristic town. **Methods:** We performed: 1) canine clinical analysis, collecting blood and tissue samples (spleen, liver, lymph node, skin with and without lesion) of 26 dogs euthanized after seropositive test result for leishmaniasis, 2) direct parasitology tests, culture of isolated parasites and PCR with primers directed to kDNA (kinetoplastDNA), to evaluate canine infection, 3) clinical examination of children among 4-10 years with potential risk of infection, 4) comparison with previous study that examined sandflies collected in the area (SUCEN provided). **Results:** The inquiring of the canine population, based in serological tests, revealing that within a population of 2,348 dogs, 66 dogs were positive for CVL (2.81%), while 26 dogs were sacrificed. From the 26 dogs euthanized, 22 (84.6%) were positive for direct parasitological test, while 21 (80.77%) had positive cultures in at least one of the collected samples. PCR analysis showed that spleen samples are the most sensible 92.30% (24/26), followed by skin lesions and lymphnodes samples, both with 88.46% (23/26) of positivity. Blood samples, despite less invasive sample, showed lower positivity 80.77% (21/26). Clinical evaluation of the human population and the epidemiological analysis of sandflies showed the absence of human disease and the main vector involved (*L. longipalpis*). **Conclusion:** The survey in Embu das Artes showed a different pattern of transmission for the CVL since the disease was confirmed in the canine population, but was not shown the classic vector or human disease. This suggests that a different vector should be involved in the transmission. The comparison of the canine clinical with the PCR results of spleen tissue (24/26), showed that the two dogs were negative for PCR and had no typical signs of CVL, suggesting false positive results of serology and unnecessary euthanasia. **Supported by** FAPESP, CNPq, FMUSP-LIM48. **E-mail:** pccotrim@usp.br

Leish017- Association between clinical signals severity and occurrence of canine visceral leishmaniasis

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The similarity of visceral leishmaniasis manifestation in humans and dogs renders the study of canine visceral leishmaniasis important. However, more susceptible dogs present more severe clinical manifestations with classical signs of visceral leishmaniasis, as well as cutaneous wounds. The clinical manifestations of canine visceral leishmaniasis consist of signs that vary in number and severity, and can bear similarities to other canine infectious illnesses. These characteristics of the dog disease support the efforts to design more reliable and sensitive methods for clinical diagnosis of canine visceral leishmaniasis. The present study aimed to perform a clinical and serological survey in dogs from an endemic area for visceral leishmaniasis in the municipality of Camaçari, Bahia, Brazil. The association of the presence and severity of the signals with the positive diagnosis of canine visceral leishmaniasis was evaluated. A cross-sectional study was performed including 22 neighborhoods in the municipality of Camaçari. The houses included in this study were chosen randomly. Five hundred dogs were included and evaluated clinically. The diagnosis of visceral leishmaniasis was determined by both ELISA and culture positivity in splenic aspirates. Associations were determined using chi-square test ($P < 0.05$). *Leishmania* infection was diagnosed in 27% of animals evaluated. Some evaluated signs, and their severity, showed a positive correlation with the positive diagnosis of canine visceral leishmaniasis. In addition, the diagnosis of canine visceral leishmaniasis positively correlate with the severity of the signs including wound on the ears (53%; OR 4,2; IC 2,1-5,1), onychogryphosis (42%; OR 3,0; IC 2,0-4,5), hyperkeratosis on the nose (36%; OR 2,6; IC 1,7-3,9), crust in the body (39%; OR 2,4; IC 1,6-3,6), depigmented nose (37%; OR 2,4; IC 1,6-3,6), lymphadenopathy (34%; OR 2,4; IC 1,6-3,7), conjunctivitis

(34%; OR 2,0; IC 1,1-2,4) and alopecia (28%; OR 1,5; IC 1,0-2,3). Together, the results indicate that there is a high association between presence and severity of disease signals with the positive diagnosis of canine visceral leishmaniasis, which is related to the odds of the animal be infected by *Leishmania* sp. These data indicate that the presence and the severity of clinical signs in dogs enhance our capacity to diagnosis canine visceral leishmaniasis. **Support by** FAPESB, INCT-CNPq, PDTIS, PST Veras' grant (CNPq:306672/2008-1). **E-mail:** dmfraga@hotmail.com

Leish018- Canine visceral leishmaniasis seroprevalence in Xakriabá Indigenous Land, Minas Gerais State, Brazil, in 2011

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Introduction: In Brazil, visceral leishmaniasis (VL) or Neotropical kala-azar is a peri-urban and rural zoonosis. This disease is potentially fatal to humans in which mortality may reach 10% without proper treatment. According to the Brazilian Ministry of Health, the expansion of this zoonosis has affected young people or groups of individuals with co-morbidities such as malnutrition and low immunity, causing high mortality rates. Brazil is endemic for VL. The dog is considered the domestic reservoir, and is responsible for endemic and epidemic presentations of this disease. In Minas Gerais state, the highest rates human cases are reported in the north, precisely where Xakriabá Indigenous Land (XIL) is situated. Studies conducted on XIL, demonstrated that there is a high prevalence of mild to severe malnutrition in the population up to twelve years old. Thus the aim of the present study was to evaluate the seroprevalence of dogs for canine visceral leishmaniasis (CVL) in XIL that is located in a region of high endemicity for VL and under the impact of children's malnutrition. **Material and Methods:** The study design was a cross-sectional census. The serological survey was performed by using the ELISA and IFI methods in blood samples dried on filter paper, from 864 dogs, distributed in 16 XIL villages. **Results:** The results of the serological investigation, using blood dried on filter paper were: 30.5% positives and 15.5% indeterminate for ELISA. From those testing positive or indeterminate for ELISA, 8.0% were also positive by IFI. The results were markedly heterogeneous between the villages. Also based in the ELISA test results, the seropositivity ranged from 43.60% (Brejo Mata Fome) to 2.50% (Riacho do Brejo). **Main Conclusions:** It can be concluded that the canine population from XIL presents a high seroprevalence of VL. This fact may hinder the control of human Kala-azar cases in this region. Thus, in this area, permanent epidemiological surveillance action is necessary, regardless of the village, especially considering the high rate of children's malnutrition in this population. **Financial support:** CNPq, FAPEMIG and CAPES **E-mail:** anasampaio-rocha@yahoo.com.br

Leish019- Canine survey conducted from two canine cases of visceral leishmaniasis in Itaipuaçu, Municipality Maricá, State of Rio de Janeiro

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Introduction: The American visceral leishmaniasis is a zoonosis that affects humans and other species of wild and domestic animals. The disease is caused by *Leishmania (Leishmania) chagasi* and transmits through bites of insects vector *Lutzomyia longipalpis*. In Brazil, this disease remains a major challenge in public health issues, since it is in the process of expansion in several regions, being human and canine cases recorded both in rural and urban areas. From two canine cases of Visceral Leishmaniasis (VL) in a period of one year in Itaipuaçu, municipality of Maricá, state of Rio de Janeiro, a canine survey was conducted. **Material and Methods:** One hundred forty-five autochthonous animals were evaluated through an active search. Four fragments of intact skin of the scapular region, using "punch" of 3mm were collected for direct and indirect parasitological examinations. Two fragments were immersed in saline in

an attempt to parasite isolation and two were immersed in 10% buffered formalin to perform histopathological and immunohistochemical tests. Marrow aspirate was obtained from the manubrium of the sternum. The bone marrow material obtained was transferred aseptically to a biphasic culture medium (NNN supplemented Schneider's medium with 10% fetal bovine serum) and in a tube with anticoagulant plus the fastener "cell block" for histological and immunohistochemical staining. **Results:** Seven animals (7/145) were positive in parasitological test. Of two dogs were isolated *Leishmania chagasi* from both bone marrow and intact skin. Amastigote forms were identified in the same two animals positive to histopathological and immunohistochemical examination. All seven dogs were positive in immunohistochemical examination of the bone marrow aspirate with fixative "cell block". Two animals were positive to *Trypanosoma caninum*. **Conclusions:** In urban areas, the domestic dog is the main source of infection for insect vectors, a fact that has directed many efforts in the identifying and elimination of positive animals for leishmaniasis, with the aim to break the transmission cycle. The notification of new cases of canine VL in this region, where previously there was no record of human or canine case, thus exposing the fragility of the disease control program and the risk of expansion in the state of Rio de Janeiro, which can be aggravated due to the fact that few knowledge about the dynamics of transmission in this area. With the geographic growth and urbanization of American visceral leishmaniasis is necessary to establish more effective control measures in order to contain the disease. **E-mail:** tuanne.abrantes@ipecc.fiocruz.br

Leish020- Canine Skin and Conjunctival Swab Samples for the Detection and Quantification of *Leishmania infantum* DNA in an Endemic Urban Area in Brazil

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Introduction: We evaluated kDNA PCR/hybridization and quantitative real-time PCR (qPCR) targeting the gene of DNA polymerase of *Leishmania infantum* for CVL diagnosis and assessment of parasite load in clinical samples obtained invasively and non-invasively. **Material and methods:** Eighty naturally infected dogs from an endemic urban area in Brazil were used. Animals were divided into two groups based on the presence or absence of CVL clinical signs. Skin biopsies, bone marrow, blood and conjunctival swabs samples were collected and submitted to *L. infantum* DNA detection. In addition, anti-*Leishmania* antibody titers were measured by Immunofluorescence antibody test. **Results:** The symptomatic dogs had increased titers compared to asymptomatic dogs ($P=0.025$). The frequencies of positive results obtained by kDNA PCR/hybridization for asymptomatic and symptomatic dogs, respectively, were as follows: right conjunctiva, 77.5% and 95.0%; left conjunctiva, 75.0% and 87.5%; skin, 45.0% and 75.0%; bone marrow, 50.0% and 77.5%; and blood, 27.5% and 22.5%. In both groups, the parasite load in the skin samples was the highest ($P<0.0001$). The parasite loads in the conjunctival swab and bone marrow samples were statistically equivalent within each group. The parasite burden in conjunctival swabs was higher in the dogs with clinical signs than in asymptomatic dogs ($P=0.028$). This same relationship was also observed in the bone marrow samples ($P=0.002$). No differences in amastigotes load in the skin were detected between the groups. **Main conclusions:** The conjunctival swab is a suitable clinical sample for qualitative molecular diagnosis of CVL. The highest parasite burdens were detected in skin regardless of the presence of VL-associated clinical signs. The qPCR results emphasized the role of dogs, particularly asymptomatic dogs, as reservoirs for CVL because of the high cutaneous parasite loads. These results may help to explain the maintenance of high transmission rates and numbers of CVL cases in endemic urban regions. **Financial support:** Conselho Nacional de Desenvolvimento Científico e Tecnológico. **E-mail:** saninoalmeida@gmail.com

Leish021- Canine leishmaniasis: current situation in Paraguay

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Introduction: Visceral leishmaniasis is a vector borne disease caused by *Leishmania infantum*, a protozoan that affects humans and its urban reservoirs, mainly dogs, and is transmitted by infected sandflies. **Objectives:** The purpose of this work was to determine the seroprevalence of canine leishmaniasis by immunochromatographic rK39 test in serum samples obtained for routine exam requested by dog's owners and veterinarians, active surveillance in areas of silence transmissions and control of human cases notified for the National Service of Paludism Eradication (SENEPA) to the National Programme of Zoonoses Control and National Rabies Center (PNCZ y CAN) in 2011. **Material and methods:** A total of 12879 canine blood samples were analyzed by immunochromatographic rK39 test (Inbios®, USA), in the Laboratory of Leishmaniasis of the PNCZ y CAN proceedings from 12 of the 17 departments of the country. The results were analyzed by Epiinfo 3.5.1 and SigmaPlot11. **Results:** The 5589 canine blood samples proceeding from routine exam from Asuncion (capital city) and the departments: Central, Boquerón, Caaguazú, Cordillera, Itapúa, Guairá, Presidente Hayes, Paraguari, San Pedro, Misiones and Caazapá showed 2708 positive serum samples with a prevalence of 48,45% of canine leishmaniasis. The active surveillance proceedings of Asuncion and the departments of Central, Itapúa, Ñeembucú and Paraguari Central departments showed that 580 out of 2884 samples resulting in a prevalence of 20,11% of canine leishmaniasis. From the 64 focus of human visceral leishmaniasis proceeding from Asunción and the departments: Central, Cordillera and Paraguari, it was observed that 920 of 4406 dogs blood samples showed a prevalence of 20,88% of canine leishmaniasis. It was observed a global prevalence of 32,67% of canine leishmaniasis. Euthanasia procedures were performed in 619 positive dogs (67,28%) proceeding from focus of human visceral leishmaniasis and 420 positive dogs (73,42%) proceeding from active surveillance in areas of silence transmission of visceral leishmaniasis. **Main conclusions:** The high canine visceral leishmaniasis shows the urgent need to continue a strict epidemiological surveillance, sanitary education and community participation by the Ministry of Public Health and Social Welfare in the control of this disease in Paraguay. **E-mail:** jorgemiret@gmail.com

Leish022- Assessment of liver histological alterations in dogs naturally infected with *L. infantum*

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Introduction: Canine visceral leishmaniasis (CVL) is a protozoan disease caused by *Leishmania chagasi*/ *infantum* and is endemic for humans and dogs in many regions in Brazil. Dogs are considered to be the main domestic reservoir. Dogs constitute an excellent model to study leishmaniasis, because they share many clinical, immunological and pathological features with humans VL. The aim of the present study was to assess liver histological alterations in dogs naturally infected with *Leishmania chagasi*. **Material and Methods:** We examined 39 animals from an endemic area, the city of Jequié, Bahia. The animals were grouped into four categories: a) 9 infected-symptomatic dogs; b) 10 infected-asymptomatic dogs; c) 9 non-infected-symptomatic dogs; d) 10 normal dogs. Histological evaluation was performed in a blind manner. **Results:** The results shows that infected-symptomatic dogs differed significantly from the others with respect to the frequency of portal inflammation ($p < 0.003$), granulomas in portal tracts ($p < 0.04$) and parasitism ($p < 0.01$). Only 1 out of 10 dogs in the infected-asymptomatic group had parasites in the liver, while 8 out of 9 infected-symptomatic animals had parasites. It is interesting to note that granulomas in infected-symptomatic animals were found to be permissive to parasites, while in the infected-asymptomatic group, only one dog had parasites. Moreover, dogs with parasites in the liver presented more splenic architectural disturbance than dogs without parasites in the liver. **Main Conclusions:** These

findings suggest that the evaluation of liver biopsies may provide important information on the evolution of CVL and indicate that the functional analysis of granulomas is required to provide relevant information regarding the factors related to the mechanisms involved in parasite survival. **E-mail:** isadora@aluno.bahia.fiocruz.br

Leish023- Occurrence of infection for *Leishmania* sp. in dogs from endemic areas of American tegumentary leishmaniasis in State of Pará, Brazil

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Introduction: Despite the records of dogs infected by *Leishmania* sp., the role of animals in the cycle of transmission of American Tegumentary Leishmaniasis (ATL) has not been elucidated. This study aimed to investigate the frequency of the canine infection in rural localities from Ulianópolis, Dom Eliseu and Rondon do Pará county, where so far no reported cases of human or canine visceral leishmaniasis (VL). **Material and Methods:** From May to December 2011, were investigated 224 dogs in rural areas of three counties. From dogs with lesions was collected material for the direct detection of parasites. For indirect immunofluorescence assay (IFA) were used as antigen promastigote of *Leishmania* (*Viannia*) *braziliensis*, being considered reactive sera with titers equal to or greater than 40. The DNA extraction from blood sample was performed using phenol: chloroform: isoamyl alcohol, which was subsequently used for molecular detection by the technique of Polymerase Chain Reaction (PCR). Primers S1629 and S1630 were used to amplify the mini-exon genes. **Results:** Of the 224 dogs studied, 6 (2,68%) had lesions suggestive of ATL, of which only one was positive for the direct detection of parasites. In the serologic survey, 89 (39,7%) dogs were reagent, while in molecular research, 12 (5,36%) had the DNA of *Leishmania* subgenus *Viannia*. Only 9 (4,01%) dogs were PCR and IFA positive. No DNA was found compatible with *Leishmania* (*Leishmania*) *amazonensis*. **Main Conclusions:** The frequency of dogs with reactive serum and the detection of DNA from *Leishmania* although low, this finding indicate the presence of infected dogs and the circulation of agent in that region. This result demonstrates the importance of dogs as possible reservoirs and the need for more studies to evaluate their participation in the transmission cycle of ATL in studied areas. **Financial support:** FAPESPA/CNPq **E-mail:** camila_alves1@yahoo.com.br

Leish024- *Leishmania infantum* infection in dogs in Latin America: a systematic review and meta-analysis

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Associated factors to *Leishmania infantum* infection in dogs in Latin America have been considered controversial or partially understood. We performed a systematic literature review to identify the best evidence on the subject in the available scientific information, to identify the role of each of the studied factors and gaps in existing knowledge. Systematic searches were carried out in four databases (Medline, Lilacs, Bank of Thesis of Capes and Google Scholar), and by consultations in reference lists of articles and experts. Theoretical discussions or Meta-analyzes of p-values and odds ratios (odds ratio) were used to pool the information for each variable. After evaluating the full text of 48 publications, 36 were included in the review. Thirty-two studies were cross-sectional, 2 cohort and 2 ecological. There was a predominance of the use of only one serological test for infection. Few studies have made some sort of control of confounding or adequately discussed the eligibility criteria and the refusals. Although not statistically significant, there was more chance of infections in male dogs and with more than one or two years old. The presence of chickens in the household was not an associated factor with the infection. There was more positivity, with statistically significant association, in dogs living around the homes, short-haired, with pure breed and that lived near forest areas. Other variables were analyzed by a small

number of studies, which prevented us from obtaining reliable information. No statistical evidence was obtained of the existence of publication bias; however there was great loss of information due to the primary studies did not provide data needed to obtain measures of association. The patterns identified in this review are useful for better understanding of the dynamics of infection and for the management of disease control. These should be further investigated in studies that consider and address the limitations identified. Further investigations should be prioritized in areas where there is no information and for variables that the knowledge is poor or inconsistent. **E-mail:** davidsoeiro@gmail.com

Leish025- Visceral leishmaniasis in dogs of municipality of Parnaíba, State of Piauí, Brazil, in the year of 2011

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Visceral leishmaniasis (VL) is a chronic infectious disease caused in the Americas by *Leishmania* (*Leishmania*) *infantum* *chagasi*, who's the main vector is *Lutzomyia longipalpis*, and domestic hosts are dogs, many of them are asymptomatics or oligosymptomatic. However, it has been shown that infected dogs, although asymptomatic, are a source of infection for the sandflies and consequently have an active role in the transmission of *Leishmania*. It is a serious public health problem and, if untreated, can have a fatality rate of 95%, currently identified as reemerging disease with high and growing prevalence in the Northeast, including the State of Piauí as a major endemic areas. This study aimed to determine the incidence of canine visceral leishmaniasis and the areas with most cases occurring in the city of Parnaíba-PI, in 2011. A survey of data from VL cases in dogs in 33 neighborhoods in the city, reported during the year 2011. Samples were collected from animals with suspected kala-azar in the Center for Zoonosis Control (CCZ) exams were performed in the Regional Health (FUNASA) of Parnaíba-PI, using the test method to evaluate the indirect immunofluorescence assay (IFA). 863 examinations were conducted in 33 districts of Parnaíba, with the positive results for canine leishmaniasis or VL of 79 dogs examined. The largest number of cases was recorded in the Planalto district, accounting for 21.5% of positive tests. And all positive dogs were euthanized as a control measure, as also recommended by health agencies. This study showed that the city of Parnaíba-PI, has a high incidence of canine visceral leishmaniasis, facilitating the spread of disease in human population and resulting problems in public health. This monitoring is required for bodies in the main affected areas. **E-mail:** mayarefortes@hotmail.com

Leish026- Visceral leishmaniasis: cases in the city of Codo, state of Maranhão, Brazil in 2011

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Introduction: The visceral leishmaniasis (VL) transmission in Brazil occurs due to bite of a sand fly species infected by *Leishmania chagasi*. The hepatosplenomegaly symptoms is most evident. Being considered a systemic disease, therefore, affects various organs such as liver, spleen and bone marrow. The World Health Organization considers endemic in 88 countries pointing the Africa, Asia, Europe and Latin America continents. The prevalence is 12 million people infected and an incidence of 500.000 cases / year worldwide. The average annual number of cases in Brazil is 3.500, and in some northeastern states the rate of disease incidence reached 20.4 cases per 100.000 inhabitants. In the State of Maranhão, the first known cases of VL occurred in 1982, currently, the municipalities have a high prevalence of cases, like Codo, which since 2003 notified cases, 18 confirmed in 2011. Based on these

data, was carried out a survey of the epidemiological profile of cases Codo. **Methods:** It was a descriptive and transversal study. The data on notification forms of the 18 patients with the diagnosis of LV were obtained by the Endemic Diseases Department, Municipal Health Secretariat of the Municipality of Codo. Was included data for the year 2011 and the variables were: gender, race, age, symptoms and geographical area. The municipality of Codo is located east in the State of Maranhao, Brazil, has a population of 118.038 in habitants spread over a land area of 4.361.318 km². **Results:** We recorded 18 cases of VL and no deaths. From the survey from reporting 12 (67%) were male, 13 (72%) mulatto, 9 (50%) were between 0-10 years of age, 16 (15%) patients had fever, 15 (14%) splenomegaly, 14 (13%) each had pallor, weakness and hepatomegaly, as more signs collected, and 15 (83%) were from urban areas. These data are possibly related to favorable climatic conditions (temperature and high humidity) and vegetation cover that the municipality has, therefore, is located in the central region of Maranhao. There are studies that emphasize that the LV has been considered an endemic rural, but since the year 1980 the disease has been gradual urbanization, occurring now in urban centers, so that, the most endemic areas 80% of cases occur in children under 10 years. **Conclusions:** VL mainly affects people living in areas whose homes are close to the abundant vegetation which facilitates the proliferation of vectors. Therefore, the human action in Codo district can be contributing to devastate the natural habitat of pathogens through the deforestation for construction of houses. It is worth mentioning that the factors that are contributing to the occurrence of new cases in the city, are not clear, therefore, the need for studies to investigate the movement and distribution of vectors in the municipality. Probably, factors related to population growth and migration may favor is for the accumulation of organic matter and poor sanitation, which favors the occurrence of the disease. **Keywords:** Leishmaniasis, Epidemiology, Brazil. **E-mail:** ftlarissabarros@hotmail.com

Leish027- Visceral leishmaniasis in Caxias, Maranhao, Brazil: the evolution of endemic disease in the last 8 years

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Introduction: Visceral leishmaniasis (VL) is a reportable disease, with clinical features of severe outcome, which remains a public health problem. This study aimed to evaluate the epidemiological, clinical, laboratory aspects and treatment of the visceral leishmaniasis, identifying the socio-demographic aspects of the disease, raising the specific laboratory tests used for diagnosis of VL and deaths during the study period, as well as investigating the situation of canine visceral leishmaniasis in the municipality of Caxias, Maranhao. **Material and Methods:** We analyzed the records of the Information System for Notifiable Diseases (SINAN) through the Department of Epidemiological Surveillance of the Health Department of the Municipality of Caxias, bank information DATASUS and Center for Zoonoses (CCZ) in Caxias, Maranhao, since January 2003 to May 2010 with a convenience sample. **Results:** During the period in question were observed 334 human cases of VL, while 10 cases resulted in death (mortality 2.9%). The annual distribution of cases was 77 cases in 2003, 37 in 2004, 54 in 2005, 21 in 2006, 48 in 2007, 42 in 2008, 34 in 2009 and 21 cases registered till May 2010. Most cases occurred in children aged between 1 and 4 years, 143 cases (42.8%), but other age groups were also affected. Prevalent among males with 56.7% of cases and observed that the LV in the city of Caxias is predominantly urban. Most patients had parasitological confirmation; the clinical and epidemiological criterion alone was not relevant. The pentavalent antimony was the drug of first choice in the first treatment in 95% of cases. In canine serological surveys conducted in the period 2005 to 2009, 47.3% of canine serum collected was positive. **Conclusion:** There were no deaths in the last three years, meaning low severity of disease in the city, suggesting that the symptoms and factors associated with unfavorable clinical course of patients with VL in Caxias been prevented and / or addressed at an early stage. It is noteworthy that still makes it necessary to raise awareness of the government, especially in regard to controlling the transmission. **E-mail:** marcosdavi2006@yahoo.com.br

Leish028-Epidemiological study of American cutaneous leishmaniasis in Boriticupu – MA – Brazil, from 2005 to 2010

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Introduction: Among the infectious diseases, leprosy is the leading cause of permanent disabilities, responsible for the discrimination of patients and their families in many societies. An estimated two million people have disabilities as sequelae of the disease (WHO, 2005). Appropriate diagnosis and treatment before the occurrence of nerve damage is the most effective measure to prevent sequelae. A measure related to physical disability is the score EHF (eyes, hands and feet) that determines the maximum degree of disability for each of these sites in the body and then joins the six numbers. The value can vary from 0 to 12. Recently, two other scales were developed for evaluation of activity limitation and safety awareness of patients: SALSA scale - Screening of Activity Limitation and Safety Awareness (EBENSO, 2007) and Social Participation scale (VAN BRAKEL et al., 2003; PARTICIPATION SCALE USERS, 2006). The SALSA scale, developed by an international group, in 2000-2002, it is a standardized instrument to measure the activity limitation and safety awareness in individuals with leprosy, diabetes and other peripheral neuropathies, both in developed and in development areas. The participation scale allow quantification of restrictions to participation experienced by people affected by leprosy or other stigmatizing problem. **Material and Methods:** We performed cross-sectional study with 69 patients discharged from leprosy between 2007 and 2009 in the municipality of Boriticupu-MA to characterize the situation of functional limitation, activity and social participation. Was applied simplified neurological assessment of neural function, EHF score, sociodemographic questionnaire and the SALSA and participation scales. **Results:** We found a predominant population of males in the economically active age group and occupation-related demands for physical effort, especially for the farmer. Percentage of 76.8% had no physical disabilities and 59.4% presented no limitation of activities, with no perception of risk for 69.6% of individuals who participated in the study. There was also a predominance of individuals without significant impediment to participation, represented by 72.5%. It was evident the relationship between the SALSA and participation scales with the EHF score and age. **Main conclusions:** The application of SALSA and participation scales revealed a population, mostly, without limitation of activity, without perception of risk and without significant constraint to participation. However, the small percentage that showed a change reflects the need for improvement in post-discharge care in the municipality. **E-mail:** eloisagoncalves@globo.com

Leish029- Updating of American Visceral Leishmaniasis (AVL) in São José do Rio Preto region, São Paulo state, Brasil

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AVL transmission in São José do Rio Preto region started in 2008, when three human autochthon cases were registered in Jales city. This evaluation was done with the purpose of updating the occurrence of this endemic disease in the region. The study region includes 101 cities that belong to São José do Rio Preto microregion (31 cities), Catanduva (18 cities), Votuporanga (30 cities) and Jales (23 cities). There were worked secondary data about vector distribution and positive dogs extracted from computerized data system of Superintendência de Controle de Endemias de São José do Rio Preto and the number of human cases inputted by Diretoria Regional de Saúde de São José do Rio Preto referred to the period of January 2008 to November 2011. Data indicate that 15 cities of the region (14,8%) present the vector or dog transmission or human transmission. *Lutzomyia longipalpis* vector presence occurred in 12 cities (Aspásia, Dolcinópolis, Jales, Marinópolis, Palmeira D'Oeste, Santa Fé do Sul, Santa Salete, Santana da Ponte Pensa, Três Fronteiras, Urânia, Valentim Gentil e Votuporanga), dog transmission occurred in 8 cities (Aparecida D'Oeste, Jales, Palmeira D'Oeste, Rubinéia, Santa Albertina, Santa Fé do Sul, Urânia e Votuporanga) and human transmission occurred in 4 cities (Jales, Santa Fé do Sul, Urânia, Votuporanga). In the cities of Aparecida D'Oeste, Rubinéia e Santa Albertina the presence of the vector was not noticed, although there is dog transmission occurrence. Percentage of positive dogs in the region

was 10,2%. The cities of Aspásia, Dolcinópolis, Marinópolis, Santa Salete, Santana da Ponte Pensa, Três Fronteiras e Valentim Gentil did not conduct dog inquiry. Jales microregion distinguishes itself as the most important for this endemic disease nowadays. The endemic disease also dislocates to the microregion of Votuporanga, in which there are 2 cities, one with vector presence (Valentim Gentil) and other with vector presence and dog and human transmission (Votuporanga). AVL situation in all these had become worse. Concerted actions must be done and intensified with the purpose of minimize the effects in the population. Studies to evaluate the sanitation conditions of residences, as well as focus points to a refined investigation and effective control measures must be searched. The educative component must be activated to the development of strategies of collaborative actions and communication actions with population so it compromises itself to participate of sand fly vigilance in the region which lives. **E-mail:** sscandar@hotmail.com

Leish030- Transmission patterns of the American Visceral Leishmaniasis in the State of São Paulo

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In the State of São Paulo (ESP) the transmission of American visceral leishmaniasis (AVL) is reported in 100 municipalities, 68 municipalities with human and canine transmission, only human in five municipalities and 27 with only canine transmission. In the late 1990s, when for the first time in the ESP (in the city of Araçatuba) the vector *Lutzomyia longipalpis* was detected in the urban areas and the first human and canine cases were reported, the AVL has shown distinct transmission patterns in the regions of São Paulo. The objective of this study was to analyze the different epidemiological profiles of the transmission of AVL in ESP in order to contribute to strategies for disease control. **Material and Methods:** The ESP was divided in Mesoregions according to the groupings of administrative health regions in the state. The data were compiled in a database containing information about epidemiological classification (under the Program for Surveillance and Control of American Visceral Leishmaniasis of the Health Department in the State of São Paulo), the year of first reporting of the vector and the first notification of canine and / or human AVL cases. **Results:** Three different patterns of the appearance of AVL cases were identified in ESP. The first one in the mesoregions of Araçatuba, Assis, Bauru, Marília, Presidente Prudente and São José do Rio Preto, where 67.0% of the municipalities were classified as transmission of canine AVL and / or human and the detection of the vector preceded the human/canine cases. The second one in the municipalities located in the regions of Campinas, Piracicaba and São Paulo Metropolitan Macro, where the detection of the vector preceded the records of autochthonous cases, but so far only canine visceral leishmaniasis (CVL) was observed. And the third one in the mesoregions of São Paulo Metropolitan Area, where were reported only LVC cases, and until the present moment, the vector *L. longipalpis* was not found, in spite of intense entomological activities conducted over the last ten years. **Conclusions:** The temporal pattern of transmission of AVL cases in ESP is characterized by the initial appearance of the vector, followed by the transmission of canine and then human transmission. This pattern is mainly related to the mesoregions located in the west of the ESP, where the transmission of AVL is older and has occurred with greater frequency than other regions. The participation of other vectors and different strains of *Leishmania chagasi*, besides the possibility of involvement of other reservoirs, are hypotheses that must be considered to understand the patterns presented in the other areas of the ESP. **E-mail:** ricardo@sucen.sp.gov.br

Leish031- Temporal analysis of the occurrence of American cutaneous leishmaniasis in Pernambuco, Brazil

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Introduction: The American cutaneous leishmaniasis (ACL) has a great relevance as a public health problem in Brazil, due to the increased incidence and prevalence of cases in populations living in poverty and greater difficulty in accessing health care services. **Objective:** This study aimed to evaluate the

temporal distribution of ACL cases reported in Pernambuco and identify areas of risk of autochthonous transmission of ACL in the field of military instruction Marshall Newton Cavalcanti (CIMNC). **Materials and Methods:** A descriptive study classified as quantitative. Were used as a database ACL cases diagnosed in the Military Hospital Area of Recife, the records of cases reported by the Information System for Notifiable Diseases in Pernambuco, for the period 1996 to 2010 and cases identified through the search active in the villages surrounding the CIMNC. The variables used were the years of occurrence and location of the case of LTA, age and gender. Data analysis was performed descriptively. **Results:** According to the SINAN were reported 9165 cases of ACL in Pernambuco between 1996 and 2010. The middle register of LTA was 611 cases reported annually. The most affected age group was 20-35 years, with masculine predominance (58.9%). 49% (n = 4493) of the reported cases were reported in Zona da Mata. In the North Forest Zone were 516 reported cases. Of these, 204 cases were reported in military training after the CIMNC. The temporal distribution cases allows of record average of 16 autochthonous cases per year. The active case finding identified 35 cases in the localities surrounding the CIMNC. **Conclusion:** The ACL showed no significant increase in incidence during the study period, maintaining a homogeneous average, but as a record high number of reported cases. The persistence of cases of leishmaniasis in the state suggests the need to obtain more efficient methodological tools for controlling the transmission of disease. **Keywords:** American cutaneous leishmaniasis, spatial distribution, epidemiology. **Financial Support:** PIBIC-AF/CNPq/UPE – 2011-2012 **E-mail:** capsandra@uol.com.br

Leish032- Main causes of case disposal of visceral leishmaniasis epidemiological investigation in a reference unit in Mato Grosso do Sul

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Introduction: Visceral leishmaniasis (VL) is a chronic and systemic disease, characterized by fever of long duration, malaise, weight loss, hepatomegaly and splenomegaly, asthenia, among other events. The suspicion of VL can be confirmed through laboratory tests, when there is amastigotes marrow blood presence in parasitological examination and / or detection of antibodies in serological tests, or through clinical-epidemiological criteria, when the patient comes from an endemic region and he /she is sensitive to the specific treatment received. Differential diagnosis requires time and skill in investigative clinical reasoning as the signs and symptoms are similar in several diseases. The fact justifies the importance of studies that report the causes most commonly seen in the VL suspicion disposal. **Objective:** To describe the causes of disposal of VL suspected cases investigated by the Hospital Service of Epidemiology, in the Reference Unit in Campo Grande, Mato Grosso do Sul. **Materials and Method:** this work is a cross sectional study, carried out through analysis of VL medical records and Chips of Epidemiological Research (CER) of the Hospital Epidemiology Service, generated from visits to the hospital and to the outpatient care in 2011. **Results:** Of 142 notified VL cases, fifteen (10.6%) were discarded for other diagnoses or no clinical improvement after treatment, of whom four (26.7%) were adults with HIV. Although the suspicion of VL were justified by signs and symptoms such as fever (93.3%), splenomegaly (93.3%), hepatomegaly (86.7%), asthenia (80%), loss of body mass (60%) , pale skin and mucosa (53.3%), edema (13.3%), cough and / or diarrhea (46.7%), infectious (33.3%), hemorrhagic phenomena (20%) and jaundice (6.7%) were frequently reported, the non-positivity in serological tests and / or parasitological led to investigation and confirmation of several diseases such as megaloblastic anemia (2 cases), liver cirrhosis (2 cases), thrombocytopenic purpura secondary to HIV, dengue, infection with hepatitis C virus, lymphoma, cholestasis transinfeciosa, disseminated tuberculosis, obstructive cardia cancer, acute CMV infection and mycobacteriosis, leading a patient to death. Only one case investigated with the diagnosis remained undetermined. **Conclusion:** the report of other diseases presenting signs and symptoms similar to those of the LV can collaborate with clinical reasoning and accelerating diagnosis, bringing benefits to the patient who receives special treatment more quickly or ceases to receive unnecessary empirical treatment. **E-mail:** joslaine.nunes @ bol.com.br

Leish033- Laboratory and Clinical Aspects of Visceral Leishmaniasis in the city of Caxias – MA

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Visceral Leishmaniasis (VL) is an anthroponosis caused by a protozoan Trypanosomatidae family, genus and subgenus *Leishmania* and specie chagasi-L. (L.) chagasi. VL is endemic in 88 countries; with 90% of cases occur in Brazil, especially in the Northeast. In VL, the age group below 10 years, especially the smallest of five years, is the most frequently affected, infection by L. (L) chagasi is characterized by a fever, pallor, weight loss, increased abdominal size, hepatosplenomegaly and edema. It was also observed other clinical manifestations such as cough; diarrhea, jaundice and bleeding that hinder the differential diagnosis with other diseases, delaying the identification, which may cause the patient to death. The diagnosis is made from clinical and epidemiological aspects, and laboratory tests such as complement fixation, indirect immunofluorescence, direct agglutination test, ELISA and Dot-ELISA, and molecular biology techniques such as polymerase chain reaction. The gold standard is the parasitological diagnosis by aspiration of the spleen, bone marrow, liver and lymph nodes. **Material and Methods:** A retrospective, descriptive study of diagnosed cases of visceral leishmaniasis in the municipality of Caxias, treated at Children's Hospital Dr. João Viana, in the period 2007 to 2011. The data analyzed were provided by the Department of Epidemiological Surveillance. Were analyzed age, sex, race, diagnostic criteria, clinical manifestations, and initial drug development. **Results:** Were observed that the total of 180 cases (100%), 70% were aged 9 years old, 108 cases were male (60%) and 72 cases were female (40%). As for race, 80.6% were done. Regarding diagnostic criteria, there was prevalence of clinical and epidemiological method (85.5%). Regarding clinical manifestations, 6% had bleeding, 21.6% of infections, 51.7% hepatomegaly, splenomegaly 76.7%, pallor 79.7%, 77.9% weight loss, cough and 51.1% / or diarrhea, jaundice 24%, swelling 25.7%, 86.4% and 95 asthenia, fever 5%. The initial drug was antimonial pentavalent in 87.2% of cases. 92.3% were cured. **Main Conclusions:** Visceral leishmaniasis is an endemic disease that has many complications, especially in children. Early diagnosis and disease control should be improved VL is a public health problem and must be fought in order to minimize damage and costs. **E-mail:** wilson-jardim@hotmail.com

Leish034- Evaluation of clinical epidemiological and laboratory profile of patients suspected of having leishmaniasis tegumentary American in the state of Pernambuco between 2006 and 2009.

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Introduction: The American tegumentary leishmaniasis (ATL) is an infectious non-contagious disease caused by protozoa of the genus *Leishmania*, which affects the skin, cartilage and mucous membranes. In Brazil, this disease has in the past 20 years, an increasing number of cases and geographic distribution, being found today in all Brazilian states under different epidemiological profiles. The ATL also focuses on all regions of Pernambuco State, highlighting the Zona da Mata and the Greater Metropolitan Recife. **Materials and methods:** The study was conducted with the secondary database side of the Central Laboratory of Pernambuco / LACEN from-2006-to-2009, with 283 individuals treated with a hypothetical diagnosis of ATL. The variables studied were age, sex, origin, clinical form, number of lesions, evolutionary duration of the disease, lesion location and efficiency of diagnostic methods, direct search for the parasite and Montenegro skin test (MST). **Results:** Of the 283 patients seen, 147 were positive for ATL, which shows that the incidence of the disease is increasing in Pernambuco State, mainly in the metropolitan area, with 69% of all reported cases. It was the highest proportion of males (61%), the age groups between 10-29 years (39.2%) and occupational activities focused on agriculture (37.4%). On the other hand, confirmation of diagnosis by the MST was 67% and only 20.6% had parasitological confirmation. **Conclusions:** Although ATL is an originally wild zoonosis, the study showed that the

disease is showing a profile of transmission around homes. The metropolitan area is highlighted with a higher incidence, alerting managers to the need for effective surveillance. The higher incidence in males suggests connection with work activity. The age group between 10-29 years is the most affected, which shows the disease's tendency to reach young adults. Early diagnosis improves patient care, controlling the disease and reducing serious and possible deformities. **Key words:** American tegumentary leishmaniasis; Laboratory diagnosis; Infection; Pernambuco, Brazil. **E-mail:** tania39@ig.com.br

Leish035- Cutaneous leishmaniasis outbreak American community in the rural area of east Teresina

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Background: Cutaneous leishmaniasis (CL) affects millions of people worldwide. It is a anthropozoonosis not contagious, caused by different species of protozoa of the genus *Leishmania*, which affects skin and mucous membranes. With increasing urbanization and peridomestic transmission of disease, the involvement of domestic animals in transmission has become a possibility. In the presence of typical lesions of leishmaniasis, the clinical diagnosis and epidemiological may be performed, and complemented by Montenegro test (MST) and possibly by positive therapeutic response. However, confirmation of diagnosis by parasitological methods is essential. The drugs of first choice in the treatment of leishmaniasis are the pentavalent antimonials. The criterion of clinical cure is being given regular monitoring for 12 months. **Objective:** To report an outbreak of cutaneous leishmaniasis in rural areas of the city of Teresina, an epidemiologically unusual event, featuring the profile of the affected population in order to assist programs to combat the disease. **Methods:** We used data from the FMS, and Center for Zoonoses SINAN. **Case description:** In the period 2007-2010, there were 122 cases of ACL, and only from January to September 2011 were 49 reported cases, 15 cases (30.6%) only in a rural area, with predominance the cutaneous form (87.75%) of males (55.10%) and productive age (71.43%). **Conclusion:** These data make up a disease outbreak, and further studies are required for an understanding of the transmission pattern of the same. **Keywords:** Cutaneous Leishmaniasis. Anthropozoonosis. *Leishmania* **E-mail:** paulapassos0205@hotmail.com

Leish036- Clinical and epidemiological aspects of cases of Cutaneous Leishmaniasis in Caxias - Ma 2007 to 2011

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Introduction: The American Cutaneous Leishmaniasis (ACL) is an infectious non-contagious disease caused by different species of protozoa of the genus *Leishmania* that affect skin and mucous membranes. It has been documented in several countries with an estimated worldwide prevalence of 12 million, with 400,000 new disease cases each year. Cutaneous leishmaniasis is situated between the major endemic diseases in Latin America and Brazil. This last one is included among the five countries that reports more than 90% of cases of visceral leishmaniasis and among the seven with 90% of cases of cutaneous leishmaniasis in the world. Methodology: The study was based on retrospective analysis, cross sectional, descriptive, and quantitative nature documentary of 104 cases of patients diagnosed with Cutaneous Leishmaniasis in the period 2007 to 2011, in the city of Caxias - MA. Data was provided by the Department of Epidemiological Surveillance. The following variables were analyzed: age, sex, race, area of residence, type of entry, the initial drug, clinical type, epidemiological classification, presence of skin scarring at initial evaluation, frequency of MRI, criterion of confirmation, evolution and education level. Ignored/blank were excluded from analysis. **Results:** Of 104 cases reported there were 70% of male patients. The mulattoes represented 69% of the cases, the black race were observed in 19% and the white one in 12% of the cases. Considering the age group, 53.8% belonged to the age of 20-49 years. The clinical-laboratory diagnosis was used in 89% of the cases. Considering the types of injury, 96.1% were of skin damage and only 3.9% of mucosal damage. Regarding the residence, 56.5% lived in urban areas. Most

patients (58%) were illiterate or had less than half of the Elementary school completed, while those who have completed higher education were minority (3%). In the initial evaluation were observed 3 (75%) patients with cutaneous scars similar to those left by the disease? The Montenegro skin test was performed in 35 individuals and was reactive in 32 (27.6%). The pentavalent antimony was used in all the 104 cases. Regarding the evolution, 92 cases (96.8%) evolved to cure. **Conclusion:** The epidemiological characteristics observed in Caxias-MA suggest an endemic area and the urbanization of ACL, and this process affects many cities today. **Email:** wilson-jardim@hotmail.com

Leish037- Assessment of knowledge regarding Leishmaniasis of the population of Formiga, Minas Gerais State, Brazil

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Introduction: the study of Leishmaniasis epidemiology may provide valuable tools for controlling disease according to the region of occurrence. In the micro-region of Formiga, Minas Gerais State, Brazil, several human cases of American Cutaneous Leishmaniasis (ACL) and Visceral Leishmaniasis (VL) have been reported since 2001 and 2004, respectively. In 2011, there was notification of the first autochthonous case of human VL. In this context, the performance of the population may be critical for promoting sustainable preventive practices. This fact together with preventive measures directed to the municipality is crucial for the effective control of the disease. Therefore, the objective of this study was to investigate people's knowledge on Leishmaniasis from the city of Formiga. **Materials and Methods:** The study was carried out on July 2011 using a validated quantitative questionnaire, covering many concepts on Leishmaniasis and transmission risk factors. Four neighborhoods were chosen based on the number of registered cases of canine leishmaniasis. Individuals (409) were interviewed and the population sample was established using probability sampling type stratified proportionally by gender and age. **Results:** Regarding the concepts of disease, 61.4% were unaware of what Leishmaniasis was, 39.5% of interviewed subjects did not know how it is transmitted, and 40.9% reported the dog as reservoir. About prevention, 25.4% did not know how to do it and 10.4% claimed that they should avoid water collections and, finally, 54.7% did not know what to use for treatment, while 14.3% would use antibiotics. The risk of transmission questions detected that 58.9% of the individuals owned a pet, especially dogs (68.8%). Of the 11.1% of dog owners that reported dog illness close to the survey, 22.7% were diagnosed with Leishmaniasis. Environmental factors such as presence of water course (34.7%) and green areas (46.4%), as well as factors associated with urban areas - the presence of mosquitoes (43.4%), rodents (41.3%) and vacant lots (64.8%) - closeness to home, indicate possible sandfly breeding areas and, consequently, local transmission of the disease. However, further studies are needed to point the correlation of these factors to the emergence of human cases of Leishmaniasis in the city. **Conclusions:** The population of Formiga has fragmented knowledge on the scientific content of Leishmaniasis. Since, this may hinder preventive practices, those preliminary data together with the health authorities intend to provide a social actions and policies against the disease. **Financing:** FAPEMIG, CPqRR/ FIOCRUZ, SMS Formiga, FUNEDI/ UEMG **E-mail:** julimenezes@yahoo.com.br or margonari@cpqrr.fiocruz.br

Leish038- Active search as a way of new case detection of American tegumentary leishmaniasis in Timbauba, Pernambuco, Brazil

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Introduction: American tegumentary leishmaniasis is an antropozoonose considered among the six major endemic diseases worldwide infectious, being present in 88 countries. In Brazil, this disease deserves attention, due to the risk of occurrence of deformities. Can produce psychological involvement

in humans, with reflections on the social and economic field, since, in most cases, can be considered an occupational disease. The disease presents with wide distribution with registration of cases in all Brazilian regions. In the State of Pernambuco, the territory that boasts the highest incidence of cases of the disease is the Zone of Mata and the municipality of Timbaúba located in this area is considered endemic to this disease. **Material and methods:** The purpose of this paper is to demonstrate the importance of active versus spontaneous demand for detecting new cases of American tegumentary leishmaniasis in the municipality of Timbaúba, Pernambuco, Brazil. Data were collected from reportable information system Notification between the years 2006 to 2011. **Results:** In the first period of the years 2006 to 2008, practiced in the municipality studied the identification of new cases spontaneous demand, taking an average of five new cases reported per year. From the year 2009 began a partnership between Timbaúba town hall and Leishmaniasis laboratory research center Aggeu Magalhães-FIOCRUZ. This partnership resulted in lectures and trainings for doctors and other employees of the health system, in addition to the aforementioned municipality active pursuit of new cases of leishmaniasis, be used. In the period of 2009 to 2011 were notified on average thirty new cases per year, significantly increasing the cases notified and treated in the city compared with the previous period, having an elevation in the year 2010, with an index of forty-four cases. **Conclusion:** Can admit with these data, the active search coupled with constant staff training in relation to illness referenced, makes it possible to meet new cases of disease in a population that, possibly does not spontaneously the health service. **Keywords:** Leishmaniasis; endemic; notification. **Funding bodies:** Fondation Sanofi Espoir **E-mail:** alberon.araujo@gmail.com

Leish039- Climate - and Land Use- Cover Changes. Impacts on two vector-borne endemic neglected diseases in South-western Amazonia, and needed adaptation

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Introduction: In Amazonia, forest burning is used to convert forested areas into pastures or plantations, emitting Green-House Gases, which in turn contribute to a regional decrease in humidity and increase in temperature - climatic conditions that foster forest fires, further increasing GHGs. emissions, closing the perverse circle. Unprecedented regional changes due to the ongoing implementation of hydroelectric dams, hydro-ways and paved roads are expected to have great impacts on the epidemiology of human diseases, over the next years, in South-western Amazonia. Land-Use and Cover Changes and the associated biodiversity-loss favour the disruption of natural cycles that impinge on vectors. Abundance, jeopardizing an Ecosystem Service known as Infectious Diseases Regulation (the ability of ecosystems to act as buffer zones between zoo noses and human populations) Increased migration and urbanization will affect the spread of transmission of vector borne diseases, by increasing the density of both people and vectors and the transit of people. The most striking changes in the epidemiology of vector-borne diseases already observed in South-western Amazonia, so far, are the neglected (re)emerging diseases transmitted by phlebotomine sand flies: American Cutaneous Leishmaniasis (ACL) and Bartonellosis (Carrion Disease). **Material and Methods:** A four-year research project aimed at developing early warning systems is being developed since 2011, encompassing secondary data analysis, ecoepidemiological field enquiries, vector's collection and identification, as well as risk mapping by Geographic Information System, multivariate statistics, and modeling tools. **Results:** The Detection Coefficient of ACL in South-western Amazonia is well above the level of 71 cases per 100,000 inhabitants considered very high infection-risk by the Brazilian Health Ministry: in Madre de Dios (Peru) it increased from 432 (1996) to 589 (2003); in the Bolivian municipalities along the tri-national borders it varied between 198 and 1,622, in 2004; and at the Brazilian municipality of Assis Brasil it reaches almost 20 times that level of very high infection-risk (average 1,335.7 between 2001-2010). The number of Peruvian Departments with Bartonellosis mounted from 4 (1995) to 14 (2004) and the number of cases increased tenfold from 1997 to 2005, amid the growing transmission importance of Cuzco (top touristic destination). In 2004, for the first time, 175 cases were reported in Madre de Dios, bordering Bolivia and Brazil countries that lack expertise to its diagnostic/treatment. **Main conclusions:** A better understanding of the

role played by unsound regional development policies in perpetuating the high risk of transmission for the above-depicted diseases will not only enable health professionals to anticipate and face the negative impacts of climate change on the spreading of (re)emerging vector-borne infectious diseases, but also represent a timing response to the urgent need to advance scientifically informed decision making in respect to climate change socio-economic impacts, health vulnerability and adaptation measures - concerns of the Global Climate Change Human Dimensions Community. **E-mail:** manuel.cesario@uol.com.br

Leish040- American Tegumentary Leishmaniasis (ATL) and Visceral Leishmaniasis (VL) vectors *Phlebotomus* in the Regional Health District of Sinop, Mato Grosso, in 2011

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The State of Mato Grosso records of autochthonous cases of ATL in 100% of its municipalities. The Regional Health of Sinop is one of the sixteen Regional Health of the State of Mato Grosso, is located in the middle-North and covers 14 towns: Vera, Itanhangá, União do Sul, Nova Ubiratã, Sorriso, Feliz Natal, Sinop, Lucas do Rio Verde, Santa Rita do Trivelato, Cláudia, Nova Mutum, Ipiranga do Norte, Santa Carmem and Tapurah. This study aimed to list the species of sandflies found in entomological research carried out in 14 counties included in this Health Region during the year 2011. Data were obtained from the reports of entomological researches conducted during 2011 in these towns. As a result, it has been identified the development of research in 11 (78.57%) of the Regional Municipalities, namely: Vera, Itanhangá, União do Sul, Nova Ubiratã, Sorriso, Feliz Natal, Sinop, Lucas do Rio Verde, Santa Rita do Trivelato, Cláudia and Nova Mutum. In six towns (42.86%) the capture methodology adopted was to ATL and five (35.7%) was for VL, as proposed by the Ministry of Health. In these towns there were captured 1.917 phlebotomineus, distributed in 28 species. *Lutzomyia whitmani* was the most prevalent with 786 specimens (41,0%), followed by *Lutzomyia longipalpis* with 558 (29,11%), *Lutzomyia migonei* 185 (9,66%), *Lutzomyia lenti* 113 (5,89%), *Lutzomyia carmelinoi* 79 (4,12%) and *Lutzomyia evandroi* 71 (3,70%). The 22 remaining species totaled 125 phlebotomineus (6,52%). *Lutzomyia longipalpis* and *Lutzomyia cruzi*, vector of VL were found in six towns and *Lutzomyia whitmani*, *Lutzomyia migonei*, *Lutzomyia umbratilis*, vectors of ATL were found in eight towns. In six towns, the vector species for the two conditions were found concomitantly. Of the total of phlebotomineus captured 29.16% belonged to the LV vector species and 50,91% to ATL. We stress the necessity of epidemiological and entomological monitoring in municipalities with presence of vectors of medical importance particularly in those with autochthonous human cases in order to minimize the contact man/vector and the reduction in the number of cases. **Financial Support:** State Department of Health of Mato Grosso, Foundation of Research in Mato Grosso (FAPEMAT). **E-mail:** sftthies@hotmail.com.

Clinical and Pathogenesis

Leish041- Ear, nose and throat impairment assessment in mucocutaneous leishmaniasis patients followed in the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HDV): longitudinal study

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Introduction: Early diagnosis of mucosal leishmaniasis is extremely important to establish the correct treatment and prevent the development of complications. In the Brazilian Amazon, due to his geographical characteristics, the access to specialized care is very difficult, leaving patients with mucosal leishmaniasis to progress during several years. The objective of our study is to describe the clinical and epidemiological aspects of patients with mucosal leishmaniasis followed in our institution (FMT-HVD)

Materials and methods: Data from all the patients followed in the outpatient office of the FMT-HVD from July 2011... to February 2012 was collected. The entire patients were provided with proper information and sign the informed consent. A survey was used to obtain the demographic, epidemiological and clinical aspects of the patient. Impairments were assessed by rhinoscopy. **Results:** A total of 40 were included in the study. The mean age was 51 (range between 25 at 88). Almost all cases occur in male sex 9 (22.5%). Geographical distribution of the cases shows a high proportion of patients from southern Amazon River lands. The activity mostly related with the disease was forest extractive tasks. 27 (58%) patients have cutaneous leishmaniasis episode in the past, 66.7% not adequately treated. Majority of cases affects the nasal region (92,59%) one patient have a According to our results 31(77.5%) complains from scab elimination and epistaxis 24(60%) from nasal obstruction 17 (42.5%). Results from the rhinoscopy reveals: 14 (35%) septum perforation, 13 (32.5%) ulcer lesions, 13 (32.5%) infiltrative lesions. Intradermal Montenegro test was positive in 18 from 20 patients (90%). Histopathologic exam, from 29 patients show 15 (52%) compatible results. **Conclusion:** Mucosal leishmaniasis affects mainly young men with activities related to the forest. Time from the cutaneous lesion to the development of the mucosal leishmaniasis is variable, but tends to be a many years. Complications appear in 40% of the patients. The majority of the patients live in cities with a time consuming boat trip to Manaus, which limit their access to diagnostic tool and proper treatment. **E-mail:** jguerra291@gmail.com

Leish042- Profile of Oral Health of 74 patients with the cutaneous form of American cutaneous leishmaniasis (ACL)

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Introduction: The clinical presentation of ATL and the response to the specific treatment may be influenced by several factors, including co morbidities. In this context, most affected individuals have an important decrease in the oral health and dental care, but it is not known its role in the evolution of infection. **Materials and Methods:** We evaluated the age, sex, lesion duration, oral hygiene, as well as the presence of caries, gingivitis, periodontitis and tooth mobility in 74 patients with cutaneous form of ATL followed at the Ambulatory of Leishmaniasis from VigiLeish (IPEC / FIOCRUZ). **RESULTS:** The number of skin lesions ranged from one to 53 lesions (3.01 + 6.75 lesions). Patients with ATL were mostly men (62.2%) and aged 15-78 years (41.11 +17.20 years). The duration of cutaneous lesions to the time of clinical diagnosis was 3.51 + 3.31 months. As regard the profile of oral health, the teeth average was 20.53 +10.96, but 2.5% were totally edentulous. The analysis of oral health showed that 24.3% had good hygiene, 28.4% regular, 32.4% had poor oral hygiene, and 14.4% did not perform oral hygiene. Caries activity was observed in 70.8% of the patients examined, and it was considered severe in 14.9%. Examination also demonstrated periodontal disease. Thirty-one patients had gingivitis (41.9%), considered mild in 25 (33.8%) and moderate in 6 (8.1%). Sixteen patients (21.6%) had periodontitis, considered severe in five (6.8 %.) In addition, 12 patients had tooth mobility (16.2%). **Main conclusions:** We observed a profile of impaired oral health and dental care among patients presenting the cutaneous form of ATL. We are now verifying the relationship between these findings and the evolution of infection and response to treatment in order to verify the necessity to indicate an associated dental treatment. However, should be noted that as poor oral and dental care is considered signal of poverty, and other factors such as nutrition, as well as other morbidities can play a role in the clinical presentation of ACL. **Support:** IPEC-FIOCRUZ, CNPq and FAPERJ, Brazil. AOS is recipient of fellowships from CNPq and FAPERJ. **E-mail:** mariana.palmeiro@ ipec.fiocruz.br

Leish043- Extensive mucocutaneous lesions in co-infection HIV-*Leishmania*: report of two cases

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Background and Objectives: Leishmaniasis is a chronic zoonotic disease that affects the skin and mucous membranes of the nose, mouth, pharynx and larynx. It is caused by a protozoon of the genus *Leishmania* and transmitted by phlebotomine insects. This is a report of 2 HIV-*Leishmania* co-infection cases with unusual extensive leishmaniasis lesions. **Materials and Methods:** Patient 1 – A 46-year-old man from countryside of Mato Grosso state, presented in 2011 reporting a history of treatment for oral mucosal leishmaniasis 2 years before, despite no history of cutaneous leishmaniasis. It was a successful treatment and his lesions healed; he could not mention which drug was used. One month before he initiated nasal pruritus, associated with signs of inflammation in face and rhinorrhea, yellow and fetid. On examination there was conjunctival hyperemia, edema and erythema in the malar region, besides yellowish and bleeding crusts throughout the nasal mucosal. Smear collected by nasal mucosal was *Leishmania* positive and Montenegro intradermorreaction resulted negative. Anti-HIV serology resulted positive. First treatment was Pentamidine (10 doses) but without response. Prescribed Amphotericin B (1.1 g cumulative dose), with improvement but not clinical cure. CD4 = 135 cells/mm³ - 9%; viral load: 124.458 copies. Patient in irregular follow up. Patient 2 – A 63-years-old man, presented in 2011 with a history of cutaneous leishmaniasis healed without treatment but after a long period. He has had nasal blockage and crusts release for 16 years, and deterioration in the last year. Biopsy was performed at that time, without diagnosis. Montenegro intradermorreaction resulted positive (9mm). Then, he was treated with pentavalent antimony and there was clinical improvement. Presented recurrence in 6 months associated with involvement of face, this time with direct examination positive for *Leishmania*. Prescribed second series of antimony, now unresponsive. He was referred to our clinic for evaluation. Physical examination revealed inflammatory signs (erythema, warmth and swelling) in malar and nasal pyramid, and nasal septum perforation, infiltration and presence of large amounts of yellowish discharge adherent to the mucosa. HIV serology resulted positive. He was treated with Anfotericine B (cumulative dose of 1 g) with good clinical response. Waiting for CV and CD4 results. **Results and Conclusion:** The diagnosis of *Leishmania*-HIV co-infection can change the choice for leishmaniasis treatment; recommend more frequent evaluation of adverse events and longer follow-up time. Co-infection can be related with irregular response to therapy and progress to recurrences. In the above cases, the clinical expression of leishmaniasis was exacerbated with concomitant cellulitis of the face, rarely observed in regular conditions. Under these conditions, the diagnosis of HIV infection should be suspected to optimize the overall suitable treatment. **E-mail:** tatianafortes@hotmail.com

Leish044- Exacerbated lesions in pregnant patients with American tegumentary leishmaniasis coincide with transient immune alteration

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: Although around 36% of the American tegumentary leishmaniasis (ATL) cases in Brazil affect women in the reproductive age group, our knowledge of the impact of infection on both mothers and children **Introduction** is very limited; there are no epidemiologic data on the incidence of disease in pregnant women or follow-ups on the development of their children. Exacerbation of cutaneous lesions in pregnant women has been reported, but the reasons remain unclear. Our objective was to identify potential mechanisms contributing to the worsening of ATL in pregnant patients and follow their evolution post-

partum. **Material and Methods:** We analyzed local (in situ immunohistochemistry) and systemic (IFN-gamma and IL-10 by Elispot) immune responses of two patients during pregnancy and after delivery. In addition, 5 non pregnant women with cutaneous ATL and 10 healthy age-matched women volunteers were enrolled as controls. ATL was confirmed by parasite detection. **Results:** When compared with ATL controls, our results demonstrate for the first time decreased levels of IFN-gamma and NOS and enhanced levels of IL-10 in exacerbated cutaneous lesions of pregnant ATL patients. Reduced frequencies of antigen-specific IFN-gamma secreting cells and enhanced frequencies of IL-10 producing cells were detected in the peripheral blood of pregnant ATL patients. Thus, the results obtained in lesions and in peripheral blood indicate changes in both local and systemic maternal immune responses. After delivery, the healing of lesions correlated with increased in situ expression of NOS2 and increased systemic and in situ IFN-gamma but decreased IL-10 expression indicating a rapidly recovering Th1 response post-partum associated with parasite control and healing of the lesions. **Main conclusions:** Our results infer that the transient modulation of maternal immune responses during pregnancy favours parasite growth and exacerbation of lesions whereas normalization of maternal immunity post-partum initiates healing, reinforcing the importance of immune regulation and the balance the cytokine network in the control of ATL. **Funding by** IOC and IPEC-FIOCRUZ and FAPERJ, Brazil and an International Joint Project Grant (2008/R3) from The Royal Society, UK. **E-mail:** fconcei@ioc.fiocruz.br

Leish045- Health Profile of 19 patients with Oral Mucosal Leishmaniasis

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Introduction: The Mucosal leishmaniasis (ML) affects the mucosal tissues of the upper digestive airways (nose, mouth, pharynx and / or larynx). Concomitantly to the ML, most patients have impaired oral health and dental care, but its influence in the clinical presentation and response to the treatment is not known. **Material and Methods:** We evaluated the age, sex, lesion duration, oral hygiene, as well as the presence of caries, gingivitis, periodontitis and tooth mobility in 19 patients with ML attended at VigiLeish (IPEC / FIOCRUZ). **Results:** We identified ML lesions in various anatomical sites of the nasal, oral, pharyngeal and / or larynx mucosa. The duration of mucosal lesions until diagnosis was 15.08 + 15.34 months. Patients were mostly men (89.5%) aged 35-75 years (54.47 +13.72). As regard the profile of oral health, the teeth average was 16.37 +9.55, but two patients were totally edentulous (10.5%). Eight patients (68.4%) used dentures. The analysis of oral health showed that 26.3% had good oral hygiene, but 31.6% presented regular, and 42.1% had poor oral hygiene. Caries activity was observed in 73.7% of the patients examined, and it was considered severe in 15.8%. Examination also demonstrated periodontal disease (gingivitis or periodontitis) in 63.2% of the cases. Six patients had gingivitis (31.6%), and six patients had periodontitis (31.6%), classified as severe in 3 (15.8%). In addition, 6 patients had tooth mobility (31.6%). **Main conclusions:** We observed a significant association between poor oral health and dental care and LM lesions. However, it was not possible to identify a direct correlation between this finding and the severity of clinical presentations and therapeutic response. Additional studies are being conducted to ascertain the importance of this in order to verify the need for dental treatment indication associated **Support:** IPEC-FIOCRUZ, CNPq and FAPERJ, Brazil. AOS is recipient of fellowships from CNPq and FAPERJ. **E-mail:** mariana.palmeiro@ ipec.fiocruz.br

Leish046- In situ immune response in American Tegumentary Leishmaniasis skin scars and its relationship to Leishmania antigens

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Background: Parasite persistence in leishmaniasis is considered a factor responsible for either protection from reinfection or lesion reactivation. However, the immunological mechanisms involved are not fully understood. **Methods:** Forty scars of ATL patients were examined to detect *Leishmania* sp. amastigote forms and antigens, as well as the expression of IFN- γ , IL-10, and FoxP3 by immunohistochemistry. **Results:** In 3 samples, the presence of *Leishmania* sp. antigens was correlated with amastigotes localization. This was confirmed by DAPI staining and TEM analysis. IFN- γ , IL-10, and FoxP3 were observed in all scars, independent of the time from healing. A positive correlation between FoxP3 and IL-10 was verified, and scars that presented amastigote parasites displayed greater FoxP3 and IL-10 expression. **Main conclusion:** A constant interaction exists between parasites and immune response in the scars of ATL patients. Detection of amastigote forms in a 3-year-old scar suggests that parasite maintenance during the infection is dynamic, with continuous release of parasite antigens even after signs of clinical cure. This could maintain IFN- γ expression and the stimulus of the inflammatory response, suggesting that parasite persistence could be related to the immunoregulation suggested by the FoxP3/IL-10 expression. This process may also play a role in protection against reinfection. **Key words:** ATL, scars, in situ inflammatory reaction, FoxP3, IL-10, IFN- γ . **Supported by:** IOC-Fiocruz [POM 2010-2011]; IPEC-Fiocruz [PA2010]; PAPES 5 [40.3474/2008-6, and 403477/2008-5]; CNPq [470886/2003-0]; and FAPERJ [E26.111.593/2008], Brazil. **E-mail:** fconcei@ioc.fiocruz.br

Leish047- Interleukin 6 and transforming growth factor beta expression in patients with active American tegumentary leishmaniasis

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Introduction: It is known that *Leishmania* infections activate an immune response, mainly characterized by the expansion of CD4⁺ T lymphocytes, and cytokines produced by Th1 and Th2 profiles may contribute to disease healing or progression. Furthermore, the influence of Th17 profile on the outcome of the disease is suggested. Once interleukin (IL-) 6 and transforming growth factor beta (TGF- β) are necessary for the development of this cell subtype, the aim of this study was to characterize the expression of these mediators in peripheral blood mononuclear cells (PBMC) from active American tegumentary leishmaniasis (ATL). **Material and Methods:** PBMC from eight patients and five healthy individuals (control group) were incubated during 24 hours in the presence of concanavalin A (2,5 μ g/ml) or phytohemagglutinin (5,0 μ g/ml) and *L. (V.) braziliensis* soluble (AgSol, 1,25 μ g/ml) or insoluble (AgIns, 2,5 μ g/ml) antigens. IL-6 and TGF- β expression was evaluated through real time quantitative PCR, with the results obtained by the comparative Ct method. Data were considered significant when $p < 0,05$ (Wilcoxon and Mann-Whitney tests). **Results:** Under mitogenic stimuli, IL-6 and TGF- β were similarly expressed in ATL patients and control group. In response to AgSol, IL-6 was expressed 4,73-fold higher in patients (Δ Ct = 12,57 \pm 1,59) in relation to healthy individuals (Δ Ct = 14,82 \pm 0,4; $p = 0,01$). The cytokine was 2,61-fold more expressed in patients (Δ Ct = 11,70 \pm 1,91) under AgIns stimulation comparing to control group (Δ Ct = 13,8 \pm 0,37), although without statistical significance. A 0,54-fold higher IL-6 expression was observed in PBMC from patients in response to AgIns in comparison to AgSol ($p = 0,01$). TGF- β was 1,54-fold more expressed in patients stimulated by AgSol (Δ Ct = 12,66 \pm 1,21) and 1,37-fold under AgIns stimulus (Δ Ct = 12,27 \pm 1,44), even without statistical difference in relation to control group (Δ Ct AgSol = 13,18 \pm 0,22; Δ Ct AgIns = 12,73 \pm 0,25). Patients exhibited a 0,75-fold higher TGF- β expression in response to AgIns comparing to AgSol, although without statistical significance. **Main conclusion:** The expression of IL-6 and TGF- β indicate the development of cellular immune response against *L. (V.) braziliensis* soluble or insoluble antigens. The results obtained here in may contribute to better characterize the role of Th17 subtype in *Leishmania* infection. **Keywords:** Leishmaniose Cutânea, Citocinas, mRNA. **E-mail:** marinaasouza@gmail.com

Leish048- The first study of clinical features and epidemiology of American tegumentary leishmaniasis in endemic area in north of Minas Gerais, Brazil

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Introduction: The American Tegumentary Leishmaniasis (ATL) has worldwide distribution and in the Americas. In Brazil, it has been reported in all states as a common dermatologic disorder which deserves greater attention. In recent years, the number of cases and geographic range is increasing. The aim of this paper is to describe the epidemiology, clinical manifestation, treatment and outcome of ATL patients enrolled in outpatient ATL reference clinics in Montes Claros, Minas Gerais, Brazil. **Material and Methods:** Medical records of patients seen at the Center for Integrated Service Tancredo Neves (CAETAN) in the period November 2009 to December 2010 were analyzed. The data were compiled at Microsoft Excel 2010 program. **Results:** ATL was confirmed in 86 patients, 77 in the cutaneous form and 9 in the mucosa form. The diagnosis was obtained in 23 (26.7%) patients through the Montenegro test (MST), 29 (33.7%) by biopsy and in 34 (39.53%) by MST and biopsy. The mean and median age of patients was 39 and 37.5 years, respectively. Geographically, 59.3% patients were from Montes Claros, the remaining were from nearby cities. The disease has prevailed in males (64%), from urban areas (72%), students (22%), and agricultural workers (18.6%). As for the previous use of medication for the lesion, 51 (59.3%) reported doing so: 37 (43%) had used antibiotics (including azithromycin, used by four patients) 10 (11.6%) had used various topical medications and 6 (7%) were treated with antimony-specific N-methylglucamine. The mean and median of time of injury until undergoing specialized care were 11.2 months and 3 months, respectively. The predominant lesion sites were lower extremities (46.3%) and upper extremities (33%). Regarding treatment, 24 (36.4%) patients were treated in another city or admitted for hospital treatment; of 62 (72%) followed at CAETAN, N-methylglucamine antimoniate was used in 56 (90.3%), Amphotericin B was used in 4 (6.4%), and azithromycin was used in 2 (3.2%). Three months post treatment, 16 patients (25.8%) achieved a criterion of cure at 3 months, 30 patients (48.38%) showed signs of clinical improvement and 16 of them (25.8%) lost follow-up. **Main Conclusions:** the results represent a sample of patients with ATL in this region. Since ATL is a public health problem, it is important to record and follow up cases of ATL in order to control the disease. **E-mail:** filan.moc@ig.com.br

Leish049- Aspects involved in the production of intracellular nitric oxide in patients with cutaneous Leishmaniasis of the Indigenous Reserve Xakriabá, Minas Gerais, Brazil

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Infections caused by *Leishmania (Viannia) braziliensis* presents many clinical manifestations and cutaneous Leishmaniasis (CL) is the most common clinical form. In CL, an important mechanism in the induction of NO production, involves signaling by receptor of low affinity for IgE (FcεRII/CD23), in a microenvironment of cytokines type 1. Seric levels of IgE and the expression of FcεRII/CD23 are increased in CL and the human monocytes express this receptor after activation with IFN-γ or IL-4. In indigenous reserve Xakriabá, located in northern Minas Gerais, autochthonous cases of LC has been reported since 2001 and the absence of immunological data about this disease in the community, reinforces the need to investigate aspects about the host immune responses. The objective of this study was to evaluate by flow cytometry, the profile of intracellular NO production, cytokines IL-4 and IFN-γ and the expression of CD23. For this, cultures of leukocytes of the peripheral blood of clinical group - LT (n=18), asymptomatic - AS (n=9) and control - CT (n=9) were evaluated for characterization of monocytes and T lymphocytes phenotypic and functional profile in the absence or presence of *L. (V.) braziliensis*. The obtained results showed that AS group had a higher percentage of monocytes NO producers in the

non-stimulated culture in relation of groups LT and CT, is possible to relate the absence of lesion in these individuals with the highest NO production expressed naturally by this group. Moreover, after stimulation AS group showed a more equilibrated, when compared to LT group that presented a significant increase of NO production after being stimulated. In AS group, was possible observe a higher production of IFN- γ (cytokine type 1) and IL-4 (cytokine type 2) showing a more balanced immune response profile. On the other hand LT group presented, in relation to group CT, a more inflammatory profile with statistically significant increase in expression of the activation marker CD23. Thus, we conclude that higher rates of NO production in the AS group was related to the best capacity to combat the parasite through the immune system and the pro-inflammatory profile observed in the LT is related to the maintenance of skin lesions. It is know that for the characterization of this immune response and other groups, is necessary to evaluate other aspects. Therefore, the quantification of seric levels of IgE is being processed, in order to be possible to analyze the data of jointly. **E-mail:** raquelcarvalho@cpqrr.fiocruz.br

Leish050- Apoptosis of CD8⁺ effector lymphocytes associated with the active phase of cutaneous leishmaniasis

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Introduction: In cutaneous leishmaniasis (CL), the interaction between the immune response and the parasite is crucial for the clinical manifestations of this disease. Results from our group have demonstrated the key role of CD8⁺ T cells and apoptosis of these as modulators of adaptive immune response during disease. Knowing that the lymphocyte population undergoes differentiation under antigenic stimulation, it becomes crucial an evaluation of naïve, effector and memory subsets. Furthermore, these functionally distinct cell categories may be subject to death by apoptosis differently, due to an antigenic stimulus. **Objective:** The goal of this research was to evaluate the frequency of CD8⁺ effector T cells, those in apoptosis and the influence of *Leishmania braziliensis* antigens in the induction of this subpopulation during and after CL. **Methods:** Peripheral blood mononuclear cells were obtained from CL patients, with active disease, during the treatment (PDT); of patients cured after the treatment (PAT); and healthy controls (HC). These cells were evaluated, by flow cytometry, ex vivo and in vitro, after 5 days of stimulation with antigens of *L. braziliensis*. The cytofluorimetric protocol included: 7-AAD and monoclonal antibodies anti-CD8, anti-CD27 and anti-CD45RA. **Results and Discussion:** Ex vivo experiments showed that the PDT have a higher percentage of effector CD8⁺ T lymphocytes compared to HC and to PPT. However it was also observed that a higher percentage subpopulation of cells undergoing apoptosis in these patients. In addition, the antigens of *L. braziliensis* induced an increase in frequency of these effector cells in both groups of patients as well as apoptosis of these cells, thus demonstrating an antigenic specificity. **Conclusion:** The impaired viability of the subpopulation of CD8⁺ effector T cells may reflect a down regulation of the role of their functional activity and may contribute to the persistence of the lesion. Thus, despite a lower percentage of CD8⁺ effector T lymphocytes from PAT, the highest percentage of viable cells may be associated with infection control and healing of the lesions in these individuals. **E-mail:** raquelferraz@ioc.fiocruz.br

Leish051- Comparative study of the in situ immune response in oral and nasal mucosal leishmaniasis

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Introduction: Mucosal Leishmaniasis (ML) may occur in both nasal and oral mucosa. However, despite the impressive tissue destruction, little is known about the oral involvement. **Material and Methods:** In order to compare some changes underlying inflammation in oral and nasal ML, we performed immunohistochemistry on mucosal tissue of 20 ML patients (nasal [n=12]; oral [n=8] lesions) and 20 healthy donors using antibodies that recognize inflammatory markers (CD3, CD4, CD8, CD22, CD68, neutrophil elastase, CD1a, CLA, Ki67, Bcl-2, NOS2, CD62E, Fas, and FasL). **Results:** A significantly larger number of cells, mainly T cells and macrophages, were observed in lesions than in healthy tissue. In addition, high NOS2 expression was associated with a reduced number of parasites, highlighting the importance of NOS2 for parasite elimination. Oral lesions had higher numbers of neutrophils, parasites, proliferating cells, and NOS2 than nasal lesions. **Main conclusions:** These findings, together with the shorter duration of oral lesions and more intense symptoms, suggest a more recent inflammatory process. It could be explained by lesion-induced oral cavity changes that lead to eating difficulties and social stigma. In addition, the frequent poor tooth conservation and gingival inflammation tend to amplify tissue destruction and symptoms and may impair and confuse the correct diagnosis, thus delaying the onset of specific treatment. **Keywords:** American tegumentary leishmaniasis; mucosal leishmaniasis; oral lesions; inflammation; immunohistochemistry; Leishmania **Support:** This work was funded by IOC and IPEC-FIOCRUZ, PAPES 5, FUNASA/MS, CNPq and FAPERJ, Brazil. M.R.P. is a fellow from CNPq. We thank to Rodrigo Mexas for the final artwork. A.O.S is recipient of fellowships from CNPq and FAPERJ. **E-mail:** fconcei@ioc.fiocruz.br

Leish052- Evaluation of the Frequency of Apoptosis and CD8⁺ lymphocytes and Natural Killer Cells in Patients of Cutaneous Leishmaniasis

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Introduction: The clinical course of cutaneous leishmaniasis (CL) depends on the interaction between the parasite and the immune response of patients. Is well known that CD8⁺ T lymphocytes play a key role in this response due to their cytotoxic potential and cytokine production. Because the natural killer cells (NK) present similar functions, becomes necessary a more detailed study of this population in Leishmaniasis. Results from our group demonstrated a marked occurrence of apoptosis in CD8 + T lymphocytes in patients LC leads to an imbalance in the immune response, leading to disease progression. Thus, a greater knowledge of the phenomena involved in this immune response is crucial to establish immunological parameters associated with the healing, progression and protective response to LC. **Material and Methods:** Peripheral blood mononuclear cells were obtained from patients with active disease LC during treatment and healthy individuals (control group). These cells were subjected to an *in vitro* experimental assay, which were cultured in the presence and absence of antigenic stimulation *L. braziliensis*; and another *ex vivo* assay. The assessment of these tests was performed using a cytofluorimetric protocol of phenotyping and apoptosis. **Results:** In *ex vivo* experiments we observed which patients with active disease had a lower frequency of CD8⁺ T cells and NK cells when compared to controls. At the same time, the percentage of these two cell populations undergoing apoptosis was higher in patients with active disease compared with controls. Regarding the *in vitro* assays, no statistically significant difference was noted in the distribution of CD8⁺ T cells and NK cells between patients and controls. Under the same conditions, the increase was evident in the percentage of CD8⁺ T lymphocytes in apoptosis of patients compared to controls. Also, the percentage of NK cells in apoptosis was more pronounced in both groups when compared to CD8 + T lymphocytes. **Main Conclusions:** Apoptosis seems to be responsible for a smaller amount of CD8⁺ T lymphocytes in patients with active LC, while this illness does not seem modify the distribution of NK cells, but viability of these cells is impaired. The *L. braziliensis* antigens seem to induce apoptosis in NK cells in both groups, which may associated with the fact that these cells do not have antigen specificity and are being activated on contact with these antigens. The CD8⁺ T lymphocytes of patients with active disease seem to be able to recognize the

antigens of *L. braziliensis*, probably because they have been previously primed *in vivo*. **E-mail:** clarissafc@ioc.fiocruz.br

Leish053- Clinical and immunopathological parameters of golden hamster (*Mesocricetus auratus*) in experimental *Leishmania (Viannia) braziliensis* infection

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Introduction: American tegumentary leishmaniasis (ATL) is widely spread in Brazil, and represents a major public health problem. In this context, *Leishmania (Viannia) braziliensis* stands out as the most prevalent species. Studies in experimental model are of paramount importance to evaluate immunopathogenesis and efficacy of vaccine candidates and new drugs. Recently we demonstrated that golden hamster (*Mesocricetus auratus*) is a suitable experimental model for *Leishmania (Viannia) braziliensis* infection. This model develops chronic lesions resembling humans in both clinical and histopathological aspects. This study aimed to determine the optimum inoculum to use in experimental trials and establish clinical and immunological parameters in hamsters infected with *L. braziliensis*.

Materials and Methods: We used 30 hamsters in two experiments, divided in 4 groups (n=5), intradermal infected with different inocula (10^4 , 10^5 e 10^6 promastigotes in stationary phase of *L. braziliensis*). Parameters such as lesions development, measured weekly with a thickness gauge; parasite load, measured by limiting dilution assay; lymphocyte proliferative response (LPR), measured by MTT reduction, anti-*Leishmania* antibodies levels, by ELISA assay and histopathology, were evaluated.

Results: Our results showed significant differences in lesions development (10^4 versus 10^5 , $p<0.01$; 10^4 versus 10^6 , $p<0.001$; 10^5 versus 10^6 , $p<0.05$; $n=30$), which median of the last lesions measurement in 10^4 , 10^5 and 10^6 groups were, respectively, 0.18, 1.33 and 2.57. Positive correlation was observed between: parasite load and lesion development ($r=0.82$, $p<0.001$; $n=30$); LPR and lesion development ($r=0.59$, $p<0.05$, $n=15$); IgG serum levels and lesion development ($r=0.71$, $p<0.01$, $n=15$); parasite load and LPR ($r=0.79$, $p<0.001$; $n=15$); IgG serum levels and LPR ($r=0.69$, $p<0.05$; $n=15$). At histopathological level, the main differences between different inocula was the absence of inflammatory infiltrate in dermis of 10^4 *Leishmania* inoculated animals, compared with 10^5 and 10^6 inoculated animals, which exhibited extensive areas with granulomatous reaction; Schaumann's Bodies were seen more frequently in 10^6 *Leishmania* infected animals compared with 10^5 group; 10^4 infected animals showed no Schaumann's Bodies, since they are associated with inflammatory reaction and presence of *Leishmania*. **Conclusions:** Our data indicate that infection with 10^5 promastigotes is the most appropriate inoculum for experimental trials. The correlation between clinical or parasitological status and immunological assays enable the use of lymphocyte and IgG assays to follow up the *Leishmania* infection in the vaccine and therapy studies. **Financial support:** FAPERJ, CAPES, CNPq. **E-mail:** alda@ioc.fiocruz.br

Leish054- Cutaneous-mucous leishmaniasis with important sequelae after treatment: a case report

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Introduction: It is estimated that 3-5% of patients with cutaneous leishmaniasis develop mucosal damage. Mucocutaneous leishmaniasis (ACL) is usually expressed by destructive lesions located in the mucosa of the upper airways. On examination of the mucosa erythema, infiltration and ulceration can be observed. Therapeutic response can be difficult to achieve, requiring higher doses of drugs. **Material and Methods:** The medical records of a patient treated in an outpatient clinic were reviewed. The data obtained were compared with the typical clinical presentation of the disease, making it clear that it was an unusual manifestation of the disease. **Results:** A 44-year-old man, residing in a rural area in Montes Claros, Minas Gerais, Brazil presented with mucocutaneous leishmaniasis. He had a 2-year history of lesions in the lips and in the oral mucosa. He had been treated regularly with an antibiotic at another clinic, with partial improvement of the lesions. However, he presented with a 6 month history of

reappearance of those lesions, and lesions involving the left nostril, the malar region and the tongue. This condition was accompanied of signs of secondary infection treated with amoxicillin and clavunilate, nasal obstruction and great difficulty in feeding. Destruction of the nasal septum and the columella were observed. The patient was admitted for investigation for ACL, blastomycosis, sporotrichosis and actinomycosis. He was undergone to Montenegro Test, with nonreactive result, serology for Leishmania, with a positive result and serology for Blastomycosis, with positive serology (1:4). Biopsy of the lesions suggested ACL, confirming the diagnosis. The positive serology for blastomycosis was likely due to the cross reaction with Leishmania. The patient was admitted for treatment with Amphotericin B deoxycholate (cumulative dose of 1694mg), used to treat ACL and blastomycosis, obtaining complete resolution of lesions. With the treatment, however, the patient's mouth opening was impaired, and he lost 23 kg. He did not regain his normal weight after hospital discharge, as the healing led to subsequent stenosis and inability to eat properly. He showed mutilation scarring, atypical for an individual without comorbidities or immunosuppression. He was referred to an otorhinolaryngologist and plastic surgery. **Main Conclusions:** This case report reflects an unusual complication of mucocutaneous leishmaniasis treatment, with oral and nasal stenosis. In chronic lesions, mutilation can cause partial or total loss of the nose, lips or eyelids. There must be a close monitoring of these patients for potential discomfort, weight loss and social stigma that the injury may cause. **E-mail:** filan.moc@ig.com.br

Leish055- Kinetics of *Leishmania* dissemination from inflammatory site to draining lymph node

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Background: The mechanisms of *Leishmania* dissemination and the phagocyte populations capable of transporting the parasite and parasite molecules from the infection site to different tissues are still controversial. **Objectives:** In this work we use an experimental model of cell migration *in vivo* to study the dissemination of *Leishmania* from inoculation site to the draining lymph nodes. **Methodology:** *L. amazonensis* promastigotes were injected into the peritoneal cavity of Balb/c mice and cell cultures were done of cells collected from peritoneal cavity, lymph nodes, spleen and lungs, at 15min and 30min; 1h, 2h, 4h, 6h, 12h and 24h. Afterwards, we tested different cell labeling reagents (CFSE, biotin and GFP) in order to choose those that allows better discrimination of intact parasites (potentially alive) from degraded parasites (parasite molecules). In these experiments parasites were injected intraperitoneally and the draining lymph nodes were collected for analysis using a fluorescence microscopy. **Results:** Peritoneal cells and lymph nodes cultures were positive 15min to 6h after infection. Parasites were detected in splenic cells cultures 15min to 1h after infection. Lungs cultures were positive after 1h of infection or, less consistently, 15min and 30min after inoculation. Parasites were viewed in the draining lymph nodes 1h after infection inside of phagocyte cells and even outside of phagocytes in their promastigotes form. **Conclusions:** *Leishmania* dissemination from peritoneal cavity to the lymph node occurs in less than 30 minutes after injection. The transit to bloodstream occurs in the first hour of infection. Parasite transport from peritoneal cavity to the spleen may use an alternative pathway not involving thoracic duct. **Keywords:** *Leishmania*, lymph node, migration. **E-mail:** mhermida@aluno.bahia.fiocruz.br

Leish056- Cytokines and their involvement in the pathogenesis of glomerulonephritis in experimental visceral leishmaniasis

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Introduction: Glomerulonephritis (GN) occurs very frequently however the pathogenesis is not clear. In previous studies, we have observed proliferative GN in dogs with VL (Costa et al. *Vet Pathol.*, 40:677, 2003) and T cell infiltrate (Costa et al., *Braz J Med Biol Res* 33: 1455, 2000). To extend the study, we

characterized BALB/c mice infected with *Leishmania (Leishmania) chagasi* as good model to study GN in VL (Prianti et al., *Braz J Med Biol Res.* 40:819, 2007). Since it is known that cytokines are important mediators of inflammation, and that play important role in the pathogenesis in glomerulonephritis, we focused on the participation of these factors in glomerulonephritis in visceral leishmaniasis. **Aim:** the present work aims to analyze the participation of CD4+, IL-4, TGF- β and MCP-1 in kidney of *Leishmania (L.) chagasi*-infected mice. **Methods:** BALB/c mice were infected through intraperitoneal route with 2x10⁷ purified *Leishmania (L.) chagasi* (MHOM/BR/72/strain 46) amastigotes. In different time periods, we evaluated by immunohistochemistry and quantified by morphometry the expression of CD4+ T cells, and analyze the expression of IL-4, TGF- β and MCP-1 in renal cells using Real-Time PCR. The level of these cytokines was also investigated in renal tissue using capture ELISA. **Results:** CD4+ T cells were present in glomeruli in greater amount at 7 days post-infection (PI) that decreased afterwards. The analysis showed higher expression level of IL-4, MCP-1 in 7 days PI, with subsequent decrease. It was observed a high expression of TGF- β in animals with 15 days post infection, than the non-infected control group. **Conclusion:** The data suggest important participation of CD4+ T cells in GN in murine VL, and show the contribution of TGF- β , IL-4 and MCP-1 to the development and/or modulation of the inflammatory process. **Supported by:** CNPq, FAPESP, FINEP and LIM-38 (HC-FMUSP) **E-mail:** mgprianti@gmail.com

Leish057- Contribution to the historical acknowledgement of Cutaneous Leishmaniasis in Brazil in the 1821-1850 years

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Introduction: Only in 1884, there was the first reference of the Cutaneous Leishmaniasis (CL) in the Brazilian southeast, held in Italy, through Breda, that described a illness on the 18 Italians coming from São Paulo. In 1885 doctor Alexandre Cerqueira observed the injuries of CL in Bahia, however he did not, at first, related it with the button Biskra or orient button, name of the CL in Asian countries. Only after the observations of the Baiano doctor Juliano Moreira, presented under the title “Is there in Bahia the button of Biskra? Clinical study”, published on the Medical Gazeta of Bahia on February of the next year, this correlation was done. Only in 1909, Adolfo Lindenberg reported the discovery of the parasite of this illness in workers from deforestation areas for the construction of highways in the interior of São Paulo, resembling it to the *Leishmania tropica*, causal agent of the orient button; however, it was Doctor Gaspar Vianna, in 1911, which indentified the parasite as *Leishmania brasiliensis* (nowadays *L. braziliensis*). In the year of 1925, it was published by Eduardo Rabello, the work: Contributions to the study of Cutaneous Leishmaniasis in Brazil. On this study, Rabello considered the occurrence of this illness in central and south regions of the country still in the XIX century, designated by regional expressions as button of Bahia, buba, or vague denominations as *ferida brava* and among others. The work objective demonstrate the existence of CL in the Brazilian southeast while the implantation of the *complexo of cafeeiro* expansion in Vassouras. **Material and Methods:** The analysis of 222 inventories *post-mortem* of the comarca of Vassouras, saved and available to the research through the Historical Documentation Center allowed the making of a small database about the heritage of the inventoried, including the health of their slaves. **Results:** Out of the 8.055 inventoried slaves, we find 808 records of illness and mutilations, in other words, 10,03%. Among these, 68 showed skin disorders, nearly 8,4% of the cases. And among the last ones, we consider 14 cases as possible occurrence of Leishmaniasis, pointed as: old Chagas, chronic Chagas, cureless Chagas, chronic wound and chronic ulcer. We even checked more 54 cases that show disorders in the skin. The analysis of the data allowed us to find some cases as the one of the slaves Manoel Comprino and Romão, the first of Banguela and the second of Cabinda, both as property of Luiza Ignacia da Conceição, and described on a first inventory in 1841 with the presence of chronic Chagas. The executrix was José d’Azevedo Ramos. In the year of 1844 it was opened a new inventory, whose executrix was Claudio Gomes Ribeiro d’Avelar. On it, new record to relate the presence of Chagas on these same slaves, now described as chronic wounds. This perennial nature of the wound is a strong evidence of leishmaniasis. **Main conclusions:** By the several arguments presented, it looks like viable to us the existence of CL in Rio de Janeiro in the beginning of the XIX century, being against several works

that suggest the arrival of the CL in Rio de Janeiro Just at the end of the XIX century or beginning of XX century. **E-mail:** gfurusawa@gmail.com

Leish058- Clinical manifestations of visceral leishmaniasis in reference unit of Mato Grosso do Sul

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Introduction: Visceral leishmaniasis (VL) manifests itself in the asymptomatic form, diagnosed in serological surveys, in the oligosymptomatic form, which shows few signs and symptoms of the disease, and in classical form, in which the clinical manifestations are exacerbated, as hepatosplenomegaly, fever, weight loss, anorexia and asthenia. The acute period of VL is defined by fever, hepatosplenomegaly and mild anemia; state period is characterized by weight loss, daily fever, cutaneous-mucosal pallor, diarrhea, anorexia, malaise, asthenia, edema, cough, and hepatosplenomegaly, and the final period by hepatosplenomegaly and severe anemia, being observed at this stage the main complications causing death. The expansion of the VL in Mato Grosso do Sul is a recent phenomenon and early recognition of signs and symptoms is the key to reducing rates of morbidity and mortality. **Objectives:** To describe clinical signs and symptoms of VL cases treated in Reference Unit of Campo Grande, MS. **Materials and Method:** this work is a cross sectional study, carried out through analysis of VL medical records and Chips of Epidemiological Research (CER) of the Hospital Epidemiology Service, generated from visits to the hospital and to the outpatient care in 2011. Results: There were 142 reported cases of VL. After epidemiological investigation, 15 (10.6%) cases were discharged, 30 (23.6%) were confirmed by clinical and epidemiological criteria and 97 (76.4%) by laboratory criteria. Of 127 confirmed cases, 75 were men (59.1%), with ages ranging from six months to 89 years and an average of 24.8 years. Eleven (8.7%) were HIV positive, and in 14 CER (11%) this data was ignored. The clinical signs and symptoms were more frequent fever (95.3%), splenomegaly (89.8%), hepatomegaly (85.8%), asthenia (78.7%), loss of body mass (70.1%), pallor (59.8%), cough and / or diarrhea (54.3%), presence of infection (25.2%) and edema (21.3%). Hemorrhagic phenomena were also reported (7.9%), jaundice (6.3%), nausea and / or emesis (5.5%) and pancytopenia (3.1%). **Conclusion:** The data reveal that the majority of patients had the classic form of VL, showing fever and hepatosplenomegaly, being these signals detected when the patient is subjected to a good clinical examination. Understanding the signs and symptoms avoids delays and errors in diagnosis and complications of the disease. It is noteworthy that all patients with febrile hepatosplenomegaly from endemic areas should be investigated for VL, and that the delay in instituting appropriate treatment increases the chances of morbidity and mortality. **E-mail:** joslaine.nunes@bol.com.br

Leish059- Comparative analysis of children and adults admitted to tertiary hospitals with visceral leishmaniasis (Kala-Azar) in Fortaleza, Brazil

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Introduction: Visceral leishmaniasis (Kala-azar) is an endemic parasitic disease that affects millions of people in tropical countries. Clinical observations have shown that there may be difference in disease pattern among children and adults. The aim of this study is to assess disease spectrum and outcome differences in pediatric and adult kala-azar in a large population. **Material and Methods:** This is a retrospective study including consecutive patients with confirmed epidemiological and laboratory diagnosis of kala-azar admitted to a tertiary hospital in Fortaleza, Ceará, Brazil in the period from December 2003 to December 2010. A comparison between children (≤ 14 years old) and adults (> 14 years) was made. Acute kidney injury (AKI) was defined according to the RIFLE ("Risk, Injury, Failure, Loss, End-stage renal disease") criteria. Statistical analysis was done by the SPSS program and p

values < 0.05 were considered significant. **Results:** A total of 432 patients were included. There were 146 (33.7%) children, with mean age 4.8 ± 3.8 years, and 286 (66.3%) adults, with mean age 37 ± 15 years. Compared with children, adults presented a higher incidence of jaundice (18.5 vs. 4.7%, $p=0.0001$), higher time between the beginning of symptoms and hospital admission (88 ± 10 vs. 37 ± 3 days, $p=0.0001$), higher levels of serum creatinine at admission (1.1 ± 1.0 vs. 0.5 ± 0.2 mg/dL, $p=0.0001$), hemoglobin (8.2 ± 1.7 vs. 7.0 ± 1.7 g/dL, $p=0.0001$) and ALT (90 ± 12 vs. 58 ± 6 , $p=0.004$). Children had higher levels of leukocytes (3420 ± 1715 vs. 2500 ± 1878 /mm³, $p=0.0001$) and a higher incidence of splenomegaly (94 vs. 86%, $p=0.009$). AKI was more frequent among children (45.8%) than in adults (32.5%), $p=0.008$. According to the RIFLE criteria, children were in Risk (67%), Injury (31%) and Failure (2%), while adults were in Risk (17%), Injury (44%) and Failure (36%), $p<0.0001$. Mortality was higher among adults (11.5 vs. 2.7%, $p=0.001$). **Main Conclusions:** Kala-azar is frequent among children and adults. The disease seem to be more severe among adults, as evidenced by higher incidence of some complications such as acute kidney injury (higher creatinine levels), liver damage (higher AST and jaundice) and a higher mortality rate. The higher time between the beginning of symptoms and hospital admission seen among adults could contribute to disease complications occurrence. AKI was more frequent and milder in children, evidenced by the higher prevalence of the class "Risk". There may be difference in immunological response between children and adults with kala-azar, which could also contribute to the worse clinical picture seen in adults. **Financial Support:** CNPq (Brazilian Research Council) **E-mail:** ef.daher@uol.com.br

Leish060- Assessment of glomerular and tubular function in patients with visceral leishmaniasis before specific treatment with pentavalent antimonials

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Introduction: Visceral leishmaniasis (VL), also known as kala-azar, is an endemic disease in tropical countries, the subtropics and southern Europe, affecting 1–2 million individuals and causing approximately 500,000 new cases and 5,000 deaths each year. Renal abnormalities caused by leishmanias have been well documented in experimental animal studies and comprise interstitial and glomerular abnormalities. The aim of this study is to investigate tubular and glomerular function before VL treatment with pentavalent antimonials. **Material and Methods:** This is a prospective study with 13 VL patients admitted to a tertiary hospital in Fortaleza city, Brazil. Urinary acidification and concentration tests were performed using calcium chloride (CaCl₂) and desmopressin (DDAVP), respectively, after a 12h period of water and food deprivation. Glomerular filtration rate (GFR), fractional excretion of sodium (FE Na) and potassium (FEK), transtubular potassium gradient (TTKG) and free water clearance (CH₂O) were measured to assess glomerular and tubular function. The SCD group was compared to a group of 15 healthy volunteers (control group). **Results:** Patient's average age was 40.8 ± 19 years, and 92% were male. VL patients and controls had higher levels of serum creatinine (0.9 ± 0.1 mg/dl vs. 0.8 ± 0.1 , $p=0.01$). Urinary volume was higher in the VL group (2025 ± 1114 vs. 1178 ± 480 ml/24h, $p=0.01$). Microalbuminuria was higher in VL group (17.3 ± 25.7 vs. 6.5 ± 6 mg/g urine creatinine, $p=0.0001$), as well as was 24h proteinuria (228 ± 39 vs. 91 ± 56 mg/24h, $p=0.0001$). GFR was lower in VL patients (80.8 ± 24.8 vs. 102 ± 17 ml/min/1.73m², $p=0.01$). Urinary acidification deficit was found in 7 VL patients (53.8%), who presented urinary pH >5.5 after CaCl₂ test. Urinary osmolality was significantly lower in VL patients (483 ± 91 vs. 818 ± 202 mOsm/kg, $p=0.0001$, after 12h period water deprivation, and 543 ± 106 vs. 825 ± 150 mOsm/kg, $p=0.0001$, after DDAVP administration). Urinary concentration deficit was found in 10 VL patients (76.9%), who presented Uosm (urinary osmolality)/Posm (plasma osmolality) ratio < 2.8 after DDAVP test. FE Na was higher among VL patients (0.1 ± 0.05 vs. 0.04 ± 0.02 %, $p=0.0002$), as well as FEK (1.22 ± 0.75 vs. 0.49 ± 0.28 %, $p=0.001$). The TTKG was similar in both groups (4.98 ± 4.3 vs. 2.7 ± 1.5 , $p=0.06$), as well as CH₂O (-0.89 ± 0.56 vs. -1.1 ± 0.3 , $p=0.21$). **Main Conclusions:** VL is associated with important renal tubular dysfunction. Both glomerular and tubular abnormalities can be found. VL patients presented decreased GFR, higher levels of proteinuria, FE Na and FEK. Urinary concentration and acidification deficits were found in a significant proportion of patients (>50%). Kidney function evaluation

should be cautiously performed in all VL patients in order to early detect these renal abnormalities and institute adequate treatment. **Financial Support:** CNPq (Brazilian Research Council). **E-mail:** ef.daher@uol.com.br

Leish061- Co-infection of visceral leishmaniasis and HIV in the Brazilian state in 2007 to 2010

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Introduction: Visceral leishmaniasis (VL) is a severe and potentially lethal disease to humans. It is caused by the interaction between the mosquito vector Phlebotomine (*Lutzomyia* and *Phlebotomus*) of *Leishmania chagasi* and a susceptible human host. It is particularly important because of its high incidence and distribution, and lethality when combined with comorbidities, having been considered, since 1980, opportunistic disease associated with immunosuppression, especially in patients with HIV / AIDS, determining the worst prognosis. According to WHO, this co-infection has already reached 35 countries, having been recorded more than 608,230 cases of HIV in Brazil in 2010, of which 3701 are related to LV. This work discusses about the epidemiology of visceral leishmaniasis co-infection with HIV / AIDS.

Materials and Methods: Retrospective epidemiological documentary research in the database and tabulations of Sistema de Informações de Agravos de Notificação. For that, it was used the descriptors Visceral leishmaniasis and HIV, which have been amended on the epidemiological characteristics specifics of the group. The data refer to the state of Piauí, area of high prevalence of LV. **Results:** Have been reported in Piauí 2660 VL cases in the study period, 412 of which were ignored as the comorbidities. The predominant mode of infection was the mosquito vector. Of the remainder, 3.78% ($X = 21.25$) was associated with HIV, the focus of this work. Between 2007 and 2008, however, there was a large increase of 633.33% in this index. In 2009, on the other hand, there was a more discreet change of 36.36%, remaining stable since then. 10.21% of this group are confirmed by laboratory tests, while only 2.61% was diagnosed based on clinical and epidemiological criteria, immunological diagnosis IFI was performed in 8.70% of cases associated LV / HIV. Were recorded only 8.12% cure for LV, 24.60% of deaths directly related to LV, there were no retirements to treatment for the group in question

Conclusion: The association VL / HIV in the state of Piauí in the study period has grown dramatically, reaching a plateau in which is (3.78%). These data point to the fragility of the ways of control and prevention of both diseases and is a predictor of increased mortality. The growing number of HIV cases in the state and its changing epidemiological profile are weighing on the prognosis of LV, resulting in recurrence and a higher prevalence of atypia, expressed in the presence of immunosuppression. Laboratory diagnosis remains the most widely used in the detection of LV, followed by IFA and clinical - epidemiological. Such information is important for guiding public health policies aimed at the recovery of sick individuals, but not only bring the light of preventive needs of these two conditions and health promotion targeted to areas of endemic disease in addition to combating the mosquito vector. **E-mail:** manoel.medufpi@gmail.com

Leish062- Visceral leishmaniasis in patients infected and not infected with HIV / AIDS in tertiary hospitals in Recife.

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Objective: This study aimed to describe the clinical and epidemiological, laboratory, treatment and outcome in patients with visceral leishmaniasis (VL) with and without infection by human immunodeficiency virus (HIV). **Materials and Methods:** A retrospective study was conducted from the National Notification and Aggravation (SINAN-PE) were removed from 22 cases (VL / HIV-AIDS) and 23 controls (LV) from the records of the tertiary hospitals Recife where he described the comparison groups from 2001 to 2010. **Results:** The review of medical records of these patients found that the male

population was the highest record in both groups, the average age of the case group was 31.5 years and control was 27.3. Regarding clinical data, variables that were statistically significant differences were diarrhea, which showed 65% in the case group and 34.8% in controls and increased liver where it was found in 91.3% in the control and 60% in cases. Some clinical characteristics could not participate in the statistical analysis by the large number of 'unregistered' between them. Myelogram was the most specific test performed. There was a significant difference in the treatments used, Glucantime was more widely used in the control group (82.6%) and amphotericin B in the case group (57.1%), Liposomal Amphotericin B was also used more in the group of cases with 23.8%. The group of cases also had higher number of deaths (27.3%). **Conclusions:** The LV is an important opportunistic disease in HIV patients. This study reveals the importance of how to perform health teams working in an area endemic for VL due to large population displacement and migration of the disease. **Keywords:** Leishmaniasis, HIV / AIDS co-infection VL / HIV-AIDS **E-mail:** gabibrito_@hotmail.com

Leish063- Immunochromatographic test using rK39 in the diagnosis of visceral leishmaniasis in HIV-infected patients.

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Introduction: Diagnosis of visceral leishmaniasis (VL) is based on parasitological and immunological methods. Parasitological methods present high specificity but low sensitivity and immunological assays that are based on detection of anti-Leishmania antibodies using total Leishmania antigen present high sensitivity but low specificity. In HIV-infected patients serological methods are reported as having low sensitivity in patients from Europe due to severe immunosuppression. Immunochromatographic test has been used in the diagnosis of VL around the World because in practice it is easier to be used in the field. The specificity and sensitivity of immunochromatographic test using Leishmania infantum_derived rK39 antigen are around 90% non HIV-infected VL patients. In this work we evaluated the performance of immunochromatographic method in the diagnosis of VL in HIV-infected patients. **Material and Methods:** Sera from patients presenting comorbidity, visceral or tegumentary leishmaniasis (TL) and AIDS, were used to detect anti-rk39 antibodies (Kalazar Detect™ rapid test/Inbios International,Inc) using immunochromatographic strips. All patients had diagnosis of leishmaniasis confirmed by parasitological method and HIV infection confirmed by serological methods according to official program from Ministry of Health from Brazil. At the same time we measured the Leishmania specific antibody levels by ELISA and indirect immunofluorescent assay (IFI), using total antigen from Leishmania major-like to compare with the sensitivity and specificity of immunochromatographic test. **Results:** Nine sera from VL patients with HIV infection and eight sera from TL with HIV infection were used. Seven from nine (66.66%) VL patients were positive with immunochromatographic method, eight (100 %) with ELISA (titer 1/160 to 1/1280) and seven (77.77%) by IFI. All sera from patients with TL were negative by rk39 while 75% were positive by ELISA and 37.5 % by IFI. **Conclusion:** In a study coordinated by WHO using immunochromatographic strips with rK39 of the same brand in sera from non HIV-infected VL patients, the sensitivity and specificity were 84.7 % and 96.8% respectively. We obtained lower sensibility in the present study, probably because HIV-infected patients present severe immunosuppression. However we suggest that the use of immunochromatographic strips using rK39 antigen is still useful for the diagnosis of VL in HIV-infected patient in Brazil. **Supported by** FAPESP, CNPq, LIM-38 (HC-FMUSP) **E-mail:** bjcelest@usp.br

Leish064- Histomorphometric study of apoptosis and renal inflammatory response associated with clinical manifestations in *Leishmania (Leishmania) chagasi* naturally infected dogs.

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Introduction: Apoptosis plays an essential role in the regulation of renal cellularity in both healthy and diseased kidneys. This process takes part in the evolution of lesions triggered by several microorganisms, including *Leishmania* sp. The aim of this study was to characterize and measure apoptosis and the renal inflammatory response in *Leishmania* (L.) *chagasi* naturally infected dogs. **Material and Methods:** Sixteen dogs with visceral leishmaniasis and seven non infected controls were used. Diagnosis of canine leishmaniasis was confirmed by RIFI and ELISA, direct visualization of the parasite (in bone marrow aspirates and *imprints* of popliteal lymph node, spleen, liver and skin), culture and PCR (only in kidney). Animals regarded as non infected controls had negative results in all tests. After anesthesia, fragments of the kidney were collected and fixed in 10% neutral buffered formalin and processed for routine histological examination. The histomorphometry of apoptosis and the inflammatory reaction was performed apart in glomeruli, tubules and inflammatory infiltrates of twenty representative histological fields. **Results:** The average area and cellularity of the inflammatory infiltrates in symptomatic dogs were higher than in other groups. Also asymptomatic animals had higher area and cellularity of the inflammatory infiltrates than controls ($p<0.05$; Tukey). The number of inflammatory foci, as well as their perimeters and extremes diameters (maximum and minimum) in infected dogs (symptomatic and asymptomatic) were higher than in controls ($p<0.05$; Turkey test). Glomerular and tubular cells (in both cortical and medullary regions) showed a higher apoptotic index in symptomatic animals as compared to control dogs ($p<0.05$, Turkey test). Apoptosis within the inflammatory infiltrates was highest in symptomatic dogs, intermediary in asymptomatic and smallest in controls ($p<0.05$, Turkey test). **Main conclusions:** Our results indicate that there is an association between apoptosis and inflammatory responses in kidney of *Leishmania chagasi* naturally infected symptomatic dogs. In this context, apoptosis may be one of the factors that contribute to the clinical and renal lesions progression in course of visceral leishmaniasis. **E-mail:** brbaravet@yahoo.com.br

Leish065- Factors associated with American visceral leishmaniasis in humans. Systematic review and meta-analysis

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Introduction: More than 30 years after the visceral leishmaniasis urbanization in Brazil, there is little understanding about its risk factors. The present study is the first systematic review about factors associated with visceral leishmaniasis in the Americas. We sought to identify the best evidence on the subject in the available scientific information to identify the role of studied factors and gaps in existing knowledge. **Material and Methods:** Searches were carried out in four databases (Medline, Lilacs, Bank of Thesis Capes and Google Scholar), together with consultations to reference lists of articles and to experts in the area. Theoretical discussions or Meta-analyzes of p-values or of odds ratios, using random or fixed effects models were used to pool the information for each variable. To analyze the occurrence of heterogeneity the Q-test and the I^2 statistic were used. The publication bias was investigated by the funnel plot, by the Egger's test, and by the statistic of "Trim and Fill". **Results:** Fifty-one studies were reviewed. Twenty-four were cross-sectional, 17 ecological, 7 case-control and 3 of cohort. Three studies were conducted in Venezuela, two in Colombia, one in Honduras and forty-five in Brazil. Regarding the associations, the male sex was associated with visceral leishmaniasis in studies which used Leishmania Skin Test for the diagnosis and in case-control studies in which the outcome was the clinical syndrome. The opposite occurred when serological tests were used. Younger individuals were more prone to illness but less positive. Although with different levels of evidence and of heterogeneity, the presence of dogs at home, the positivity in dogs, the highest rates of vegetation and lowest socioeconomic levels were associated with human visceral leishmaniasis. This did not occur with the presence of chickens in the household and with nutritional status. Susceptibilities to bias and limitations in analysis and in the description of the results were often identified in the studies analyzed. No statistical evidence was obtained about the existence of publication bias; however there was great loss of information due to the primary studies did not provide the necessary data for obtaining measures of association. **Conclusions:** The results showed the existence of consistent standards for some of the factors analyzed and should be

taken into account to the developing of control measures more effective and well-targeted. Studies in new areas of the continent, with improved methodological quality, giving priority to the investigation of the trends identified and their causes, as well as variables for which the knowledge is poor or inconsistent must be developed. The limitations found in the publications indicate that the quality of the studies must be greatly improved. **E-mail:** viniciusbelo4@hotmail.com

Diagnosis

Leish066- Critical analysis of laboratory techniques for diagnosis of the canine visceral leishmaniasis in Sentinel Pole University Center, in São José do Rio Preto, São Paulo, Brazil

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Introduction: Leishmaniasis are zoonotic diseases caused by protozoa of the genus *Leishmania* that can affect humans. Visceral leishmaniasis (VL) is a serious public health problem and Brazil accounts for 90% of reported cases on the continent. WHO estimates indicate a contingent of more than 350 million people at risk of transmission of leishmaniasis, and more than 12 million individuals infected with *Leishmania*. **Objective:** Analyze the performance of the immunoassay Chembio DPP ®/ Biomanguinhos-Fiocruz, suitable for a rapid, sensitive and specific for LVcanina. **Material and Methods:** Were examined 86 serum samples from dogs treated at a routine in a pole sentinel for the active search for cases of LVcanina, linked to the University Center located in Sao Jose do Rio Preto, in São Paulo. After identification and registration of their origin, from an endemic area or not, all animals were submitted to clinical evaluation. Laboratory diagnosis was performed by use of serological techniques, DPP, ELISA and IFI for detection of anti-*Leishmania* antibodies; parasitological technique for the detection of *Leishmania* shaped amastigote from a sample of lymph node aspirate. **Results:** Finally, 27 samples were positive for at least one of four laboratory techniques. DPP single recognized 19 positive samples. Considering the parasitological diagnosis with the gold standard, DPP test showed 100% sensitivity and specificity since it recognized all positive samples for the presence of shaped amastigote of *Leishmania*. The association DPP and ELISA had a sensitivity of 83% and a specificity of 94%. The association of IFI and DPP sensitivity was 84% but the specificity was less than 35%. **Conclusion:** The results of this study indicated the association of the DPP rapid test with ELISA proved suitable for the purposes of a service established in a sentinel pole for the diagnosis and identification of dogs naturally infected by *L. (L.) infantum chagasi*. **E-mail:** dmbbertollo@ial.sp.gov.br

Leish067- A serological survey for canine visceral leishmaniasis in the city of Teresina, Piauí, by indirect immunofluorescence (IF) and enzyme linked immunosorbent assay (ELISA)

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Introduction: The evaluation of the domestic reservoir through serological surveys is part of the measures recommended by the Ministry of Health of Brazil for the control of canine visceral leishmaniasis. In Teresina, the diagnosis is made by the Department of Zoonoses (GEZOON) in partnership with the Federal University of Piauí (UFPI). The present study aims to analyze the serological profile of canine visceral leishmaniasis (CVL), and the concordance of the diagnostic tests used in routine. **Materials and Methods:** Samples were collected by venipuncture in different neighborhoods of Teresina by agents of the GEZOON, from October 2011 to January 2012, and processed in the laboratory of parasitology of the UFPI. We used the enzyme immunoassay (ELISA) for screening of samples and indirect immunofluorescence (IFAT) to confirm the initial positive test, both using kits FIOCRUZ/Biomanguinhos. We used the following criteria to conceptualize the results of agreement: values $\leq 40\%$ are considered poor, from 40.1 to 79.9% regular, 80 to 89.9% are considered good and $\geq 90\%$ are considered as excellent. With respect to seroprevalence of the disease in dogs is considered high if it exceeds the 25%. **Results:** During the study period were collected and processed 5096 serum samples, and were reactive in ELISA 1471 (28.87%) samples. Analyzing the positive ELISA by IFAT, it was observed that 1349 (91.71%) agreed with the first result. It should be noted that 27 samples (2%) analyzed by IFAT were inconclusive due to limitations of the technique. **Conclusions:** Given the methodology used, there was a high correlation of IFAT compared to ELISA and high seroprevalence neighborhoods worked. **E-mail:** fassisle@gmail.com

Leish068- Assessment of serological tests for the diagnosis of canine visceral leishmaniasis

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Introduction: Although euthanasia of seroreactive dogs is used since the 50s, it is still a controversial issue, because it does not seem to contain the spread of the disease. Also, the accuracy of the tests employed in diagnosis of dogs is being discussed. This study assessed indirect immunofluorescence (IIF), immunoenzymatic assay (ELISA) using different antigens and TR DPP canine visceral leishmaniasis (DPP) immunochromatographic test for the diagnosis of canine visceral leishmaniasis (CVL) from an endemic area in the municipality of Rio de Janeiro. **Material and Methods:** Serum samples from 144 domestic dogs euthanized by the leishmaniasis control program were assessed. Initially, those animals were evaluated through parasitological culture (reference method). Each serum sample was tested using one protocol that used the IIF-Canine-Visceral-Leishmaniasis commercial kit (Bio-Manguinhos, Rio de Janeiro, Brazil), which employs promastigote forms of *L. major*-like (MHOM/BR/76/JOF) as antigen (IIF-*L. major*-like). The other protocol (IIF-*L. chagasi*) was performed with same kit, substituting the antigen by promastigote forms of *L. chagasi* (MHOM/BR/74/PP75). As IIF, the samples were tested through two immunoenzymatic assays: EIE-Canine-Visceral-Leishmaniasis kit (Bio-Manguinhos, Rio de Janeiro, Brazil), which employs a soluble antigen from promastigotes forms of *L. major*-like (ELISA-*L. major*-like), and the other was performed with the same kit, substituting the antigen by promastigotes forms of *L. chagasi* (ELISA-*L. chagasi*). TR DPP Canine Visceral Leishmaniasis (Bio-Manguinhos, Rio de Janeiro, Brazil) is an immunochromatographic test, which employs recombinant antigens K26 and K39. All tests were performed according to manufacturer's instructions. Sensitivity and specificity were calculated using parasitological culture as reference test. Simple kappa statistics (k) was used in order to compare the concordance among the serological tests, according to classification: $k = 0.00$ to 0.10 (virtually no reliability); $k = 0.11$ to 0.40 (low); $k = 0.41$ to 0.60 (discrete); $k = 0.61$ to 0.80 (moderate) and $k = 0.81$ to 1.0 (substantial). **Results:** Thirty-nine percent ($n=56$) were seroreactive to *Leishmania*, independent of the test or antigen used. Twenty-eight percent, 10%, 22%, 17% and 17% of serum samples were positive in IIF-*L. major*-like, IIF-*L. chagasi*, ELISA- *L. major*-like, ELISA-*L. chagasi* and DPP, respectively. The sensitivity of the serological tests was 93%, 100%, 73%, 60% and 93%, with specificity of 87%, 92%, 77%, 96% and 92% for ELISA-*L. major*-like, ELISA-*L. chagasi*, IIF-*L. major*-like, IIF-*L. chagasi* and DPP, respectively. Kappa agreement among the different serological tests varied from low to moderate. **Main Conclusions:** Based on results, ELISA-*L. chagasi* was the best test for the diagnosis of CVL. However,

we suggest that DPP might be an alternative for CVL diagnosis with advantages such as storage and transportation form, allowing a simple and rapid diagnostic without the need of a laboratorial specialized structure when compared to conventional serological tests. **E-mail:** denise.silva@ipec.fiocruz.br

Leish069- A β -mercaptoethanol-ELISA for the diagnosis of canine visceral leishmaniasis

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Following procedures of antigen preparation similar to those described for the direct agglutination test (DAT), an IgG ELISA employing promastigotes of *Leishmania infantum* was developed for the diagnosis of canine visceral leishmaniasis (CVL). The newly developed β -ME-ELISA was also compared with an ELISA version using instead of β -ME-treated, trypsin-treated promastigote (TRYP-ELISA) antigen. While in comparison with DAT (31/31 = 100%) slightly lower sensitivity (29/31 = 93.5%) was demonstrated for the β -ME-ELISA developed in dogs with confirmed CVL, evidently higher sensitivity was estimated in the same canine population by comparison with the TRYP-ELISA (27/31 = 87.1%). When challenged against conditions other than CVL: including Babesiosis, ehrlichiosis and Chaga's disease, a specificity of 97.5% was estimated for β -ME-ELISA as compared to 100% or 94.1% for DAT and TRYP-ELISA respectively. In an endemic population manifesting CVL clinical suspicion (n=38), results obtained with the β -ME ELISA were more concordant (33/38 = 86.3%) with those of the DAT than the TRYP-ELISA (27/38 = 71.1%). In this group of unconfirmed CVL, the incorporated β -ME treated promastigote antigen has demonstrated also higher sensitivity (29/31 = 93.5% or 62/69 = 88.9%) in comparison with that of its trypsinized equivalent (27/31 = 87.1% or 54/69 = 78.2%). Our current results implicate the application of β -ME ELISA in the laboratory to support results obtained in the field with the DAT. **E-mail:** andrade@ufpe.br

Leish070- Comparison of the press imprint method with histopathological test for the direct diagnosis of cutaneous leishmaniasis

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Background: Cutaneous leishmaniasis presents a broad clinical spectrum and may be confused with other diseases affecting the skin. Thus, it is of greatest importance the rapid and accurate diagnosis of the disease. Parasitological diagnosis is the gold standard in laboratory confirmation of the disease, including several techniques such as imprint, aspirate lesions, histopathology and immunohistochemistry of biopsy. Despite its high specificity, the parasitological diagnosis has low sensitivity (50% -70%) that depends on several factors, such as the degree of parasitism, the type of the biological material collected, the processing and staining, and the ability of the observer. As a result, it becomes necessary to search for alternative techniques for the diagnosis of leishmaniasis. A modified technique of the imprint, the press imprint, was developed and it allows a better representation of the lesion. In this study, it was compared the performance of the press imprint with histopathology, in direct diagnosis of leishmaniasis. **Material and Methods:** Using punch 2mm thick, two biopsies were collected from the skin lesion of each patient. One fragment was fixed in 10% buffered formalin and processed, and sections were stained with Giemsa and Hematoxylin-Eosin and examined under a microscope. Another fragment was subjected to press imprint technique, which consists of macerate the biopsy sample between two glass plates under pressure. After the procedure, the slides were stained with Giemsa and examined under the microscope. **Results:** Of 100 patients tested, 74 showed the presence of *Leishmania* sp. in one or in both methods. Of

the 74 positive patients, 67 (91%) were positive by the press imprint method. The histopathological examination showed the presence of *Leishmania* sp. in 32 patients (43%). **Conclusion:** The press imprint showed better performance than the histopathologic for the direct diagnosis of cutaneous leishmaniasis. **Financial support:** CNPq (grant 480083/2008-8). **E-mail:** claudenia1313@hotmail.com

Leish071- Development of DNA electrochemical biosensor: analysis of selectivity for detection of Leishmaniasis.

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Leishmaniasis is a chronic infectious and parasitic disease that every year affects 500.000 people, with an estimate 50.000 deaths per year. In contrast to the methods currently used in leishmaniasis diagnosis, biosensors provide sensitive, selective and rapid response. A biosensor is an analytical device that allows measuring biological signals from a recognition event that occurs on the surface of a transducer, showing high selectivity, simplicity and low cost. Thus, our study aimed to determine the specificity and selectivity of DNA onto pencil graphite electrode (PGE) for detection of Leishmaniasis. Firstly, the PGE was pretreated applying a potential of 1.8 V for 5 min to obtain a more sensitive and stable analytical signal. Then, the specific probe for *Leishmania* sp (0.5 µM) was immobilized on the activated PGE using a potential of 0.5 V for 5 min. The selectivity was analyzed by hybridization with different sequences (complementary target, non-complementary and mix). The electrochemical behavior of the PGE surface was studied by the guanine oxidation signals present in the probe and targets sequences using the differential pulse voltammetry (DPV). The results of hybridization between probe and complementary target presented a decrease in the guanine signal (from 272 nA to 109 nA), because hybrid formed. However, the interaction between probe and no-complementary target did not lead to a significant decrease in the guanine oxidation (223) due to absence of entire hybridization. On the other hand, the interaction between probe and mix (target and non-complementary) sequences showed a current peak similar to the complementary target (108 nA). This demonstrates that the presence of non-complementary sample does not interfere with the specificity of the biosensor. The data demonstrated that this biosensor was able to distinguish between the complementary target and non-complementary. Also specificity was not changed even in the presence of non-complementary samples. The data demonstrated that this biosensor was able to distinguish between complementary and non-complementary sequences. The biosensor proposed showed a good promise for leishmaniasis diagnosis. **Key-words:** Leishmaniasis, DNA probes, electrochemical biosensor **E-mail:** wessullas@yahoo.com.br

Leish072- Effectiveness of diagnosis for Canine Visceral Leishmaniasis carried out at the Center of Zoonosis Control of Camaçari, Brazil

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According to World Health Organization, visceral leishmaniasis (VL) is a serious public health problem. The disease is caused by protozoa of the genus *Leishmania* and transmitted by *Lutzomia longipalpis*. The dog is considered the main reservoirs at urban areas, probably by facilitating the transmission for men and other animals. The control strategies recommended by the Health Department in Brazil are based mainly on identification and euthanasia of seropositive dogs. Until December 2011, ELISA was used to screen and IFA to confirm the diagnosis of visceral leishmaniasis, as recommended by Brazilian Health Department. A cross-sectional study was conducted from May to August 2011 in order to compare the efficacy of three diagnosis tests: IFA conducted by the Center of Zoonosis Control (CZC) of the municipality of Camaçari (Bahia) and culture of splenic aspirates and ELISA performed at the Laboratory

of Pathology and Biointervention (LPBI), FIOCRUZ. Blood samples were collected to perform ELISA and IFA, as well as splenic aspirates to precede culture. The positivity rate for IFA was 40.31%, for splenic aspirate cultures was 11.22%, and for ELISA was 25.51%. Using culture as the gold standard, several parameters were calculated: i) for IFA sensitivity 73% (16/22), specificity 64% (111/174), positive predictive value (PPV) 20% and negative predictive value (NPV) 95%. When positivity at ELISA or culture was considered, the results were combined to compare with IFA, thus the other parameters calculated revealed: ii) for IFA sensitivity 78% (39/50), specificity 73% (106/146), PPV 49% and NPV 91%. These results show that using the diagnostic tests performed in Camaçari by the CZC, dogs were incorrectly diagnosed, leading to the maintenance of false negative and euthanasia of false positive in this endemic area. To prevent this situation, is necessary to develop more accurate diagnostic tests with higher specificity and sensitivity. **Support by** FAPESB, INCT-CNPq, PDTIS, PST Veras' grant (CNPq:306672/2008-1) **E-mail:** dmfraga@hotmail.com

Leish073- ELISA-rK39 and PCR for the detection of asymptomatic *Leishmania* infections in people living in endemic foci of visceral leishmaniasis (VL) in Carabobo State, Venezuela

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Introduction: Visceral leishmaniasis (VL), which is fatal without treatment, is considered as public health problem worldwide. Epidemiological monitoring of *Leishmania* infection in people living in endemic areas is useful in order to stratify the prevalence of infection and prioritize the control actions. So far, no gold standard technique is available for this purpose. In Venezuela, in the VL Control Program, the rK39-ELISA test is routinely used for diagnosis in suspected VL patients and to quantify the prevalence of asymptomatic VL carriers of the infection in epidemiological studies. We compare here the ELISA- rK39 and the PCR-ITS1, as markers for the detection of potential *Leishmania* sp. infection in endemic VL foci in Carabobo State, Venezuela. **Materials and Methods:** 181 blood samples were screened using the ELISA with the recombinant K39, 0.5 µg/mL, serum dilution 1/100, title alkaline phosphatase conjugate 1/10.000, and reading at 405 nm. Nested PCR was standardized for the amplification of the *Leishmania* sp. intergenic spacer 1 (ITS1). The reaction was performed with dNTP's 0,1 mM; MgCl₂ 2,0 mM; primers 0,2 µM and Taq polymerase 1,0 U, using in the first reaction LITSR: 5' CTGGATCATTTTCCGATG 3' and L5,8S: 5' TGATACCACTTATCGCACTT 3' as forward and reverse primers respectively. In the 2nd reactions were used VAN2: 5'TCC CAT CGC AAC CTC GGT T 3' and SAC: 5' AAA GCG GGC GCG GTG CTG 3' as forward and reverse primers respectively. **Results:** ELISA-rK39 and PCR-ITS1 were positive in 22.1%, and 53.6% of the samples respectively. The agreement of both techniques was observed in 14.9% of positive and 31.5 % of negative samples. 7.2% of positive results by ELISA-rK39 were negative by PCR-ITS1 and 27.1% of positive by PCR-ITS1 were negative by ELISA-rK39. **Conclusions:** PCR-ITS1 was more efficient than ELISA-rK39 in the detection of asymptomatic *Leishmania* infections, probably because it highlights the more recent ones which would indicate active transmission. This information might be useful to optimize and prioritize VL prevention and control actions. **Financial support:** Projects FONACIT, MC-2008000911-2 and STREP-FP6_INCO-CT2005-015407 **E-mail:** dcannova@gmail.com

Leish074- Evaluation of rK39 rapid test for diagnosis of visceral leishmaniasis in a large urban setting

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Introduction: Visceral leishmaniasis (VL) is an endemic disease in Belo Horizonte (BH), Brazil, where it has an urban transmission pattern with a high case fatality rate. In order to foster early diagnosis of VL, a rapid dipstick immunochromatographic test was implemented in 2010 in emergency care units and referral hospitals of BH, a large city with around 2.5 million inhabitants. We aimed to evaluate the performance of a rapid test for detection of VL in a large urban setting. **Methods:** Patients that performed rK39 dipstick rapid test for detection of VL (Kalazar-Detect™) from May 2010 to December 2011 in eight public emergency care units and five referral hospitals from BH were included in the analysis. Serum samples from these patients were also sent to a referral laboratory to perform indirect immunofluorescent reaction (RIFI) to detect antibodies against *Leishmania*; RIFI was considered to be positive if a titer greater or equal than 1:80 was observed. Demographic and clinical information were obtained from case report forms. According to the recommendations from the Brazilian Ministry of Health, a confirmed VL case was defined as a patient with symptoms compatible with VL that had either a positive IFI, a positive direct examination or culture for *Leishmania*, or a good clinical response to treatment). **Results:** During the study period, 476 suspect cases of VL performed both RT and RIFI. RT was positive for 114 (23,9%) patients and RIFI was positive for 134 (28,2%). Comparison of these two tests yielded a Kappa of 0.71 (CI95% 0.62-0.80). Clinical information was obtained from 381 patients and 145 (38%) of those patients met criteria for confirmed LV. Sensitivity and specificity of RT was 72.4% and 99.6%, respectively. Predictive positive and negative values for RT were 99.1% and 85.5%, respectively. **Conclusion:** RT and RIFI had a moderate agreement for the diagnosis of VL. RT showed a high specificity and patients with clinical symptoms compatible with VL that have a positive RT might be treated without further investigation; however, a negative RT warrants further investigation. RT might be a good option for the initial evaluation of suspect cases of VL in different levels of care. **E-mail:** alexandresmoura@gmail.com

Leish075- Immunoglobulin G avidity test in diagnosis of visceral leishmaniasis

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Introduction: Immunoglobulin G avidity assays have been widely used to discriminate recent and past infections in diseases as rubella, cytomegalovirus and toxoplasmosis. In visceral leishmaniasis (VL) there are few reports showing the antibody avidity profile in infected subjects or evaluating the use of these tests for early diseases' diagnosis. **Methods:** This study assessed the anti-leishmania IgG avidity in patients with classic VL (group 1; N=10), subjects treated for the disease that presented clinical cure (group 2; N=19) and asymptomatic subjects with at least one positive anti-leishmania serological test (group 3; N=20) living in the endemic areas of Porteirinha and Janaúba, Minas Gerais. Serum samples were obtained in two different time points for each subject. A *Western blotting* assay was performed using *Leishmania chagasi* promastigote antigen. The presence of low avidity antibody was verified by the completely loss of reactivity after strips treatment with 8M urea. **Results:** Serum samples in group 1 reacted with a wide range of antigens, giving multiple bands, mostly of high avidity. Sera obtained from the same patients six months after specific treatment showed a similar reactivity pattern, but in 4 patients there was an increase in low avidity bands. Subjects in group 2 had serum samples collected in different time points after clinical cure: <1 year (5); 2-3 years (8); >4 years (6). The sera showed a variable reactivity pattern with a predominance of low avidity bands. Samples of these subjects collected after 2-3 years of the first assessment had a similar pattern. Finally, in group 3, 14 individuals had reactivity and avidity patterns analogous to those observed for group 2, while 6 subjects had results resembling VL patients. The second serum samples of these subjects were obtained after 3-4 years, and none of them evolved to classic disease. The reactivity profile and low avidity bands proportion were similar to the first serum sample in the subgroup of 14 subjects, comparable to group 2. The remaining 6 individuals had a few increase in high avidity bands percentage. We compared the proportion of high avidity bands and found a significant difference between groups 1 and 2 or 3 ($p<0,0001$), but not between groups 2 and 3. **Conclusions:** The presence of high avidity IgG antibodies in VL patients is consistent with the long incubation period usually observed for the disease. However, the high proportion of low avidity bands in cured individuals was not expected. We thought that the low antibody levels could be "simulating" low avidity. Results observed in the asymptomatic individuals suggest that IgG avidity tests aren't suitable to

identify cases that may progress to disease. Further studies are needed to evaluate the use of these tests in VL diagnosis. **E-mail:** monique_tiburcio@yahoo.com.br

Leish076- Interobserver and intraobserver agreement for the MST technique directly on the skin of the patient and compared to a paper overlay

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Montenegro skin test (MST) is used for diagnosis and epidemiological surveys of American cutaneous leishmaniasis. This study assessed the degree of Interobserver and intraobserver agreement for the MST technique directly on the skin of the patient and compared to a paper overlay. This is a prospective, longitudinal, descriptive study, with 51 patients who performed MST for diagnosis at the Leishmaniasis Surveillance Laboratory, Evandro Chagas Clinical Research Institute (IPEC), Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, between October 2010 and April 2011. The antigen was applied in the right forearm and 48 hours later three readings were made with an interval of 15 minutes among each other. The first and third readings were taken by observer 1, and the second by observer 2. At the first reading the infiltrated area was demarcated with a pen and the decal paper soaked in alcohol was then applied upon it; it was subsequently read by both observers. The simple agreement and kappa index were calculated for measurements made in vivo by the same observer and for both observers, and for reading in vivo and in the overlay by either observers, using or not a tolerance of 1 mm of difference between readings. We calculated the specificity (Sp), sensitivity (S), positive predictive value (PPV) and negative predictive value (NPV) of the test. The first in vivo reading showed higher values of Ep and PPV when compared with the second and third readings. The technique of demarcation with a pen, when performed more than once within short periods of time (for example, an hour), may result in false positive results, and repetition is not advisable. The safety of decal reading can be demonstrated by the high correlation found between the readings in the overlay by two observers, as well as between in vivo reading and reading in the overlay. The use of one millimeter of tolerance induced a significant increase in the agreement, especially in Interobserver variability of the reading of the forearm with the reading of the overlay. However, we must be careful when the patient has a threshold value between the negative and positive test (4 and 5 mm), because it may cause a change in the outcome of the MST. **E-mail:** maria.pimentel@ipec.fiocruz.br

Leish077- Lyophilized and ready-to-use Direct Agglutination Tests in the serodiagnosis of canine visceral leishmaniasis: performance comparison

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In the south of Europe, more than 2.5 million dogs are infected with *Leishmania infantum* and suffer of canine visceral leishmaniasis (CVL). More than half of these are asymptomatic and as infectious to phlebotomine sandflies as the symptomatic animals. Nowadays, there are no serological tests to apply in field conditions, sensible enough to signal these dogs. In this way, hoping to develop a sensitive, specific, easy-to-use and cheap serological tool, capable of monitor CVL and meet recommendations of World Health Organization (WHO, 2010) our group tried, in this study, to adapt a direct agglutination test (DAT) able to offer, same or better benefits, as the one already developed for the human host (Harith et al., 1995). So, we compared the performance of a DAT, where its antigen was prepared from intact *Leishmania infantum* promastigotes treated with [3-mercaptoethanol (B-ME-DAT), with another one, conventional, trypsinized, but prepared with intact promastigotes (TRYP- DAT). The [3-ME-DAT showed sensitivity and specificity values of 100% and the TRYP-DAT, respectively, 93.5% and 100%. The predictive positive value (PPV) was 93.9% in the [3- ME-DAT and 87.8% in the TRYP-DAT; the predictive negative value (PPN) was respectively 100% and 97.5%. The concordance ($1 \leq 0.879$, $p=0.000$) and correlation (0.935) obtained between the two tests with these different antigens were optimal. Despite the

fact that more studies are needed to better evaluate our findings, in our opinion, this new [3-ME-DAT, has the potential to detect CVL at its early stages, prior to appearance of clinical signs and parasite proliferation and consolidation. This achievement suggests new research lines directed towards early diagnosis of CVL and subsequent quick treatment, possibly using less toxic and cheaper medicines. WHO (2010) Control of the leishmaniasis. Technical Report Series 949. Report of a meeting of the WHO Expert Committee on the Control of Leishmaniasis, Geneva, 22-26, ISBN: 978 92 4 120949 6. **E-mail:** saulix@gmail.com

Leish078- Montenegro skin test and evolution time of the lesion prior to treatment as predictors of treatment failure in cutaneous leishmaniasis.

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This study aimed to examine the association between Montenegro skin test (MST), evolution period of the lesion prior to treatment and therapeutic response in patients with cutaneous leishmaniasis (CL) treated at the Evandro Chagas Clinical Research Institute (IPEC), Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil. We conducted a case-control study in which for each case with poor response to therapy two controls that have evolved with lesion healing after treatment, paired by sex and age, were randomly selected. All studied patients underwent treatment with intramuscular meglumine antimoniate (Sb⁵⁺) with a dose of 5 mg Sb⁵⁺/ kg / day for 30 continuous days. Patients with CL were approximately five times more likely to fail when the lesions had less than two months of evolution time at the beginning of the treatment. Patients with treatment failure showed less intense MST reactions than those who progressed to clinical cure. Every 10 mm of increase in MST response corresponded to a 26% reduction in the chance of occurrence of failure. The treatment with less than two months of evolution time of the lesion and a poor cellular immune response, reflected by a less intense MST, contributed to the occurrence of treatment failure in cutaneous leishmaniasis. **E-mail:** lilianedefatima@gmail.com

Leish079- Preliminary results of a new approach based on a nanocomposite for the detection of Leishmania (Leishmania) infantum

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Visceral leishmaniasis (VL) is a serious public health problem in Brazil, where it was initially regarded as a rural disease. In the past decades, the disease has spread to urban areas of medium and large cities. Because VL is often fatal if untreated, rapid diagnosis and early treatment is essential. Thus, the development of rapid, accurate diagnostic tests is a priority for the management of this disease in endemic areas. Conventional tests routinely used for the VL diagnosis are useful in particular situations, but they may have limitations, including low sensitivity and low specificity for detection of antibodies. Currently, molecular methods based on detection of parasite DNA have shown excellent performance in the diagnosis of visceral leishmaniasis. In particular, the PCR has advantages over conventional methods as it allows the detection of small amounts of parasite DNA in different types of samples, thus providing a rapid and reliable diagnosis. However, PCR-based techniques might be expensive as well as require skilled professionals and sophisticated equipment. Hence, the development of new tools to provide a rapid, easy-to-perform, and accurate diagnosis is desirable. The ELINOR test is based on the use of hybrid fluorescent-magnetic nanoparticles, which allows producing a luminance signal that can be detected with a fluorescence microscope at low concentrations of DNA or RNA, without the need for amplification prior genetic material to be tested. The objective of this study was to compare the performance of ELINOR test with a conventional PCR protocol used in the Reference Service on Leishmaniasis of Fiocruz Pernambuco. Twelve samples (i.e., 7 blood and 5 bone marrow samples) from VL patients and six blood samples from healthy individuals were tested. In brief, DNA extraction was

performed with a commercial kit and the primers RV1 and RV2 were used to perform both tests (PCR and ELINOR). All the 12 samples of VL patients were positive by PCR and two of them were negative by using the ELINOR test. Moreover, all six samples from healthy individuals were PCR-negative whereas two were ELINOR-positive. These preliminary results suggest a good level of concordance between ELINOR and the PCR protocol used. These findings suggest that ELINOR could represent an important advancement in the field diagnostic in endemic areas of VL. **Financial support:** CAPES and Pibiti-CNPq-Fiocruz. **E-mail:** edeneide@gmail.com

Leish080- Preparation of information materials related to the microscopic images obtained during direct examination of leishmaniasis

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Introduction: The demonstration of the parasite is important for accurate diagnosis of the Cutaneous Leishmaniasis (CL), although largely used in this context, the direct examination of the parasite requires skilled personnel for their performance, considering that the diagnosis is given by viewing the parasite in the material examined. However, such forms do not always present themselves in typical ways. The objective of this study was to identify fungal structures and / or artifacts that are commonly found in slides prepared for direct examination of the CL, and also identify typical and atypical forms of *Leishmania* sp. in order to prepare a chart of figures to be used on the preparation of a technical manual aimed to training health professionals. **Materials and Methods:** The glass slides were selected from the archive of the Laboratory of Leishmaniasis Surveillance obtained of patients treated between 2009-2011 whose diagnosis had been defined by direct examination or culture. Slide reading was performed by using an oil immersion objective (×1000) and the slides were photographed using the software Motic Image Plus 2.0 for capture, measurement and processing of the image. **Results:** Four chart of figures (measuring 20cm x 20cm) were made containing images related to: (1) typical amastigotes: structures whose shape, color and presence of the kinetoplast, defined the diagnosis of leishmaniasis with assurance, (2) atypical amastigotes : structures whose shape or color not defined amastigotes of *Leishmania* sp. with assurance (3) fungal structures: structures found in slides from patients with a diagnosis of sporotrichosis and histoplasmosis that resembled with amastigotes and (4) artifacts: different structures present in glass slides that could induce mis identification with amastigotes of *Leishmania* sp. **Key findings:** The lack of training to perform the direct examination of the CL still being a challenge in different regions of the country. During slides examination it was possible to select structures that could easily confuse the viewer. The photo-documentation of these structures and composition of chart of figures will be of great value during the training activities and contribute to a more accurate diagnosis of Leishmaniasis. **E-mail:** fatima.madeira@ipec.fiocruz.br

Leish081- rK39 immunochromatographic test for the diagnosis of human visceral leishmaniasis immunocompetent patients

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Visceral leishmaniasis (VL) is an endemic disease that affects mostly people with low socio-economic status. When untreated the infection can be fatal, and the drugs currently in use are expensive and toxic. Therefore, confirmation of diagnosis before starting therapy is crucial. Brazil has experienced a sharp increase in the number of cases of visceral leishmaniasis since 1999. Rapid diagnosis of VL provides the correct therapeutic for infected individuals. The diagnostic tests include demonstration of the parasite, either by microscopy of tissue smear, molecular diagnosis or demonstrating the host immune response against infectious agents by means of serological tests. Currently the demonstration of the parasite in smears of tissue is considered the gold standard for diagnosis of

VL. However, this procedure is invasive, risky and requires experienced professionals, which is not always possible under field conditions. The analysis by molecular biology requires costly equipment and availability of large physical space. Therefore is essential searching solutions to an efficient diagnostic, fast and with least damage possible to the patient. In this context, our laboratory undertook to analyze the feasibility of rk39 immunochromatographic rapid test as tool for the diagnostic of the disease. The principle serological along with its reduced cost, easy use with minimal training required and small amounts of biological sample in the analysis make rk39 a promising laboratory technique.

Methods: We analyzed 33 plasma samples from patients with clinical suspicion of VLH from the HCFMUSP. Serological response analyzed by rk39 antigen (DiaMed-IT LEISH and Chembio DPP Leishmania Rapid Assay) was compared with parasitological and molecular findings. **Results:** Preliminary results showed that of 22 samples analyzed by rk39, 10 samples were positive for LVH and these results were confirmed by parasitological and molecular tests. Twelve samples tested were negative for VLH, which also showed results consistent with the molecular and parasitological findings. And 11 samples from HIV patients presented negative rk39, unlike of the parasitological and molecular results. **Conclusions:** The rapid test rk39 demonstrated high potential as a predictor of VL, which is useful in field situations, where the shortage of professional and resources demands a rapid and efficient action. But still has limited applicability in immunocompromised individuals, since the immune response in which is based the test is affected in these patients. **Supported by** FAPESP **E-mail:** LMABRAZ@usp.br

Leish082- Standardization of swab method to American cutaneous leishmaniasis by PCR-based diagnosis in patients of Pernambuco State, Brazil

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American cutaneous leishmaniasis (ACL) is a neglected disease caused by protozoans of the genus *Leishmania*, which cause to their patients chronic morbidity due to severe cutaneous lesions. In the Americas, the maintenance of the transmission cycle is tightly provided by the eco-epidemiology aspects, including climate and wide variety of host reservoirs and sand fly vectors. In this context, Brazil is an important country in terms of incidence and larger endemic geographical extension area. The diagnosis of ACL is relatively complex, being necessary expertise for the identification of the parasite by the direct visualization in microscopy, isolation in culture media or DNA detection in samples of tissue obtained in the edge of lesion by the procedure of biopsy. The later one is notably an invasive method, which allowed secondary infections and cause a great discomfort to the patients. The aim of this work is the standardization of biological samples collection by swab method in ACL patients of Pernambuco State, Brazil. Samples of cutaneous exudate and biopsy were collected in 75 ACL patients from Pernambuco. Before collections was done local anesthesia with prilocaine hydrochloride (3%) and felypressin, and then, sterile swabs were used to the collection of the exudate and punches (4-8mm of diameter) to the biopsy. The swab was slightly passed on all parts of the lesion (center and edge). There was no use of scalpel before swabs collection. The samples were packed on 1,5mL tubes and after were done the purification of DNA using a commercial kit (Qlamp® DNA Mini Kit - Qiagen, Valencia, USA). The PCR was performed using the LEIB1 (GGGGTTGGTGTAAATATAGTGG-3') and LEIB2 (5'-CTAATTGTGCACGGGGAGG-3') primers specific to *Viannia* subgenus. Amplified products were observed on agarose gel (1%) stained by ethidium bromide. Among samples of 75 patients analyzed, it was observed a concordance of 88% (66/75) in the results. Seven patients presented positive result just on the biopsy and two presented the same, but just on the swab. Our results confirm that the swab method is feasible and show great efficacy when compared with biopsy. The use of this non-invasive method as auxiliary diagnosis of ACL is relevant due to its practicality and improvement in the attainment of biological samples. **Financial support:** CNPq and FACEPE (APQ-0630-2.13/08) **E-mail:** isabele@cpqam.fiocruz.br

Leish083- Evaluation of the swab in saliva method for the diagnosis of cutaneous leishmaniasis in patients from endemic areas of Pernambuco State, Brazil

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Leishmaniasis comprises a spectrum of diseases endemically distributed in tropical and subtropical regions, caused by different species of protozoans of the genus *Leishmania*. In the Americas, leishmaniasis are zoonotic diseases caused by different species of subgenus *Leishmania* and *Viannia*. The improvement of the diagnosis is related to the availability of accurate methods with high sensitivity and specificity for detection of *Leishmania* spp., which can provide information on the epidemiology of the disease. Currently, clinical sample for the diagnostic analysis provides critical care to the patient, considering dealing with invasive procedures, liable to inflammation and secondary infections, which may cause resurgence of the cutaneous lesion in some cases. The aim of this study is to improve the molecular diagnosis of American cutaneous leishmaniasis (ACL) by the combination of the swab method, using the human saliva as new collection site, with the PCR technique for identification of *Leishmania* (*Viannia*) spp. in patients of Pernambuco State, Brazil. Among 31 patients suspected of ACL from endemic areas of the State, were collected of sublingual salivary secretions and oral mucosa with cotton swabs. DNA was extracted using a commercial kit to done the PCR technique using the LEIB1 (GGGGTTGGTGTAATATAGTGG-3') and LEIB2 (5'-CTAATTGTGCACGGGGAGG-3') primers specifics to the subgenus *Viannia*. We found positivity in 54.83% of the samples of salivary secretions and 33.3% of oral mucosa samples. Compared the present method with direct smear and biopsy-PCR, it was observed 50% of compatibility. The usefulness of the method of collection using swab salivary fluid demonstrated a new alternative for the diagnosis of ACL, especially in endemic regions, due to the lack in local health services for parasitological diagnosis. To the patient it will promote simplicity, comfort and convenience to the collection of parasites. Moreover, it provides a new discussion about the nature of these findings on issues relevant to the tropism of *Leishmania* spp. in saliva. **Financial support:** Pibic-CNPq and FACEPE (APQ-0630-2.13/08) **E-mail:** joannabiomed@gmail.com

Leish084- Real-time PCR as a tool for accessing *L. (V.) braziliensis* tissue parasitism load

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Tegumentary leishmaniasis (TL) comprises a group of protozoan disease transmitted to mammals, including humans, by phlebotomine sandflies (Desjeux, 2001). In Brazil, the majority of infection is due to *Leishmania* (*Viannia*) *braziliensis*. Cutaneous lesions (CL) are the most common clinical presentation, however about 1 to 10% of whom develop metastatic mucosal (ML) or mucocutaneous (MCL) lesions. It is well known that *L. (V.) braziliensis* lesions exhibit a low number of parasites (Ridley et al., 1989) and kDNA PCR has been considered as a higher sensitive tool for diagnosis than conventional methods (Pírmex et al, 1999). Not only detection, but also determination of parasite load is crucial for the knowledge of clinical and epidemiological aspects of the disease. In order to analyze tissue parasitism in different clinical samples (LCL n=23; CL reactivations n=14; ML n=13; skin MCL n=6; mucous MCL n=6 and scars n=7) we took advantage of real time PCR technique based on *Leishmania* small subunit RNA (SSR) gene. TL diagnosis of patients was confirmed using routine tests, as well as kDNA PCR. CL showed a significantly higher tissue parasitism than other clinical forms. Mucous infection displayed low parasite load in both ML and MCL. These results indicate that the anatomical site much more than the clinical form could be associated with parasite load. In relapses CL cases, we observed no or little difference in tissue parasitism before and after therapy. However, in scar samples it was observed scarce

or undetectable parasite levels. SSR could be considered a very useful target to access parasitism load in lesions since it was able to detect *Leishmania* DNA in some samples previously negative according to kDNA PCR. Our data indicate that quantification of parasite load by real-time SSR PCR can be an important molecular tool for clinical management of TL patients. **Supported by:** POM FIOCRUZ and PROEP – FIOCRUZ/CNPq **E-mail:** luizaper@ioc.fiocruz.br

Leish085- Using qPCR for diagnosis of canine visceral leishmaniasis and quantification of *Leishmania* sp in different tissues of naturally infected dogs

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Because infected dogs are considered to be the main domestic reservoir for *Leishmania infantum* (syn *L. chagasi*) in Brazil, the diagnosis of canine visceral leishmaniasis must be made both accurately and promptly. Serologic techniques are very sensitive but present risk of cross-reaction with other diseases. First, the authors standardized a previously described qPCR protocol, targeting the kinetoplast DNA of the parasite. Using this protocol, the authors aimed to determine which tissue confers the most accurate detection of parasite DNA. In a serological surveillance in the municipality of Jequié, an endemic area in Bahia, Brazil, forty-six dogs were randomly selected and classified according to the number of clinical signs of canine visceral leishmaniasis. Dogs with one to three signs were considered oligosymptomatic, and those that presented more than three signs were considered polysymptomatic. All dogs were euthanized and splenic and blood aspirates, as well as lymph node fragments were obtained during necropsies. Aspirates and tissue samples were immediately frozen and stored at -80°C until use. ELISA and parasite culture of spleen aspirates were performed to confirm parasite infection. For each qPCR reaction, a serial dilution containing DNA from *L. infantum* in concentrations varying from 10⁵ to 10⁻² parasites was used to generate a standard curve for gene expression quantification. Each gene's expression values were normalized against the respective value of the eukaryotic 18S rRNA constitutive gene of host tissue and parasite loads were expressed as the number of parasites per concentration (□g) of 18S rRNA gene. A ROC curve was generated to determine the positivity limit of the test. Differences between parasite loads of each tissue from oligo and polysymptomatic dogs were evaluated using Friedman test ($p < 0.05$). Using qPCR, all the 46 dogs showed positivity for the presence of parasite DNA, considering at least one of the tissues evaluated. ELISA was positive in 78% (36/46), and culture in 30% (14/46) of the dogs. Regarding the comparison of tissue analyzed, parasite DNA was highly detected in splenic aspirates, which showed positivity in 45 out of the 46 samples (97.5%, $p < 0.05$). Positivity in qPCR was detected in 78% (36/46) of blood samples, and 50% (23/46) of lymph node fragments. Using qPCR, parasite DNA was better detected in splenic aspirates in comparison with the other tissues in both polysymptomatic ($p < 0.0001$) and oligosymptomatic ($p < 0.0001$) dogs. In conclusion, splenic aspirates related to other tissues analyzed showed to be the most sensitive tissue for the detection of parasite DNA using qPCR. In our experience, spleen aspirate procedure is a well-tolerated technique, even in the most severely affected dogs. Finally, the authors recommend the use of this dog tissue to a more accurate detection of *Leishmania* infection. **Support by** FAPESB, INCT-CNPq, PDTIS, PST Veras' grant (CNPq:306672/2008-1). **E-mail:** pveras@bahia.fiocruz.br

Leish086- Polymerase chain reaction as method of diagnosis of visceral leishmaniasis in dogs

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Introduction: Leishmaniasis comprises a spectrum of diseases caused by members of the genus *Leishmania*. The visceral leishmaniasis (VL) is caused by *Leishmania chagasi* and has emerged as a new epidemiological model of disease in peri-urban cities. The most important reservoirs of the parasite are dogs, due to its close relationship with men. Given the above, this study aims to investigate the use of

PCR as a method of molecular diagnosis for canine VL in samples of peripheral blood of seropositive dogs collected by the Center for Zoonosis Control Mossoró-RN. **Material and Methods:** It was chosen a population of 23 dogs with clinical signs described for leishmaniasis. The animals were recruited from various sectors of the Mossoró-RN, in the period from September to December 2011. For each dog were collected 10ml of peripheral blood, after consent from their owners. The genomic material was extracted as described by (Grimberg et al., 1998). The PCR method was developed using primers complementary to DNA sequences of kinetoplast minicircle from *Leishmania* as described by (Smyth et al., 1992). The study was approved by the Ethics Committee of UFRN (094/06). **Results:** The detection of *Leishmania* circulating antibodies was previously confirmed by serological methods of ELISA and RIFI (1:80). The investigation was enhanced by PCR, which amplified a fragment of DNA (805pb), gel electrophoresis revealed 0.1% in all samples, confirming the presence of kinetoplast minicircle from *Leishmania* DNA. **Main Conclusions:** While it is only an indirect method of measuring the titles of canine infection, serology is still the main method used for canine VL, given its use in the national surveillance program. The major limitation of these methods is the occurrence of false positives reported. Given the above, PCR is configured as an alternative in the diagnosis of infection, independent of immunocompetence, which may predict an early diagnosis. Added to this, molecular methods contribute for identification of individual species from distinct geographical regions. **Keywords:** Visceral Leishmaniasis. PCR. Molecular Diagnosis. Immunodiagnosis. **E-mail:** wogel.uern@gmail.com

Leish087- PCR screening of visceral leishmaniasis by conjunctival swab samples in domiciled dogs of a district of Belo Horizonte, Minas Gerais, Brazil

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PCR assays have greatly improved the sensitivity of visceral leishmaniasis (VL) diagnosis in dogs. Various canine tissues (including blood, urine, skin biopsies, lymph node, bone marrow and spleen) have been used for PCR detection of the parasite. However, the non invasive samples assume great importance in this context because they are simpler, painless and more easily allowed by the dog-owners. Non invasive samplings would represent an essential tool in mass-screening survey for interventional programs. An interesting approach in this context is the conjunctival swab (CS), a method for sample collection that uses a sterile swab for sampling the dog conjunctivas. This method was shown to be highly sensitive when used for diagnosis of symptomatic and asymptomatic dogs. The purpose of this study was to evaluate the molecular diagnosis of canine visceral leishmaniasis using conjunctival swab (CS) samples in dogs living in a highly endemic area of leishmaniasis and to compare the results with those obtained by the conventional serological tests used by the Brazilian VL control program. Seventy-two dogs domiciled in the area covered by the health center of the Jardim Felicidade II district, north of Belo Horizonte city (Minas Gerais state, Brazil) were screened randomly. In parallel to the annual canine serological survey, in which blood samples were collected in filter paper, CS were also collected from both dogs conjunctives. The dogs were also clinically evaluated by a veterinarian. The DNA purification from CS was carried out by the phenol/chloroform method and after extraction DNA from both conjunctivas of the same dog was mixed constituting a single sample for the PCR assay. *Leishmania infantum* indirect fluorescent antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA) and internal transcribed spacer 1 nested PCR (ITS-1 nested-PCR) of CS samples were performed and the results were compared. All animals showed negative serological diagnosis in ELISA and IFAT tests whilst the positive PCR percentage in CS samples was 13.3% (10/72). Three of PCR positive dogs were symptomatic at clinical examination and the other seven had no apparent clinical signs suggestive of disease. In conclusion, the results demonstrate that ITS-1 nested-PCR using CS samples were able of detecting *Leishmania* infection in symptomatic or asymptomatic dogs with negative serological diagnosis. **E-mail:** rleite2005@gmail.com

Leish088- Optimization of a single tube nested PCR (STNPCR) for the diagnosis of visceral leishmaniasis

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Introduction: Nested PCR Conventional is a PCR very sensitive and specific for the diagnosis of visceral leishmaniasis. Moreover, this type of PCR is notorious for contamination problems in the product passing from the first to the second round. In order to have a PCR as efficient, but without the risk of contamination, we propose the optimization of a single tube nested PCR (STNPCR). **Materials and Methods:** We utilize the targets of the small-subunit rRNA (SSUrRNA) in the first step and ribosomal internal transcribed spacer (ITS-1) in the second stage. The STNPCR performance was compared with the nested-PCR for detection of DNA from *Leishmania (L.) chagasi*. For STNPCR, the inner primers were immobilized on the internal interface of the cap by adsorption microtubes, followed by solubilization after the first reaction. This procedure eliminated the opening of the microtube, which could lead to false-positive results by cross-contamination. **Results:** The detection limit of *L. (L.) chagasi* purified DNA was 1 fg by Nested-PCR and 10 fg STNPCR. We also tested the specificity of the system against other parasites, and observed amplification with DNA of *Trypanosoma cruzi*, reaching up to 1 pg detection. **Conclusion:** This work not only presents a promising tool for the diagnosis of visceral leishmaniasis, as well as a new target to be exploited in the diagnosis of Chagas' disease, either in mono-infection with *T. cruzi*, or co-infection with *Leishmania* spp. **E-mail:** almerice_lopes@yahoo.com.br

Leish089- Evaluation of polymerase chain in blood and lesion for the diagnosis of American cutaneous leishmaniasis

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American cutaneous leishmaniasis (ACL) can occur in the cutaneous and mucosal forms, causing disfiguring injuries. The laboratory diagnosis of ACL includes immunological methods and detection of the parasite, which present limitations. The polymerase chain reaction (PCR), due to high sensitivity, has been employed for diagnostics of ACL. The objective was to evaluate the PCR in lesion (PCR-L) and in peripheral blood leukocytes (PCR-S), compared to conventional techniques, such as parasite direct search (PD), Montenegro skin test (MST) and indirect immunofluorescence (IIF). We investigated 223 patients on suspicion of ACL, from the northwestern Paraná. For PCR, the material of lesion was collected by scraping the inside edge; the blood was added to a solution of Dextran 6% and EDTA 10% for obtaining leucocytes. The DNA lesion was obtained by boiling and the blood, by Guanidine-phenol. For amplification, we used MP3H/MP1L primers, which generate a minicircle fragment with 70 base pairs of *Leishmania (Viannia)* kDNA. The amplified products were analyzed by agarose gel electrophoresis. The positivity of the PD was 29.4% (58/197), of the IIF was 40.0% (88/220), of the MST was 43.7% (73/167), of the PCR-L was 41.6% (81/195), and of the PCR-S was 27.0% (31/115). The cutaneous form of the ACL was the most frequent, with 89.7% (200/223). The mucosal form was observed in 23 patients, and among those who performed the tests, the PD was negative (0/4), the PCR-L was positive in 50.0% (3/6), and the PCR-S in 38.1% (8/21). Five patients had positive result only in PCR-L, which 4 were cases of relapse, while others 2 patients showing only PCR-S positive. These results show that PCR techniques have good performance positivity when compared to PD, and shows advantage over MST, for not being intrusive. Since the DNA detection of the parasite gives high security to its presence, we found that PCR techniques can be a valuable tool for the ACL diagnosis, especially in patients with chronic lesions and who have received a specific treatment, or in patients with recurrent or reinfection with *L. (V.) braziliensis*. Our results suggest that the PCR-S is also important for detecting dissemination cases, that can generate serious mucosal forms, with the PCR-S using the technique of leucocytes separation presented greater positivity than other PCR techniques in blood. The methodology of PCR-S described here, employing peripheral blood leukocyte shows sensitivity comparable to that presented by the PD, and can be an alternative for those cases in which you cannot obtain material from lesion, with the advantage of

providing information about the species infecting organism. **Support:** Fundação Araucária, CNPq, CAPES. **E-mail:** mateus_ve@hotmail.com

Leish090- Evaluation of DNA extraction methods and PCR sensivity on detection of *Leishmania (Viannia) braziliensis* DNA from *Lutzomyia (Nyssomyia) intermedia* s.l.

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American Cutaneous Leishmaniasis (ACL) is an important zoonosis. The method for investigation of *Leishmania* from vector is the microscopic visualization after dissection of the digestive tract of the insect. The polymerase chain reaction (PCR) is characterized by its high sensitivity in detecting *Leishmania* in sandflies regardless of parasite load in the sample. The objective of the current study was to optimize the DNA extraction techniques and to determine the sensitivity of the PCR technique in detecting *Leishmania (Viannia) braziliensis* DNA from sand fly *Lutzomyia (Nyssomyia) intermedia* s.l. For this study, sand flies were obtained from July through October, 2010, from Adrianópolis city, situated around the Ribeira Valley, in Parana State, southern Brazil. CDC light traps were installed peridomicile inside the houses and forest for 12 hours (18 pm to 6 am). After the capture, the insects were identified and separated in vials containing ethanol 80%. Promastigotes were obtained by growing *L. (V.) braziliensis* in RPMI culture. To analyze the PCR sensitivity, four groups of 1, 3, 6, and 10 male sand flies were formed and to them 10, 50, and 100 *Leishmania* promastigotes were added. The DNA extraction was performed by three methods: Chelex 100 (BioRad), phenol/chloroform and commercial kit ChargeSwitch®. The PCR results were evaluated through horizontal electrophoresis in 1.5% agarose gel. The extraction methods showed different results. With the phenol/chloroform method, despite the lower cost, the PCR was not efficient as it showed unspecific bands and delay in its implementation, about three days to run. The ChargeSwitch® kit appeared to be an efficient technique for DNA extraction maintaining the integrity of DNA. The Chelex technique presented satisfactory results in addition to being an easier technique to conduct with labor time of approximately one hour and it was of low cost. Despite the good results obtained with the kit ChargeSwitch®, the cost was higher and the extraction time was twice as higher in contrast with Chelex. Thus, Chelex was the best technique for DNA extraction allowing satisfactory detection of DNA from *L. (V.) braziliensis*. The PCR sensitivity in detecting DNA from *L. (V.) braziliensis* was positive for all groups.
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Leish091- Development of a new Duplex Real Time PCR for Visceral Leishmaniasis diagnosis with sample quality control by TaqMan probe technology

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Introduction: Visceral Leishmaniasis (VL) is a severe disease which can lead to death, and its early detection is important to avoid severe damage to the individuals affected. Molecular methods to detect *Leishmania* can be considered as alternatives to overcome the limitations presented by conventional methods. Real-time PCR is an improved PCR system with numerous advantages compared to the conventional gel-based assays as higher sensitivity and specificity, the quantitative determination of pathogen loads, the shorter running time and the significantly reduced risk of contaminations. To exclude false negative results caused by PCR inhibitors or loss of DNA during the sample storage or extraction step, an internal control (IC) system can be included in a duplex PCR setup. This option of independent amplification of an IC increases the reliability of the test results and is time and cost saving in the long run. **Objective:** The aim of this study is to develop duplex PCR system able to detect small amounts of target DNA of *Leishmania infantum* and the gene coding for glyceraldehyde-3-phosphate dehydrogenase

(G3PD) in mammals by TaqMan probe technology, enabling quality evaluation of the sample simultaneously with detection of the specific target. **Materials and Methods:** With the aid of the Primer Quest© software, was designed 4 probes, compatible with the system LINF 1B (Paiva-Cavalcanti et al. 2009). Based on the melting temperature (T_m) one of them was chosen to be synthesized with the TaqMan probe technology (Applied Biosystems). The specificity analysis was performed by MEGA 4.0 software. The set formed (LINF 1B primers and probe) will be combined with the kit TaqMan® Reagents GAPDH Control (Human) (Applied Biosystems) and tested by keeping the standardized conditions by Paiva-Cavalcanti et al. (2009). **Results:** The probe sequence chosen is 5'-AAATGGGTGCAGAAATCCCGTTCAAA - 3', T_m 59.2 °C, compatible with the amplified sequence by LINF 1B and cycling conditions previously standardized. The alignment of the chosen sequence showed specificity for the conserved region of kDNA of *Leishmania infantum* amplified by the LINF 1B system. **Conclusion:** The observed parameters during the construction of the probe demonstrate potential success in the standardization of duplex PCR, generating a new technological tool for the diagnosis of leishmaniasis. Standardization of a duplex PCR with IC amplification simultaneously with the target will limit the maximum possibility of false-negative results, fulfilling the need for a technique with quantitative ability. This technique will allow monitoring of patients, and contribute to the enhancement of knowledge related to parasite load and parasite-host interaction, providing useful information to improve the treatment and control measures. **Financial Support:** FACEPE, CPqAM-FIOCRUZ **E-mail:** mp@cpqam.fiocruz.br

Leish092- Detection of *Leishmania infantum* by conventional and real time PCR in animals and their ectoparasites in Pernambuco, Brazil

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Introduction: Visceral leishmaniasis (VL) is a parasitic zoonosis caused by *Leishmania infantum*, which constitutes a serious public health problem worldwide. The only proven vectors of *L. infantum* are phlebotomine sand flies, but scientific evidence indicate that other vectors might eventually be involved, including ticks and fleas. There has been a growing scientific interest about the possible vectorial competence of the tick *Rhipicephalus sanguineus*, which has been found naturally infected by *L. infantum*, being able to transmit, under experimental conditions, the parasites to rodents, opening new perspectives in the epidemiology of zoonotic VL. This study was aimed to verify the involvement of domestic animals and their ectoparasites in the epidemiology of VL in Pernambuco State, Brazil. **Material and Methods:** Two rural areas from Pernambuco were selected (Site A and B). Genomic DNA was extracted from blood samples and ectoparasites (ticks, fleas, and lice) using different commercially available DNA extraction kits. DNA from blood samples and ectoparasites were tested using a conventional PCR and a quantitative real time PCR. **Results:** A total of 282 (five cats and 277 dogs) blood samples and 117 ectoparasites from dogs were collected. Ectoparasites were found only in dogs (35.74%). Overall, 76.24% of the animals and 43.59% of ectoparasites were positive for *L. infantum* in at least one technique, with parasite DNA load averages of 738.83 fg and 9.12 fg, respectively. Concerning the relationship between the positivity in dogs and their ectoparasites, 12.32% of the positive dogs were parasitized by positive ectoparasites. **Conclusion:** The high prevalence of animals positive for *L. infantum* indicates the potential risk for possible zoonotic VL outbreaks in the studied areas. The results show that new paradigms are emerging in the epidemiology of VL in Pernambuco, reinforcing the need for studies to the direction and implementation of prevention and effective control measures. **Financial Support:** FACEPE, CPqAM-FIOCRUZ **E-mail:** mp@cpqam.fiocruz.br

Leish093- Comparison of molecular methods and clinical samples for visceral leishmaniasis diagnosis in asymptomatic dogs

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Introduction: Visceral Leishmaniasis (VL) is a serious public health problem in Brazil. In the urban area the dog is the main reservoir and VL control in Brazil involves the elimination of infected dogs. Serological tests are used for routine surveys, but they present problems of specificity and sensitivity. In addition, serological test performance depends on infection status and an important limitation in VL control programs is the inability to identify asymptomatic dogs because these tests are insufficiently sensitive. Molecular methods as the kDNA PCR PCR-hybridization are useful in the diagnosis and identification of *Leishmania* species. The kDNA PCR-hybridization uses radioactive probes to improve the sensibility of the PCR assay and to allow the discrimination between *Leishmania* species. The aim of this work was to compare the kDNA PCR-hybridization sensitivity with other three molecular methods used for VL diagnosis, in different clinical samples of asymptomatic dogs. **Material and Methods:** Bone marrow, peripheral blood, conjunctival swabs and skin biopsies were analyzed by the methods kDNA PCR-hybridization, kDNA semi nested PCR (kDNA snPCR), *Leishmania* nested PCR (LnPCR) and *Internal Transcribed Spacer 1* nested PCR (ITS-1 nPCR). Thirty positive asymptomatic dogs with positive serological and parasitological tests were used. Six non infected dogs were used as controls. The DNA extraction from swabs was performed by Phenol-Chloroform method. Commercial kits were used for DNA extraction from peripheral blood, bone marrow and skin biopsies. **Results:** The kDNA PCR-hybridization detected 5/30 (16.7%) positive dogs for peripheral blood samples, 17/30 (57%) for skin biopsies, 19/30 (63.3%) for bone marrow and 21/30 (70%) for conjunctival swabs. The kDNA snPCR found 7/30 (23.3%) positive dogs using peripheral blood, 17/30 (57%) for skin biopsies, 12/30 (40%) for bone marrow and 24/30 (80%) by conjunctival swabs. The LnPCR method detected 9/30 (30%) positive dogs for peripheral blood samples, 15/30 (50%) for bone marrow, 13/30 (43.3%) for skin samples and 19/30 (63.3%) for conjunctival swabs. The ITS-1 nPCR analyses showed 19/30 (63.3%) positive dogs for peripheral blood, 19/30 (63.33%) for skin biopsies, 29/30 (97%) for bone marrow and 29/30 (97%) for conjunctival swabs. **Main Conclusions:** The higher positivity (97%) were obtained using the ITS-1 nPCR for samples of conjunctival swab and bone marrow. Interestingly, conjunctival swabs which are non-invasive and easily obtained produced similar sensitivity than invasive bone marrow specimens. **E-mail:** antero@cdtn.br

Leish094- Comparison of traditional methods and PCR for American Cutaneous Leishmaniasis diagnosis in Instituto de Infectologia Emilio Ribas, São Paulo, SP- Brazil.

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Introduction: The routine diagnosis of American cutaneous leishmaniasis (ACT) is based on epidemiological data, clinical examination, parasitological laboratory methods and the immunological test. Unfortunately, the ACT present a wide spectrum of clinical manifestations resulted from the combination of the immune response of the host, species of the infecting *Leishmania* and the intrinsic characteristic of the parasite. All these factors difficult ACT diagnosis and limit the sensibility of the diagnosis methods. Besides this, none of them are able to discriminate the leishmania species implicate in the pathological process, one of the actual clinical questions for directing a better therapy. The technique of polymerase chain reaction (PCR) using specific primers (as the ones direct to kDNA) has been reported as a useful tool for ACT diagnosis. Thus, here we present a comparison between the traditional methods and the PCR for ACL diagnosis. **Material and methods:** 130 skin or mucosal biopsies were collected from clinically suspected ACL patients attended in IIER, São Paulo, Brazil. Epidemiologic information, clinical

data, progress of the disease and intradermal reaction of Montenegro result were obtained by consulting medical reports. Microscope examination with biopsy imprinting slides, *in vitro* isolating, culturing of the parasites and PCR were performed in IMT-USP, SP. DNA samples were amplified with kDNA primers and posterior restriction fragment length polymorphism (RFLP) technique allowed us to detect the patients infected with *Leishmania (Viannia) braziliensis*. **Results:** PCR was able to detect the DNA of *Leishmania* in 90% of suspected ATL patients, whether traditional methods had the following percentage of positivity: 60.93% for MST, 60.52% for DI and 18.3% by *in vitro* culture. The molecular method was able to detect all positive samples for the traditional method. The RFLP technique detected the *Leishmania (V.) braziliensis* species in 85 infected patients. **Main Conclusions:** The PCR was easier to execute, more efficient and faster than other traditional methods. Molecular method was not influenced by type of clinical manifestation, secondary infections present in some lesion, immune response of the host or age of lesion as occurred for other traditional methods. The identification of *Leishmania (V.) braziliensis* by RFLP technique can contribute in the prognostic of disease and direct the treatment. Thus, PCR-RFLP technique could be applied as an alternative laboratory method for confirmation of the disease and further studies must be done in order to validate it as a specific tool for ACL diagnosis. **Supported by:** CNPQ, LIM 48 and FMUSP **E-mail:** pccotrim@usp.br

Leish095- Comparison of the Real-time Polymerase chain reaction (qPCR) and Conventional PCR to detect the genome of *Leishmania* sp in biological samples

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Introduction: Visceral leishmaniasis is caused by *Leishmania* sp and affects a growing number of people in Brazil. The metropolitan region of Belo Horizonte is considered one of the endemic regions for this disease. The diagnosis of leishmaniasis consists mainly of four groups of tests: i) parasitological ii) immunological tests, iii) molecular diagnosis and iv) clinical tests. The Polymerase Chain Reaction (PCR) has proven to be effective in detecting kDNA (kinetoplast DNA of the parasite) present in peripheral blood and fragments of bone marrow lesions, presenting high sensitivity and specificity. In this study, we compared the results of conventional PCR and Real-Time PCR and the application of these techniques in molecular diagnosis of visceral leishmaniasis, using the peripheral blood as a biological sample of the patients. **Objective:** The objective is to offer the patient of the (SUS) Unified Health System funded by the Brazilian government a differential diagnosis by molecular biology, aiding the clinician in completing the diagnosis of leishmaniasis. **Methodology:** This project is approved by the Ethics Committee (CEP) of the Santa Casa de Belo Horizonte Hospital, with the protocol number 021/2010. The genus-specific conserved region of kinetoplast DNA (kDNA) comprising 120 bp, was the target of amplification. For this purpose we used the primer pair 150-152 in both PCR techniques. We studied 20 samples from patients with proven leishmaniasis who were treated by the Medical Clinic of Santa Casa de Belo Horizonte Hospital and 10 healthy people as control group. Results: Preliminary results show that both techniques showed the same ability to detect the genome of *Leishmania* sp. The Conventional PCR has a lower cost than the real-time PCR. Meanwhile, the Real-Time PCR has the advantage of being able to obtain the result in a day, whereas the conventional PCR requires almost two days for reading the result. **Conclusions:** These results demonstrate that both PCR techniques and tools are important and should be standardized for the diagnosis of leishmaniasis, especially in cases where other clinical and laboratory tests cannot make the diagnosis. **Support:** FAPEMIG/CNPq **E-mail:** rachelbc@santacasabh.org.br

Leish096- Canine Visceral Leishmaniasis in the State of Rio de Janeiro: Diagnosis by PCR and Genetic Study of Parasitic Populations.

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Introduction: Canine visceral leishmaniasis (VL) caused by infection with *Leishmania (Leishmania) chagasi* is endemic in the Municipality of Rio de Janeiro. The PCR technique allows detecting the presence *Leishmania* DNA directly from clinical samples, showing advantages over conventional methods. Different PCR-based technique has enabled, besides detection and typing, the study of genetic diversity of *Leishmania (Leishmania) chagasi*. **Materials and methods:** 43 biopsies, such as: fragments of intact skin, spleen, liver and lymph nodes (cervical and popliteal), cutaneous lesions naturally infected dogs and sororretores to *L. (L.) chagasi* were used. Biopsies were submitted to PCR assays using specific primers directed to the variable region of kDNA minicírculos of members of the *L. donovani* complex. Low- stringency single-specific primer PCR (LSSP-PCR) and restriction fragment length polymorphisms-PCR (RFLP-PCR) analyses were employed in order to detect genetic polymorphisms of natural clones, in relation to tissue tropism in the different anatomical sites. Restriction fragment length polymorphisms-PCR analyses were performed using Hinf I and Rsa I restriction enzymes. The matrix of genetic profiles was transformed into a tree using the UPGMA algorithm and the Simple Matching coefficient of similarity. **Results:** 43 biopsies of different anatomical sites from a total of seven dogs were analysed. The presence of *L. (L.) chagasi* DNA was detected by PCR in 100% of the fragments of skin, spleen and lymph nodes (cervical and popliteal), only in two dogs the presence of *L. (L.) chagasi* DNA was detected in mesenteric lymph nodes. Two dogs with cutaneous lesions showed to be positive for *L. donovani* complex. Through the LSSP-PCR and RFLP-PCR techniques it was possible to note that parasite populations present in different anatomical sites exhibited distinct genetic polymorphisms. However, identical profiles were detected in different anatomical sites in the same dog. The genetic profiles generated by LSSP- PCR and RFLP-PCR were grouped into 3 and 2 separate clusters with coefficients of similarity ranging from 0.55 to 1.00 and 0.55 to 0.96, respectively. The present study demonstrates intraespecific genetic variability of *L. (L.) chagasi* in the distinct organs. **Conclusion:** It was possible to demonstrate that *L. (L.) chagasi* infection can be manifested by parasite spreading with genetically distinct subpopulations in different tissue tropism. **Support:** CNPq and Instituto Kinder do Brasil. **E-mail:** rpacheco@ioc.fiocruz.br

Leish097- Comparing of four DNA extraction methods for analysis of urine from patients with leishmaniasis visceral

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Efficient and reliable methods of diagnosis are necessary for successful control program of visceral leishmaniasis. However, parasitological techniques are invasive, and difficult to apply in field studies. In relation immunological assays are not entirely satisfactory. Those do not discriminate between disease and asymptomatic infection, and the risk of having cross-reactions. PCR has been seen as an alternative diagnosis when microscopy and serology are negative or indeterminate. This approach has been standardized in various body fluids, including urine. Urine has the advantage of collecting non-invasive, inexpensive and easy to perform. For good performance of PCR is essential that the DNA extraction is effective, producing a DNA pure and free of inhibitors. The purpose was to compare three methods of DNA extraction for PCR analysis in urine. Using samples with known amounts of DNA from *Leishmania infantum*, the following extraction protocols were compared: the commercial kits illustra blood genomicPrep Mini Spin Kit (GE), QIAamp DNA Mini Kit (QUIAGEN) and extraction of phenol-chloroform preceded by precipitation with ethanol. Then, the protocols are tested on samples of urine of subjects presenting with parasites in bone marrow aspirate. PCR was performed using the primer pairs RV1/RV2 that amplify 145 bp products from the region of kDNA. Both extractions with phenol-chloroform as the QIAamp DNA Mini Kit (QUIAGEN) showed a good sensitivity for extracting DNA to less than one parasite. Of the ten urine of patients with VL, five were positive for extracting phenol-chloroform, two samples positive by QUIAGEN kit and three samples by GE kit. There was no agreement on positive results by the three extraction protocols in specimen. However, in two specimens, all the three protocols resulted in no amplifications. Despite the poor performance of the GE kit on the sensitivity test, it was able to purify DNA from three samples of subjects with LV that other protocols could not. The method of phenol-chloroform, introduced by Sambrook et al (1989) is still used in research and diagnostics. This would be more suitable for purification of DNA in urine. It is noteworthy that, although lower cost than the commercial kits, this method uses toxic solvents. **E-mail:** almerice_lopes@yahoo.com.br

Leish098- Comparison of PCR, Direct Agglutination Test (DAT) and *rK39* test (rapid diagnosis) for diagnosis of visceral leishmaniasis in immunocompetent patients

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Introduction: The major features of visceral leishmaniasis (kala azar) are intermittent fever, enlargement of the spleen, and pancytopenia. The disease leads to death if it is not treated. The agents responsible for kala azar are *L. infantum*, *L. chagasi*, and *L. donovani*. However, parasitological techniques are invasive, and difficult to apply in field studies. In relation immunological assays are not entirely satisfactory. Those do not discriminate between disease and asymptomatic infection, and the risk of having cross-reactions. PCR has been seen as an alternative diagnosis when microscopy and serology are negative or indeterminate. **Materials and Method:** A PCR assay amplifying a repeated sequence from the *Leishmania infantum* genome was compared with Direct Agglutination Test (DAT) and *rK39* test (rapid diagnosis) for the diagnosis of visceral leishmaniasis in immunocompetent patients. PCR was performed using the primer pairs RV1/RV2 that amplify 145 bp product from the region of kDNA. Of 25 patients with suspected for visceral leishmaniasis by physicians, 11 had an indisputable diagnosis of visceral leishmaniasis by direct examination of bone marrow aspiration. **Results:** Both serological tests performed 100% sensitivity for diagnosing visceral leishmaniasis. PCR exhibited sensitivity of 54.5% in comparison with bone marrow aspirate, DAT and *rK39*. The specificity values for PCR, DAT and *rK39* were 64.3%, 54.5% and 78.5% respectively. **Main Conclusions:** Our results differed from other authors, because PCR was not the best tool for the diagnosis of VL. Further studies should be made to adapt the PCR into our reality. According to this previous study, the rapid test would be the best choice to replace the bone marrow aspirate for diagnosis of VL in our region. **E-mail:** almerice_lopes@yahoo.com.br

Leish099- The Diagnostic Accuracy of Serologic and Molecular Methods for Detecting Visceral Leishmaniasis in HIV Infected Patients: Meta-Analysis.

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Background: Human visceral leishmaniasis (VL), a potentially fatal disease, has emerged as an important opportunistic condition in HIV infected patients. In immunocompromised patients, serological investigation is considered not an accurate diagnostic method for VL diagnosis and molecular techniques seems especially promising. **Objective:** This work is a comprehensive systematic review and meta-analysis to evaluate the accuracy of serologic and molecular tests for VL diagnosis specifically in HIV-infected patients. **Methods:** Two independent reviewers searched PubMed and LILACS databases. The quality of studies was assessed by QUADAS score. Sensitivity and specificity were pooled separately and compared with overall accuracy measures: diagnostic odds ratio (DOR) and symmetric summary receiver operating characteristic (sROC). **Results:** Thirty three studies recruiting 1489 patients were included. The following tests were evaluated: Immunofluorescence Antibody Test (IFAT), Enzyme linked immunosorbent assay (ELISA), Immunoblotting (Blot), direct agglutination test (DAT) and polimerase chain reaction (PCR) in whole blood and bone marrow. Most studies were carried out in Europe. Serological tests varied widely in performance, but with overall limited sensitivity. IFAT had poor sensitivity ranging from 11% to 82%. DOR (95% confidence interval) was higher for DAT 36.01 (9.95-130.29) and Blot 27.51 (9.27-81.66) than for IFAT 7.43 (3.08-1791) and ELISA 3.06 (0.71-13.10). Although specificity was generally high for all serological tests, there is unexpectedly high variation in specificity among studies evaluating the same test. PCR in whole blood had the highest DOR: 400.35 (58.47-2741.42). The accuracy of PCR based on Q-point was 0.95; 95%CI 0.92-0.97, which means good overall performance. **Conclusion:** Based mainly on evidence gained by infection with *Leishmania*

infantum chagasi, serological tests should not be used to rule out a diagnosis of VL among HIV-infected, but a positive test at even low titers has diagnostic value when combined with the clinical case definition. Considering the available evidence, tests based on DNA detection are highly sensitive and may contribute to a diagnostic workup. **Financial support:** FAPEMIG **E-mail:** glauciacota@uol.com.br

Treatment

Leish100- Treatment of mucosal lesions of the upper respiratory and digestive tracts of patients with american tegumentary leishmaniasis with low doses (5mg Sb⁵⁺/Kg/DAY) of meglumine antimoniate

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Introduction: Pentavalent antimonials in 10 to 20mg Sb⁵⁺/kg/day doses are still the first drug of choice for American tegumentary leishmaniasis (ATL) treatment despite reports of low efficiency and high number of adverse effects, including death. This study aims at describing the efficiency and safety of 5mg Sb⁵⁺/Kg/day of meglumine antimoniate in treating patients with mucous or mucocutaneous (ML/MCL) ATL over sixteen years at the Evandro Chagas Clinical Research Institute (IPEC), Oswaldo Cruz Foundation, Rio de Janeiro, Brazil. **Material and Methods:** A retrospective and descriptive study was conducted with a longitudinal surveillance of a series of cases. Data were obtained from medical records of patients with ML/ MCL treated at IPEC Otorhinolaryngology Department between January 1st, 1989 and December 31, 2004 with low doses of meglumine antimoniate applied continuously or intermittently (in series with rest intervals). **Results:** From a total of 1667 patients with ATL treated over that period, 148 (8.9%) presented ML/MCL. From those, 98 (66.2%) treated with 5mg Sb⁵⁺/Kg/day of meglumine antimoniate were included in the present study. Most patients were men who performed activities with risk for ATL in Southeastern Brazil. The most frequent clinical presentation was late mucosal impairment with nasal involvement. Patients undergoing serial treatment were mostly older and possibly with more co-morbidities. Both therapeutic plans presented good efficiency with few adverse effects. Even in patients needing retreatment, these therapeutic plans were well tolerated and efficient. Patients treated with amphotericin B or high dose of meglumine antimoniate, after treatment with low dose, presented an adequate response. **Conclusion:** Treatment of ML/MCL with 5mg Sb⁵⁺/Kg/day meglumine antimoniate was effective and well tolerated, with 91.7% healing, even when more than one treatment was needed. **E-mail:** claudia.valete@ipec.fiocruz.br

Leish101- Influence of chemical composition of meglumine antimoniate on its pharmacokinetics and efficacy by the oral route in murine model of visceral leishmaniasis

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Introduction: Meglumine antimoniate (MA) is the first choice drug for the treatment of leishmaniasis in Brazil. Despite its efficacy, this drug has disadvantages such as requirement for parenteral administration, long-term therapy and side effects like pain and cardiac complications, which favors the abandonment of

treatment. An oral drug could facilitate the treatment, decrease costs with hospitalizations and improve therapeutic compliance. Therefore, the aim of this work was to determine the physicochemical properties, pharmacokinetics and efficacy by oral route of MA obtained from two different sources: synthetic MA prepared at UFMG (sMA) and commercial drug (Glucantime Aventis®) (Glu). **Materials and Methods:** The physicochemical characterization was performed through osmolality measurements, elemental analysis of C, Na⁺, K⁺, Cl⁻ and Sb and the permeation studies across regenerated cellulose membrane MWCO of 3000 daltons. In pharmacokinetic study, Swiss mice received sMA or Glu by oral or intravenous route at 300 mg Sb/kg and the serum was collected at different time intervals for quantification of Sb by atomic absorption spectroscopy and pharmacokinetic parameters were determined (R-STRIP software). To evaluate the efficacy, female BALB/c (n = 5/group) infected with *L. (L.) chagasi* were treated orally at 300 mg Sb/kg/12h for 30 days. The control group received saline (CONT) and the positive control received Glu (80mg Sb/kg/day) by intraperitoneal (IP) route. Parasite loads were determined in spleen and liver by limiting dilution analysis. **Results:** sMA was found to consist in a zwitterionic 2:2 Sb-meglumine complex, while Glu complex showed a 2:3 Sb-meglumine exhibiting a net positive charge. sMA showed significantly greater permeation efficiency through cellulose membrane than Glu. Pharmacokinetic parameters for sMA and Glu in serum given orally were respectively 22.051 and 12.653(mg/l) for C_{max}, 33.549 and 54.537 (min) for T_{max}, 2013 and 1885 (mg.min/L) for AUC and 9.87 and 5.97% for bioavailability, indicating a higher absorption rate of sMA. Following treatment of infected mice, both compounds showed significant reduction of parasite load in the liver compared to CONT. On the other hand, only sMA promoted significant reduction of parasite load in the spleen. The level of reduction of parasite load after oral treatment with sMA was equivalent to that obtained after IP administration of Glu. **Main conclusions:** sMA showed higher efficacy than the commercial drug after oral administration in a murine model of visceral leishmaniasis. This result can be explained by the lower molecular weight and zwitterionic character of the sMA, resulting in higher absorption by oral route. **Financial Support:** CNPq / FAPEMIG / CAPES / FIOCRUZ-CPqRR **E-mail:** kellykato@yahoo.com.br

Leish102- Effectiveness of low dose antimony long time after therapy for cutaneous leishmaniasis in Rio de Janeiro State, Brazil

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Introduction: Cutaneous leishmaniasis (CL) is the most frequent clinical form of American tegumentary leishmaniasis in Brazil, whereas mucosal disease (ML) is rare. CL is characterized by a painless ulcerated lesion and *Leishmania (Viannia) braziliensis* is the only isolated specie in Rio de Janeiro (RJ) metropolitan region. Pentavalent antimony (Sb⁺) is used for treatment since 1912 and may cause heart and kidney adverse effects. The Brazilian Health Ministry recommends 10-15mg of Sb⁺/kg/day by 3 weeks. Low Sb⁺ doses (5mg/kg/day/30 days) could cure CL. If this therapy increases relapses or evolution to ML long time after therapy is not known. Our goal was to show if low doses are as effective as regular doses long time after therapy. **Methods:** 175 CL patients treated with 5mg Sb⁺/kg/day/30 days from IPEC/Fiocruz-RJ that have finished treatment at least 5 years ago were included. Field visits were done for clinical evaluation. **Results:** after 26 field visits 45 patients were found and examined 11-25 years after treatment. During active disease all patients had typical CL lesions and positive Montenegro Skin Test (mean 25.8mm). Patients' age ranged from 5-76 years-old. Mean time of evolution was 3 months with 1.7 lesions per patient. Limbs were affected in 68%. Parasitological diagnosis was possible in 28 cases (PCR, imprints, culture and/or histopathology). After treatment, 41 (91.1%) cured and 4 patients (8.9%) needed a second treatment with regular doses of 15-20mg Sb⁺/kg/day. One patient (2.3%) developed ML after one year and was cured with 20mgSb⁺/kg/day and Amphotericin-B. The mean accumulated Sb⁺ in the low doses patients was 8,574 mg/patient. If they were treated with regular doses it would be 18,479 mg/patient. The others 130 patients were not found due to: not localized address (35%), unknown in the address (17%); moved to unknown place (12%); others causes - death, dangerous neighborhoods, not found at home - (36%). **Conclusion:** these results suggested that low Sb⁺ dose therapy was as efficient as regular doses even long time after therapy (11-25 years). No resistance was noted to regular doses applied after an initial low dose treatment. The cure was reached in 91% of cases with half of the recommended dose, which may cause less adverse effects and are of easier

administration. A mean economy of US\$93.00/patient was achieved. One of our patients developed ML. Anyhow; this form occurs in 3-5% of all CL cases. We believe that CL resistance to Sb⁺ may occur independent of the used dose since regular dose therapy may occasionally fail. We believe that low Sb⁺ doses are effective even very long time after therapy at least with *Leishmania* species of RJ State. **E-mail:** ricardog@ioc.fiocruz.br

Leish103- A prospective observational study of adverse drug reactions in patients using meglumine antimoniate to treat visceral leishmaniasis at a hospital in Brazil

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Introduction: In Brazil, visceral leishmaniasis (VL) is a prevalent disease and represents an important public health issue. According to the Ministry of Health, 3,526 cases were confirmed in 2010, including 469 patients in the southeast state of Minas Gerais. Meglumine antimoniate is the first choice drug to treat VL and may exhibit high toxicity to the patients. Close monitoring and early detection of adverse drug reactions (ADR) are a key element to prevent patients from adverse events related to this drug. This study aimed to investigate the meglumine antimoniate-related ADR in VL inpatients. **Material and Methods:** A prospective observational study was performed to follow up VL cases treated with meglumine antimoniate at a private hospital, from December 2010 to June 2011. Patients of both genders without age limit were enrolled if they presented positive serology for VL. Patients who refused to participate during the follow up were excluded. Data were collected by chart review and patient interview. Cardiotoxicity, pancreatitis, liver/kidney dysfunction and skin reactions were considered as outcomes. The Naranjo algorithm was used to evaluate the causality of the suspected ADR. This research project was approved by the Institutional Ethics Committee and all participating patients signed a written informed consent. **Results:** A total of 14 patients were included in this study. Their age ranged from 14 to 60 years and nine patients were male. Suspected ADR were reported for seven cases. The first patient complicated with cardiotoxicity and the second patient had liver dysfunction, pancreatitis and skin allergy. For both cases, meglumine antimoniate was replaced by amphotericin B deoxycolate. The third and fourth patients had liver dysfunction and the fifth patient presented a skin allergy. The sixth patient had liver dysfunction and pancreatitis and the seventh patient presented kidney dysfunction. For the latter two cases, meglumine antimoniate was replaced by liposomal amphotericin. The Naranjo algorithm classified all reactions as probable. **Main conclusions:** This case series including VL inpatients treated with meglumine antimoniate suggests that this drug is probable to be associated to high toxicity. Half patients presented clinical complications during the follow up. The observed reactions may be frequent during drug therapy and cause severe clinical complications. Pharmacovigilance strategies should be performed to minimize the morbidity and mortality related to the use of meglumine antimoniate. **E-mail:** aquila.serbate@gmail.com

Leish104- Mucosal leishmaniasis (ML) with low adherence to pentavalent antimonials (Sb⁵⁺), intolerance to amphotericin B and with good response to pentamidine

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Introduction: In Brazil, 20mg Sb⁵⁺/kg/day for 30 days is considered to be the first choice regimen for the treatment of ML, despite reports of low efficacy and high number of side effects. At IPEC - FIOCRUZ, the dose of 5mgSb⁵⁺/kg/day have been an effective and well tolerated treatment for patients with ML. In the absence of a therapeutic response, the second drug of choice (amphotericin B) is used, although it is poorly tolerated sometimes. The third drug of choice is pentamidine that is known for the possibility of

development of diabetes mellitus. **Material and Methods:** The case of a man with ML caused by *Leishmania (Viannia) braziliensis* with multiple recurrences over 13 years of follow-up in IPEC is reported. **Case Report:** Thirteen years ago, a man, 50 years old, had rhinorrhea, nasal obstruction and dysphonia, infiltrative lesions in the nasal septum, palate, uvula and pillars, ulcer-destructive lesions in inferior turbinates, without skin lesions or scars, MST 15mm, histopathology with chronic granulomatous inflammation with the presence of amastigotes and *Leishmania sp.* promastigotes were isolated in culture. Treatment with 5mgSb⁺/kg /day was discontinued and abandoned in the fourth dose after laryngeal edema. Restarted treatment with improvement of the lesions, there was another drop in the thirty-dose. After six years, relapse occurred with partial destruction of the nasal septum and inferior turbinates, the uvula and epiglottis, with good response to therapy with 47 doses of 5mgSb⁺/kg/day intermittent schedule. One year later, the second recurrence was treated successfully with 3g of amphotericin B. Another relapse after two years was treated with 80 doses of 5mgSb⁺/kg /day, with no significant improvement. He was treated with amphotericin B deoxycholate, interrupted with 50mg because of nephrotoxicity and was replaced by liposomal amphotericin B, also stopped by with 200mg because of nephrotoxicity. The patient was submitted to pentamidine (4mg/kg) with improvement of symptoms and healing of the lesions. **Conclusion:** The administration of pentamidine was well tolerated in a patient with ML with poor adhesion and resistance to meglumine antimoniate and intolerance to amphotericin B and the outcome was satisfactory. **Support:** IPEC-FIOCRUZ, CNPq and FAPERJ, Brazil. **E-mail:** claudia.valete@ ipec.fiocruz.br

Leish105- Antimicrobial peptides isolated from tree frog *Phyllomedusa hypochondrialis* are effective against *Leishmania* and *Trypanosoma* parasites

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Among the tropical parasitic diseases, those caused by protozoan parasites present a major challenge to public health, being represented by leishmaniasis and Chagas disease. Considering the high toxicity and the lack of effective drugs, the need for novel treatments is urgent. Amphibian secretions represent a myriad source of effective antiparasitic peptides, and if adequately studied, could provide novel drug prototypes. In this study, by using bioguided fractionation, we isolated five anti-parasitic peptides from the secretion of tree frog *Phyllomedusa hypochondrialis*, and studied their effectiveness against *Leishmania infantum* and *Trypanosoma cruzi* parasites. The antiparasitic fractions were characterized by mass spectrometry and sequencing by Edman degradation leading to the identification of bradykinin, dermaseptins 1 and 4 and phylloseptins 7 and 8. *Leishmania (L.) infantum* showed susceptibility to peptides, with bradykinin and phylloseptin 7 presenting EC₅₀ values of 11.36 µM and 10.06 µM, respectively. *Trypanosoma cruzi* were more sensitive to peptides, showing EC₅₀ values in a range concentration of 0.25 to 0.68 µM, with little or no cytotoxicity to mammalian cells. The lack of mitochondrial oxidative activity of parasites suggested that peptides were leishmanicidal and tripanocidal at the highest tested concentrations. By using the fluorescent probe Sytox Green, it was observed that dermaseptins 1 and 4 and phylloseptins 7 and 8 caused pores to the plasma membrane of *T. cruzi* and *Leishmania*, leading to parasite death. The present study demonstrated the potential of peptides of amphibian, and if adequately studied, may contribute as prototypes of new drugs for neglected diseases. **Supported by:** FAPESP, **Keywords:** Amphibians. Poison. *Phyllomedusa hypochondrialis*. Peptides. Visceral, leishmaniasis. Chagas disease **E-mail:** erikagp@usp.br

Leish106- A new treatment for cutaneous leishmaniasis: drug repositioning, development of a topical agent, and combination therapy.

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Introduction: Treatments available for cutaneous leishmaniasis (CL) are far from optimal; the antimonials currently in use are toxic, with resistance reported in many foci, particularly in CL caused by *L. tropica*. Efficient treatment requires a combination of a drug to kill the parasite, a wound-healing agent and an immuno-stimulant in order to achieve rapid healing with minimal scar formation. **Material and Methods:** Published reports have shown all *Leishmania* species tested to be susceptible to Amphotericin-B. Two topical formulations of Amphotericin-B, a nano-liposomal formulation and an ointment, have been developed and tested *in vivo* against CL in mouse and hamster models using *L. major* and *L. amazonensis*, respectively. In a drug repositioning approach, existing drugs were screened *in vitro* in an intra-macrophage assay using recent isolates of *L. tropica* and *L. braziliensis*. **Results:** The results of topical Amphotericin-B formulations are very encouraging, and pre-clinical development is expected to be completed for initiating clinical trials by 2013. Approximately 50 drugs used for other indications have been screened *in vitro* against new isolates of *L. tropica* and *L. braziliensis*. Several highly active drugs, including azoles and allylamines with an IC₅₀ of <50nM, have been identified. An expert group will determine which drug(s) will be tested in clinical trials alone and in combination. An appropriate immuno-stimulant (TLR-9 agonist) and a wound-healing agent are being selected. A clinical development plan, in line with EU and US regulations, is in place for stepwise evaluation of a three-component treatment modality. Clinical trials against both Old World and New World CL are expected to start by 2013. **Main conclusions:** We are seeking to develop topical treatments that can be effective against CL caused by wide range of parasites. Initial results from studies on two different Amphotericin-B formulations, together with screening of existing drugs, show encouraging results. These drugs will be used stepwise with an immuno-stimulant and a wound healing agent to develop combination therapies for CL. The goal is to develop a treatment that is safe, efficacious, affordable and easily adaptable to field conditions with minimal burden on health systems. **E-mail:** fmodabber@dndi.org

Leish107- Antileishmanial activity of α -bisabolol against *Leishmania amazonensis* *in vitro*

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Leishmaniasis is a group of chronic diseases caused by an obligate intracellular parasite of the *Leishmania* genus. Parasites can cause skin lesions or, depending on the parasite species, affect internal organs such as liver, spleen and bone marrow. Leishmaniasis treatments have several issues that involve lack of effectiveness, due to cases of drug resistance, and numerous side effects. These problems have encouraged the search for new antileishmanial compounds. Therefore, the use of natural products, derived from medicinal plants, has raised the interest of researchers in the quest for an alternative treatment for cutaneous leishmaniasis. Some essential oils or isolated compounds obtained from plants have shown an effective antileishmanial action in experimental studies, making these compounds promising options for the treatment of cutaneous leishmaniasis. So, the purpose of the present study was to evaluate α -bisabolol cytotoxicity and antileishmanial activity. For cytotoxic analysis J774.G8 cells treated or not with α -bisabolol (60 to 1,86 μ g/mL) were used. Neutral red dye was used to measure α -bisabolol concentration able to inhibit 50% of cell number. The 50% cytotoxic concentration (CC₅₀) was determined by regression analysis. Antileishmanial activity of α -bisabolol (60 to 1,86 μ g/mL) was tested using promastigotes for 24h. The parasite amount was determined by counting in a Neubauer chamber and the concentration that inhibited 50% (IC₅₀) of parasite growth was determined after 24h by regression analysis. The α -bisabolol antileishmanial activity was evaluated also with intracellular amastigotes. Results showed significant antileishmanial activity of α -bisabolol able to inhibit 50% of promastigotes forms (8.07 μ g/mL) or intracellular amastigotes (4.29 μ g/mL). α -bisabolol cytotoxic concentration able to inhibit 50% of cells was (14.8 μ g/mL). Ultrastructural analysis of α -bisabolol treated promastigotes showed total destruction of parasite: membranes rupture, subpelvic microtubules destruction and increase of lipidic inclusions. Intracellular amastigotes also presented ultrastructural alterations like mitochondria destruction for e.g and no changes in host cell morphology was observed. Our study confirmed the

antileishmanial action of α -bisabolol. This compound was able to enhance the inhibition of promastigotes but was more effective against amastigotes. These results suggest that intracellular amastigotes were more sensitive to α -bisabolol treatment, indicating that besides the direct action of α -bisabolol there is also an indirect action through the activation of macrophages. In conclusion, the α -bisabolol present cytotoxic effect against *Leishmania*, *in vitro*, showing that α -bisabolol has promising antileishmanial properties. These results open new prospects for research that can contribute to the development of products based on essential oils or isolated compounds from plants for the treatment of cutaneous leishmaniasis. **E-mail:** calabrese@ioc.fiocruz.br

Leish108- Antileishmanial activity of red and brown algae from Southern Portugal

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Introduction: Pervasive around the globe and affecting millions of humans and dogs in developed and developing countries, leishmaniasis are neglected diseases that urgently need new, effective and non-toxic therapies. Because of their diversity, availability and variety of secondary metabolites, marine organisms are nowadays recognized as a source of novel products to be used alone or in combination therapy with available drugs, being a promising alternative to anti-Leishmania therapy and control. As a result, in the mid-90's, the scientific community started to search for antileishmanial activity in MO, and promising results were obtained with different species against both forms of several *Leishmania* species. Although the majority of the reports are in sponges, several seaweed species, namely from brown and red macroalgae revealed anti-Leishmanial activity. Having this in mind, this work aimed to evaluate the antileishmanial activity of different organic extracts of three brown and three red algae on *L. infantum* promastigotes. **Material and Methods:** Brown (*Cystoseira baccata*, *C. nodicaulis* and *C. tamariscifolia*) and red (*Jania* sp, *Peyssonnelia* sp and *Bornetia secundiflora*) seaweeds collected in Atlantic Ocean on Southern coast of Portugal (Algarve) were extracted sequentially with hexane, ethyl acetate, ether and methanol. *L. infantum* promastigotes, maintained in RPMI medium with 20% FBS, were incubated with serial dilutions of each fraction, and viability determined by microscopy and MTT assays. Amphotericin B was used as control drug. Drug activity of each extract was expressed as % reduction of parasite burdens compared to untreated infected controls and the IC50 values were determined with GraphPad Prism. Microscopical assessment of parasites morphology upon exposition to different seaweed fractions was carried out on Giemsa stained smears. **Results:** Decreased viability of *L. infantum* promastigotes was observed after treatment with all the extracts of brown algae species, but the hexane and ether fractions of *C. baccata* were the most bioactive with IC50 values of 7.93 ± 0.09 and 5.18 ± 0.27 $\mu\text{g/mL}$, respectively. Conversely, most of the extracts from the red algae were not active, and for the bioactive ones the IC50 values varied between 150.70 ± 4.56 and 391.10 ± 7.85 $\mu\text{g/mL}$ for the ethyl acetate extract of *B. secundiflora* and hexane extract of *Peyssonnelia* sp. respectively. **Main conclusions:** Our results suggest that these *Cystoseira* algae, *C. baccata* in particular, are potential candidates as biological sources for antileishmanial compounds. Further works are in progress to isolate and characterize its active compounds, determine cytotoxicity on macrophages and intracellular activity of the extracts. Study supported by FCT project (SEABIOMED-PTDC/MAR/103957/2008). Bruno de Sousa C., Custódio L., Maia C. and Cortes S. thanks to FCT for the PhD (SFRH/BD/78062/2011) and post-doctoral (SFRH/BPD/65116/2009, (SFRH/BPD/44082/2008 and SFRH/BPD/44450/2008) grants, respectively. **E-mail:** calsousa@ualg.pt

Leish109- Antileishmanial Activity of Diamines

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Introduction: Leishmaniasis is one the most significant of the neglected tropical diseases, it is endemic in 98 countries, over 350 million people are at risk and 2 million new cases arise every year. The treatment of leishmaniasis requires the administration of toxic and poorly tolerated drugs, as pentavalent antimonials, pentamidine or amphotericin B. Thus, there is an urgent need for effective drugs to replace or supplement those in current use. Diamines have shown antiparasitic activity, including against *Leishmania*. In rational trials we analyzed the leishmanicidal effects of diamines, including: (1) 1,3-*N,N'*-Bis(*p*-metoxi-benzil)propanodiamina, (2) 1,4-*N,N'*-Bis(*p*-metoxi-benzil)butanodiamina, (3) 1,6-*N,N'*-Bis(*p*-metoxi-benzil)hexanodiamina and (4) 1,8-*N,N'*-Bis(*p*-metoxi-benzil)octanodiamina. **Material and Methods:** All the compounds were assayed against *L. amazonensis*, *L. braziliensis*, *L. chagasi* and *L. major* promastigote forms and were tested for cytotoxic effects on mammalian cells. In both cases the viability of the cells was checked using the MTT colorimetric method, after three days incubation period. The active compounds were assayed against amastigote forms and the antileishmanial activity was determined by counting the number of intracellular parasites after 72 hours of treatment. The supernatant was also collected to measure of the nitrite, by-product of nitric oxide (NO), by the Griess method. **Results:** Among all the species assayed, only *L. major* promastigote forms were sensible for compounds tested (IC₅₀ values of 2.60 µM, 12.25 µM, 67.79 µM and 23.36 µM for compounds 1, 2, 3 and 4, respectively). Against intracellular parasite only the compounds 1 and 4 showed activity, with IC₅₀ values of 17.97 and 57.07, respectively. None of compounds tested showed toxicity against mammalian cells, indicating high selectivity. It is interesting to emphasize that were observed a relationship between activity and carbons number of the compounds. None of compounds induced significant nitrite production in the culture medium in all concentration used compared to untreated controls. **Main Conclusions:** These results have shown a good in vitro antileishmanial activity of diamines, but the leishmanicidal effect observed on amastigote forms is unlikely due to NO production by macrophages. **Supported by** FAPEMIG, CNPq and UFJF **E-mail:** patriciamachado12@yahoo.com.br

Leish110- Antileishmanial activity of 4-aminoquinoline derivatives

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Introduction: Alternative drugs against leishmaniasis are urgently needed. Most of the few available treatments cause highly toxic side effects and are no longer effective due to potential emergence of parasite resistance. Quinolines are an important class of compounds with promising effectiveness in the treatment of leishmaniasis. Continuing our search of potent antileishmanial based on quinoline derivatives we report the evaluation of new 4-aminoquinoline against promastigotes and amastigotes of *Leishmania* species. **Material and Methods:** Antipromastigote activity and cytotoxicity in macrophages were determined using the tetrazolium-dye (MTT) colorimetric method after 72 hours of treatment. The antiamastigote activity was determined by counting the number of intracellular parasites and the percentage of infected macrophages after 72 hours of treatment. The results in promastigotes and amastigotes were expressed as IC₅₀ (concentration inhibiting parasite growth by 50%). **Results:** Among four compounds assayed, two 4- aminoquinoline derivatives 4-(phenylhydrazinyl)-7-chloroquinoline (3) and 4-(benzohydrazide)-7-chloroquinoline (4) exhibited significant antileishmanial activity. The compound 3 showed high activity against promastigotes of all *Leishmania* species tested with IC₅₀ values of 6.2 µg/mL, 6.5 µg/mL and 7.1 µg/mL for *L. amazonensis*, *L. braziliensis* and *L. chagasi*, respectively. The compound 4 displayed activity against only *L. braziliensis* and *L. chagasi* promastigotes (IC₅₀ values of 11.4 µg/mL and 14.0 µg/mL, respectively). Both compounds 3 and 4 showed a significant activity against intracellular amastigotes of *L. braziliensis* (IC₅₀ values of 0.8 µg/mL and 2.4 µg/mL, respectively) and selectivity indexes (the ratio of IC₅₀ on macrophages to IC₅₀ on *L. braziliensis* intramacrophage amastigotes) values of 187.5 and 62.5, respectively. **Main Conclusions:** The in vitro leishmanicidal activity can be related with the presence of benzene ring. Further experiments are being carried out in order define a mechanism of action for the rational design of new leads for antileishmanial compounds. **Supported by** : FAPEMIG, UFJF and CNPq. **E-mail:** lucianantinarelli@yahoo.com.br.

Leish111- Anti-inflammatory effect of 1,25(OH)2D3 on *Leishmania mexicana* BALB/c mice infected

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Introduction: The most active metabolite of vitamin D, 1,25 (OH)2D3 is a steroid hormone implicated in a wide range of cell functions such as differentiation, proliferation and apoptosis. The actions of 1,25 (OH)2D3 are mediated by its binding to the vitamin D receptor, which acts as a transcription factor to modulate the expression of a lot of amount of genes in a tissue-specific manner and is constitutively expressed in a variety of immune cells. Different and complex clinical effects have been associated with 1,25 (OH)2D3; it has been suggested that could be one of the key factors in infectious diseases. Leishmaniasis is an important health problem in tropical and subtropical regions. *Leishmania mexicana* causes two kinds of cutaneous leishmaniasis localized or diffuse. In this work we explored the effect of treatment of 1,25 (OH)2D3 on a susceptible leishmaniasis mice model. **Material and Methods:** Four mice (Balb/c) groups were integrated; They were inoculated as follows: Group1, *L. mexicana* (1X10⁶ promastigotes in footpad) + 1,25 (OH)2D3 (0.5 µg/kg/2 days/IP); Group2, *L. mexicana*; Group3, *L. mexicana*+vehicle and Group4, PBS in footpad+vehicle. Each week we quantified the lesion size and body weight. At week 12 post-infection, animals were sacrificed and the cytokines production was determined *in situ* using immunohistochemistry. The histopathological analysis was performed and collagen production was determined by Masson staining. Finally, we determined the number of parasites present in each condition. **Results:** A significant reduction in the lesion size was found in animals treated with 1,25 (OH)2D3. Well preserved tissue and presence of large numbers of eosinophils and fibroblasts was found in the group treated with 1,25 (OH)2D3, in the other infected groups destroyed epidermis was observed with large amount of neutrophils and epithelioid macrophages. The amount of pro-inflammatory cytokines in mice infected and treated with 1,25 (OH)2D3 was lower than the animals only infected. Interestingly, there no differences in the number of parasites were found. Finally, the amount of collagen was higher in animals with treatment. **Conclusion:** In a mouse model susceptible to *L. mexicana* 1,25 (OH)2D3 treatment significantly reduces the inflammatory phenomenon induced by this parasite. **E-mail:** espi77mx@yahoo.com

Leish112- Activity of new derivatives of amodiaquine against *Leishmania braziliensis*

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Introduction: *L. braziliensis* is the main cause for the high annual incidence of the cutaneous leishmaniasis in Brazil. As no efficient vaccine is available, chemotherapy constitutes the main tool for the control of the disease. The quinoline derivatives can be considered promising series of antileishmanial drugs, which can be exemplified by the activity of the antimalarial drug Amodiaquine against different species of *Leishmania*. In this work, we report the antileishmanial evaluation of a series of amodiaquine derivatives against promastigote and amastigote forms of *L. braziliensis*. **Material and Methods:** Antipromastigote activity and cytotoxicity in macrophages were determined using the tetrazolium-dye (MTT). The anti-amastigote activity was determined by counting the number of intracellular parasites and the percentage of infected macrophages after 72 hours of treatment. The results in promastigotes and amastigotes were expressed as IC₅₀ (concentration inhibiting parasite growth by 50%). **Results:** Among

six compounds assayed, only compounds **PQUI 1**, **PQUI 2** and **PQUI 3** displayed a significant activity against promastigotes with IC₅₀ values of 2.2 µg/mL, 3.4 µg/mL and 5.5 µg/mL, respectively. All compounds were more active against intracellular amastigotes (IC₅₀ values of 1.1 µg/mL, 0.8 µg/mL, 0.6 µg/mL, 29.3 µg/mL, 3.3 µg/mL and 1.0 µg/mL for compounds **PQUI 1**, **PQUI 2**, **PQUI 3**, **PQUI 4**, **PQUI 5** and **PQUI 6**, respectively). Furthermore, all compounds exhibited high antiamastigote activity and low toxicity presented selectivity indexes (the ratio of CC₅₀ on macrophages to IC₅₀ on *L. braziliensis* amastigotes) values of 29.7, 47.4, 25.6, 5.1, 150.0 and 187.5 for compounds **1**, **2**, **3**, **4**, **5** and **6**, respectively. **Main Conclusions:** Some compounds affected intracellular amastigotes but were completely inactive against promastigotes, suggesting a host cell-dependent mechanism of action. The results provide evidence that the amodiaquine derivatives are promising candidates to be considered as lead compounds for leishmanicidal drugs since they are effective against intracellular amastigotes at concentrations in the low sub microgram/mL range. Further studies are underway to investigate the possible intracellular targets of this series. **Supported by** FAPEMIG and UFJF **E-mail:** lucianantinarelli@yahoo.com.br.

Leish113- Deaths associated to cutaneous and mucocutaneous leishmaniasis treatment, Minas Gerais, Brazil

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Introduction: The pentavalent antimonial drugs have been the most widely used for cutaneous and mucocutaneous leishmaniasis (CL) treatment. The meglumine antimoniate (Glucantime®) is one of the options with maximum efficacy in CL treatment, however, serious side effects, as arrhythmias and sudden death, may occur. The purpose of our study was to evaluate the death rate during the CL treatment and the risk factor associated with death. **Material and methods:** This study was carried out in the State of Minas Gerais, located in southeastern Brazil, endemic area for leishmaniasis. Data were obtained from the Reportable Disease Information System –SINAN (Brazilian Ministry of Health and Minas Gerais State) and complemented with data of the Mortality Information System and from application forms used to investigate deaths associated to CL. Demographic and clinical data were evaluated as well as the causes of death obtained from death certificates. **Results:** During the years of 2010 and 2011 were notified 3584 cases of CL. Among these cases, 95.6% were notified as new cases and 4.4% as relapses. The mean patient age was 39.1±20.7 years and 62.4% of the patients were men. The cutaneous form accounted for 93.5% of the cases, while the mucocutaneous form corresponded to 6.5% of the cases. Glucantime® was used as treatment in 94% of the cases. During the period studied were reported 53 deaths. Among the patients who died, 49 (92.5%) aged greater than or equal to 50 years old and 47 (88.6%) were treated with Glucantime®. Multivariate regression analysis identified age ≥ 50 years as a factor associated with death (OR 26.8; 95% CI 9.6-74.3) as well as female sex (OR 2.2; 95% CI 1.2-3.8) and year of diagnosis 2011 (OR 1.7; 95%CI 0.9-3.0). Stratifying the patients by smaller age groups we observe a trend of increase mortality associated with increasing age. Patients aged until 49 years old had a death rate of 0.15%. Patients aged from 50 until 59 years old had a death rate of 1.3% (OR 8.6; 95%CI 2.3-34.9), from 60 to 69 years old a death rate of 2.8% (OR 18.8; 95%CI 5.5-70.5), from 70 to 79 years old a death rate of 7.7% (OR 54.4; 95%CI 16.8-194.3) and, finally, patients aged equal or more than 80 years old had a death rate of 18.3% (OR 146.9; 95%CI 44.1-539.6). Through the records of death investigations we could realize that the main cause of death from patients investigated was cardiac events as arrhythmias and sudden death. **Conclusions:** Despite the low number of deaths during the study period, our findings suggest a significantly increased risk of death associated with the systemic use of (Glucantime®) in patients over 50 years. Alternative treatments, as topical agents, deserve to be urgently studied and employed in clinical practice. We also concluded that the current Brazilian guide of cutaneous and mucocutaneous treatment should be more restrictive regarding the use of systemic Glucantime® in older patients. **E-mail:** andrea.dias@saude.mg.gov.br

Leish114- Effects of CXCL10/GAMMA interferon-inducible protein 10 treatment in BALB/C mice infected with *Leishmania Infantum Chagasi*

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Introduction: Visceral leishmaniasis caused by *Leishmania infantum chagasi* is characterized by the loss of the ability of host to generate an effective immune response. In this study was investigated the role of CXCL10 chemokine in controlling *L. infantum chagasi* infection in vivo. **Material and Methods:** Groups of BALB/c mice were treated or not with recombinant CXCL10 (5 µg/kg) at 1, 3 and 7 days of infection. After 1, 7 and 23 days of treatment, some parameters were evaluated: parasite load, levels of IFN-γ, IL-4, TGF-β and IL-10, and histopathological alterations in the liver. **Results:** After 23 days of treatment, CXCL10 induced a significant reduction on the number of parasites in the spleen as compared to control group ($p=0,027$). In the liver, the parasite load decreased between the 7th and 23th day post treatment ($p<0,05$) in treated group. IFN-γ was induced most significantly in treated than in control group, and reached its maximum production (100pg/mL) on day 23 after treatment, correlating with parasite burden reduction in the target organs ($p<0,05$). IL-4 was produced in low doses in both groups, although treated animals had shown higher levels than control group. In treated animals, IL-10 levels were smaller than in control group ($p=0,0029$) at 23th day of treatment. After 7 days of treatment, TGF-β production was 2 times lower in treated than in control group ($p=0,0092$), and after 23 days of treatment, this cytokine remained with lower levels than those observed in control ($p=0,0069$). In the histopathological analysis of the liver was found after the 1st day of treatment, in both groups, more immature granulomas (IG) than non-granulomatous infiltrate (NG), and some few mature granulomas (MG) were only observed in treated group. After 7 days of treatment, the amount of NG infiltrates decreased, and IG were still the most frequent in both groups, besides a slight increase of MG was observed in treated group. **Main conclusions:** At the light of the found results, it is possible to suggest an important leishmanicidal role to CXCL10 in BALB/c mice infected by *L. infantum chagasi*, which seems to be mediated by a significant IFN-γ production, and suppression of IL-10 and TGF-β, opening the hypothesis that this would be associated to a decrease in the frequency of regulatory T cells induced by CXCL10 in these animals. **Keywords:** *L. infantum chagasi*, CXCL10, IFN-γ, IL-4, IL-10, TGF-β, granuloma. **E-mail:** sayomelo@gmail.com

Leish115- Efficacy of Photodynamic Antimicrobial Chemotherapy (PACT) to *Leishmania braziliensis*: an in vitro study

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Introduction: Leishmaniasis is a neglected tropical disease considered caused by parasites of the genus *Leishmania*. Affects about 12 million people in around 80 countries with 2 to 3 million cases per year. *Leishmania braziliensis* is the main species that causes cutaneous leishmaniasis in Brazil. Despite many achievements in research, the first-line chemotherapy is still based on antimonials, which are toxic and prone to the phenomenon of drug resistance. Despite recent advances in the understanding of the disease and promising programs for drug discovery, the treatment of leishmaniasis still remains a serious public health problem and the phenomenon of resistance to drugs is a major concern for the future. PACT is a potentially applicable technical, economical and safe, which has been used to treat cancer and other microorganisms. **Material and Methods:** The cytotoxicity evaluation of new PS through the technique of crystal violet, the concentrations determined safe (nontoxic) to animal cells. For PACT, semiconductor laser ($\lambda = 660\text{nm}$, 40mW, $4.2\text{J}/\text{cm}^2$, CW) associated to phenothiazine's derivatives (5 and 10 µg/ml, TBO, Methylene Blue or Phenothiazine) on the promastigotes form of *Leishmania braziliensis* in a single

session was used. Viability of the parasites was assessed in quadruplicates of each group. The samples were removed and analyzed in a haemocytometer 72h after PACT. We found an important decrease in the number of viable parasites on all treated groups in comparison to their controls. **Results:** We found an important decrease in the number of viable parasites on all treated groups in comparison to their controls. The results of present study showed significant percentage of lethality (above 95%) of the protocol. The 99.23% of lethality was achieved with 10 µg/ml of TBO. No lethality was seen on groups treated neither with laser nor with each compounds separately. **Conclusions:** The results are promising and indicative that the use of PACT may be a powerful treatment of leishmaniasis when compared to already available ones. **E-mail:** arturfelipes@gmail.com

Leish116- Evaluation of activity of resveratrol analogues on promastigote and amastigote forms of *L.braziliensis*

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Introduction: Leishmaniasis is a neglected infectious disease caused by an obligate intracellular parasite the *Leishmania* genus that affects mainly developing countries. The annual incidence is 2 million new cases per year, and 350 million people live in endemic areas. The treatment is based on the use of pentavalent antimonials that have high toxicity. However, it is necessary research the new drugs. Resveratrol have shown antiprotozoal activity which led us to test various compounds derived from this compound, as hydroxylamine stilbenoides. The compounds 4-hidroxi-1-*N*-(4'-metoxi-benzilideno)aniline (1), 4-hidroxi-1-*N*-(4'-hidroxi-5'-metóxi-benzilideno)aniline (2), 4-hidroxi-1-*N*-(3',4',5'-tri-metóxi-benzilideno)aniline (3), 4-hidroxi-1-*N*-(benzilideno)aniline (4), 4-hidroxi-1-*N*-(4'-hidroxi-benzilideno)aniline (5), 4-hidroxi-1-*N*-(2'-hidroxi-benzilideno)aniline (6), 4-hidroxi-1-*N*-(4'-dimetilamino-benzilideno)aniline (7) and 4-hidroxi-1-*N*-(4'-nitro-benzilideno)aniline (8) were tested in promastigote forms of *L. braziliensis*.

Material and Methods: The cytotoxicity in promastigote forms and mammalian cells were determined by MTT colorimetric method after 72 hours incubation with the compounds. The active compounds were assayed against amastigote forms and the antileishmanial activity was determined by counting the number of intracellular parasites after 72 hours of treatment. The supernatants were collected after 48 hours incubation with these compounds to measure of nitric oxide by Griess method. The results were expressed as the concentrations inhibiting parasite growth by 50 percent (IC₅₀). **Results:** Among the compounds tested, the compounds 1, 2, 3 and 4 showed activity against promastigote forms of *L. braziliensis*. Against intracellular amastigotes of *L. braziliensis*, among the resveratrol analogues assayed, the compound 3 exhibited the best activity (IC₅₀=20.31 µM). It did not induce significantly the production of nitric oxide in any of compounds tested when compared with the untreated controls. **Main Conclusions:** The compounds were not toxic for macrophages cells at the maximum concentration tested. These results have shown the biological activity of the resveratrol analogues and these compounds would be a promising matrix for developing a new class of leishmanicidal agents. **Supported by** FAPEMIG, CNPq and UFJF **E-mail:** danielatasp@yahoo.com.br

Leish117- Evaluation of raloxifene, toremifene and tamoxifen as potential drug candidates for visceral leishmaniasis: activity as single drugs and in drug combination schemes

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Introduction: Leishmaniasis is endemic in 98 countries, with about 12 million people infected worldwide. Visceral leishmaniasis (VL), given its high incidence and mortality, is considered by the World Health Organization as one of the most important infectious diseases nowadays. Leishmaniasis treatment relies on a limited therapeutic arsenal, with toxic drugs administered by the parenteral route such as pentavalent antimonials, amphotericin B and pentamidine. The toxicity, high cost and route of administration of these drugs, coupled with parasite resistance to antimonials in some regions, indicate

that the discovery of new alternatives for leishmaniasis treatment is essential, either as new drugs or as new chemotherapeutic regimens using drug combinations. We have recently described the antileishmanial activity of tamoxifen, a drug already in use for the treatment of breast cancer for over 30 years. Tamoxifen has been shown to be effective in several *in vivo* experimental models of visceral and cutaneous leishmaniasis. Tamoxifen is a Selective Estrogen Receptor Modulator (SERM) acting as an anti-estrogen in the mammary tissue, and as an estrogen in bone density, cell proliferation in the endometrium and cholesterol metabolism. The aim of this work was to characterize the antileishmanial activity of the tamoxifen-related compounds raloxifene and toremifene, alone and associated with antileishmanial standard drugs, in a VL model. **Methods:** The effect of single drugs and drug combinations of tamoxifen and raloxifene with the antileishmanial standard drugs, pentavalent antimony, amphotericin B and miltefosine was determined using promastigotes or intracellular amastigotes of *Leishmania infantum chagasi*. The drug interactions were assessed with a fixed ratio isobologram method and the fractional inhibitory concentrations (FIC); FIC sum (Σ FIC) and overall mean Σ FIC were calculated for each combination. **Results:** The single drug experiments indicate that raloxifene and toremifene are active *in vitro* against *Leishmania (L.) infantum chagasi* promastigotes and intracellular amastigotes, with Effective Concentration 50% values in the micromolar range. Preliminary results on the *in vitro* drug combinations indicate Σ FIC values in the range of 0.5 to 4, which defines additively or indifference. **Main conclusions:** Other SERMs apart from tamoxifen are active against *Leishmania* and may be useful in drug combination schemes. Data obtained from this work might provide the basis for a clinical trial design, given that tamoxifen and raloxifene have well established clinical safety profiles. **Supported by** FAPESP, CNPq and CAPES **E-mail:** juliana_reimao@yahoo.com.br

Leish118- Evaluation of topical treatment of experimental cutaneous leishmaniasis with formulations containing pentamidine in swiss albino mice

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Introduction: Leishmaniasis is an endemic disease caused by species of the genus *Leishmania*, characterized by lesions on the skin or viscera, producing cutaneous, muco-cutaneous or visceral clinical forms. The transmission occurs when the sandflies injects metacyclic promastigotes in their mammalian hosts that bind to macrophages and are rapidly phagocytosed. Treatment of cutaneous leishmaniasis (LC) is difficult due to scarce number of drugs capable to eliminate completely the parasite intracellular form. It is realized with pentavalent antimonials (PA) or pentamidine isethionate (PE). However, these drugs have various adverse effects: malaise, myalgia, headaches and changes in liver, kidney and pancreas. Additionally, patient may not respond to the treatment. For toxicity reasons, PE is experimentally tested for the LC treatment of topical formulation as directly onto the lesions. **Materials and Methods:** Studies were carried out using 12 Swiss albino mice (*Mus musculus*) that were infected with amastigote forms in right footpad and, after appearance of the lesion (72 days), the animals were divided into four groups and treated with two applications/day for eight days. Was used a cream containing 10% PE, vegetable oil base and dibenzofurane compound from Nordic lichen (Group ACPU), or with 10% PE cream with the above base (Group ACP). Control animals were treated with cream containing only the oil base and Nordic lichen (Group ECU) or without cream administration (Control Group). Impression smears were made from the cutaneous lesions of experimental groups fixed and stained (Giemsa). The evolution of the lesions was accompanied by measurements of the skin lesions in mice treated and untreated during the investigation. **Results:** The aim of this work is evaluation of topical PE treatment of experimental LC caused by *L. amazonensis*. No reduction was observed of the size of lesions measurements during the experimental course of infection between the groups. Thus, in the ACP group was observed crust formation. Portions of the cutaneous lesions from the ACP group showed necrosis and a reduction of tissue nodule. Impression revealed amastigotes in all groups, with morfological deformation in amastigotes and severe cells destroyed proximal the epidermis. **Conclusions:** Treatment optimization and additional evaluations of topical treatment with PE and the toxicity are in process mainly with this

variety of *Leishmania* that has proved more resilient and more able to spread. Pathologically, in all infected groups, the epidermis showed focal ulceration. **Keywords:** Cutaneous Leishmaniasis, Topical Treatment, Pentamidine, *Leishmania amazonensis* **Financial support:** INPA/ Academy of Finland, project N°133153 **E-mail:** klaudiadcw@gmail.com

Leish119- *In vitro* activity of antimalarial drugs against *Leishmania amazonensis*

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Introduction: Leishmaniasis comprises a broad spectrum of diseases caused by *Leishmania* parasites. Treatment with the first-line drugs for leishmaniasis, the pentavalent antimonials, can be complicated by side effects, less sensitivity or resistance of some *Leishmania* species, variations in pharmacokinetics, drug-host immune response interaction and high cost. Therefore, it is pivotal the development of safer, cheaper and more effective treatments for leishmaniasis. Drug repositioning is a strategy to identify and develop new uses for existing drugs, reducing expenditures and research time. In this context, the aim of this work was to evaluate the antileishmanial activity of antimalarial drugs. **Methods:** Promastigotes of *Leishmania amazonensis* were incubated in the presence of increased concentrations of artesunate, chloroquine, hidroxichloroquine, mefloquine or primaquine, ranging from 0.6 to 50 μ M, in order to determine the concentration that inhibits in 50% the parasite growth (IC_{50}). The cell cycle was assessed in drug-treated promastigotes using propidium iodide incorporation in flow cytometry analysis. Cytotoxicity to mammalian cells was evaluated in BALB/c mice splenocytes by incorporation of [*methyl*-³H]-thymidine, allowing the calculation of lethal concentrations for 50% of cells (LC_{50}). Peritoneal macrophages from CBA mice were infected with promastigotes of *L. amazonensis* and treated with antimalarial drugs in various concentrations to determine the IC_{50} against amastigotes. Infected and treated macrophages were analyzed by transmission electron microscopy. **Results:** Chloroquine and hidroxichloroquine did not significantly affect promastigote growth at 50 μ M. At this concentration artesunate and primaquine significantly inhibited parasite growth, although less than 50%. Mefloquine at 50 μ M inhibited completely the growth of *L. amazonensis*, compared to controls, with an IC_{50} of $8.4 \pm 0.7 \mu$ M. Incubation with mefloquine at the IC_{50} concentration for 48 h caused a G2/M cell cycle arrest. Only chloroquine, hidroxichloroquine and mefloquine were active against amastigotes at a maximum tested concentration of 5 μ M and presented an IC_{50} of $0.78 \pm 0.08 \mu$ M, 0.67 ± 0.12 and $1.56 \pm 0.18 \mu$ M, respectively. There was no cytotoxicity effect of these drugs at the concentrations tested. The ultrastructural analyses by transmission electron microscopy showed that, after treatment with chloroquine parasites, showed a Golgi complex damage and increased in vacuolization in the cytoplasm, while membrane blebbing was observed after mefloquine treatment of infected cells. **Conclusion:** Chloroquine, hidroxichloroquine and mefloquine showed a promising antileishmanial activity, low toxicity and may constitute an alternative therapy to conventional treatment of cutaneous leishmaniasis. *In vivo* assays will be performed to validate the effect against *L. amazonensis* of these antimalarial drugs. **E-mail:** vinicius@aluno.bahia.fiocruz.br

Leish120- *Leishmania* specific multifunctional CD4+ T cells increases after therapy in American cutaneous leishmaniasis patients

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Introduction: Currently, the most accepted approach to control human leishmaniasis is a prophylactic vaccine. To develop such a vaccine it becomes necessary to understand the factors that participate in the healing process and protection during and after natural infection. T helper 1 (Th1) immune response is important for the protection against intracellular parasites and many studies have evaluated this response only by measuring IFN- γ production, although the evaluation of this single parameter is not always

sufficient to predict protection. **Material and Methods:** Using multiparametric flow cytometry, as well as IFN- γ ELISA, we evaluated the quality of the Th1 response stimulated by crude antigen extract of *Leishmania braziliensis* promastigotes (LbAg) in peripheral blood mononuclear cells (PBMC) obtained from cutaneous leishmaniasis (CL) patients before (BT) and 140 days after therapy (AT - after healing). **Results:** Analysis of the T lymphocytes subpopulations did not show significant variations in the percentage of CD4+, CD8+ and CD25+ T cells induced by LbAg between the groups. The integrated MFI (frequency x MFI) for IFN- γ , TNF- α and IL-2-producing CD4+ T cells did also not differ between CL groups but were higher in the healed patients when compared to a control group of healthy individuals from non-endemic areas. The quantification of IFN- γ by ELISA showed that LbAg induced higher production of this cytokine in both groups of CL patients when compared to the control group ($p < 0.005$). Although IFN- γ production appeared to be higher AT when compared to the group of patients analyzed BT, no statistically significant difference was observed. However, by assessing the contribution of the CD4+ phenotypes producing 3, 2, or a single cytokine in the total Th1 response evaluated, we observed that healed patients show an increase in multifunctional CD4+ T cells (producing IL-2, TNF- α and IFN- γ , simultaneously) when compared to patients BT and healthy controls ($p < 0.05$). Before therapy multifunctional T cells contributed with 10% of the total Th1 response evaluated, while IL-2 single positives contributed with 22%. In healed patients, multifunctional T cells were the major phenotype observed (28%) and IL-2 single positives contributed with only 1% of the total Th1 response. On the other hand, in healthy controls, multifunctional T cells comprise only 3% of the total Th1 response and the major phenotypes observed were those single positives for IL-2 (28%), IFN- γ (24%) and TNF- α (13%). Multifunctional CD4+ T cells also had the highest mean fluorescence intensity for the three cytokines studied. **Conclusions:** Our results call attention to the importance of studying a Th1 response regarding its quality, and not just its magnitude, and indicate that this kind of evaluation might help understand mechanisms involved with cure and protection in human tegumentary leishmaniasis, that will certainly help in the development of vaccines and/or immunotherapeutical strategies against the disease. **E-mail:** pmdeluca@ioc.fiocruz.br

Leish121- Leishmanicidal activity of extracts from cerrado Brazilian plant

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Introduction: Leishmaniasis is a group of tropical diseases caused by species of protozoan parasites of the genus *Leishmania*. The drugs currently available for treatment of leishmaniasis are unsatisfactory because of their limited efficacy, frequent side effects, and increasing drug resistance. In order to find new drugs against leishmaniasis, we have studied extracts and natural molecules of Brazilian plants such as *Arrabidaea brachypoda* (Bignoniaceae), a typical tree in Brazilian Cerrado, rich on secondary metabolites, with biological activities as antioxidant, trypanocidal, cytotoxic, antimicrobial, antifungal and antitumoral properties. **Objective:** To obtain extracts from *A. brachypoda* leaves: crude, hexane, ethyl-acetate, butanolic and aqueous and to evaluate them to the leishmanicidal activity against forms of *Leishmania (Leishmania) amazonensis*. **Methods:** In all tests, 0.6% dimethyl sulfoxide (DMSO) was the concentration used to dissolve the extracts. The promastigote forms of *L. (L.) amazonensis* in log phase of growth were grown on a 24-well plate in Schneider's medium in the absence or in the presence of different concentrations of the extracts at 25°C, to evaluate parasite survival. After 72h, promastigote forms were counted in Neubauer chamber and the results were expressed as percentage of inhibition in relation to the control. The 50.0% inhibitory concentrations (IC₅₀) were determined by regression analysis of the data obtained. The controls and reference drug used were the DMSO and Amphotericin B, respectively. Murine peritoneal macrophages were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin at 37°C in 5% CO₂. Cells (8x10⁵ cells/well) were cultured in 24-well chamber slides, on the glass slides of 13mm diameter, and infected with late log-phase promastigotes at a multiplicity of infection of 10:1 (parasite/macrophage). The drug dilutions were administered. After 72 hours, chamber slides were fixed in absolute methanol, stained with 10% Giemsa, and examined under the oil immersion objective of the light microscope. At least 200 macrophages were counted per well for calculating the percentage of infected macrophages, and the percentage inhibition was calculated in relation to the DMSO-only control, for the determination of IC₅₀.

value. **Results:** As much the crude, hexane, ethyl-acetate, butanolic and aqueous extracts inhibited growth of the promastigote forms, with IC_{50} of 5.10, 1.45, 2.82, 15.74, and $> 40.00 \mu\text{g/mL}$, respectively, after 72h of incubation. Whereas the crude, hexane, ethyl-acetate and butanolic extracts inhibited growth of the amastigote forms with IC_{50} 6.61, 3.48, 3.83, $8.51 \mu\text{g/mL}$, respectively. The activity is correlated to the non-polarity having been the hexanic the most apolar extract and aqueous the most polar. In addition, Amphotericin B showed IC_{50} of $4.32 \mu\text{g/mL}$. **Conclusion:** In the present study, we report for the first time a novel pharmacological activity of the extracts from *A. brachypoda*, which showed important activity against *L. (L.) amazonensis*. **Acknowledgements:** Capes, CNPq, Fapemig, Finep, Unifal-MG. **E-mail:** kfa.karina@yahoo.com.br

Leish122- Leishmanicidal potential of derivatives benzophenones

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Introduction: Leishmaniasis is a tropical neglected disease that occurs mainly in poor countries. That disease is caused by protozoan parasite of the genus *Leishmania*, family *Trypanosomatidae*, and it's transmitted by the bite of female phlebotomine sandfly infected with the pathogen. At present, the chemotherapies associated with all forms of leishmaniasis have several drawbacks including poor efficiency, bioavailability, and toxicity to humans, high cost and the emergence of drug resistant parasitic strains. This makes urgently necessary the development of new drugs. Recent studies have evaluated the activity in *Leishmania*- derived benzophenones, and found that these derivatives exhibit leishmanicidal action. Therefore, new semi-synthetic derivatives of benzophenones aiming to action on parasites of the genus *Leishmania* were tested. **Objectives:** the objective of this study was to evaluate the action of semi-synthetic derivatives of benzophenones against *Leishmania (Leishmania) amazonensis*. **Methods:** The promastigote forms of *L. (L.) amazonensis* were transferred to a ratio of 1×10^6 cells/mL in 24-well plates in Schneider's medium in the absence or in the presence of different concentrations of the derivatives of benzophenones at 25°C , to evaluate parasite survival. In all tests, 0.6% dimethyl sulfoxide (DMSO) was the concentration used to dissolve the derivatives. The controls and reference drug used were the DMSO and Amphotericin B, respectively. After 72h, promastigote forms were counted in Neubauer chamber and the results were expressed as percentage of inhibition in relation to the control. The 50.0% inhibitory concentrations (IC_{50}) were determined by regression analysis of the data obtained. To assess the cytotoxicity were used murine peritoneal macrophages maintained in RPMI 1640 at 37°C and 5% CO_2 , arranged in 24 well plates at a ratio of 8×10^5 per well, to which are added substances to be evaluated at various concentrations and incubated for 72 hours. After the incubation period is added $50 \mu\text{L}$ of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-difeniltetrazolium bromide) to each well, with a further incubation for 4 hours, then the cells are solubilized with DMSO and analyzed in a spectrophotometer UV/VIS Shimadzu, to 570 nm to determine the 50% cytotoxicity concentrations (CC_{50}) by comparison to the control without addition of compounds. The compounds tested are called LFQM-115, LFQM-116 and LFQM-117. **Results:** The compounds LFQM-115, LFQM-116 and LFQM-117 inhibited growth of the promastigote forms, with IC_{50} of 4.90, 9.80 and $7.05 \mu\text{g/mL}$, respectively, in relation to cytotoxicity activity they exhibited CC_{50} of 24.60, 40.60 and $140.06 \mu\text{g/mL}$, respectively. The starting compound exhibited IC_{50} and CC_{50} of 29.00 and $>160.00 \mu\text{g/mL}$, respectively. The compounds LFQM-116 and LFQM-117 showed good leishmanicidal activity. Then the chemical modifications are justifiable, because in spite of the derivatives being slightly more cytotoxic (compared to the starting compound), their promastigote activities were enhanced. Further studies will be conducted. **Acknowledgements:** Capes, CNPq, Fapemig, Finep, Unifal-MG. **E-mail:** leticia.almeida.le@gmail.com

Leish123- Characterization of American Tegumentar Leishmaniasis patients treated with liposomal Amphotericin B, Central West Region, Brazil, in 2010

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Introduction: American Tegumentar Leishmaniasis (ATL) is a chronic, infectious disease, not contagious, caused by protozoa of the genus *Leishmania*. This chronic disease can affect the skin and mucous membranes. **Objective:** The aim of this research was to determine the frequency of ATL in the Central West Region of Brazil, and to correlate characteristics of the symptoms of patients who had been treated with Liposomal Amphotericin B. Data this research was obtained, in 2010. **Methodology:** The quantitative study examined records of patients who had requested the release of liposomal Amphotericin B, as a treatment option, from the Ministry of Health, and had being treated with the drug. **Results:** A total of 28 patients with ATL was examined, 22 (78.6%) were men and six (21.4%) were women, 22 (78.6%) were older with age above of 50 years. 28 (100%) of patients with leishmaniasis were of the Central West Region and worked in different functions, however, of the three professions were predominant (10.7%) mason, five (17.9%) were housewives, eight (28.6%) were retired. Clinical symptoms of patients with ATL were evaluated, and the three most common were: injury in the face (10.7%), three with an ulcer in the ear (10.7%), and 11 (29.3%) were asymptomatic. The criteria used for release and use of liposomal Amphotericin B drug of choice for the treatment of the disease was: 19 (67.9%) with renal failure, four (14.3%) with resistance to Amphotericin B, three (10.7%) were age and heart disease. **Conclusion:** We observed positive results in the treatment of ATL with liposomal Amphotericin B, and this drug has few collateral effects. Thus, liposomal Amphotericin B can be used successfully in the treatment of American Tegumentar Leishmaniasis. **Key words:** American Cutaneous Leishmaniasis, liposomal Amphotericin B, Central West Region, Brazil. **E-mail:** liviaulopes@gmail.com

Leish124- Liposomal amphotericin B to treat visceral leishmaniasis in São Paulo state, Brazil

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Introduction: Visceral leishmaniasis (VL) is in frank expansion in Sao Paulo state and a high lethality is observed. Liposomal amphotericin B (LAmB) has been used as treatment of choice in children under 10 years and adults above 50 years of age, pregnant women, and patients with associated severe illness or immunosuppressive status. In this study we evaluated the treatment response using LAmB for VL patients in Sao Paulo state, comparing by age group. **Materials and Methods:** A survey at database of Zoonosis Center of Epidemiological Surveillance Center "Prof. Alexandre Vranjac" of São Paulo Health Department (CVE/CCD/SES-SP) was done to obtain data from all VL cases among 2007-2010, regarding to their clinical and epidemiological characteristics of VL, especially to their treatment. A number of 344 notified patients were analyzed and it was included only HIV-negative patient. **Results:** Database showed approximately 1220 cases of VL. Confirmed parasitological diagnosis and HIV-negative results were present in 550 patients, from whom 60.72% (334/550) were treated by liposomal amphotericin B as preconized by SES-SP at a total dose of 20mg/Kg (4-5mg/Kg/day for five days). Male were 55.38% (185/334) and 68.86% (230/334) were white. At about 59.28% (198/334) were children under ten years and 21.55% (72/334) were 50 years or more. 307 patients had their outcome reported and were included in the analysis of therapeutic response. The mean total cure rate was 93.81% (288/307) and lethality was 5.21% (16/307). Relapses were only observed in 2.28% (7/307) of cases without relationship with age. Concerning relapses we observed 1.6% (3/180) in children until 10 years and 2.89% (2/69) in patients above 50 years or more. Related to lethality we observed 1.6% (3/180) until 10 years and 10.14% (7/69) above 50 years or more. Surprisingly, we observed lethality of 17% in the group between 20-49 years.

Conclusions: Our data suggest that the use of LAmB for treatment of VL in children below ten years may contribute to the low lethality in this age group. High lethality in older than 50 years may be due to drug toxicity or pre-existing comorbidities in this population group. Although meglumine antimoniate is the drug of choice in individuals aged between 20-49 years, treatment with LAmB showed higher mortality, probably due to more severe cases in this casuistic. **E-mail:** igorthiago@usp.br and jlindoso@usp.br

Leish125- Mortality and risk factors associated with death from visceral leishmaniasis in patients treated with Liposomal Amphotericin B, Minas Gerais State, Brazil.

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Introduction: Studies on treatment outcomes in patients with visceral leishmaniasis (VL) treated with liposomal amphotericin B in Brazil are scarce. The use of liposomal amphotericin B is restricted to patients with severe disease and specific groups such as patients with renal failure. The aim is to evaluate the mortality and identify risk factors for death in patients with VL treated with liposomal amphotericin B. **Methods:** This historical cohort study was carried out in the Minas Gerais State, located in southeastern Brazil, in a period of 2008 to 2010. Data were obtained from the Reportable Disease Information System–SINAN (Brazilian Ministry of Health and Minas Gerais State) and complemented with data of the Mortality Information System and from application forms used to request liposomal amphotericin B. Demographic, clinical and laboratory data were evaluated. The primary endpoint was mortality at day 60 after initiation of liposomal amphotericin B treatment. Multivariate logistic regression was performed to identify variables associated with death from VL. **Results:** Two hundred seventy-three patients were included (68.5% male; median age, 42 years). The 60-day mortality rate was 28.2%. During the study period there was an increase in requests for liposomal amphotericin B. The number of treatments required during the years 2008, 2009 e 2010 were 58, 92 and 123, respectively. The main cause for liposomal amphotericin B request was renal failure (68.9%). Among the studied patients, 23.1% of treatments were carried out for HIV positive patients, 39.6% reported history of renal disease and 16.1% of cardiac disease. The factors independently associated with increased risk for death were age \geq 60 years (OR 5.8; 95%IC 2.5-13.6), presence of bleeding (OR 2.3; 95%IC 1.1-4.6), jaundice (OR 2.7 95%IC 1.4-5.7), presence of bacterial infections (OR 2.1 95% IC 1.1-4.2), serum urea \geq 40 mg/dl (OR 6.2 95%IC 2.6-14.9), white blood cells \leq 1000 or \geq 10000 cells/mm³ (OR 2.6 95%IC 1.2-6.2) and liver disease (OR 4.1 95%IC 1.6-10.4). **Conclusions:** The 60-day mortality rate in this group of patients was higher than overall case fatality rate reported in Brazil. Although a specific group of patients was evaluated, the risk factors associated to death were similar to those found in studies conducted with other patients groups. Studies should be conducted to assess whether a less restrictive strategy for the use of liposomal amphotericin B is able to reduce mortality. **E-mail:** bruna.tourinho@saude.mg.gov.br

Leish126- Phytochemical screening and *in vitro* leishmanial activity of crude extract of *Morinda citrifolia* fruit (noni)

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In the search for new alternatives of leishmaniasis treatment, the discovery of therapeutic properties of active substances present in plant extracts has attracted the interest of research into new treatment options using medicinal plants. *Morinda citrifolia*, commonly known as noni, stands out since it has several active substances that have antimicrobial, antiviral and antifungal and potential activity against

protozoa such as the members of *Leishmania* genus. We carried out the phytochemical study and evaluated, *in vitro*, the cytotoxicity of crude extract of *M. citrifolia* fruit, using macrophage cells and antileishmanial activity against *L. amazonensis*. The crude extract was obtained from the release of the liquid by the fruits and was carried out the phytochemical screening and the high performance liquid chromatography with diode array detector and light scattering. Antileishmanial activity was tested against promastigotes, axenic amastigotes and intracellular amastigotes. The crude extract showed a yield of 6.31% and the phytochemical screening showed the presence of anthraquinones, flavonoids, alkaloids, terpenoids, saponins, coumarins, phenolic compounds, tannins, anthocyanidins and chalcones. The high performance of liquid chromatography with diode array detector and light scattering identified phenolics and aromatics compounds as the major constituents. The extract showed a dose-dependent activity and the IC₅₀ was of 204.1 µg/mL for promastigotes, 137.0 µg/mL for axenic amastigotes, and 63.6 µg/mL for intracellular amastigotes. The cytotoxicity assay showed that the extract is toxic for the protozoan and was not to the cell. Transmission electron microscopy was performed to evaluate the ultrastructural alterations in promastigotes treated with 15 to 120 µg/mL of crude extract, for 24 hours and results showed cytoplasmatic vacuolization, lipid inclusion and increased activity of exocytosis. The crude extract of *M. citrifolia* fruit is active against *L. amazonensis* in the *in vitro* model and promising for further researches of new treatments for leishmaniasis. **E-mail:** anabreu@ioc.fiocruz.br

Leish127- The anti-trypanosome drug fexinidazole was effective in pre-clinical evaluation of cutaneous and visceral leishmaniasis caused by New World *Leishmania* species

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Currently, the available drugs to treat leishmaniasis have significant shortcomings, including high toxicity, long therapeutic regimens, parenteral administration and high cost. In the search for new alternatives, the Drugs for Neglected Diseases initiative (DNDi) has rediscovered the nitroimidazole fexinidazole, which is currently undergoing testing in phase 1 clinical trial for development against African sleeping sickness. This study evaluated the *in vitro* and *in vivo* activity of fexinidazole against three *Leishmania* species of New World: *L. braziliensis*, *L. amazonensis*, and *L. infantum*. In the amastigote-murine macrophage model, fexinidazole was effective against *L. braziliensis*, *L. amazonensis* and *L. infantum*, with IC₅₀ (IC₉₀) values of 16.0 µM (49.8 µM), 31.9 µM (324.7 µM) and 14.7 µM (157.3 µM), respectively. The *in vivo* activity of fexinidazole was evaluated in Balb/c model for *L. infantum* and *L. amazonensis*. Seven days after infection with *L. infantum* and 20 days after infection with *L. amazonensis*, the animals of each group received intraperitoneal injections, once daily for five days (*L. Infantum*: 25, 50 and 100mg/Kg/day) or 15 consecutive days (*L. amazonensis*: 50, 100 and 200mg/Kg/day). The effective doses of Glucantime® for *L. amazonensis* (300mg/Kg/day) and *L. infantum* (80mg/Kg/day) were used as reference. The cutaneous lesions of animals infected with *L. amazonensis* were measured on days D0, D7, D14 during treatment and D+7 post treatment. Parasite load in the liver (*L. infantum*) and spleen and cutaneous lesion (*L. amazonensis*) was determined by the positive parasite culture until limiting dilution. For the visceral leishmaniasis model, *L. infantum* parasite load in liver was significantly reduced ($p \leq 0.05$, compared to the untreated group), being as effective as the reference dose of Glucantime® ($p \leq 0.05$). For the cutaneous leishmaniasis model, a dose-response curve was observed with the tested doses (50, 100 and 200mg/kg/day) with incremental activity, significant decrease in lesion size and parasite load in the cutaneous lesions and spleen, as compared to the untreated group ($p \leq 0.0001$). The dose of 200mg/Kg/day of fexinidazole promoted a 100% parasite clearance in all dilutions tested in the lesion and spleen and complete healing of lesions after the treatment. The dose of 100mg/Kg/day was as effective as the reference dose of Glucantime® ($p \leq 0.05$). These observations provide support to the clinical development of fexinidazole for the treatment of cutaneous and visceral leishmaniasis caused by New World *Leishmania* species. **Email:** cfrossard@dndi.org

Leish128- Transmission potential and skin parasitism of symptomatic and asymptomatic dogs with visceral leishmaniasis treated with allopurinol and levamisole

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The objective of this study was to evaluate the parasitism in the skin of dogs with visceral leishmaniasis (VL) and the potential of transmission to *Lutzomyia longipalpis*, before and after treatment with a combination of allopurinol and levamisole. We used seven adult dogs, males, naturally infected with *Leishmania (L.) chagasi* and an uninfected dog, from the Management Zoonosis Control in Teresina, state of Piauí and of the Center for Zoonosis Control of Timon, MA, Brazil. The dogs were classified into three groups: five symptomatic dogs with serology and parasitology (bone marrow and/or popliteal lymph node) positive for LV and with three or more clinical manifestations, two asymptomatic dogs, one with serological and parasitological examination (culture) positive and the other with serological positive (IFAT/ELISA), without clinical manifestations, and one non infected dog. Before, during and after treatment the dogs underwent clinical examination. Smears were made of skin, bone marrow and popliteal lymph node for direct examination and quantification of the parasite load. Infected dogs were treated with the combination of allopurinol (20 mg/kg orally two times daily) and levamisole (0.5 mg/kg, orally, every other day) for five months. Xenodiagnosis was performed in all dogs before and after treatment. We used 60 female *Lutzomyia longipalpis*, packed in plastic containers with the end screened. The containers were placed on the ventral edge of both ears of each dog. Dogs were monitored for 45 minutes. In the sixth and seventh days after the blood meal, the insects were dissected and the gut was removed for observation by light microscopy, of *Leishmania* promastigotes. Three symptomatic dogs infected sandfly prior of the treatment, but not infected after treatment. Asymptomatic dogs not infected sandflies neither before nor after treatment. It was observed clinical improvement and reduction of parasitic load (n = 1) and absence of amastigotes in the skin of symptomatic dogs (n = 4) after treatment. There were no amastigotes in skin biopsies from asymptomatic dogs before and after treatment. It was concluded that transmission of amastigotes to the vector is dependent on the clinical condition of the dog and of the anti-*Leishmania* treatment. **E-mail:** fassisle@gmail.com

Leish129- Treatment of VL-HIV Co-infected Patients in São Paulo State, Brazil

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Introduction: Co-infection *Leishmania*-HIV/AIDS has increased worldwide, as well as relapse and lethality by visceral leishmaniasis (VL) in some regions. Resistance to pentavalent antimonial has been reported in India and it is not related to co-infection, contrary to Brazil where there is no related resistance to anti-*Leishmania* drugs. However, we have observed high rates of relapses and lethality in adults presenting VL-HIV/AIDS co-infection, due to interposition of areas of HIV/AIDS and VL. Here we evaluated the response to anti-VL drugs in co-infected patients in São Paulo state. **Materials and methods:** A survey at database of Epidemiological Surveillance Center “Prof. Alexandre Vranjac” of São Paulo Health Department was done to obtain data from all VL-HIV/AIDS co-infected patients of São Paulo state among 1999-2010, especially to their treatment. A number of 101 notified patients were analyzed. **Results:** Database showed approximately 1769 notified cases of VL. One hundred and one (5.70%) was HIV+ and had confirmed parasitological diagnosis, being included in this analysis. Male were 74.25% (75/101) and 79.20% (80/101) were young adults (19-49y). The mean lethality was 19.80% (20/101) and relapse was 10.89% (11/101) in VL-HIV/AIDS co-infected. In 99 treatments rescued, 41.41% (41/99) were done with meglumine antimoniate (MA) and 51.48% (52/101) with amphotericin B formulations. Almost 82.92% (34/41) co-infected patients were treated with MA, 76.47% (13/17) with amphotericin B deoxicolate (AmBd) and 71.42% (25/35) with amphotericin B liposomal (LAmB) were young adults (19-49y). Unfortunately, only 87.80% (36/41), 70.58% (12/17) and 91.42% (32/35) of outcomes with MA, AmBd and LAmB were rescued respectively. Cure was obtained in 69.44% (25/36) of treatments with

MA, 41.66% (5/12) with AmBd and 78.12% (25/32) with LAmB. Relapses were 5.55% (2/36) with MA, 8.33% (1/12) with AmBd and 25.00% (8/32) with LAmB. Regarding to deaths, lethality was 16.66% (6/36), 50.00% (6/12) and 21.87% (7/32) when treated with meglumine antimoniate, amphotericin B deoxycolate and amphotericin B liposomal respectively. **Conclusions:** Our data suggest higher lethality and relapse in co-infected patients compared to VL alone. Comparing the three drugs used, AmBd (elected by Brazilian Ministry of Health to treat VL-HIV/AIDS co-infected patients) had higher lethality than two others and LAmB had better outcomes (more cures) than MA and AmBd. As proposed by Sao Paulo State, amphotericin B liposomal might be the drug of choice to treat coinfecting individuals countrywide and efforts to that should be done by authorities. **E-mail:** igorthiago@usp.br and jlindoso@usp.br

Leishmania Diversity

Biochemical and Immunology

Leish130- Diversity of dermatropic *Leishmania* species in Santarém, Brazil

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Introduction: Seven dermatropic *Leishmania* species cause human tegumentary leishmaniasis (TL) in the Brazilian Amazon: *Leishmania (Leishmania) amazonensis*, *L. (Viannia) braziliensis*, *L. (V.) guyanensis*, *L. (V.) shawi*, *L. (V.) lainsoni*, *L. (V.) naiffi* and *L. (V.) lindenbergi*. The first two are associated with the most severe forms of the disease i.e., diffuse-cutaneous and mucocutaneous, respectively. Exploitation of the mineral riches of western Pará has produced accelerated economic growth in the city of Santarém and the region is undergoing environmental transformations that may favour increased TL incidence. In this study we describe the etiology and geographical distribution of new TL cases treated in the Centro de Controle de Zoonoses in Santarém, a reference centre for primary health assistance in support of 21 municipalities of western Pará. **Material and methods:** Sixty-one patients were recruited from January 2010 to July 2011. The research protocol received ethical approval from the Instituto Evandro Chagas (CEP/IEC 018/09 & 024/09). PCR products ITS1rDNA and *hsp70-234*, obtained from DNA extracted from patient skin samples and promastigotes of seven reference strains of *Leishmania*, were digested with restriction enzymes (PCR ITS1rDNA: HaeIII and MboI; PCR *hsp70-234*: HaeIII and BstUI) and submitted to high performance electrophoresis in 12.5% polyacrylamide gel. Band profiles were compared between patient samples and controls, allowing six species to be identified: *L. (V.) shawi*, *L. (V.) amazonensis*, *L. (V.) lainsoni*, *L. (V.) naiffi*, *L. (V.) braziliensis* and *L. (V.) guyanensis*. G6PD PCR confirmed the presence of the subgenus *Viannia* and species *L. (V.) braziliensis* in hybrid profiles from PCR-RFLP products. **Main Results:** All patients had TL based on parasitological (77%) or clinico-epidemiological (23%) diagnosis. PCR allowed identification to species in 21 (35%) of the samples: *L. (V.) braziliensis* (12; 20%), *L. (V.) lainsoni* (3; 5%), *L. (V.) naiffi* (2; 3%) and *L. (L.) amazonensis* (4; 6%). Identification was only possible to genus *Leishmania* in 23 (38%) of samples and to subgenus *Viannia* in 17 (28%). Seven samples presented hybrid profiles after enzymatic digestion (RFLP ITS1: 3 and *hsp70-234*: 4), of which five were positive with PCR using G6PD (*Viannia*: 3; *L. (V.) braziliensis*: 2) and two negative. Autochthonous cases from Santarém represented half of all samples, with four species detected, whereas allochthonous cases were from seven municipalities in the Brazilian states of Pará (6) and Amazonas (1) or French Guiana. **Conclusion:** The PCR-RFLPs ITS1, *hsp70-234* and G6PD together identified four *Leishmania* species among only 21 samples from Santarém, revealing great species diversity. Detection of hybrid patterns by PCR-RFLP suggests that new *Leishmania* lineages are circulating in the region. Most of the cases investigated in the western Pará were from municipalities in

the region of the lower Amazon River, where molecular epidemiological studies could provide the basis for planning of TL surveillance, prevention and control measures. **E-mail:** lourdesgarcez@iec.pa.gov.br

Leish131-The duration of mucosal disease and the time of clinical cure influences the *in vitro* cytokine production by peripheral mononuclear cells from long term healed mucosal leishmaniasis patients

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Introduction: Several reports have unequivocally shown that an exacerbated type 1 immune response to leishmanial antigens is the basis of severe tissue destruction observed in active mucosal leishmaniasis (ML) patients. Furthermore, persistent production of high levels of inflammatory cytokines post therapy can confer a bad prognosis. The present study investigated whether the conditions defined during active disease of ML could influence the magnitude of anti-*Leishmania* immune response. **Material and Methods:** Twenty-one clinical cured ML patients were studied. Peripheral blood mononuclear cells (PBMC) were cultured with *Leishmania braziliensis* antigens (Lb-Ag), *Toxoplasma gondii* antigens (Tg-Ag), Concanavalin A (Con-A) or medium alone, and measured the lymphocyte proliferative response (LPR) and cytokine secretion by ELISA (IFN- γ , TNF, IL-18, IL-10 and IL-5). Retrospectively, medical records of the patients were reviewed for clinical and laboratorial parameters as: delayed type-hypersensitivity response during diagnosis (Montenegro skin test, MST), duration of clinical cure after treatment or duration of ML disease. **Results:** Trend of positive correlation between duration of ML disease and MST induration ($r=0.46$, $p=0.06$, $n=16$) showed us an evidence that duration of ML illness could influence the immunopathogenesis. The IFN- γ production in response to Lb-Ag was positively correlated with duration of illness ($r=0.55$; $p=0.02$; $n=17$). In contrast, no association was observed when PBMC of the same patients were stimulated with Tg-Ag or Con-A. Also we observed a negative correlation between the duration of ML disease and production levels of IL-10 ($r=-0.77$, $p=0.04$, $n=7$). It is interesting to note that the magnitude of MST was negatively correlated with the IL-5 production after clinical cure ($r=-0.76$, $p=0.01$, $n=10$). The IFN- γ /IL-10 ratio was negatively correlated with the duration of clinical cure ($r=-0.78$; $p=0.02$; $n=8$). **Conclusions:** Together, these results suggest that the prognosis of patients seems to be determined, at least in part, by the duration of ML disease. Also, the time of clinical cure can contribute to the better modulation of immune response essential to the control of parasite replication without triggering tissue damage. **SUPPORT:** FIOCRUZ, FAPERJ e CNP-q **E-mail:** alda@ioc.fiocruz.br

Leish132- The use of metagenomics in the search of new leishmanicidal drugs

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Introduction: Soil microorganisms have been the most valuable source of natural products, providing important active biomolecules. However, the most soil microorganisms cannot be cultured using traditional cultivation techniques. The metagenomics is a promising approach that allows access to the genome of these uncultivable microorganisms and is based on the environmental DNA cloning to access the whole genome of a given microbial population. This approach involves the construction of libraries of clones that contain genetic information relating to the production of bioactive secondary metabolites. This study shows the standardization of the screenings of two soil metagenomics libraries in search of clones that produce biologically active compounds against protozoa of the *Leishmania* genus. **Material and Methods:** The screening was performed in two soil metagenomics libraries containing 250,000 clones in cosmid pJSS using two different hosts: *Escherichia coli* and *Ralstonia metallidurans*. The clones were plated, incubated for 5 days at 22°C and the leishmanicidal activity of clones were determined using a

overlay assay. If the metagenomics clone produces a leishmanicidal active compound, the parasites will not proliferate around the colony and can be identified by a halo. **Results:** Our initial screening in *E. coli* library identified 35 clones that produce a prominent halo around the colonies. The screening of *R. metallidurans* metagenomics library showed no colonies with leishmanicidal activity. **Main conclusion:** The screening of libraries derived from the soil metagenome provides opportunities to explore the genetic and metabolic diversity of soil microorganisms and this strategy may result in the isolation of novel bioactive molecules. **Supported by FAPESP E-mail:** izaltina@ursa.ifsc.usp.br

Leish133- The use of lectins to purify metacyclic promastigotes *leishmania*

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Background: In experimental leishmaniasis infections, the use of metacyclic enriched inoculum is very important, because it can simulate the natural infection and avoids the inflammatory response induced by the high density parasite inoculum. The surface of different evolutionary forms of *Leishmania* is coated by several glycoconjugates that can be recognized specifically and reversibly by lectins, proteins that have affinity with carbohydrates. The use of lectins in order to purify metacyclic *Leishmania* is a great goal. In this study it was evaluated if lectins of different specificities would be able to agglutinate *L. amazonensis* promastigotes of different evolutionary forms. **Material and Methods:** It was used *Artocarpus integrifolia* (AIL), *Erythrina fusca* (EFL), *Erythrina velutina* (EVL), *Vatairea macrocarpa* (VML), that bound to D-galactose and *Canavalia brasiliensis* (ConBr), *Cratylia floribunda* (CFL) e *Dioclea violacea* (DVL) that bound to D-glucose/D-mannose. The binding specificity was analyzed by agglutination tests. DVL agglutinated 78% of *L. amazonensis* promastigotes from logarithmic phase culture and 52% from stationary phase, so it was selected for *in vivo* tests. Stationary phase promastigotes were incubated with DLV to evaluate if the agglutination was stage-specific and it was purified the agglutinated and non agglutinated fractions. Golden hamster were infected with 10⁶ promastigotes and grouped as: Agglutinated fraction (AF) (n=8); Non agglutinated fraction (NAF) (n=9); Control (C) (n=8). The lesion size was measured along 6 weeks. The parasite load of regional lymph node was quantified by limiting dilution and histopathological analysis of the lesions were performed. **Results:** The lesions began at the third week in all groups. The lesion's size was similar in all of them, except at the fourth and fifth weeks that NAF presented a transitory reduction (p<0,01). There was not difference concerning the parasite load and histopathologic changes among the groups. **Main Conclusions:** These data suggest that DLV did not select effectively infective forms of *L. amazonensis*, although it agglutinates promastigotes from the two culture growth phases. **E-mail:** mpompeu@gmail.com

Leish134- T_H17, T_H1 and T_H2 cytokine production in patients with active American cutaneous leishmaniasis

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Introduction: American cutaneous leishmaniasis (ACL) is an anthroponotic disease caused by protozoans from the genus *Leishmania* and transmitted to the man by the bite of *Lutzomyia* phlebotomines. *Leishmania (Viannia) braziliensis* is the causative species of most cases of ACL in Brazil and Pernambuco. These infections induce a specific activation of the host immunological response, specially characterized by T cells expansion, with production of various cytokine profiles. The classical T_H1 and T_H2 subsets have been joined recently by the T_H17 lineage. IL-17 producing T cells were identified as important drivers of autoimmune pathology and allergic inflammation. Only a few studies have assessed the role of IL-17 in human infectious diseases and it is not known whether this cytokine participates as a defense mechanism or in the pathology of these diseases. **Material and Methods:** A peripheral blood mononucleated cells (PBMC) ring was obtained from 11 patients with active lesions (AT) and 6 healthy controls (CT). The cells were washed twice with PBS, centrifuged and resuspended in

culture medium. The cells were then adjusted to a concentration of $0,5 \times 10^6$ per well and then incubated with PMA and Ionomycin for 6 hours. Brefeldin A was added to the culture 4 hours prior to the end of the incubation period. After the incubation time, EDTA was added to the culture and incubated for 10 minutes. The cells were washed and added to polystyrene tubes containing monoclonal antibodies anti-CD4 and anti-CD8, both labeled with FITC, and incubated for 30 minutes. The cells were then permeabilized and stained with cytokine specific antibodies against IFN- γ , TNF- α , IL-4, IL-10, IL-17, IL-21, IL-22 and IL-23 labeled with PE, PeCy7, APC and AF647. The cells were washed and analyzed using a FACSCalibur cytometer. **Results:** A significant increase in the production of IL-4 by CD4⁺ and CD8⁺ T lymphocytes respectively was observed in AT (18.53 ± 14.44 and 11.21 ± 8.28) in comparison to CT (3.71 ± 1.42 and 3.59 ± 1.19). A discreet increase was observed in the production of IL-22 (AT= 0.29 ± 0.18 and CT= 0.14 ± 0.07), and in the simultaneous production of IL-21 and IL-22 by CD4⁺ lymphocytes (AT= 0.06 ± 0.05 and CT= 0.019 ± 0.017). It was also observed a discreet decrease in the production of IL-17 and IL-23 by these cells in AT (0.11 ± 0.10) in comparison to CT (0.20 ± 0.16). **Main Conclusions:** The significant increase in the production of IL-4 by CD4⁺ and CD8⁺ T lymphocytes suggest that this cytokine plays an important role in the immunopathology of cutaneous leishmaniasis. IL-4 induces a Th2 response in *Leishmania* infections, an immune profile related with the resistance to the disease. Additional studies, including different clinical phases of the disease, are under development to further contribute in the immunologic knowledge about the mechanisms involved in the cure of ACL. **E-mail:** amandafa@cpqam.fiocruz.br

Leish135- Proteins differentially expressed in plasma of patients infected with *Leishmania (Viannia) guyanensis*

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Introductions: Biomarker is any molecule or biological characteristic that can be detected and measured revealing normal biological processes, pathogenic or pharmacological response after therapeutic intervention. The identification of protein markers of *Leishmania* infection in humans has importance for both diagnostic confirmation and curing of the disease, but also to the search for alternative treatments and vaccines. **Material and Methods:** To pursue these markers was performed a preliminary comparative analysis of two-dimensional maps generated by 2D-I electrophoresis of plasma from normal individuals and patients (both sexes) infected with *Leishmania (Viannia) guyanensis*, before and after treatment with pentavalent antimony (Glucantime). **Results:** The number of spots expressed between the three conditions used was 250 in average, and a larger number observed with active infection in a patient (male/294 and women/271). The majority of protein spots were expressed in the acid region between 77 and 30kDa. When comparing patients' plasma before and after treatment with normal subjects the number of differentially expressed spots was 19 in men and 34 for women plasma. **Conclusions:** The expression of a greater number of spots provided before treatment indicates the presence of groups of proteins and peptides expressed specifically in this condition may be indicated as a marker protein of *Leishmania* infection. **Key-words:** Leishmaniasis, 2D, proteome, infection. **Financial support:** FAPEAM/INPA **E-mail:** luandafigueira@yahoo.com.br

Leish136- Preliminary evaluation of Treg cells in American Cutaneous Leishmaniasis patients

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Introduction: In Brazil, the etiological agent of American cutaneous leishmaniasis (ACL) with the highest incidence is *Leishmania (Viannia) braziliensis*. The clinical manifestations of ACL are diverse and, in all its clinical forms, susceptibility or resistance may depend in part on the T cell response. More recent studies have implicated Tregs in infection control and maintenance of a long lasting immune response in mice infected with *L. major*. These cells have been shown to be crucial for the control of T cell autoimmunity and in reducing inflammation. However, there isn't a good demonstration of its direct role in individuals infected with *Leishmania* or on the mechanisms involved in the disease development. Therefore, the objective of this project is to begin evaluating Treg cells in PBMC of individuals with active ACL. **Material and Methods:** The proportion of Treg cells was determined by *ex vivo* labeling of PBMCs from five patients with active ACL from an endemic area in the state of Pernambuco. Cells were incubated with surface and intracellular antibodies (anti-CD3, -CD4, -CD25, CD45RA, -CCR7, -CCR4, -CTLA-4, -FOXP3 and -IL-10). The samples were analyzed (20,000 events / tube) in a flow cytometry (FACSCalibur, BD) and identified by the software "CellQuestPro" linked to the cytometer. **Results:** It was observed the presence of active (aTreg, CD4⁺FOXP3⁺CD45RA⁻, ±66,82%) and resting Foxp3 (rTreg, CD4⁺FOXP3⁺CD45RA⁺, ±29.5%) regulatory T cells (from the total of ±0.52% of CD4⁺FOXP3⁺ Treg cells) in the samples from the patients with ACL. Within these subpopulations, ±54.67% rTreg(+) expressed the marker CCR7, and had low expression of IL-10 (±3.65%). When considering the aTreg cells we noted that they were present in a proportion in the peripheral blood of these patients. This subgroup had high expression of the markers CD25 (±70.27%), CCR4 (±68.08%) and CTLA-4 (±52,15%), consistent with its expansion, activate phenotype and high suppressive capacity. **Conclusions:** The results support the hypothesis that regulatory T lymphocytes are activated during the immune response of patients with ACL, being the aTreg more present in these individuals, and acting by direct cellular inhibition mechanisms. These studies aim to cooperate in the development of appropriate strategies for future immunological interventions and vaccination. **Financial Support:** CAPES, FACEPE, FIOCRUZ-PE. **E-mail:** carolina.brelaz@gmail.com

Leish137- Preliminary approach to the intracellular localization of Nicotinamide Mononucleotide Adenylyltransferase in *Leishmania braziliensis*

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Introduction: The Nicotinamide Adenine Dinucleotide (NAD⁺) plays essential roles in cell metabolism, especially in processes related to energy transduction due to its electron transfer potential. Nevertheless, recent works have unveiled a whole new spectrum of functions of NAD⁺, in which the molecule is used as a substrate rather than as a cofactor. Among others, these processes relate directly to cell death pathways, calcium mobilization, circadian rhythm control, caloric restriction and ageing, in several model organisms. Given the importance of NAD⁺ in energy metabolism and cell signaling, it is not a surprise that two pathways are involved in its biosynthesis: a *de novo* pathway and a salvage pathway, both converging at a step catalyzed by the Nicotinamide Mononucleotide Adenylyltransferase (NMNAT; EC 2.7.7.1), the central module in the NAD⁺ synthesis. NMNATs from several model organisms have been experimentally characterized, including archaeobacteria, bacteria, yeasts, insects, plants and mammals. Our research group focuses on the study of the energetic metabolism in intracellular parasites of global importance, such as *Plasmodium falciparum* and *Leishmania braziliensis*. Specifically, we work towards the identification and functional and kinetic characterization of their NMNATs, aiming to establish the molecular basis for the future design of rational control measures. **Materials & Methods:** Previously, we have accomplished the identification, cloning, overexpression, purification and enzymatic characterization of a recombinant version of *L. braziliensis* NMNAT (His-LbNMNAT). In order to continue the study of the metabolism of NAD⁺ in *Leishmania*, the His-LbNMNAT purified protein was used for polyclonal antibody production in murine models. The obtained antibodies were implemented in the standardization of Western blot and immunofluorescence protocols using cell extracts and promastigotes of different *Leishmania* species. **Results:** This experimental approach allowed us to reveal the presence of a protein of lower molecular weight compared to the expected weight in the soluble fraction of the extracts. Currently, the identity of the identified signal is being confirmed by mass spectrometry.

Immunolocalization assays revealed a cytoplasmatic distribution and a partial overlaying between the NMNAT and the parasites mitochondria, as detected by using mitochondrial probes. **Conclusion:** The above results expand the knowledge related to the energy metabolism of *Leishmania*, by establishing an initial experimental approach in determining the subcellular location of the NMNAT in the mobile phase of the parasite. **E-mail:** lecontreras@unal.edu.co, mhramirez@unal.edu.co

Leish138- Potential antigenicity and conservation of CD8 T cell epitopes of Kinetoplastid membrane protein-11 (KMP-11) of *L. amazonensis*: An immunoinformatics approach.

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Introduction: Kinetoplastid Membrane Protein-11 (KMP-11) is a candidate molecule for a vaccine against leishmaniasis. **Material and Methods:** We used an immunoinformatics approach to identify and characterize protein regions with potential antigenicity and promiscuity. We used the *L. amazonensis* KMP-11 sequence (GenBank: AAG60608.2) in FASTA format to predict CD8+ T cell epitopes and to perform epitope conservancy analysis using algorithms available in Immune Epitope Data Base and Analysis Resource (<http://www.iedb.org>). Physical and chemical properties of KMP-11 sequence were analyzed using the Expert Protein Analysis System (ExPASy/ProtScale). The results were analyzed using the R software statistical environment. **Results:** We found that among the *Leishmania* genus exist four conserved sequences representing 21 nonamers of the *L. amazonensis* KMP-11 sequence. Our analysis revealed three nonamers with high potential to be CD8+ T cell epitopes: N65 (KMHEHSEHF), N22 (KMQEQNAKF) and N54 (MIKEHTDKF). Using the concept Total Score, we observed that the nonamers N65 (KMHEHSEHF), N23 (MQEQNAKFF), N22 (KMQEQNAKF) and N54 (MIKEHTDKF) presented the highest values meaning that high amount of these peptides could be presented by MHC molecules on the cell surface. Among the 85 nonamers considered, we observed two conserved regions among the *Leishmania* genus: N11 (KLDRLDEEF) and N65 (KMHEHSEHF). The most promiscuous nonamers were N54 (MIKEHTDKF), N22 (KMQEQNAKF), N65 (KMHEHSEHF) and N73 (FKQKFAELL). **Main Conclusions:** In this study using an immunoinformatics approach we identified and described some conserved sequences of KMP-11 of *L. amazonensis* with potential antigenity for CD8+ T cells. This could constitute an interesting strategy to identify potentially immunoprotective sequences of this protein to be tested in immunological studies for vaccine development. **Keywords:** Bioinformatics, antigenicity, KMP-11, class I HLA, Leishmaniasis, *Leishmania amazonensis*. **Financial Support:** FAPERJ, CNPq, CNPq/PEC-PG. **E-mail:** jucasaar@ioc.fiocruz.br

Leish139- Multilocus analysis supports the existence of inter-species recombination in an endemic area of cutaneous leishmaniasis where *Leishmania* species are sympatric

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Cutaneous leishmaniasis (CL) in Pará, Brazil, has been associated to all *Leishmania* species described in the Country so far causing CL: *L. (Leishmania) amazonensis* and several *L. (Viannia)* species. Some studies have demonstrated that the transmission cycle complexity and the co-existence of two or more *Leishmania* species living in sympatry affect the level of genetic polymorphism in natural *Leishmania* populations. In this study twenty-two *Leishmania* isolates were typed by multilocus analyses employing enzyme electrophoresis (MLEE) and/or DNA sequences (MLSA), after PCR targeting different genes. The results confirm the circulation of *L. amazonensis*, *L. braziliensis*, *L. guyanensis*, *L. naiffi* and *L. lainsoni* in the studied area. Two isolates were typed as *L. naiffi* by MLEE, but the *hsp70* gene sequence of one of

them presented high identity with *L. braziliensis* sequences. Another isolate was typed as *L. guyanensis* by all enzymes assayed and DNA sequences. However, this isolate presented a heterozygotes profile for the enzyme 6PGDH, indicative of hybrid between *L. guyanensis* and *L. braziliensis*. Of note is the fact that the hybrid and the putative parentals are circulating in the same region. Although hybrids between these two species were already observed in other endemic regions, this is the first evidence of this hybrid in a Brazilian endemic region. One isolate was typed as *L. amazonensis* by some markers employed. For some markers, like hsp70 gene, this isolated was typed as *L. mexicana* complex, because the sequence divergence does not allow the identification of *Leishmania* species belonging to this complex. The profile obtained for G6PDH after MLEE assay indicate a mixed profile between *L. braziliensis* and *L. amazonensis* and MPI gene sequence analysis typed this isolate as *L. braziliensis*. This isolate was inoculated in hamsters, which presented disseminated lesions. The isolates obtained from the hamsters were typed again by MLEE and all of them exhibited the same *L. amazonensis* and *L. braziliensis* mixed profile. It is not possible to infer the mode of reproduction based on our results. Whether *Leishmania* parasites presenting hybrid profiles are contributing to or are a result of the genetic diversity detected in the area is another point that needs to be addressed. The results presented here indicate that the studied region is complex in which relatively pure populations and hybrids are circulating and probably adapted to multifaceted transmission cycle. **E-mail:** boitemc@ioc.fiocruz.br

Leish140- mRNA Expression for tumor necrosis factor Alpha (TNF- α) in patients with American tegumentary leishmaniasis

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Introduction: In all forms of ATL, the susceptibility or resistance to disease is dependent on T cell responses, which are characterized by an increase of CD4 + T cells, showing a Th1 or Th2 profile. In Th1 responses, TNF- α plays an important role in the healing of the disease, contributing to the intracellular killing of the parasite and disease control. Thus, this study aimed to quantify mRNA expression of TNF- α using peripheral blood mononuclear cells (PBMC) of patients with active leishmaniasis. **Material and Methods:** Cells from seven patients and five controls were incubated for 24 hours in the presence of the concanavalin A (2.5 mg / ml), phytohemagglutinin (5.0 ug / ml) and *L. (V.) braziliensis* soluble (AgSol, 1,25 μ g/ml) or insoluble (AgIns, 2,5 μ g/ml) antigens. The expression of TNF- α was evaluated using real time quantitative PCR, and the results were obtained through the comparative Ct method. Data were considered significant when $p < 0,05$ (Wilcoxon and Mann-Whitney tests). **Results:** Under the mitogen stimulation, TNF- α was significantly expressed in patients and controls. In response to AgSol, TNF- α expression was 3.45 fold higher in patients (Δ Ct = 14.47 ± 1.36) in comparison to healthy individuals (Δ Ct = 12.26 ± 0.66 , $p = 0.01$). In patients (Δ Ct = 13.04 ± 1.67), this cytokine was 3.78 fold more expressed in cells stimulated with AgIns when compared to control (Δ Ct = 13.8 ± 0.37). A significant mRNA production 0.3 fold higher for TNF- α in PBMC of patients was promoted by AgIns in relation to AgSol ($p = 0.01$). **Main conclusions:** TNF- α is a proinflammatory cytokine that although is related to an improved immune response, overproduction may contribute to tissue damage and lesion formation. Thus, this study may help to better understand the role of TNF- α in the process of healing of the disease. **Keywords:** American tegumentary leishmaniasis, cytokines, mRNA, qPCR. **E-mail:** thays_malmeida@hotmail.com

Leish141- LRV detection by nested rtPCR in tegumentary leishmaniasis patients from Brazil

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Tegumentary leishmaniasis (TL) comprises a spectrum of clinical manifestations caused by a group of protozoan, *Leishmania* spp., transmitted to humans and other mammals by phlebotomine sandflies. Clinical manifestations of the disease range from localized cutaneous lesions to disseminated leishmaniasis, including mucosal. More than ten species of *Leishmania* from subgenera *L. (Viannia)* and *L. (Leishmania)* cause TL in South America (Reithinger et al 2007). However severe forms regarding to mucosal commitment are mainly associated to *L. (V.) braziliensis*. From 1-10% of TL infections result in mucosal leishmaniasis 1-5 years after the cutaneous lesion has healed (Davies et al 2001; Marsden 1986). Many host factors such as gender, age and immune status are associated with disease progression. Recently it was suggested that metastasizing parasites exhibiting high *Leishmania* RNA virus (LRV) 1 load subverted the host immune response and promoted parasite persistence (Ives et al 2011). LRV is a RNA virus that persistently infects different *Leishmania* lineages as *L. (L.) major* (Widmer & Dooley 1995) and also strains of *L. (V.) braziliensis* and *L. (V.) guyanensis*, circulating within specific regions in South America (Salinas et al 1996). Nevertheless many strains must be examined to support the hypothesis that those containing the virus are associated with the subsequent development of mucocutaneous or relapse leishmaniasis (Scott 2011). In this study we used nested rtPCR targeting conserved virus sequence to investigate the presence of LRV in Leishmaniasis lesions from Brazilian patients predominantly diagnosed with *L. (Viannia)* infections. No LRV infection were observed in samples collected in endemic regions located in the southeast region (n=0/31), mainly from Rio de Janeiro, independently of the severity of the disease observed in these patients. It is known that *L. braziliensis* is the principal species causing TL in these regions. However, LRV was detected in some samples collected from patients from the North region (n=2/8). Both LRV positive cases developed reactivations around one year after the first lesion. Sequencing of *Leishmania* *hsp70* gene was conducted to determine the *Leishmania* species causing TL. The two samples presenting LRV infection were identified as *L. guyanensis*. These results corroborate other studies demonstrating LRV infection in *L. guyanensis* strains circulating in the Amazon River basin (Saiz et al 1998; Salinas et al 1996). **Supported by** POM FIOCRUZ and PROEP – FIOCRUZ/CNPq **E-mail:** marciapo@ioc.fiocruz.br

Leish142- *Leishmania chagasi* immunosensor piezoelectric using recombinant antigen immobilized in nanogold nafion film

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Introduction: Visceral leishmaniasis (VL) is among the main neglected diseases around the World, and the detection of specific antibodies to *Leishmania sp* and effective diagnostics of VL is of fundamental importance with regard to the efficient and appropriate treatment of the disease and quality of life of the patient, becoming a challenge for the scientific community. **Material and Methods:** Exploring the properties of nanomaterials, an immunosensor sensitive, rapid and selective was developed for diagnosis of VL. Nafion polymer was used to modify the electrode surface of quartz-crystal, acting as a platform for linked-gold nanoparticles on gold surface of the piezoelectric crystal. The *L. infantum* (rLci2B-NH6) recombinant antigen was previously immobilized on self-assembled monolayers (SAM) on the surface of gold nanoparticles, and then added to the nafion film. **Results:** The proposed immunosensor reacted well to VL canine serum showing good linearity $r = -0.98899$ ($p < 0.0001$, $n = 4$), with a low relative error = 5 %, **Conclusions:** The results obtained indicate that it may be a promising alternative tool for the diagnosis of VL, being able to distinguish positive and negative canine serum to *L. infantum*. **E-mail:** Joiramoss@gmail.com

Leish143- *Leishmania braziliensis*: differential lipid profiles of promastigotes and amastigotes

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Introduction: *Leishmania spp.* is an intracellular parasite that belongs to the Trypanosomatidae family. This protozoa is transmitted by phlebotomine sandflies as metacyclic promastigotes that infect macrophages in the vertebrate host where they multiply as amastigotes. In Argentina, the coexistence of *L. braziliensis*, *L. guyanensis* y *L. amazonensis* has been detected, being the major causative agents of human leishmaniasis. The limitation of initial infection depends on the activation of the innate immunity. Since there is a growing body of evidence pointing to an important modulatory role of microorganisms' lipids in this process, herein we focused on the study of the lipid profiles of *L. braziliensis* promastigotes (PRO) and intact or autolysin amastigotes (AMA). **Material and Methods:** *Parasites.* *L. braziliensis*, clon MHOM/BR/75M2904, PRO and AMA were axenically cultured in LIT media at 24°C or 37°C, respectively. Autolysin AMA was prepared by freezing-thawing and subsequent overnight incubation at 37°C. *Lipid profile analysis.* Lipids were extracted from PRO and intact or autolysin AMA according to Bligh & Dyer, analyzed by thin layer chromatography and identified by comparison with authentic standards after charring. Densitometric analysis was performed with Gel-Pro® Analyzer 4.0 software. *Nitric oxide (NO) detection.* NO production was determined in J774 cell line stimulated with *L. braziliensis* lipids by the Griess assay. **Results:** Quantitative differences in the phospholipid (PL) fraction of *L. braziliensis* PRO and AMA were determined. The latter presented higher amounts of lysophosphatidylcholine and phosphatidylethanolamine with respect to PRO (~ 4 and 1.2 fold higher, respectively). Besides, PRO presented higher levels of sphingomyelin and phosphatidic acid with respect to AMA (~ 2-fold). Considering that autolytic processes can generate inflammatory factors, we also performed the lipid analysis of autolysin AMA. Results showed a significant parasite PL degradation with an important increase in free fatty acids (FFA), thus suggesting the action of Phospholipase A, enzymatic activity previously described in this protozoa by our group. On the other hand, we determined that total lipids from each parasite stage were able to induce nitric oxide, a soluble factor implicated in parasite growth inhibition. **Conclusion:** The quantitative differences observed between the lipid profiles of *L. braziliensis* PRO and AMA, could be related to the biological function of each stage. In autolysin AMA, PL degradation generated FFA, second messenger that might participate in several signaling pathways related to inflammatory processes. **Supported by** FONCYT/CONICET **E-mail:** paradife@fmed.uba.ar

Leish144- *Leishmania (Leishmania) chagasi*-infected hamster as a model to study pancytopenia and the role of IGF-I in visceral leishmaniasis

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Introduction: Pancytopenia is an important clinico -pathological alteration in visceral leishmaniasis (VL) but the mechanism is not completely known yet. Insulin-like growth factor-I (IGF-I) plays a role in the development of the infection by *Leishmania*. In addition IGF-I is considered an important promoter of growth of hematopoietic cells *in vitro* although the role of the endogenous IGF-I in the hematopoiesis is not clear. *Leishmania (L.) chagasi*-infected hamster (golden hamster) is a good experimental model to study the alterations in the VL and in this model we evaluated the development of the pancytopenia and the expression of IGF-I in the bone marrow. **Material and Methods:** We used hamsters infected (N=10) with 2×10^7 amastigotes of *Leishmania (Leishmania) chagasi* and non-infected hamsters as controls (N=10). We maintained for 90 days and the blood was collected every 30 days and processed in an ABC VET automated veterinary hematology analyzer and for differential counts. IGF-I expression was quantified by Real Time PCR relative quantification method. **Results:** Ninety days post-infection infected hamsters presented hepatosplenomegaly and hematological alterations. The leucocyte count was

8.11±0.99 (mean ± standard deviation) $\times 10^3/\text{mm}^3$ in the infected animals and, 12.20±1.73 $\times 10^3/\text{mm}^3$ (p=0.0079, Mann-Whitney test) in non-infected animals. The red blood cell count was 7.4±0.24 $\times 10^6/\text{mm}^3$ in the infected animals and 7.8±0.38 $\times 10^6/\text{mm}^3$ in non-infected animals. The platelets count was 451.14±13.02 $\times 10^3/\text{mm}^3$ in the infected animals and 527.4±81.88 in non-infected animals. Analysis of mRNA expression of IGF-I in bone marrow revealed 2.7 times higher expression in the infected animals than in control animals. **Conclusion:** These results suggest that *L. (L.) chagasi* –infected hamster is a good model to study pathogenesis of pancytopenia of VL, and higher expression of IGF-I may suggest its role in an attempt to recover hematopoiesis during VL. **Supported by:** FAPESP, CNPq, CAPES, FINEP, LIM-38 (HC-FMUSP) **E-mail:** amandaratorres@usp.br

Leish145- kDNA Minicircle Signature: A Molecular Marker to Evaluate Genetic Polymorphisms of *Leishmania (Viannia) braziliensis* in Cutaneous and Mucosal Leishmaniasis Patients

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Introduction: *Leishmania (Viannia) braziliensis* is the main widespread species responsible for human cases of American tegumentary leishmaniasis (ATL) in Brazil. The disease is characterized by chronicity, latency, and metastatic tendencies. In some cases, after the appearance of an initial cutaneous lesion, mucosal involvement can occur as a consequence of the dissemination of the parasite through the blood and lymphatic systems. **Material and methods:** A total of 61 samples obtained from cutaneous and mucosal lesions of patients with different clinical manifestations of ATL, including HIV co-infected subjects, proceeding from Rio de Janeiro, Brazil were analyzed. We have used polymerase chain reaction (PCR) and low-stringency single-specific primer PCR (LSSP-PCR) analyses to detect *L. (V.) braziliensis* and to investigate kDNA signatures of parasite populations. The intraspecific polymorphisms in the variable region of the kinetoplast DNA (kDNA) minicircles of *L. (V.) braziliensis* in ATL patients has been investigated by our group using LSSP-PCR. Phenetic analysis was performed to evaluate the degree of heterogeneity of the kDNA minicircles. **Results:** Similar and divergent kDNA signatures have been observed in parasites recovered from mucosal (nasal and/ or oral) and cutaneous lesions of the same patient. No correlation was observed between the genetic profiles of *L. (V.) braziliensis* and either the clinical forms of the disease (mucosal, mucocutaneous and disseminated) or the location of the lesion (cutaneous, nasal or oral). In order to evaluate the impact of immunosuppression on the genetic structure of the parasite, the genetic polymorphism of *L. (V.) braziliensis* detected in cases of mucosal leishmaniasis from HIV-infected and non-HIV-infected patients has been evaluated. Samples obtained from human cases of *Leishmania*/HIV co-infection were found to be genetically divergent. The clinical and epidemiological implications of such results will be discussed. **E-mail:** rpacheco@ioc.fiocruz.br

Leish146- Isolation and identification of *Leishmania chagasi* recombinant antigens by immunoscreening of cDNA library

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Introduction: The leishmaniasis are endemic in more than 88 countries and represent an important health problem in Brazil. Early diagnosis, vector control and, until recently, dog culling, constitute the only approach to fight visceral leishmaniasis. Human and canine serodiagnosis, however are impaired by cross reactions to other diseases and by antigen standardization when large scale production is needed for public health programs. Recombinant antigens can potentially reduce both constraints and production costs. Nowadays, there are only two recombinant antigens on the market for kala-azar diagnosis. Issued or pending foreign patents for a dozen other antigens and a clear trend to explore the potential market

represented by kala-azar in a worldwide basis may severely restrict the opportunities for the local development of new royalties-free diagnostic tests, vaccines or immunotherapy for leishmaniasis, creating a detrimental dependence on foreign products and increasing control programs costs in Brazil. The aim of this work was the isolation and characterization of novel antigens recognized by either human or canine visceral leishmaniasis sera. **Material and Methods:** The cDNA library was constructed on pSport1 plasmid and *Escherichia coli* DH10B was used as the host. Cloning was performed in Sal I and Not I sites. Human and dog sera were adsorbed against a recombinant HSP70 fragment previous to their use in immunoscreening. Sequencing was done in ABI 3100 sequencer. **Results:** The cDNA library immunoscreening allowed the isolation of clones expressing both HSP83 and K39 antigens and other antigens, belonging to two categories: hypothetical proteins or proteins with known function, either enzymes or structural proteins; except for one case, the proteins were not previously described as antigens in leishmaniasis. Excluding the immunodominant HSP83 and K39, there were no antigens common to both screenings, using either human or canine sera. Some of the 10 novel antigens have a few or many amino acid repeats, which may explain their ability to be strongly recognized by kala-azar antibodies. Domains and motifs could be assigned to some antigens, as well as putative functions and cellular localization. Epitopes were mostly situated in the c-terminal domain. All antigens were intracellular proteins, either cytoplasmic or associated to the cytoskeleton. **Main Conclusions:** These novel antigens are candidates to be employed in the development of commercial diagnostic tests for kala-azar, vaccines or immunotherapeutic formulations, in a national patent basis, therefore reducing the risk of a total dependence of public health services on previously patented foreign products. **E-mail:** marcia.melo@pq.cnpq.br

Leish147- High IL-10, IL-17, TNF- α , IFN- γ and iNOS expression in sera, spleen and liver from asymptomatic naturally infected dogs by *Leishmania chagasi*

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Introductio: Visceral leishmaniasis (VL) is a systemic, chronic and potentially fatal disease caused by *Leishmania chagasi* in Brazil. Dog is the main domestic reservoir, being the most important link between the parasite and the man. Cytokines are key elements of the host immune response against *Leishmania* spp., and thus has been widely studied for better understanding of protective immunity as well as the pathogenic mechanisms triggered by the parasite. The development of Th1 response is associated with protection against these parasites, and IL-10 to susceptibility to infection. But recent data suggest a positive relationship between the IL-17 production and protection in human disease. The aim of this study was to correlate the clinical status of 22 *Leishmania infantum* naturally infected animals with the cytokines expression. **Material and Methods:** In this study were correlated clinical manifestations of canine visceral leishmaniasis (CVL) with cytokines profile in serum, spleen and liver in animals naturally infected by *Leishmania chagasi* of rural areas at Mossoró-RN, Brazil. Twenty two dogs were clinically evaluated and classified in asymptomatic (n=11) and symptomatic (n=11). Six normal dogs were used as negative controls. The cytokine profile was analyzed in terms of IFN- γ , IL-10, IL-17, TNF- α and iNOS mRNA expression in liver and spleen tissues using real time-PCR, and IL-10, TNF- α , IFN- γ in sera using ELISA. **Results:** Onychogriphosis, weight loss, localized alopecia, furfuraceous dermatitis, skin lesions and mucosal paleness were the most pronounced symptoms. The mRNA expression of IL10, IL17, IFN- γ , iNOS in spleen and liver tissue from asymptomatic dogs are higher than in symptomatic animals. A higher IL-10, TNF- α e IFN- γ production was also detected in sera from asymptomatic dogs than in symptomatic. Also, a high parasite load was observed in symptomatic animals. **Conclusions:** These results show a higher expression of Th1, Th2, and Th17 cytokine profile in asymptomatic *Leishmania*-infected dogs. However, the symptomatic dogs displayed cellular immunity impairment, with symptoms of canine visceral leishmaniasis and following death. **E-mail:** pauloguedes@cb.ufrn.br

Leish148- Evaluation of the persistence of *Leishmania braziliensis* infection in BALB/C mice

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Introduction: Leishmaniasis caused by *Leishmania braziliensis* is distinguished from other forms of leishmaniasis by its chronicity, latency, and tendency to metastasize. BALB/c mice infected by *L. braziliensis* develop small and non-ulcerative skin lesions that heal spontaneously. It is uncertain whether *Leishmania* parasites ever disappear after clinical cure of cutaneous leishmaniasis. **Material and Methods:** In this study was characterized the persistence of *L. braziliensis* in murine model by evaluating parasite load, lesion size and cytokines in different times of the infection. BALB/c mice (n=36) were infected by *L. braziliensis* and evaluated for 90 days of infection. **Results:** Parasite load disappeared gradually in footpad, while it was sustained in draining lymph node, even with 90 days post-infection. *L. braziliensis*-infected mice produced more IL-4 and TGF- β than IFN- γ in the firsts 15 days of infection. IFN- γ levels increased significantly after 30 days of infection followed by decreasing of parasite load in footpad and reduction of the lesion size. IL-10 and TGF- β production were significantly higher in the draining lymph node at 30 days post-infection and continued prevalent on the 90^o day of infection, followed by IFN- γ . **Main conclusions:** The present study showed *L. braziliensis*-infected BALB/c mice produced high levels of IL-4 and TGF- β in the initial phase of infection allowing the disease progression until the 30^o day of infection, when IFN- γ production elevated and the lesions decreased, also coinciding with the disappearance of the parasites in the footpad. However, some parasites persisting in the lymph node and this was mediated by IL-10 and TGF- β , which levels continued high even at 90^o day post-infection. IFN- γ production was also elevated, suggesting that the balance between these cytokines is determinant in parasite persistence and low parasite load maintenance in the lymph node, avoiding a future recurrence of the disease. **Keywords:** *Leishmania braziliensis*, BALB/c mice, persistence, IL-4, IFN- γ , TGF- β and IL-10. **E-mail:** priscilag_88@hotmail.com

Leish149- Effect of zidovudine (AZT) in the rate growth of *Leishmania (Viannia) braziliensis* promastigotes

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Introduction: The worldwide situation of leishmaniasis has been aggravated in recent years due to the increase on cases of *Leishmania*-HIV co-infection, which do not present a characteristic clinical profile. Therefore this new epidemiological profile (*Leishmania*-HIV co-infection) and the widespread use of nucleoside derivatives such as AZT, the antiretroviral therapy. This study evaluated the anti-Parasitary activity of this antiretroviral drug on *Leishmania (V.) braziliensis* promastigotes, the most prevalent species in Brazil. **Material and Methods:** The *L. (V) brasiliensis* (MHOM/BR/1975/M2903) promastigotes were cultivated in Grace's liquid medium (Grace's Insect Medium - Gibco ®) supplemented and incubated at 26 ° C with AZT at 1, 10, 20, 30, 40, and 50 μ M, which was diluted in DMSO. The microplates were daily examined for 7 days, the promastigotes were counted through the use of a Neubauer chamber. The experiment was conducted in quintuplicate and the results are represented as mean plus standard deviation. Statistical significance was considered when $p < 0.05$ (ANOVA and Student's t test). **Results:** Statistical differences were observed when comparing the control group with: day 1- 40 and 50 μ M AZT, day 2- 10 μ M and 40 μ M AZT, day 3- 30 μ M and 50 μ M AZT, day 4- 30 μ M and 40 μ M AZT, days 5, 6 and 7 - AZT 40 and 50 μ M. **Main Conclusions:** the main differences were observed in the groups tested with concentrations of AZT above the macrophage LD50 of 30 μ M, confirming what was described previously. Also it was observed that there is a reduction of the promastigotes growth rate on the second day of culture when exposed to the concentration of 10 μ M of AZT, showing that the action of the drug occurs mainly in the proliferative phase of the parasite growth which corresponds of intensive energy production and mitochondrial activity of the parasite. **E-mail:** carolinaaguia@gmail.com

Leish150- Effect of *Leishmania* infection on the expression of CD39 and CD73 in macrophages

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Introduction: Macrophages (MØ) act as the major reservoirs of *Leishmania* parasites and are the principal effector cells that determine the fate of the host parasite interaction. Studies have shown that cellular damage or injury in the sites of inflammation usually promotes release of elevated levels of the extracellular nucleotides which act on a variety of immune cells. Extracellular ATP is quickly hydrolyzed to adenosine in a coordinated two-step enzymatic pathway by nucleotide triphosphate diphosphohydrolase-1 (CD39) and the ecto-5'-nucleotidase (CD73) expressed on activated immune cells. Adenosine has been implicated as a potent anti-inflammatory and is a strong immunosuppressive molecule. **Methodology:** In this study, we looked at the expression of CD39 and CD73 on murine MØ infected with metacyclic forms of *Leishmania amazonensis* and the percentage of cells expressing these surface molecules was analyzed by flow cytometry. The percentage of infection and surface expression of ectonucleotidases were studied in MØ treated with cadmium chloride (CdCl₂) in normoxic conditions. **Results:** Our preliminary results showed that control resident MØ, cultured at 33°C in complete cultured medium for 24 hr, down expressed both CD39 and CD73 whereas *Leishmania amazonensis* maintained the levels of the expression in infected MØ population. We found that MØ were the major cell populations when analyzed in the total peritoneal cell population and they showed a higher percentage of infection followed by increased CD39 and CD73 expression. Furthermore, we investigated the effects of CdCl₂, inhibitor of HIF-1α, on expression of CD39 and CD73 as well as its influence on the percentage of infection compared to untreated MØ. We observed that addition of CdCl₂ reduces expression of CD39 and/or CD73 as well as number of infected populations in thioglycollate (TG) stimulated MØ and bone marrow derived MØ (BMDM). The expression was low in uninfected F480 positive and also for uninfected F480 negative populations in all groups of MØ under all conditions. Resident MØ expressed the highest CD39 /CD73 followed by TG stimulated MØ and the least expression by BMDM. **Conclusion:** our present data show that *Leishmania amazonensis* infected MØ maintain high level of CD39 and CD73 expression suggesting that they may participate in extracellular ATP metabolism. When MØ are treated with inhibitor of HIF-1α and then post infected with *Leishmania amazonensis*, percentage of infected MØ is reduced which is followed by decreased CD39 and CD73 expression. Therefore, it may be possible that these parasites may have a role in modulating CD39 and CD73 with a possible regulatory link over HIF1α. **Supported by** TWAS, CNPq and FAPEMIG **E-mail:** bjbajra@nupeb.ufop.br

Leish151- Effect of intrinsic insulin-like growth factor-I on *Leishmania* (*Leishmania*) major growth within macrophages

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Introduction: In *Leishmania* infection specific and non-specific immune factors contribute to its evolution, including growth factors such as insulin like-growth factor I (IGF-I). We have shown that extrinsic IGF-I favors the *Leishmania* proliferation and the infection development. However, IGF-I is constitutively present in macrophages whose effect is not known in leishmaniasis. **Aim:** To study the role of intrinsic IGF-I in *Leishmania* infection, we studied the IGF-I expression and parasite growth using two different strategies to inhibit the IGF-I expression. We used interferon-γ stimulus known to inhibit the IGF-I production and the small interfering RNA (siRNA) for IGF-I silencing. In addition, we showed the localization of IGF-I during parasite-macrophage interaction by confocal laser scanning microscopy. **Material and Methods:** Mouse macrophage cell line RAW 264.7 (5x10⁵ cells) was infected with stationary phase *L. major* promastigotes (8 parasites/cell) and incubated for 48 hours. The cells were either treated with IFN-γ (200U/mL) or were transiently transfected after the infection with 150µM IGF-I-siRNA using Lipofectamine 2000 for 6 hours. IGF-I RNA expression was quantified by Real Time PCR

relative quantification method. Confocal analysis of IGF-I and *Leishmania* co-localization was also performed using anti-IGF-I antibody (1:75) and anti-mouse-IgG Alexa Fluor546 (1:200). The parasitism was evaluated under light microscopy. **Results:** Upon IFN- γ stimulus IGF-I RNA expression decreased 6.9 times in macrophages as well as the parasitism, from 44 to 24 parasites/100 cells (median) ($P < 0.05$). Silencing of IGF-I with siRNA resulted in 70% inhibition of IGF-I mRNA expression, and the parasitism decreased from 100 (median) to 54 parasites per 100 cells ($p < 0.05$). The level of IGF-I expression were analyzed using confocal microscopy. Initially, localization of IGF-I and *Leishmania* was analyzed within macrophages. IGF-I was seen in cytoplasmic region and on intracellular *Leishmania*, revealing a direct interaction between the parasite and IGF-I. In free promastigotes in culture IGF-I staining was absent. Then when the cells were either stimulated with IFN- γ or transfected with IGF-I siRNA we observed a decrease in IGF-I immunostaining under confocal microscopy. **Conclusion:** These results strongly suggest the importance of IGF-I in *Leishmania* infection with its apparently direct role on the growth of *Leishmania* within macrophages. **Supported by** FAPESP, CNPq, CAPES, FINEP, LIM-38 (HC-FMUSP). **E-mail:** luizareis@usp.br

Leish152- Effect of ciprofibrate on the lipid fractions and on the parasite load of *Leishmania (Leishmania) chagasi*-infected hamster

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Introduction: Visceral leishmaniasis caused by the parasite *Leishmania (Leishmania) chagasi*, in its active phase, presents changes in lipoprotein metabolism with reduction in HDL and increase in triglyceride (TG) levels. Some studies suggest that lipids are important energy source for *Leishmania* amastigotes. Furthermore there are drugs that reduce plasma lipoprotein levels such as ciprofibrate. **Aim:** In this project, in the hamster model of VL we evaluated the plasma lipoprotein fraction alterations and the effect of the drug ciprofibrate on the parasite load. **Material and Methods:** Hamsters (*Mesocricetus auratus*) were infected with 2×10^7 purified amastigotes of *L. (L.) chagasi*. After 60 days post-infection, in part of experiments, we performed the treatment for 15 days with 0.5 mL of ciprofibrate (7.6mg/mL in sorbitol) by gavage. The animals were divided in six groups: (A) 5 uninfected and untreated ; (B) 5 uninfected treated with sorbitol; (C) 5 uninfected treated with ciprofibrate; (D) 7 infected and untreated; (E) 9 infected and treated with sorbitol; (F) 9 infected and treated with ciprofibrate. After the treatment, we collected sample of whole blood to measure the lipoprotein fractions by enzymatic colorimetric method. Furthermore, we collected samples of liver and spleen to analyze parasite load by Stauber's and Real-Time PCR methods. **Results:** We observed that triglyceride levels doubled in infected and non-treated animals (group D) when compared with non-infected and non-treated animals (group A) as well as the cholesterol level. In the infected and non-treated animals (group D) the HDL levels was 50% of the level in the non-infected and non-treated (group A). Upon treatment with ciprofibrate the infected animals (group F) had the cholesterol levels decreased 50% when compared with the infected and non-treated animals (group D). Upon treatment (group F) the parasite load in the spleen decreased to 25% of that in the infected and non-treated animals (group D) using two methods. **Conclusion:** The infection with *L. (L.) chagasi* promoted changes in lipid profile affecting triglyceride, cholesterol and HDL levels, and the treatment with ciprofibrate normalized the alteration of lipids and reduced the parasite load in hamster with VL. **Financial Support:** FAPESP 2011/03768-1, CNPq, LIM-38 (HC-FMUSP) **E-mail:** ivemcd@usp.br

Leish153- Differential protein expression in infective and non-infective promastigote forms of *Leishmania (Leishmania) amazonensis*

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Introduction: the outcome of *Leishmania* infections is determined by both species, host, parasite and their genetic make-up. Although much has been learned about the immune response to this parasite, there is still a lack of knowledge concerning the factors deriving from the parasite itself. The recent completion of genome sequences for *L. major* projects and *L. infantum*, as well as other species of the genus, in accord with the simultaneous advance of proteomics, has greatly accelerated and favored the pursuit of new *Leishmania* proteins. **Material and Methods:** using a proteomic approach based on studies addressing related *Leishmania* protein infectivity, 2-DE proteomics maps to determine protein expression profiles of promastigotes infected and uninfected forms of *L. amazonensis* were developed in this study. **Results:** About 251 and 145 different protein spots were detected in infective and non-infective forms, respectively. While the vast majority of spots had similar distribution and intensity, few were defined as computationally and preferentially expressed in non-infective *Leishmania* as compared with the infective kind, or vice versa. **Conclusions:** This data confirms the feasibility of creating an array of protein-based 2-DE protein profiles related to the infectivity/virulence of *Leishmania* species by providing a framework for the future design of studies about the virulence factors in the proteome of *Leishmania*. **Financial support:** INPA/FAPEAM/LNLS/FINEP/SECT-AM. **Keywords:** *Leishmania amazonensis*, Leishmaniasis, Proteomic analysis, Infectivity, Two-dimensional gel electrophoresis, mass spectrometry. **E-mail:** lilianecr76@gmail.com, afranco@inpa.gov.br

Leish154- Differences in parasitism and response to insulin-Like Growth factor (IGF)-I in the human macrophage cell line (THP-1) infected with different *Leishmania* species

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Introduction: Leishmaniasis is caused by protozoa of the genus *Leishmania*, an obligate intracellular pathogen that preferentially establishes and proliferates within macrophages. Early events in the host-parasite interactions are crucial for the establishment and outcome of the disease where several factors take part. Among them insulin-like growth factor ('insulin-like growth factor'= IGF)-I has participation showing direct effect in the induction and proliferation of the parasite during *Leishmania*-macrophage interaction, both in vivo and in vitro in animal model. This effect results in an alternative activation of macrophages leading to increased parasitism. To better understand the mechanism of human leishmaniasis an appropriate model of the study of *Leishmania*-macrophage interaction is necessary and important although it presents some problems. Donor-related variability is an obstacle when we use monocytes derived from peripheral human blood. Then human monocyte cell line THP-1 infected with *Leishmania* appears as an interesting model since it expresses main features of mature macrophages following differentiation and may grow in culture to obtain large batches maintaining reproducible characteristics. Then it allows studies to evaluate the differences in the development of *Leishmania* infection involving different species. **Aim:** To study the parasite-macrophage interaction, intracellular parasite growth and the effect of IGF-I on parasitism using human differentiated monocyte cell line THP-1 infected with *Leishmania* (*L.*) *amazonensis*, *L.* (*L.*) *major* and *L.* (*Viannia*) *braziliensis*. **Material and Methods:** 2.5×10^5 THP-1 cells were differentiated into macrophages by 20 nM phorbol 12-myristate 13 acetate (PMA) then infected with the stationary phase promastigotes of the different species (10 parasites: one cell ratio) and maintained for 24 h at 37°C in 5% CO₂ atmosphere. Part of culture was stimulated with rIGF-I (50 ng/mL). The parasitism was evaluated by light microscopy and the results presented as the number of parasites per 100 cells. **Results:** In culture without any stimulation we observed 47.6 ± 1.5 (mean \pm standard deviation) per 100 cells with *L.* (*L.*) *amazonensis*, 30.1 ± 1.5 with *L.* (*L.*) *major* and 35 ± 2.9 with *L.* (*V.*) *braziliensis*. When IGF-I was used as stimuli the parasitism increased in all species but differently and it reached 55 ± 5 with *L.* (*L.*) *amazonensis*, 37.3 ± 0.6 with *L.* (*L.*) *major* and 39 ± 2.2 with *L.* (*V.*) *braziliensis*. **Conclusion:** Different species of *Leishmania* presented differences in parasitism and in the response to rIGF-I, *L.* (*L.*) *amazonensis* presenting higher parasitism and better response to IGF-I that may be related to the disease manifestation with this species. Further we consider *Leishmania*-infected THP-1 cell line a useful model to study the differences in the pathogenesis of the

disease caused by different parasite species. **Supported by** CAPES, CNPq, LIM-38 (HC-FMUSP) **E-mail:** amandalima@usp.br

Leish155- Characterization of Exosomal Proteins of *Leishmania* spp. with human Plasminogen binding capacity

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Exosomes are small membrane vesicles (50-100 nm) secreted by a multitude of eukaryotic cell types. Silvermann *et al.*, 2010 demonstrate, in *L.(L.) donovani* and *L.(L.) major*, that the exosome release is a general mechanism for protein secretion in these organisms, and found exosomes and exosomal proteins in the cytosolic compartment of infected macrophages associated with parasite-macrophage communication. In this sense, we carried out a study with causal agents of different clinical manifestations of Leishmaniasis: *L.(L.) mexicana*, *L.(V.) braziliensis* y *L.(L.) infantum* with the purpose to characterize exosomal proteins with human plasminogen binding capacity to contribute to the understanding of the mechanisms of invasion and dissemination of *Leishmania* into the host. Promastigotes of *Leishmania* spp. were grown in Schneider's medium until reach exponential phase and were maintained in RPMI-1640 medium during 24 hours for secretion process. Exosomes were purified by differential centrifugation and ultracentrifugation at 110.000 g and were submitted to SDS-polyacrilamide electrophoresis followed by Ligand blotting for plasminogen. Mass spectrometry and Western blotting were used to identify the plasminogen binding proteins. In these exosome preparations, the proteins enolase and LACK were present. Additionally, another plasminogen binding protein with a molecular mass of 14 kDa was visualized corresponding to a small calpain like cysteine peptidase these findings indicate that *Leishmania* is able to secrete proteins in exosomes that interact with the host plasminogen, the zymogenic form of the serin-protease plasmin. These secreted proteins could be involved in degradation of fibrin network or other extracellular matrix proteins into the host, suggesting a possible association with the mechanisms of invasion and dissemination of *Leishmania*. **Keywords:** *Leishmania*, exosomes, plasminogen. **E-mail:** lourdesfiguera2268@yahoo.com

Leish156- Canine Visceral Leishmaniasis Experimental Model in Beagle Dogs

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Introduction: Leishmaniasis experimental infection in animal models is not standardized due to the low consistency of the disease development after infection, infective stage, amount of parasites or the route of administration. The aims of the study were to develop an animal model with dogs and to demonstrate the efficacy of the method measuring immunological parameters, parasitological evolution, clinical signs and histopathological aspects. **Material and methods:** Twenty-five beagle dogs (10 males/15 females; 7-34 months) were purchased, bred under vector-borne infection-free conditions and kept for 30 days before the experimental infection. *Leishmania infantum* amastigotes (M/CAN/ES/96/BCN150/MON-1 strain) were cultured and inoculated in golden hamsters. After 60 days hamsters were euthanized and their spleen homogenized, cultured for 2 days in Schneider's medium and promastigotes isolated. 500,000 parasites/0.5 mL were intravenously administered in the cephalic vein. Efficacy of the infection was tested. IFAT, direct ELISA and western blot were used to investigate the production of specific IgG and IgG2a, delayed type hypersensitivity (DTH) was investigated by intradermal skin prick test, parasite evolution was performed by Real-time PCR and clinical signs were classified according to the presence/number of signs. After 12 months dogs were sacrificed and histopathological studies done in liver, kidney and spleen. **Results:** DTH was positive in 10 dogs and IgG detected in all animals. Serum samples recognized different reactive bands from a parasite extract. Parasites were detected in 100% of

the infected dogs. Promastigotes forms were cultured from lymph nodes (80% of the animals) and spleen (68%). Amastigotes were observed in lymph nodes (76% of the animals) and spleen (80%). Parasites were visualized by direct microscopic observation in 80% of the animals and DNA in 96%. Clinical signs were clearly visualized. A total of 36% dogs had 1 to 3 signs of infection and 56% more than 4. Splenomegaly, hepatomegaly and chronic nephritis were the most common manifestations. **Main conclusions:** A consistent reproducible model of Leishmaniasis infection has been developed. The model reproduces the course of natural infection and can be used for the investigation of drugs or vaccines developed for canine or human Leishmaniasis. **E-mail:** jcarnes@leti.com

Leish157- Calmodulin intergenic spacer: useful marker to characterization of *Leishmania* strains.

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Introduction: Calmodulin gene modulates the calcium metabolism in various cellular activities on tripanosomatids. Depending of the *Leishmania* spp strain, this gene has two or three copies in the genome, separate one from another by the intergenic spacer which has the intergenic region and untranslated regions (UTR) 3' UTR and 5' UTR. Even during evolution this gene is much conserved, is known that the 3' UTR regions in *Tripanosoma cruzi*, presents genetic variations able to regulate the expression and also allow the classification. In this work we analyze the intergenic spacer of *Leishmania* spp., in order to evaluate the useful as molecular marker to identification and classify *Leishmania* to specie level. **Materials and methods:** We use ten reference strains and ten strains isolate from lesions of panamenian patients. The primer 3utrcal and 5utrcal were designed to amplify the intergenic spacer. The PCR products were cloned and transformed. Two colonies per each strain were picked and the plasmids were purified and sequenced. Informatics softwares were used to analyze the results. **Results:** All the *Leishmania* species which were evaluated amplified a product of about 1,200 pb corresponding to one of two intergenic spacer. For the different species this region presents genetic variations which distinguish the strains the subgener *Vianna* has mutations in the intergenic spacer which can differentiate the strains belonging to this Subgener for *Leishmania amazonensis* and *Leishmania mexicana* there are two important mutations that allow the identification of both strains. *Leishmania chagasi* has a characteristic sequence which allows distinguishing from the other strains the ten strains isolate from lesions of panamenian patients, had the same mutations of the *Leishmania panamensis* reference strain and were classified as *Leishmania panamensis*. **Main conclusions:** The accurate identification of the specie involve in human leishmaniasis is very important for epidemiology and clinic in order to design treatment measures and appropriate control or document the distribution in a region. In Panamá the main etiological agent of leishmaniasis is *Leishmania panamensis*, even also has been reported occasionally *Leishmania amazonensis*. Up to now the visceral manifestation of the disease caused by *Leishmania chagasi* has not been described, but have been collected in some areas of the country sandflies *Lutzomya longipalpis*, it natural vector. Also Panama is a transit country and has a high risk of imported cases caused by strains very virulent that are present in other countries. Our preliminary results indicate that the intergenic spacer of calmodulin gene is a useful molecular marker in order to genotify *Leishmania* strains. **E-mail:** ara04@yahoo.com

Leish158- Association between the emerging disseminated leishmaniasis and polymorphisms in *Leishmania braziliensis* strains

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Abstract: We have previously described a multiclonal population structure among genotypically polymorphic *L. braziliensis* from an area with high endemicity for American tegumentary leishmaniasis (ATL) in Bahia, named Corte de Pedra. Based on RAPD (*Randomly amplified polymorphic DNA*) profiles, we also found an association between clinical outcome of ATL and parasite genotypes in this region, indicating a role for the intra-species variability among these microorganisms on form of disease. In order to further explore the hypothesis of association between form of ATL and strain of *L. braziliensis*, we cloned, sequenced and compared homologous RAPD bands previously explored for genotyping the *L. braziliensis* of Corte de Pedra. With this strategy we found six genomic loci that were polymorphic between representatives of the different clades (i.e. subpopulations) of parasites described. PCR primer sets were designed for the specific targeting of each locus identified. Using these primers each locus was re-amplified electrophoresed and had the band corresponding to the amplicon gel extracted and cloned into pCRII vectors. Then six clones of each locus were sequenced per leishmania isolate. The cloned amplicons permitted identify that the SNPs and indels defining the polymorphisms at each locus segregate within the population of *L. braziliensis* in Corte de Pedra according to consistent haplotypes. Furthermore, several SNPs, indels and haplotypes displayed significant associations with disseminated leishmaniasis (DL). In particular, patients infected with *L. braziliensis* containing certain SNP genotypes and haplotypes found in the locus Lb CHR 28/425451 presented significantly increased risk ratios for developing DL. Thus this rapidly emerging form of ATL may have its outcome driven in part by the infecting *L. braziliensis* strain. **E-mail:** aschriefer@globo.com

TOXOPLASMOSIS

Toxo001- Seroepidemiology of human toxoplasmosis in the urban area of Metropolitan Region of Belém, Pará State, Brazil

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Introduction: Toxoplasmosis is one of the most common and prevalent infections worldwide. *Toxoplasma gondii* can infect several animal species and in humans, and can cause severe complications, especially in congenitally infected children and immunocompromised individuals. Its prevalence varies according to the geographic and climatic characteristics of each region, as well as to its population's cultural behavior. In Brazil, the prevalence is high among humans and domestic animals, especially in the Amazon, which is considered endemic. The aim of this study was to perform a seroepidemiological evaluation for human toxoplasmosis in an urban area of Metropolitan Region of Belém (MRB), Pará State, Brazil. **Material and Methods:** During the period of 2002 to 2009 a total of 2,740 individuals of both sexes, aged 02-89 years, from the municipalities of Ananindeua and Belém, both in the Metropolitan Region of Belém, Pará State were included in this study. After following the ethical procedures and having the individual consent, we filled a form containing personnel and epidemiological data, and collected venous blood. Serum samples were tested for anti-*T. gondii* IgG and IgM antibodies by an indirect fluorescent antibody test (IFAT) and/or immunoenzymatic assay (ELISA). **Results:** The overall seroprevalence was 77.9% for IgG and 11.9% for IgM. Human were divided into five groups and the seroprevalence obtained was: Pregnant women (IgG: 78.9%, IgM: 2.9%), candidates for renal transplant (IgG: 85.5%, IgM: 2.9%), HIV-positive patients (IgG: 83.7%, IgM: 3.8%), patients with ocular symptoms suggestive of toxoplasmosis (IgG: 83.5%, IgM: 6.4%), patients with lymphadenopathy and febrile illness (IgG: 67.6%, IgM: 27.2%) and individuals from spontaneous demand of Instituto Evandro Chagas (IgG: 72.0%, IgM: 10.4%). An association between different variables and seroprevalence was also performed. It was observed that, for IgG antibodies, a significant difference between age groups, with a tendency of increasing soropositivity as the age group increased ($P<0,0001$) and the consumption of undercooked meat was the only variable related with seropositivity ($P<0.05$).

Conclusion: Based on these results, we conclude that the high prevalence of toxoplasmosis corroborates the findings obtained in the late 90th, indicating that the transmission remains intense in the urban area of MRB. **Keywords:** Seroepidemiology, human toxoplasmosis, Pará State. **Financial Support:** CNPq (Universal: N° 484537/2006-2007), IEC/SVS/MS. **E-mail:** edicleicarmo@iec.pa.gov.br

Toxo002- High serum prevalence of *Toxoplasma gondii* antibodies in human population in communities settled alongside the Purus River in the municipality of Lábrea, State of Amazonas, Brazil

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Introduction: Historically, the serum prevalence of *T. gondii* antibodies in the Brazilian population is high. In the North of Brazil, in five different states of the Brazilian Amazon, prevalence's above 50% were found. **Material and Methods:** This survey estimated the prevalence of infections by *T. gondii* in the population of four (4) communities settled alongside Purus river, in the municipality of Lábrea (7°15'90'' S and 64°47'57'' W), state of Amazonas, Brazil. Blood samples were collected in order to check for IgG and IgM antibodies against *T. gondii* by immunoassay method (ELISA) (ELECSYS Toxo - ROCHE DIAGNOSTICS). **Results:** 235 individuals of 4 communities that represent typical population with characteristic habits and peculiarities of the inhabitants of areas alongside the rivers in the interior of the state of Amazonas. The overall prevalence infection rate by *T. gondii* was 55.7% with no difference by gender. It was noticed a growing prevalence according to the age bracket, that is, 43.43% in the group aged from 0 to 14 years old, which reached up to 70.5% in the group over 14 years old. **Main Conclusions:** The researchers believe that, although the prevalence is significantly high, it will not reach the levels of other Amazonian populations that live in urban areas, as Purus river, with its flood season may reduce the environment contamination, and therefore the exposure of the inhabitants. Complementary studies on the genotyping of strains and clinical characterization of this parasitological infection is been carried out. **Support FAPESP:** 2010\12371-5. **E-mail:** spider@icbusp.org

Toxo003- Serological survey of *Toxoplasma gondii* infection in a rural area of Brazilian Amazon: Preliminary results

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Introduction: Toxoplasmosis is a cosmopolitan zoonotic infection caused by the protozoan *Toxoplasma gondii*, a parasite that can infect birds and mammals, including humans. Serological surveys have showed a high worldwide prevalence of this infection. In northern Brazil, especially in Pará State, there is not much information about toxoplasmosis epidemiology, a clear majority of it restricted to the urban area in Belém City, where the prevalence is above 70%. This study aims to present preliminary results of a serological survey conducted in Novo Repartimento, a rural municipality in Pará State. **Material and Methods:** A descriptive cross sectional study was performed on a sample of the population from Novo Repartimento. The Subjects included in this study were randomly chosen out of the demand at a local clinical analysis laboratory. After ethical procedures and getting individual consent, we had filled in a form with personal and epidemiological data, and collected venous blood in order to have serum that was tested for anti-*T. gondii* IgG and IgM antibodies using an indirect fluorescent antibody test (IFAT). **Results:** From July 2010 to December 2011, 325 subjects of both sexes were included in, aged 01-78 years. The serological analysis showed that 85.54% (278/325) had a serological profile of previous infection (IgG+/IgM-), 13.8% (45/325) were negative for both immunoglobulin and only 0.6 (02/325) had a

profile suggestive of recent infection (IgG+/IgM+). **Conclusion:** Preliminary data indicate a high seroprevalence of *T. gondii* infection in Novo Repartimento, as in other areas of Brazilian Amazon. Increasing the sample size and analyzing the found variables, we can have a better understanding of toxoplasmosis epidemiology in this area. **Keywords:** Serological survey, toxoplasmosis, Amazon. **Financial Support:** CNPq (Universal: Nº 484537/2006-2007), IEC/SVS/MS, FAPESPA. **E-mail:** edicleicarmo@iec.pa.gov.br

Toxo004- Serological screening and risk factors for Toxoplasmosis in pregnant and postpartum women attended in public health units in Niteroi, Rio de Janeiro, Brazil

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Introduction: Toxoplasmosis has a wide geographical distribution. Congenital transmission occurs when a woman acquires the infection for the first time during pregnancy and transmits it to the fetus. It is a serious health problem in Brazil and its diagnosis is crucial for pregnancy assistance programs. Identifying risk factors for the infection is also important to improve primary prevention among non-immune pregnant women. **Objective:** establish the rate of toxoplasmosis seroprevalence in pregnant and postpartum women, assisted by Public Health System in Niterói-RJ, and its correlation with risk factors for the disease. **Material and Methods:** 276 pregnant and 124 postpartum women, with ages from 14 to 45 (mean 29,5), assisted by Public Health System (SUS) in Niterói-RJ, answered a short questionnaire, had peripheral blood sample collected and received information about the disease. Blood samples were stored at -20°C until assay. The seroprevalence was determined by quantitative detection of specific IgG and IgM antibody against *Toxoplasma gondii*, using Indirect Immunofluorescence test (IFAT) and Enzyme Linked Immunosorbent Assay (ELISA). The results were analyzed by means of the Statistical Package for the Social Sciences 10.0, and the Chi-Square test, determination of Odds Ratio (OR) value with 95% of confidence interval (95% CI) and a logistic regression model were performed. **Results:** Considering IFAT and/or ELISA, the seroprevalence of anti-*T.gondii* IgG antibodies was 58.5%. A pregnant woman was positive for IgM anti-*T.gondii* antibodies and had intermediate IgG avidity test. The patient received treatment and was followed until the end of gestation. The newborn showed no signs or symptoms of congenital toxoplasmosis and was tested negative for IgM and positive for IgG anti-*T.gondii* antibodies. The analysis of risk factors showed a significant association ($p<0.05$) between seropositivity and age, contact with cats and presence of rats at home. This association was confirmed for age and contact with cats through the logistic regression model, with the inclusion of high school education as a protective factor. **Conclusion:** seropositivity was high in this population. Considering that many childbearing age women are susceptible, the adoption of preventive measures is recommended in order to avoid toxoplasmosis. **E-mail:** patriciariddell@vm.uff.br

Toxo005- Serological evaluation for toxoplasmosis in newborn of mothers with positive serology in chronic phase in public maternity hospitals of Goiânia-Goiás

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Introduction: *Toxoplasma gondii* infections cause serious damage to human and animal species, depending on the host's immune system and transmission mechanisms. In most individuals is a chronic infection in an asymptomatic form, but when occur a congenital transmission can cause irreversible damage to the fetus, explaining the importance of monitoring pregnant women during prenatal care, and newborns of mothers chronically infected. Our objective was to analyze and monitor a group of infants who had higher serological titers than their mothers, on the day of birth, for confirmation and/or exclusion of infection during pregnancy. **Materials and Methods:** We collected 188 samples of peripheral blood of newborns and their mothers who were seropositive for toxoplasmosis (IgG positive) during the prenatal

period. The collection of peripheral blood was performed at 24 hours of birth in two maternity hospitals in Goiânia – Goiás. Serology was performed to identify and confirm the presence of anti-*T. gondii* by immunofluorescent antibody test (IFAT) with serial dilutions to obtain the final titration. Those samples were considered positives with titers higher than 1:40. In all samples of newborns who had titers higher than their mothers, was performed a technique of polymerase chain reaction (PCR) from the leukocyte cream to identify the presence of DNA fragments of *T. gondii*. These children were followed for a period of one year, and new blood samples collected ranging in age from 6 to 9 months for repeat serological testing by IFA. **Results:** In all samples collected on the day of birth was confirmed the passage of maternal antibodies anti-*T. gondii* IgG antibodies to their newborns. In 22.34% (42/188) samples, the serum titers of anti-*Toxoplasma gondii* (IgG) detected by the IFA technique were higher than detected in the serum of their mothers. It was possible to collect in the 2nd 64.28% (27/42) children. The PCR reaction performed on the first 42 samples were negative. The 27 samples collected after six months of life of children, also presented themselves seronegative by IFA technique (IgM and IgG). **Conclusions:** The programs of care to pregnant women are increasingly valuing primary care for the control of congenital infections, this study shows that 22.34% of infants born to mothers chronically infected with *T. gondii*, although there has been no confirmation of transmission in 27 children together, the data does not rule out the need for prenatal and neonatal periods, when there is suspicion of the possibility of congenital toxoplasmosis, in order to treat and consequently a better prognosis congenitally infected children. **E-mail:** jubavelar@hotmail.com

Toxo006- Serum prevalence of anti-*Toxoplasma gondii* antibodies in the canine population of the municipality of Lábrea, State of Amazonas, Brazil, indicating high environmental contamination

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Introduction: Scientific investigations on toxoplasmosis are fundamental because its pathogenicity to humans and the lack of epidemiological information in Brazil. The dog, although it is not a definitive host of *Toxoplasma gondii*, plays an important epidemiologic role as an indicator of environment contamination by the protozoa. In Brazil, the infection prevalence by *T. gondii* in dogs is high, and rises from 50% to 80%. The information on this problem is scarce in the Amazon region. **Material and Methods:** This survey is aimed to report the serum prevalence of toxoplasmosis infection in dogs from the communities settled alongside the Purus river and urban area in the municipality of Lábrea (7°15'90" S and 64°47'57" W), state of Amazon, Brazil. Blood samples of 99 dogs were collected, 33 from the urban area, and 66 dogs from the communities settled alongside the Purus river. The research on IgG class antibodies against *Toxoplasma gondii* was carried out by means of IIR - indirect immunofluorescence reaction, and were considered positives when antibody titers were 1:16 or above. **Results:** The results showed the presence of anti-*T. gondii* antibodies in 72.77% (24/33) in urban dogs, and 56.06% (37/66) in dogs living alongside the Purus River, which are similar to other Brazilian studies. **Main Conclusions:** The data obtained from this work allows the conclusion that the environment contamination is high, and the difference of prevalence between the two places may be caused by the annual variation of the water level of the Purus River (flood season), that may reduce the environment contamination in the areas alongside the river. FAPESP PROCESS NO. 2010\12371-5 **E-mail:** spider@icbusp.org

Toxo007- Seroprevalence of toxoplasmosis among Arthritis Rheumatoid patients

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Introduction: Arthritis Rheumatoid is a chronic inflammatory disease that leads to immune complex-mediated damage against self-antigens and deposition in the joints. Arthritis Rheumatoid is a autoimmune systemic disease and Researches indicated the 1.% of the people are infected with this kind of disease. Infectious diseases among rheumatoid arthritis patients have a high prevalence in compare with the other people. Toxoplasmosis is an Opportunistic infections disease with Global outbreak among immune suppression and immune deficient patients. The aim of this study was to determine the prevalence of specific Toxoplasma antibodies among patients with rheumatoid arthritis and control group. **Methods:** This study was established in a Case-control pattern on 110 serum samples including 55 of rheumatoid arthritis patients and 55 of healthy volunteer. The level of toxoplasmosis antibody was measured among affected patients with Arthritis Rheumatoid and control group. ELISA kit was used for measuring of IgG&IgM antibody. **Results:** Based on findings through this study the rate of toxoplasmosis prevalence among Arthritis Rheumatoid patients was higher in comparison with control groups. The rate of Toxoplasma IgG antibody seropositivity among Arthritis Rheumatoid group was %58.18 and in control group was %27.27. IgM antibody was not positive in none of the patients and control group. **Conclusion:** In Arthritis Rheumatoid patients using immunosuppressive drugs, especially steroids, can provide a context for opportunistic infections. Therefore, it is recommended timely screening more accurate and management of decrease prevalence of Toxoplasmosis among Arthritis Rheumatoid patients. **E-mail:** babak80@gmail.com

Toxo008- Seroprevalence of *Toxoplasma gondii* (IgG) in pregnant women attended in department of obstetrics and gynecology on Hospital das Clínicas in Goiânia – GO, Brazil, during 2010 and 2011

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Introduction: *Toxoplasma gondii* is a parasite with wide distribution in nature with high prevalence in human population, under the form of asymptomatic chronic infection. The parasite can reach the fetus through the placenta, causing malformation which can lead to abortion or even to systematic and neurological problems such as blindness, mental retardation, deafness and seizures. The congenital transmission of *T. gondii* can occur when a woman becomes infected during pregnancy or if she is chronically infected and the prognosis for the fetus and thus for the newborn, dependent on the gestational period in which it acquired infection. Our objective was to evaluate the prevalence of chronic toxoplasmosis in pregnant women treated at the Department of Gynecology and Obstetrics, Hospital das Clínicas -HC-UFG, which is a public service that addresses pregnancy considered as a high risk, in Goiânia, Goiás. **Materials and Methods:** We analyzed the serological data in the medical records of pregnant women attended at Hospital das Clínicas, Federal University of Goiás in Goiânia-GO, HC-UFG, in the years 2010 and 2011. **Results:** During the two years were seen, between consultations, hospitalizations and returns, 2463 women in the service of Gynecology and Obstetrics, HC-UFG. Of these, 416 patients were admitted to conduct births. It was possible to analyze 296 records for the investigation of cases of chronic toxoplasmosis. The mean age of patients with toxoplasmosis in the chronic phase was 30 years. The data analysis showed that 57.8% (171/296) of pregnant women are reactive IgG, 29.4% (87/296) with negative serology, 2.7% (8/296) with serology compatible infection acute and 10.1% (30/296) without information on blood tests. **Conclusions:** The seroprevalence of 57.8% (171/296) of the women is in agreement with the literature, which draws attention in this survey is that almost half of all pregnant women should be considered at risk, therefore, 29.4% were seronegative, 2.7% are in the acute phase and 10.1% of pregnant women had no information about the serological tests for toxoplasmosis. These results demonstrate the importance of primary care to pregnant women, thus minimizing irreversible consequences in many newborns. **E-mail:** lo_storchilo@hotmail.com

Toxo009- Role of TLR4 Asp299Gly Polymorphism in Ocular Toxoplasmosis Susceptibility

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Introduction: Toxoplasmosis is a worldwide zoonosis which prevalence ranges from 15 to 85%. In most cases the infection is asymptomatic. Ocular toxoplasmosis can cause vision alterations and retinochoroiditis. Host immune response plays an important role in the course of *Toxoplasma gondii* infection and toll like receptors (TLR) initiate a signaling intracellular cascade that stimulate host defense by induction of nitrogen species and pro inflammatory cytokine production leading to a parasite infection control. Polymorphisms in genes involved in immune response have been associated with susceptibility or resistance to infectious and parasitic diseases. The aim of this study was to analyze the association of TLR4 Asp299Gly polymorphism with ocular toxoplasmosis in IgG specific-*T. gondii* serum positive individuals with or without ocular lesions. **Materials and Methods:** The participants were divided in two groups named as ocular and control groups. The study included 34 individuals with ocular toxoplasmosis in ocular group and 134 individuals in the control group from a rural population at Rio de Janeiro. The participants of ocular group had only cicatrized ocular lesions. Polymorphisms genotyping were analysed by ARMS-PCR. **Results:** Ocular and control groups were in Hardy-Weinberg equilibrium ($p=1$ for both groups). There was no statistically significant difference in genotype or allele distribution in TLR4 Asp299Gly polymorphism when compared ocular and control group. **Conclusions:** We concluded that in this studied population there is no association between this polymorphism and susceptibility to ocular toxoplasmosis. **Supported by:** Faperj; Fiocruz. **E-mail:** albuquerque.maira@gmail.com

Toxo010- Prevalence of *Toxoplasma gondii* infection in chickens (*Gallus domesticus*) from McComb, Mississippi, USA

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Introduction: *Toxoplasma gondii* is a parasitic protozoan which is intracellular. Its definitive host is the cat. But it has the propensity to infect many animals including birds and humans. While its prevalence has been reported in pigs and goats in Mississippi, it has not been done in chickens. **Purpose:** The purpose of this study was to find out if *T. gondii* is prevalent in chickens from Mississippi and to compare its frequency of occurrence with those in pigs and goats. **Materials and Methods:** During the month of October 2010, chicken blood samples were collected from the Sanderson Chicken Plant in McComb, Mississippi and taken to the Alcorn State University laboratory for processing. The collected blood samples were centrifuged and the sera were put in vials, labeled and stored in the freezer at -20 degrees Celsius until tested for *T. gondii* antibodies the modified direct agglutination tests (MAT) were performed at serial dilutions of 1:25, 1:50 and 1:500. A titer of 1:25 was considered to be seropositive. **RESULT:** Of a total of 612 chicken blood samples tested, 68(11.1%) were seropositive for *T. gondii*. **Conclusion:** The seroprevalence was comparatively lower than reported in pigs and goats from Mississippi. The blood samples were collected from a commercial indoor farm. Previous reports show that the prevalence of *T.gondii* is usually low in commercial indoor farms and that the ingestion of meat from these chickens is considered a low risk of transmission to humans. So, the result obtained is in agreement with previous studies. It is recommended that this kind of study be carried out in backyard chickens. **E-mail:** chiefacholonu@alcorn.edu

Toxo011- Mortality rate and prevalence of *Toxoplasma gondii* in free raised chicken (*Gallus gallus domesticus*) in communities settled alongside Purus River in the municipality of Lábrea, state of Amazonas, Brazil.

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Introduction: In the toxoplasmosis transmission epidemiologic chain only the cats and some sylvatic felines are definitive hosts. Nevertheless, the parasite has been widely spread on domestic animal species. Free raised chicken from small outdoor chicken farms may carry *T. gondii* tissue cysts that place a risk of infection for humans and also represent a significant indicator of environmental contamination.

Material and Methods: This survey estimated the occurrence of *T. gondii* in chickens (*Gallus gallus domesticus*) raised outdoors alongside the river Purus in the municipality of Lábrea (7°15'90'' S and 64°47'57'' W), state of Amazonas, Brazil. Fifty chickens raised outdoors in 4 communities settled alongside river Purus were submitted to euthanasia for collecting tissues from their hearts and brains. From each chicken approximately 50g of tissue were collected and homogenized in a saline solution at 0.85% added with acid pepsin. The homogenized compound was injected in mice for mortality rate estimation. The mice that survived for up to six weeks were examined for the presence of *T. gondii* antibodies by Modified Agglutination Test. **Results:** Two communities were found to be positive. The results showed that 10% of the chicken died within 3 weeks, and in the remaining, 40% were found to have *T. gondii* antibodies. **Main Conclusions:** the results demonstrate the high prevalence of this protozoon in chickens and oocysts in the environment, results that correspond to the data found in Brazilian literature. Complementary genotypic studies are being performed with the isolated specimens.

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Toxo012- Frequency of toxoplasmosis in pregnant women attending in public health centers, from Samambaia city, Brasília, Distrito Federal, Brazil

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Introduction: Toxoplasmosis is an infectious disease caused by a protozoan parasite called *T. gondii*, where the prevalence is around from 20 to 90% of the world population, obtained in pregnant women. The fetal infection cases are around of the 40%. **Objective:** The aim of this study to verify the frequency and risk factors of toxoplasmosis in pregnant women receiving care at health in the Public Health Centers, from Samambaia City, Distrito Federal, Brazil, from 2009 to 2011. **Methodology:** It was a field research, exploratory and quantitative. Data were collected through interviews with application of a questionnaire with objective questions relating to socioeconomic conditions and risk factors of toxoplasmosis in pregnant women. The collection of data to verified the frequency of anti-*T. gondii* in pregnant women who received prenatal care during the period 2009 to 2011 was collected from medical records of pregnant women, kept on Archival Computerized System of Public Health Centers. **Results:** Of the 214 medical records from pregnant women tested, 83 (39%) of pregnant women had IgG and IgM negative for toxoplasmosis, 73 (34%) were IgG anti-*T. gondii* positive, 58 (27%) IgM anti-*T. gondii* positive, indicated a parasitic disease acquired during pregnancy. One patient (0.5%) was illiterate, 86 (40%) had complete primary school, 199 (55.5%) had completed high school and eight (4%) were graded. 159 (74.5%) began prenatal care after three months of pregnancy, 54 (25%) began three and six months, one after the seventh month. 210 (98%) women were HIV negative. For information about the risk factors for acquisition of toxoplasmosis were interviewed 200 pregnant women who received prenatal care in Public Health Centers from Samambaia city, DF. 189 (94.5%) women said that had little or no knowledge about *T. gondii*, 11 (5.5%) knew what was toxoplasmosis and were informed about this disease in clinical prenatal care. Of the 189 women who had no knowledge about toxoplasmosis, 48

(24%) had a dog, 18 (9%) had cats and 13 (6.5%) had a dog and cat, 181 (90.5%) did not work with gardening or garden, 98 (49%) washed their hands with water only, 86 (43%) washed with soap and water, five (2.5%) did not wash their hands, 49 (24.5%) consumed meat to the point, 129 (64.5%) ate meat as well cooked, and 11 (5.5%) ate raw meat. All pregnant women (100%), regardless of whether or not knowledge about the importance of gestational toxoplasmosis transmission to the fetus had access to basic sanitation. **Conclusion:** It is necessary that health professionals in the program of prenatal care, to present and illustrate activities and services focused on health promotion and prevention of toxoplasmosis. **Keywords:** toxoplasmosis, pregnant women, prevalence of infection, the Federal District. **E-mail:** liviaulopes@gmail.com

Toxo013- Assessment of the first epidemiological profile of Toxoplasmosis in Jataí, Goiás, Brazil

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Introduction: Zoonosis caused by *Toxoplasma gondii*, characterized by being an opportunistic infection affecting immunodeficient individuals and also cosmopolitans. It is estimated that one third of the world population is carrying antigens for invading agents, with a prevalence rate of 6.7 to 98%. The transmission is fecal-oral route, via the placenta and by carnivorism. In Brazil, the infection rate is around 50 to 80% in the adult population, pointing to a positive association between socioeconomic status and increasing prevalence due to ageing. In the Midwest region data are scarce; with a seroprevalence of 64% of women at reproductive age in the city of Goiânia and 74% of women undergoing prenatal in 2005 in Rio Verde. The present study aimed to conduct the first assessment of the epidemiological aspects and infection by *T. gondii* seroprevalence in the city of Jataí, southwest of Goiás, Brazil. **Material & Methods:** Data were collected through a partnership established with the Health Department of the Municipality and Health System, for the period from January, 2005 to December, 2010. We analyzed the data on pregnant women of the Maternity Protection Program (MPP), the number of hospitalizations, laboratory tests and patients with HIV (HIV +) seropositive for *T. gondii*, participants of the Specialized Assistance Service (ASS). **Results:** The seroprevalence (IgG+) of pregnant women (n = 6077) was of 84.04% with a mean positivity of 83.96% during the studied period. Thirty-four patients presented IgM+ (0.56%), 82.35% of with avidity >60% and 8.82% to <30% and 30-60, respectively. According to the information concerning age of patients, 51.82% of visits in the MPP, were of age group ranging from 21 to 30 years old. Followed by intervals of 11-20, 31-40 and 41-47 years of age, with 33.09, 13.69 and 0.67% respectively. The socioeconomic analysis pointed to the profile of care for the majority of the residents of low income areas. (65.78%). The total number of serological tests for *T. gondii*, conducted by public health laboratories network for the period of 2007 to 2010, was 689, as information obtained from DATASUS indicated a seroprevalence of 53.50% IgG+ (n =243) for the same period. **Main Conclusions:** A high rate of seropositivity for *T. gondii* among pregnant women in the city of Jataí without significant presence in recent infections was identified. The assistance profile was composed mostly of patients from low socioeconomic status and aged between 21 and 30 years old. Data on HIV+ patients are, in average from asymptomatic infections for patients in South America (41.9 to 72%). Laboratory data from the public network showed no information about patients and their clinical situation, preventing further analysis of these data. The hospital does not discriminate on the etiologic agent responsible for the injuries. This failure is based on the DATASUS that has no specific code for the disease parasite. The lack of information pointed to an information system that is not systematized and is poorly integrated. The results corroborate the discussion of action planning and increase the flow of information between the Department of Health and DATASUS. **E-mail:** ricardo.santarita@pq.cnpq.br

Toxo014- Assessment of knowledge and preventive behavior for *Toxoplasma gondii* infection in pregnant women diagnosed with toxoplasmosis

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Introduction: Congenital toxoplasmosis occurs after the acute infection with *T. gondii* during pregnancy and may cause fetal death or serious injury to the newborn. Primary prevention is important, because the most cases the maternal infection is asymptomatic. The early diagnosis and specific treatment of infected pregnant women avoid or attenuate fetal injury. **Materials and Methods:** In order to assess maternal perception of the risk and preventive behavior on toxoplasmic infection, a survey was conducted among 86 pregnant women diagnosed with acute toxoplasmosis, referred to a referral center for toxoplasmosis in Rio de Janeiro during the year 2007. **Results:** Only one pregnant woman did receive appropriate guidance in source center. We observed by means of the maternal response to epidemiological questions a significant deficit of knowledge about toxoplasmosis, especially with regard to risk factors. Preventive measures were known but not practiced, especially in relation to the oocyst-borne infection. Despite the diagnosis of acute toxoplasmosis in the health facility of origin, only 16 (18.6%) women where they were treated after the diagnosis of toxoplasmic infection, which suggests a lack of knowledge by the professional team of prenatal care. **Main conclusions:** As oocysts are resistant to disinfectants and may resist solutions of sodium hypochlorite and acetic acid and mechanical cleaning is not fully guaranteed, pregnant women should be advised not to drink unfiltered water or boil the water and avoid the ingestion of raw vegetables and fruits with their peels. The contact with cats was not a risk factor for these women. The current health systems have difficulties in the care and counseling of pregnant women. We emphasize the importance of early prenatal serological tests, and preventive measures to susceptible pregnant women. We highlight the need to expand the knowledge of toxoplasmosis to society and to the medical professionals and obstetric nurses, responsible for prenatal care, since congenital toxoplasmosis is a preventable disease. **Keywords:** Toxoplasmosis; *T. gondii*; knowledge preventive; pregnancy. **E-mail:** wendyfbueno@yahoo.com.br

Toxo015- Knowledge of Toxoplasmosis among pregnant and postpartum women attended in public health units in the city of Niterói, Rio de Janeiro, Brazil

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Introduction: Infection with *Toxoplasma gondii* during pregnancy can lead to severe fetus and infant illness. Congenital toxoplasmosis is a serious public health problem and is considered a “silent” disease with difficult clinical diagnosis. Many congenital *T. gondii* infections acquired during gestation are preventable by simple precautions during pregnancy. The correct and early laboratory diagnosis and the transmission of information about the disease are essential for prenatal assistance programs. **Objective:** Determine the knowledge about *T. gondii* infection in pregnant and postpartum women assisted by public health system in Niteroi-RJ. **Material and Methods:** We surveyed pregnant and postpartum women assisted by public health system in Niteroi-RJ, who had already been tested for igG anti- *T. gondii* antibodies, to determine their knowledge about Toxoplasmosis. A questionnaire was applied with questions covering general knowledge about the disease, risk factors, symptoms, prevention knowledge and preventive behavior. The questionnaire took approximately 20 minutes to complete and after that the patients received information about *Toxoplasma gondii* infection. The results were analyzed by means of the Statistical Package for the Social Sciences 10.0, the Chi-Square test was performed and the value of the Odds Ratio (OR) with 95% of confidence interval (95% CI) was determined. **Results:** From the 400 women that answered the survey, 276 were pregnant and 124 were postpartum. The median age was 29 (range 14–45). The knowledge about toxoplasmosis and how to prevent the infection was deficient, having most of the surveyed women reported uncertainty about the disease. Only 111 (27.8%) of the total participants who answered the questionnaire reported knowing toxoplasmosis and 289 (72.2%) did not know about the disease. There was a statistically significant association between knowledge about the disease and the presence of toxoplasmosis infection ($p=0.013$; $OR=0.57$; $95\% CI= 0.36 -0.89$). Many errors have been reported, such as the parasite transmission through the feces of pigeons 29 (26.1%) and dogs, rabbits, rats and birds 41 (36.9%). Although all the respondents were being followed or had recently been followed by a prenatal program, 340 (85.0%) did not know about toxoplasmosis prevention. **Conclusion:** knowledge about toxoplasmosis, its transmission and prevention in this population was very low. Considering that many childbearing age women are susceptible and did not have enough information

about the disease, adoption of preventive measures is recommended in order to avoid toxoplasmosis. **E-mail:** patriciariddell@vm.uff.br

Toxo016- Incidence of cerebral Toxoplasmosis how the HIV and AIDS – defining illness deaths registered in the Epidemiological Surveillance Center of University Hospital Alcides Carneiro in Campina Grande – PB – Brazil

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Introduction: The cerebral toxoplasmosis is the cause of toxoplasmic encephalitis focal more common in patients with HIV and is one of the AIDS-defining diseases. The worldwide distribution of the disease is around 25% to 70% in patients' seropositive leading to significant neurological sequelae and infection if not diagnosed early and treated properly will lead the individual to death. It is more prevalent in individuals with CD4 counts below 200 cells/mm³ and its incidence was reduced by the introduction of antiretroviral therapy and prophylactic therapy with *trimethoprim-sulfamethoxazole*, thus reducing morbidity and mortality of HIV-positive patients with the disease. **Materials and methods:** Data regarding deaths was collected through medical records of patients of infectious diseases ward at the Hospital Universitário Alcides Carneiro in the period June 2006 to December 2010 for the students' participants of the extension project for the surveillance of the hospital in question. **Results:** In a total of 53 deaths in which of the causes of death recorded is AIDS (Acquired Immunodeficiency Syndrome) 10 of them, ie, 5.3% of total neurotoxoplasmosis also appears as a major cause of death showing the occurrence of this severe form of toxoplasmosis in HIV-positive patients as well as the relationship between the onset of cerebral toxoplasmosis with the observed mortality in these patients. Even among these deaths, 8 (4.24%) were male and 2 (1.06%) were female. **Main conclusion:** The toxoplasmosis is a severe form of toxoplasmosis that affects mainly pregnant women and immunocompromised individuals, among them HIV-positive individuals. With early diagnosis and treatment decreases the incidence, morbidity and mortality of cerebral toxoplasmosis in seropositive is recommended that prophylactic treatment with *sulfamethoxazole* and *trimethoprim* in all seropositive, in order to avoid the high mortality caused by the disease in these subjects. **E-mail:** deilanazevedo@hotmail.com

Toxo017- Comparison between techniques for toxoplasmosis serologic diagnosis in pregnant and postpartum women

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Introduction: Toxoplasmosis is a widely-distributed zoonosis caused by *Toxoplasma gondii*. Although there is a high prevalence of unapparent infections, toxoplasmosis can develop into a severe systemic illness when in its congenital form. The study of specific antibodies remains an essential parameter for diagnosing maternal-fetal infections and to establish the time of infection. The indirect immunofluorescence technique (IFAT) and the Enzyme Linked Immunosorbent Assay (ELISA) have been reported to be more sensitive and specific than indirect hemagglutination (HAI). However, HAI is cheaper, easier than the others and does not need special equipment. **Objective:** The aim of this study was to compare the data quantified by IFAT, ELISA and HAI for the detection of IgG antibodies against *Toxoplasma gondii* in pregnant and postpartum women sera. **Material and Methods:** Samples of 276 pregnant and 124 postpartum women were collected in the maternity and ambulatory at the Antonio Pedro University Hospital, at the Sergio Arouca Community Polyclinic and Modules of the Family Doctor Program in Niterói, RJ, Brazil. After coagulation, the sera were separated and frozen at -20°C for subsequent analysis. The samples were tested for IgG anti- *Toxoplasma gondii* antibodies by the Indirect Immunofluorescence, ELISA (BioKit®) and Indirect Hemagglutination (TOXO-HAI KIT®, Analisa) techniques. The Indirect Immunofluorescence and ELISA tests were performed at the Toxoplasmosis

Laboratory of the Oswaldo Cruz Institute and the Indirect Hemagglutination test was performed in the Immunoparasitology Laboratory of the Federal Fluminense University. The results were submitted to statistical analysis and the agreement between the tests was measured by the Kappa coefficient. **Results:** from the 400 samples analyzed, 217 (54.3%) were positive for IgG anti- *T.gondii* antibodies in the IFAT, 233 (58.3%) in the ELISA test and 122 (30.5%) in HAI. Indirect Immunofluorescence and ELISA had an almost perfect agreement, Kappa= 0.89, and Indirect Immunofluorescence and Indirect Hemagglutination tests showed moderate agreement, kappa= 0.52. **Conclusion:** The Indirect Hemagglutination technique exhibited a low quantity of positive samples when compared to Indirect Immunofluorescence and ELISA tests. The use of this technique for serological screening in pregnant women is questionable, and Indirect Immunofluorescence and/or ELISA techniques are the most suitable. **E-mail:** patriciariddell@vm.uff.br

Toxo018- Molecular diagnostic laboratory and in pregnant women of *Toxoplasma gondii* island São Luís do Maranhão

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Introduction: *Toxoplasma gondii* is an obligate intracellular protozoan that can cause problems in humans in cases of congenital infection or immunosuppressed individuals. Can be gained in the first trimester of pregnancy. The detection of *Toxoplasma gondii* in venous blood from the placenta is assessed by immunological tests; among them some are evaluated as false positive for IgM antibodies to *Toxoplasma gondii*. To determine the prevalence of acute toxoplasmosis in pregnant women from the island of São Luís do Maranhão, with performance of serological tests ELISA IgM and IgG avidity testing and confirmation by molecular diagnosis by polymerase chain reaction (PCR). Moreover, far will the analysis of variables such as age, gender, residence, occupation, education level, marital status, number of children and family monthly income, by completing a questionnaire determining this way the epidemiological profile and geographic areas of highest incidence of acute toxoplasmosis in the São Luís Island-MA. **Methodology:** In the patients was collected from 08 ml of blood to test for serological screening by ELISA (determination of IgM and IgG) in the laboratories of Basic Health Units and the samples were taken to the Laboratory of Molecular Biology UNICEUMA for molecular diagnosis by (PCR). **Results:** Of 120 blood samples collected, 36 (26.7%) were positive for IgM and IgG, and 32 of these show high avidity suggesting positive for toxoplasmosis infection and a longer time with the parasite. Of this group, only one sample that was positive for both immunoglobulins showed no eagerness, perhaps being an equivocal result. Thus our samples, only one patient had a recent infection by the parasite since it showed a low avidity and positivity for IgM and IgG. Half of the samples, 36 (50%) was positive only for IgM, perhaps indicating a recent infection. Of the 120 samples 14 (11.7%) were negative for toxoplasmosis. All samples are having their DNA extracted using a commercial kit is used and this DNA in PCR amplifications for parasite detection. **Conclusion:** Through these results can become more reliable and rapid diagnosis of congenital toxoplasmosis is thus an important tool in the evaluation of prenatal care. **E-mail:** costarosa.fernanda@gmail.com

Toxo019- The effect of glucocorticoids on *Toxoplasma gondii* infection in rat peritoneal macrophages

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It is well known that toxoplasmosis can be life-threatening to immunocompromised individuals such as AIDS and organ transplant patients. Glucocorticoids (GCs) are widely used clinically for the treatment of autoimmune diseases and organ transplantation resulting in acute toxoplasmosis in these patients. However, the interaction and mechanism causing the development of acute toxoplasmosis in GC therapy is still unknown. This study was therefore designed to investigate the possible mechanisms of the effect of GCs on the infection by *Toxoplasma gondii* (RH strain) of rat peritoneal macrophages. Our results showed that *T. gondii* could significantly increase their growth rate in the rat macrophages treated with GCs by comparison with those of non-GC treatment. We also demonstrated that significant inhibition of nitric oxide (NO) production was observed in the rat peritoneal macrophages at 12, 24 and 36 hrs post-treated with GCs. Further results demonstrated that GCs could inhibit the expression of inducible nitric oxide synthase (iNOS) mRNA and protein in the rat peritoneal macrophages both in vivo and in vitro. Our results strongly demonstrate that the decreasing NO product in the rat peritoneal macrophage might be tightly linked to the cause of acute toxoplasmosis in a host that is naturally resistant to *T. gondii* infection. They provide valuable data to better understand the interaction and mechanism of the development of acute toxoplasmosis with GC therapy in patients who have been administered GCs. (This work was supported by the National Basic Research Program of China (973 Program. #2010CB530000). **Keywords:** glucocorticoids, rat peritoneal macrophage, *Toxoplasma gondii*, nitric oxide. **E-mail:** lsslzr@mail.sysu.edu.cn

Toxo020- Lymphoproliferative response to P30 and ROP18 peptides in ocular and congenital Toxoplasmosis

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Background: We determine the specific Lymphoproliferative response and cytokines production to peptides derived of the P30 and ROP 18 proteins in ocular and congenital Toxoplasmosis. **Methods:** Six patients with chronic inactive chorioretinitis, six patients with an active inflammatory episode by ocular Toxoplasmosis, five with chronic infection without ocular pathology and four with congenital Toxoplasmosis were analyzed. 50 µl of whole blood were incubate in sterile polypropylene tubes with concavalin or soluble *Toxoplasma gondii* antigen (ST-Ag), the peptide 2017 of the P30 protein, or ROP 18 peptides for the clonal lineages I, II or III of *Toxoplasma* or phosphate-buffered saline (PBS) for the negative controls. Incubation time for optimal cellular responses was five days. Culture supernatants were collected from each tube and flow cytometry analysis was performed using FACScan flow cytometer (Beckman-coulter FC5000). Specific positivity of T cells (CD3+ or CD4+ cells) was estimated by subtracting the value obtained for negative-control culture supernatant. **Results:** Patients with ocular Toxoplasmosis showed specific lymphocyte response to the total antigen and to P30 and ROP18 protein derived peptides. **Conclusions:** most of the immune response was due to CD4+. Specific immune cellular response appeared against ROP18 peptides, a protein involved in virulence of *Toxoplasma*. **E-mail:** etorres@uniquindio.edu.co

Toxo021- ROS-mediated trophoblast apoptosis in ER stress via activation of caspase12- CHOP-JNK pathway in *Toxoplasma gondii* infection

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Oxidative stress is described as an imbalance in the production of reactive oxygen species (ROS) and the ability of antioxidant defenses to scavenge them, leading to damage to cellular components and programmed cell death. Pregnant women infected with toxoplasmosis may result in abortion or stillbirth, or give birth to offsprings with severe mental retardation and retinal or neurologic damage later in life. Generally, pregnancy is a state of mild oxidative stress arising from increased placental mitochondrial activity and production of reactive oxygen species (ROS). *Toxoplasma* infection would deteriorate oxidative stress of placenta; finally contribute to oxidative damage to trophoblast. We hypothesized that

Toxoplasma infection would increase ROS generation followed by subsequent apoptosis in maternal-fetal interface. In the present study, we used the mice models based on pregnant infection of RH tachyzoites, tried to explore the role of ROS and downstream activation by ROS in the mechanisms of placental trophoblast apoptosis induced by auto Toxoplasma infection. To this aim, on day 7 after fertilization, pregnant females were divided into Toxoplasma infection (group A), antioxidant intervention (group B, and control groups (group B) randomly with mice each and maintained in the animal care facility until treated. Mice of group B were infused with 100mg/kg N-acetyl cysteine (NAC). On day 8 in group A and NAC pretreated group, mice were injected intraperitoneally with 200 tachyzoites in 0.1ml 0.9% saline solution. Mice of group C were exposed to only saline solution. Mice were killed by cervical dislocation under anesthesia on day 10, 12, 14, 16, 18 after gestation. Placentas were carefully dissected out and weighed, and processed into cell suspension, or fixed in Bouins fluid for immunohistochemical analysis, and snap-frozen in liquid nitrogen for further different analysis. We investigated the oxidative and anti-oxidative molecules by the Mouse Oxidative Stress and Antioxidant Defense RT² Profiler™ PCR Array(SABioscience Company), trophoblast apoptosis index was detected *in situ* by TUNEL and/or by annexin V/propidium iodide and FCM, oxidative stress circumstance of placenta tissues through examining increased malondialdehyde (MDA) by the thiobarbituric acid method, 8-OHdG by ELISA and reduced glutathione (GSH) levels of placenta tissues, and simultaneously observed ER stress markers, p38 and JNK pathway in placenta tissues of mouse congenital toxoplasmosis models by real-time RT-PCR and western blotting, as well as in primarily cultured trophoblast in vitro based on the transwell co-culture system. Our studies discovered that twenty-seven genes were at least 2-fold up regulated, and 9 genes were at least 2-fold down regulated in toxoplasma infection group, compared to the uninfected group. NADPH oxidase 1(Nox1) and glutathione peroxidase 6(Gpx6) were most significantly increased, about 25 times as the result of *Toxoplasma gondii* infection. The above mentioned genes were almost recovery to the levels of control groups when subjected mice were pretreated on G8 and G10 by 100mg/kg NAC injected intraperitoneally. Additionally, local oxidative stress of placenta tissues exhausted GSH. The level of GSH decreased from 9.29±2.26uM/gprot in control group to 4.18±1.54 uM/gprot in infection group (p<0.01). Severe oxidative stress also resulted in peroxidation of lipids and DNA, presenting with increased levels of MDA, 8-OHdG. The levels of MDA and 8-OHdG increased from 0.41±0.16 uM/gprot and 1.77±0.65 ug/gprot in control group to 0.52±0.23 uM/gprot and 3.88±0.89 ug/gprot in infection group respectively (p<0.05). Usage of NAC could effectively alleviate the peroxidation of lipids and DNA, especially in the early stage. The levels of GSH, MDA and 8-OHdG in pretreatment of NAC group were 9.42±2.40 uM/gprot, 0.45±0.21 uM/gprot and 3.42±1.63 ug/gprot respectively (p<0.01, 0.05 and 0.05 respectively, compared to those of infection group). *T.gondii* infection also contributed to the significantly increased apoptosis level of placenta trophoblast. The levels of total apoptosis, early apoptosis and late apoptosis in control group were 11.04±0.91%, 9.77±0.99% and 1.27±0.43% respectively, but in infection group, apoptosis rate significantly increased (60.71±16.96%, 38.57±14.66% and 22.14±3.20% respectively). However, pretreatment of NAC could significantly decrease the total apoptosis level (40.28±8.96%, p<0.05), especially late apoptosis level (4.71±2.81%, p<0.05), when compared with these of infection group. The above results were further confirmed by *in situ* TUNEL detection. By real time RT-PCR and western blot analysis, we found that ER-stress markers, such as GRP78, CHOP and caspase-12, and JNK/ASK1 pathway were up-regulated or activated by acute toxoplasma infection; pretreatment of NAC could inhibit the expression of these genes and activation of these signaling cascades. However, the up-regulation or activation of p38 was not detected in this process. In summary, this study demonstrates that placental trophoblast apoptosis can be initiated mainly by ROS-mediated ER stress via activation of caspase12, CHOP and JNK pathway in acute *T.gondii* infection. **E-mail:** jishen@ahmu.edu.cn

Toxo022- Purinergic P2x7 receptor deficiency increases susceptibility to *Toxoplasma gondii* – induced ileitis

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Introduction: Orally *T.gondii*-infected susceptible hosts develop extensive acute intestinal inflammatory responses which imitate several events of human inflammatory bowel disease (IBD), and results in increased morbidity and mortality. During *T.gondii* infection, diverse pro-inflammatory mediators contribute to pathological responses in the intestinal compartment. Purinergic P2x7 receptor stimulates pro-inflammatory cytokines and directly control intracellular pathogen killing. In toxoplasmosis, P2x7 receptor polymorphisms influence susceptibility to *T.gondii*-induced retinochoroiditis in humans, and exert control of *T.gondii* killing in both in vitro and in vivo animal models. However, P2x7 receptor mediated responses in *T.gondii*-induced intestinal inflammatory responses have not been addressed yet. **Objective:** To determine the role of purinergic P2x7 receptor-mediated signaling in intestinal inflammatory response during *T.gondii* infection. **Material and Methods:** Wild type (WT, C57BL/6 background) and P2x7 deficient (*P2x7*^{-/-}, C57BL/6 background) mice orally infected with 50 and 100 cysts of ME49 strain of *T. gondii* were weighted and bleed daily. Animals sacrificed at different time points during infection had plasma and tissues collected for cytokine and histopathological analyses, respectively. T-test and Log-rank statistical analyses were performed using GraphPad Prism 5.0 (CA, USA). **Results:** P2x7 deficient mice orally infected with 50 cysts presented increased mortality rates when compared with WT counterparts ($p < 0.01$). After oral infection with a lethal dose of *T.gondii* (100 cysts/animal), *P2x7*^{-/-} mice also had increased susceptibility to *T.gondii* infection and early death when compared to WT ($p < 0.01$). To evaluate morbidity, we determined the weight loss in both groups. *P2x7*^{-/-} infected mice showed a slight decrease of weight at day 7 - 8 post infection (*P2x7*^{-/-} x WT, mean, 15.8 ± 0.74 x 17.37 ± 0.76 , $p < 0.05$). To study the intestinal compartment, we measured the length of uninfected and infected intestines followed by histopathological analysis. Both WT and *P2x7*^{-/-} infected mice presented intestinal shortening, but deficient mice were significantly different from WT (*P2x7*^{-/-} x WT, mean, 22.16 ± 13.42 x 13.35 ± 14.46 , $p < 0.01$). At day 7-8 post-infection, histological analysis showed preserved intestinal architecture and no inflammatory reaction in the ileum of WT mice. However, *P2x7*^{-/-} mice presented intestinal cell extrusion, blunting of the villi and mild mononuclear infiltration of lamina propria without any necrosis. **Conclusion:** The findings suggest that P2x7 mediated response contributes with early protective events in *T.gondii*-induced ileitis in susceptible hosts. **E-mail:** cavalcanti.marta66@gmail.com

Toxo023- Invasion kinetics of human endothelial cells by *Toxoplasma gondii* RH and ME49 strains

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Introduction: *Toxoplasma gondii* may cause congenital infection in the developing foetus. Invasion of endothelial cells lining the placental blood vessels is supposed to be the main vertical transmission route. Genotypic analysis of *T. gondii* isolates identified a population structure of 11 haplogroups, from which three clonal lineages, type I, II, and III are well known. Although, type II strains are more prevalent in Europe and North America, type I strains are over-represented in congenital toxoplasmosis; also, type I strains are highly virulent to mice and traverse epithelial barriers more effectively than type II. The aim of this study was to compare the invasion kinetics of *T. gondii* RH and ME49 strains in human microvascular endothelial cells (HMEC-1) and umbilical vein endothelial cells (HUVECs). **Material and methods:** RH and ME49 *Toxoplasma gondii* strains were expanded in Balb/c and C57BL6-RAG2^{-/-} mice, respectively. After one replication cycle in VERO cell cultures, tachyzoites were seeded at 10:1 parasite: cell ratio in 24-well plates containing slides with monolayers of either HMEC-1 or HUVECs, at 100,000/well and incubated for 30 min to 4h. The slides were fixed and stained with Wright to count percent of infected cells and number of parasitic vacuoles per cell. **Results:** In both cell types invasion with either strain increased along time; however, proportion of infected cells was lower for HUVECs than for HMEC-1. Also, the strains differed in invasion kinetics: ME49 parasites were faster than RH ones, regardless of cell type. Finally, both HMEC-1 and HUVECs showed higher number of parasitic vacuoles per cell when infected by ME49 tachyzoites than by RH protozoan, i.e. ≈ 30 vs ≈ 20 at 4 hours, respectively. **Main conclusions:** Results suggest that HMEC-1 cells are more susceptible to infection by *T. gondii* than HUVECs. This

might be related to cell cycle-progress -which is usually badly regulated in cell lines- and *T. gondii* is more invasive during the G1-S phase. The unexpected observation that RH parasites are slower than ME49 ones might be related to their higher ability to survive out of the cell. **E-mail:** bel_alegria@yahoo.com.mx

Toxo024- Host cell Rho and Rac GTPases are involved in *Toxoplasma gondii* invasion

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Summary: GTPases are a large family of hydrolases that bind and hydrolyze guanosine triphosphate. Immunity-related GTPases and ADP-ribosylation factor-6 GTPase in host cells were reported accumulating on the Parasitophorous vacuole membrane (PVM) of *Toxoplasma gondii* and took critical roles in this parasite infection. In our study, we found RhoA, Rac1 and Rac2 GTPases of host cells were activated and recruited to the PVM following *Toxoplasma gondii* tachyzoites invasion, and this involvement is dependent on their endogenous GTPase activity. A real-time photography of *Toxoplasma gondii* tachyzoites invading COS-7 cells overexpressing CFP-tagged RhoA WT showed that this GTPase was recruited to the PVM either through host cell membrane or through diffusion from the cytosol. Sequentially truncated RhoA mutants by ten amino acids were used to identify the motif(s) that is critical for efficient recruitment to the PVM, which revealed that the GTP/Mg²⁺ binding site, the GTPase-activating protein interaction site, and the Rho kinase effector interaction site and G5 box were potential important motifs. In COS-7 cells, the recruited CFP-RhoAWT was sequestered on the PVM and became more abundant upon epithelial growth factor (EGF) activation, unlike the unassociated RhoA in the cytosol that migrated towards the cell membrane and the fluorescence intensity impaired following EGF activation. The infection rates of *Toxoplasma gondii* were compared among different groups of COS-7 cells. RhoAWT or Rac1WT overexpressed cells had a significantly higher infection rate than mock cells. In contrast, the dominant negative mutants RhoA-N19 or Rac1-N17 transfected cells and RhoA or Rac1 siRNA-treated cells showed a significantly lower infection rate than mock cells. The RhoGTPases are important for cytoskeleton reorganization of host cells, and are indispensable for efficient recruitment of *Toxoplasma gondii* tachyzoites. **Keywords:** *Toxoplasma gondii*; Parasitophorous Vacuole Membrane; RhoA; Rac1; Rac2; GTPase. **E-mail:** floriapeng@hotmail.com

Toxo025- Pidotimod: preventive treatment against reactivation in a murine model of reactivated toxoplasmosis

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Introduction: patients with immunosuppression are at risk of developing reactivated toxoplasmosis. The synthetic dipeptide pidotimod is a potent immunostimulating agent of improving the immunodefence. Herewith we explored the preventive efficacy of pidotimod in mice with activated toxoplasmosis induced by cyclophosphamide (CY). **Methods:** Mice were infected with *T.gondii* PRU strain by oral ingestion. Cysts were noted in the brain tissues on day 20 post infection. The mice were treated with CY, the cytotoxic agent which is usually used for immunosuppression of patients with autoimmune disorders or malignant tumors. Pidotimod was administrated to the subjected mice to investigate its preventive and therapeutic efficacy for activated toxoplasmosis. **Results:** Pidotimod administration significantly increased the body weight, index of spleen, the survival time and decreased the parasitaemia in CY-immunocompromised mice. Cytokine profiles and CD4⁺ T cells subpopulation analysis by CBA

(Cytometric Bead Array) and flow cytometry demonstrated that pidotimod resulted in a significant up-regulation of pro-inflammatory cytokines (IFN- γ , TNF- α and IL-2) and Th1 cells, together with up-regulation of the Treg cells, while the reduction of Th2 and Th17 cells and the corresponding cytokines (IL-4, IL-10 and IL-17) did not significantly increase in CY-induced mice. Additionally, histological findings in brains and livers revealed that mice treatment with pidotimod had low histological score. **Conclusion:** The present study demonstrated that immunosuppressive mice treated with pidotimod remarkably increased Th1-type cellular immune response, prolonged survival time and decreased parasitaemia of the subjected mice. The therapeutic efficacy of pidotimod for preventive treatment against reactivation of *T. gondii* should be further investigated in clinical trials. **E-mail:** shenjilong53@126.com

CRYPTOSPORIDIOSIS

Crypto001- Study Mussels *Perna* diagnosed with *Cryptosporidium* spp. intended for human consumption indicating environmental

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Introduction: The *Cryptosporidium* genus has species capable of infecting various host animals, both domestic and wild as well as man, and the only way to transmit the infective oocysts intake, which can occur through the contaminated food and water. Sources of contamination such as water drainage of the animal faeces, the use of organic fertilizers and the release of part or untreated sewage contamination favor of various aquatic environments by this parasite since the oocysts are eliminated in the feces of the host. In the seas the presence of *Cryptosporidium* spp. directly affects the quality of fish such as mussels present in the Brazilian coast and is therefore limiting the consumption of food. The study aimed to: diagnose and characterize genetically type (s) and / or genotype (s) of *Cryptosporidium* in mussels taken from rocky shores at two locations, Lage Preta and Saco's Beach, in the Mangaratiba city, State of Rio de Janeiro, performing the sequencing and phylogenetic analyzes, including the deposit of *Cryptosporidium* sequences from GenBank, to correlate the presence of the parasite with the index of rainfall in the region and to establish possible risks of eating mussels, by identifying the genotype (s) and / or specie (s) with zoonotic potential. **Methods:** Were collected monthly from March 2009 to February 2010 totaling 12 samples. During data collection, 30 animals were separated from each location and divided into three groups of 10 animals each, totaling 72 samples. For the analyzes, the DNA extracted from tissues of mussels was used in the amplification of sequences *18SSU rRNA* by nested-PCR technique. **Results:** For species identification, the amplicons were sent for sequencing. During all the study samples was possible to diagnose mussels *Cryptosporidium* positive for at least one of the study sites. It was possible to identify three species *C. andersoni*, *C. meleagridis* and *C. parvum* in samples obtained from two locations of mussels, by observing the similarity of 99% when compared to existing sequences in GenBank. It is possible the occurrence of human cryptosporidiosis by the consumption of mussels, raw or partially cooked, from the city of Mangaratiba. Statistical analysis showed no influence of rain in positivity of the samples of mussels for *Cryptosporidium*. **Conclusions:** With these results we conclude that there is likelihood of human exposure through ingestion of mussels from the region studied. **Financial Support:** Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). **E-mail:** tcbb@ufrj.br

Crypto002- Prevalence and detection of *Cryptosporidium* spp. from feces using micrometry and 18s rRNA gene

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Abstract: The protozoa *Cryptosporidium* is an important intestinal pathogen of livestock with worldwide distribution. It is the causative agent of one of the most important parasitic diseases which can be transmitted between humans and animals. To date several different species of *Cryptosporidium* has been reported. The present study was carried out in cattle farms and a number of 246 fecal samples from were collected. *Cryptosporidium* oocytes were isolated and stained with Zell Nelson technique. *C. Andersoni* and *C. parvum* were differentiated based on their morphologic and morphometric criteria. The presence of *C. parvum* was confirmed by amplifying a 1225 bp fragment of 18s rRNA gene. Based on microscopic findings 91.67% and 8.33 % of collected oocytes belonged to *C. parvum* and *C. andersoni* respectively. 11.7% of examined cattle were infected with both species of *Cryptosporidium*. The prevalence of Cryptosporidiosis in calves under 1 year old was significantly higher than cattle above 3 years old ($P=0.025$). The results also revealed that the infection level in males were significantly higher than females ($P=0.026$). There was not seasonal difference in the prevalence of Cryptosporidiosis. **Email:** m.yakhchali@urmia.ac.ir

Crypto003- Occurrence of *Cryptosporidium* spp in treated water: A meta-analysis.

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Introduction: The transmission of waterborne cryptosporidiosis is a major cause of morbidity and mortality worldwide. Knowledge of the occurrence of *Cryptosporidium* spp. and ways of detecting the parasite can help decision making for an appropriate method, sensitive and inexpensive, identifying the level of risk of transmission of this pathogen. **Material and Methods:** We conducted a review of the scientific literature Held by a through a review of the scientific literature the occurrence and what methodologies used for concentration and detection of *Cryptosporidium* spp. in treated waters. The search was performed by two independent researchers initially marked lists of titles and abstracts were selected and included or excluded in cases of discrepancy, the differences were resolved by consensus or a third reviewer, a total of 650. **Results:** The selection was made by the proper forms compounds of relevance tests that I was reading the abstracts by selecting 66 items. By relevancy test II, by reading the full articles were included 21 articles. We included only articles that mention together the concentration and detection method being used is that microfiltration membrane filtration with porosity of micrometers (MF), Separation Immunogenetics (SIM), flocculation (FL), Ultrafiltration (UF), and all PCR other variations (Nested-PCR, qPCR and SYBR Green PCR and Real Time, Immunofluorescence indirect or direct immunofluorescence (IF) staining for Optical Microscopy (OM), Cytometry (CIT) and Ultrafiltration (UF). **Main conclusions:** Countries that stand out with the occurrence of *Cryptosporidium* spp. were Portugal, Spain and Brazil. Among the selected articles, the methodologies associated with the greatest positivity were MF + SIM + PCR with 70.8% positivity, followed by MF + 54.3% MO positivity. The Membrane Filtration plus immunofluorescence was the concentration and detection methodology most used, with nine studies (18.6%). Through this review it was observed that *Cryptosporidium* spp. is present in the treated water in several countries and also in Brazil. **E-mail:** mctulianglobal@gmail.com

Crypto004- Identifying the sources of environmental contamination by *Cryptosporidium* spp

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Cryptosporidium spp. oocysts contaminating water are potential cause of important waterborne outbreaks that impact world health and economies. Knowing the sources of the contamination allows for management methods directed at limiting oocyst entry into waters. In the case of *Cryptosporidium*, besides knowing the source location, one must also determine the oocyst identity to ascertain if the humans or different animals are the sources responsible. The objectives of this study were to explore the prevalence of oocyst contamination along with seasonal variation and potential host factors in Thailand of

water from the Chao Phraya River and coastal seawater of Bang Pu Nature Reserve pier, Samut Prakan Province. Altogether, in 2010-2011, 144 water samples were collected from Chao Phraya River (72) during the summer, rainy, and cold season, and 72 samples from sea water before, after, and during the presence of migratory seagulls. Due to birds being potential sources of oocyst contamination, 70 fecal samples from Bangkok's pigeons and 910 from migratory seagulls at Bang Pu Nature Reserve pier were also collected. There were 11.1% (8/72) and 5.6% (4/72) of samples positive by nested-PCR from river and sea water, respectively. The highest river contamination was in the cold season (29.2%), and seawater contamination was highest when seagulls were present (12.5%). Oocysts were being shed by many birds, 14.3% Bangkok's pigeons and 15.4% sampled seagull fecals. The sequencing and phylogenetic analysis revealed all oocysts from Chao Phraya River were *C. parvum*. For seawater, 50% of identified oocysts were *C. parvum*, 25% *C. serpentis*, and 25% *C. meleagridis*. The one positive sample of Bangkok's pigeons was *C. meleagridis* and all of positive seagulls were *Cryptosporidium* avian genotype III. Oocyst from both water and birds samples were 55.6% viable using a dye permeability assay. Humans, farm animals, and wildlife were suspected as the sources of *C. parvum* and *C. serpentis* in the water of this study. Rainwater runoff likely carries the oocysts of *C. meleagridis* from Bangkok's pigeons and other birds into the ocean. Migratory seagulls are potentially importing *Cryptosporidium* avian genotype III into Thailand from China. This report is the first study to identify molecularly the species *Cryptosporidium* in Thai waters and the first report of *C. serpentis* and *Cryptosporidium* avian genotype III in Thailand. **E-mail:** tmymv@mahidol.ac.th

Crypto005- Epidemiologic aspects of infection *Cryptosporidium* spp. in calves' dairy and genetic characterization of species and subtypes

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Introduction: Protozoa of the genus *Cryptosporidium* are described as important pathogens in the gastrointestinal tract and respiratory systems of various hosts, among these we can include farm animals, companion, wild and epidemiological chain of some species in this genus can infect humans. The bovine cryptosporidiosis is caused mainly by four different species, *Cryptosporidium parvum*, *C. bovis*, *C. ryanae* and *C. andersoni*. The first one is of great concern for both livestock and public health. With regard to public health, the species is the subject of several studies due to its high zoonotic potential. The study aimed to: Perform the genotypic characterization of *Cryptosporidium* species and subtypes obtained from fecal samples from calves under one year of age, from dairy farms in the State of Rio de Janeiro, Brazil, establishing the potential for zoonotic species *C. parvum* through diagnosed subtype. **Methods:** The aim of this study is to determine the occurrence of *Cryptosporidium* species and subtypes in calves up to one year of age, throughout PCR technique using *18S* and *GP60* as gene target. The occurrence of *Cryptosporidium* species in calves up to 1-year-old was determined for 143 animals on three dairy farms on the state of Rio de Janeiro, Brazil. A fecal samples collected directly from each calf rectum was processed to concentrate oocysts using the centrifugal flotation technique in saturated sugar solution before being evaluated microscopically. **Results:** Of the 28 positive samples in microscopy, 23 were confirmed by Nested-PCR using gene *18SrRNA*. After each PCR-positive specimen was sequenced, the presence of three species of *Cryptosporidium* was observed infecting calves at different ages. Pre-weaned calves were infected with *C. parvum* (7%), whereas post-weaned calves were infected with *C. andersoni* (15%) and *C. ryanae* (1%). All positive samples are being submitted to a second Nested-PCR using gene *GP60* as target. A new sequencing will be made for *C. parvum* positive samples, to observe the most prevalent subtype in the area. **Conclusions:** Were diagnosed by means of molecular techniques *C. parvum* and zoonotic subtypes, *C. andersoni*, species of importance for dairy production and *C. ryanae*, this species is the first report infecting calves in the state of Rio de Janeiro and the second description of the species in Brazil. **Financial Support:** Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). **E-mail:** tcbb@ufrj.br

Crypto006- *Cryptosporidiosis and cyclosporidiasis, two major infections in HIV positive patients, in Bukavu, D.R. Congo*

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Introduction: The magnitude of coccidian parasites infection in HIV positive patients is largely unknown in the developing world. **Material and Methods:** A prospective study was conducted at the Opportunistic Infections Clinic of the Provincial Hospital of Bukavu, from April 2010 to October 2011. HIV positive patients attending the clinic, aged 15 years and above were included after obtaining their consent, the CD4 count and patient details (age, sex, etc.) were recorded, stool samples collection was realized according to the WHO standard procedure. A single stool sample was collected and conserved in formalin – ether 10% and transported to the Provincial Laboratory of Bukavu, Unit of Microbiology for analysis within 1 to 2 hours of collection. We had a randomized sample of 108 patients. Smears were stained according to the modified ziehl neelsen procedure and examined at x100 magnification using an oil immersion microscope. Data were analyzed with EPI-INFO 3.1. Differences with P-values < 0.05 were considered significant at 95% confidence interval (CI). **Results:** Females represented 58.3% (48.5 – 67.7). Mean age 40 ± 15.7 years old (15 – 66). Stools were diarrheic in 66.7% (56.9 – 75.4). Overall prevalence of *Cryptosporidium* spp was 30.6% (22.1 – 40.2), *Cyclospora cayetanensis* 16.7% (10.2 – 25.1). The majority were on ARV therapy 63.9% (54.1 – 72.9). Patients with CD4 counts < 50cells/μL, presented with a higher prevalence of either *Cryptosporidium* spp (16.7%) or *Cyclospora cayetanensis* (11.1%) (P = 0.0000, P<0.05, OR= 7.9). In diarrheic stools we recorded 90.9% (30/33) *Cryptosporidium* spp (P = 0.0001), 83.3% (15/18) *Cyclospora cayetanensis* (P = 0.08). Dual infections were much more noted in the CD4 count range of <50 – 100 cells/ μL, 66.7% (18/27) for CD4 count <50cells/μL and 33.3%(9/27) for CD4 50 - 100cells/ μL (P=0.0000). On ARV only subjects with 100 – 200 cells/ μL of CD4, had a good clinical evolution (P=0.0000). **Conclusions:** *Cryptosporidium parvum* and *Cyclospora cayetanensis* are major causes of diarrhoea in this study. Dual infection is related to very low CD4 count. The ARV therapy shows best clinical evolution in subjects with at least 100 cells/μL of CD4. Early screening of these coccidian parasites in HIV positive patients would help reducing diarrhea and offer a better quality of life. **E-mail:** andybulabula@gmail.com

Crypto007- *Occurrence of Cryptosporidium spp oocysts in stool samples of child from day care center in Ituiutaba city, Minas Gerais state*

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Introduction: *Cryptosporidium* eliminates oocysts in the faeces of the host and cause of human infections. In environments such as day care centers, the risk of infections increase because of oral phase development by children, the vulnerability of the immune system and the accumulation of feces in diapers for long periods of time. Immunocompetent individuals present a profile of diarrhea that can evolve to a possible spontaneous cure. However, it can cause severe enteritis and is often fatal in immunocompromised and immunosuppressed patients. **Objective:** To evaluate the occurrence of *Cryptosporidium* sp. Oocysts in stool samples of child from day care center in Ituiutaba city, Minas Gerais State and to characterize the hygiene habits of their families. **Material and Methods:** This research included 118 children from zero to 10 years, from two day cares center. A meeting with the school community and child's family was organized to discuss how to prevent *Cryptosporidium* infections by improving basic hygiene habits and best practice with water, food and environment. Each child had an average of two stool samples collected on alternate days. Were performed Ritchie's methods (formol-ether) and Ziehl-neelsen modified. Slides with positive fecal smears were subjected to Ritchie's methods and Hoffman, Pons & Janer's methods to verify the presence of other intestinal parasites. We applied 118

questionnaires to parents and 62 employees of day care center assessing the living conditions and hygiene habits of the same. **Results:** Was reported 1 (2%) case positive for *Cryptosporidium* sp. in child, female with 10 years of age, without diarrhea and absence of other intestinal parasites. As for the hygiene habits described in the questionnaire, 1.7% doesn't wash their hands after using the bathroom, bite their nails 21.7%, 31.1% puts objects haven't been cleaned in the mouth and 6.1% didn't sanitize the food before consuming them. About 21.1% said they had presented adults of *Ascaris lumbricoides* and 10.6% of *Enterobius vermicularis*. **Conclusions:** The finding of a stool sample positive for *Cryptosporidium* sp. in an asymptomatic child with negative parasitological tests and absence of diarrhea emphasizes the importance of the asymptomatic individual, who can become a disseminator of the disease in environments such as day care centers. **Financial Support:** FAPEMIG/UFU. **E-mail:** renata_gfm@hotmail.com

Crypto008- Microsporidiosis and chronic diarrhea in patients with acquired immunodeficiency syndrome (AIDS) in Buenos Aires, Argentina

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Introduction: Microsporidia are eukaryotic, intracellular obligate parasites, spore forming grouped and comprising more than 1200 species classified into approximately 100 genera that were recently reclassified from protozoa to fungi. Microsporidia have emerged as important opportunistic parasites in patients with human immunodeficiency virus (HIV) infection. The species *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are the first and second most frequent microsporidia isolated from patients with AIDS, respectively. The most common clinical syndromes associated with *E. bieneusi* and *E. intestinalis* infection is chronic diarrhea, malabsorption and cholangitis. The purpose of the present study was to determine the incidence and clinical features of intestinal microsporidiosis in diarrheal HIV/AIDS patients. **Materials and Methods:** The studied group included adult 132 patients from both sexes, HIV-infected, presenting chronic diarrhea, that attended the Hospital Fco. J. Muñoz and the Hospital J. M. Penna. Each patient was evaluated with a clinical history in order to describe the symptoms and signs. Routine laboratory analyses were performed (blood cells count, liver function tests, amylase serum level) and CD4 cells count or serum viral load were determined. Stool examination for spores identification and video endoscopy with specimen collection for routine histology and Azur II-stained semi-thin sections were performed to each patient. The identification to species level was performed by transmission electron microscopy and molecular analysis PCR for *E. bieneusi* was carried out employing the Eb.gc::Eb.gt primer set to amplify the ITS of the rRNA genes. In order to determine the presence of *E. intestinalis*, the PCR protocol was performed with the SINTF1::SINTR primers based on the small subunit rRNA gene. **Results:** We selected 132 cases with chronic diarrhea and diagnosed microsporidiosis in 14 patients. They were adults within 23 and 40 years old with CD4 lymphocyte counts lower than 278 cells/mm³ (range 26-278 cells/mm³). Tissue stages of Microsporidia were identified in 14/14 cases. Spores in feces were present in 11/14 cases. *E. bieneusi* was identified in twelve cases and *E. intestinalis* in four patients. All cases of *E. intestinalis* were co-infection with *E. bieneusi*. **Main conclusions:** Microsporidiosis caused by *E. bieneusi* and *E. intestinalis* species are important causes of chronic diarrhea in patients with AIDS. **E-mail:** jorgeysilvana@speedy.com.ar

Crypto009- Diagnosis of Intestinal parasitosis and Molecular identification of *Cryptosporidium* sp. (*Cryptosporidium parvum* and *Cryptosporidium hominis*) in HIV/AIDS patients followed up at Tropical Medicine Foundation "Dr Heitor Vieira Dourado"

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Introduction: It is estimated that infections Caused by intestinal parasites protozoa and helminths Affect 3.5 billion people around the world, Causing disease in Approximately 450 million people, mostly children. Molecular methods for the diagnosis of *Cryptosporidium* have been increasingly used to provide sensitive and specific detection of the oocysts in clinical and environmental samples, setting the taxonomic position of the various species and outlining the major genotype of *Cryptosporidium* sp. **Objectives:** Intestinal parasitosis diagnose and identify by molecular *Cryptosporidium* sp. (*Cryptosporidium parvum* and *Cryptosporidium hominis*) in HIV / AIDS patients attended at Tropical Medicine Foundation "Dr Heitor Vieira Dourado". **Materials and Methods:** Fecal samples were examined using the methods of Lutz, and Ritchie techniques derived from Kinyoun staining. The extraction of genomic DNA was obtained with a commercial kit and its amplification was performed by PCR. We tested two pairs of primers to amplify a conserved region of the gene 18SSU, AWA. The PCR product was visualized on agarose gel stained with 1% ethidium bromide, based on the molecular marker of 100bp. Genomic DNA concentration was obtained with the aid of Nanodrop-2000 Spectrophotometer®. **Results:** We examined 50 fecal samples and the highest prevalence was for protozoa *Entamoeba histolytica/dispar*, with nine (32%) carriers, followed by *Entamoeba coli* and *Endolimax nana* with four (14%), respectively, *Giardia lamblia*, with two (7%) and with one *Isospora belli* (4%). Among the helminths, the prevalence was higher for larvae of *Strongyloides stercoralis* with five (18%) carriers, followed by *Ascaris lumbricoides* with two (7%). Quantification of genomic DNA samples used for the PCR amplification ranged between concentrations of 0.2 ng/ul and 76.0ng / ul. *Cryptosporidium* sp genomic DNA amplification samples showed to be negative. **Conclusion:** The intestinal parasites affect HIV / AIDS patients followed up at FMT-HVD. There was no amplification of genomic DNA of *Cryptosporidium* sp. in the assessed fecal samples. **Keywords:** intestinal parasites, *Cryptosporidium* sp, PCR. **E-mail:** marcosaugustosilva@gmail.com

Crypto010- Cryptosporidiosis: the use of the polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) for molecular monitoring in clinical trials

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Introduction: *Cryptosporidium hominis* and *Cryptosporidium parvum* are the major species responsible for human disease. *C. hominis* is primarily a human pathogen, although it has been detected infrequently in other primates, neonatal gnotobiotic pigs and in immunosuppressed gerbils, whereas *C. parvum* has a broad host range including humans and livestock. Molecular methods for the species *C. parvum* and *C. hominis* have been used to understand the clinical manifestations and epidemiology of cryptosporidiosis, to identify reservoirs of infections, and to track this group of organisms in the environment but not for monitoring treatment. We performed a longitudinal study and described the clinical manifestations at the gastrointestinal tract and the biliary system and response to treatment of *Cryptosporidium* in patients with AIDS by microscopy and molecular methods. **Material and Methods:** The study included five adult HIV-infected patients with chronic diarrhea, sclerosing cholangitis and oocyst of *Cryptosporidium* sp. in feces. Parasite diagnosis was carried out by serial stool samples stained by Kinyoun. Biopsy specimens from duodenum, peripapillary duodenum and papilla were stained with Giemsa, hematoxylin–eosin and Azur II.

The identification to species level was performed by small subunit-rRNA-based PCR-RFLP. Automated sequencing of amplicons was performed. We selected cases coinfecting with *Cryptosporidium* species and monitored the response to highly active antiretroviral therapy by clinical manifestations, microscopy in feces, species identification by PCR-RFLP and sequencing at two and five months after the initial diagnosis. **Results:** We selected two patients with co-infection with *C. hominis* and *C. parvum*. The first case was asymptomatic at two months, persistence of oocysts in feces and two species detected by PCR-RFLP and sequence analysis. After five months, the patient was symptomatic, without oocysts in feces but two species of *Cryptosporidium* by PCR-RFLP and sequencing. The second case was symptomatic at two months, with oocysts in feces and two species detected by PCR-RFLP but only *C. hominis* by sequence analysis. At five months the patient was still symptomatic, but oocysts were not detected in feces although two species of *Cryptosporidium* were present by PCR-RFLP and sequencing. **Main conclusions:** The use of the small subunit-rRNA-based PCR-RFLP was a helpful tool for the diagnosis and identification of *Cryptosporidium* species but they are not related to different clinical manifestations. **E-mail:** jorgeysilvana@speedy.com.ar

Crypto011- Comparative evaluation of immunological and molecular techniques to detect *Cryptosporidium* spp. in treated waters.

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Cryptosporidium spp. is an emerging pathogen responsible for a large number of outbreaks of diarrhea in humans throughout the world. However, the occurrence of outbreaks caused by this pathogen in Brazil is poorly known and need further attention. The lack of sensitive techniques, specific, easy to use and adaptability, and offering alternatives for detection of microorganisms in treated water hinder enlightenment and consequently there is underreporting of cases. Thus, it is important to know standard techniques that enable the identification and quantification of this agent which is worrisome from the standpoint of the quality of treated water. The study aimed at evaluating and comparing immunological techniques for detection of antigen and a real-time PCR for detection and differentiation of *Cryptosporidium* spp. in samples of treated water. Samples were taken directly from the input taps of the housing and concentrated by membrane filtration positively charged. The oocysts were detected by the techniques of direct immunofluorescence (DIF), ELISA and real-time PCR. The results were positive in 56.3% (18/32), 28.1% (9/32) and 50.0% (16/32) respectively for the techniques employed. The oocysts of *Cryptosporidium* spp. are present in treated water from the city of Goiania. As to the cost / benefit, the technique cheaper and had better performance while sensing was DIF. While real-time PCR is more expensive the advantage of permitting identification of the species. Probably the ideal is the use of both techniques, the own inference. The result showed that the microbiological indicators are not correlated with the presence of *Cryptosporidium* spp. **E-mail:** mctulianglobal@gmail.com

Crypto012- Genotyping and subgenotyping analysis of cryptosporidiosis infection in Egypt

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Introduction: *Cryptosporidium* is a gastrointestinal parasite that is recognized as a significant cause of non-viral diarrhea worldwide. It is believed that management and control of cryptosporidiosis in human requires knowledge of *Cryptosporidium* species contributing to human disease. Different analytical approaches have been developed to detect and differentiate *Cryptosporidium* at the species/genotype and subtype levels. Currently, the highly polymorphic 60-kDa glycoprotein gene (gp60) is the most suitable and widely used genetic marker for subgenotyping of *Cryptosporidium* species that infect

humans. **Material and Methods:** Twelve *Cryptosporidium* positive isolates from human cases in Cairo, Egypt were included in this study. In this study, we determined the genotypes of the *Cryptosporidium* species based on the RFLP analysis of the PCR products of the small subunit (SSU) of rRNA gene, while subgenotyping was determined based on the RFLP analysis of the gp60 gene and was confirmed by sequencing of the amplified products. **Results:** The isolates were confirmed positive using both the immunochromatographic detection kit (the stick Crypto-Giardia; Operon, Spain), in addition to microscopic examination of the modified Ziehl-Neelsen (mZN) stained stool smears. RFLP analysis of the SSU rRNA, revealed that the majority of cases were infected with *C. hominis* (75%) compared to *C. parvum* (25%). The RFLP analysis of the gp60 PCR product divided *C. hominis* isolates into 4 subgenotypes [Ia; (3), Id; (3), Ie; (2) and Ib; (1)]. On the other hand, the 3 *C. parvum* isolates belonged to the anthroponotic subgenotype (IIc). These data were further confirmed by sequencing the gp60 amplified products. Furthermore, the sequencing data revealed the presence of a new subtype in the Ia family (IaA7R1). **Conclusions:** Despite the limited number of isolates that were included in this study, we can conclude that anthroponotic transmission of *Cryptosporidium* infection has proved to be the predominant mode of transmission in Cairo, Egypt. The infectivity of the new subtype, its distribution in greater Cairo and vicinities needs further investigation to determine the significance of this finding. **E-mail:** jmrubio@isciii.es

Crypto013- Genotyping of *Cryptosporidium* isolates from different groups of children of Bahia state

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Introduction: *Cryptosporidium hominis* and *C. parvum* are the most common *Cryptosporidium* species causing infections in humans. Microscopical examination and coproantigen detection in feces only identify the *Cryptosporidium* genus. The identification of protozoan species is important to determine the route of transmission of the parasite. Data regarding the genotypes/species associated with cases of cryptosporidiosis in Brazil is still very limited. This study aimed to identify the genotypes of *Cryptosporidium* isolates from different groups of children of Bahia. **Materials and Methods:** The detection of *Cryptosporidium* in stool samples was performed by modified Ziehl-Neelsen staining and/or by detection of coproantigens using an ELISA kit (*Cryptosporidium* II, TECHLAB). Stool samples from the following groups were examined: 574 day care children of Salvador; 312 children from Conde, a small town on the coast, Linha Verde road, Bahia; 181 children with diarrhea and 79 with severe malnutrition, hospitalized in the Pediatric Center of the Prof. Edgar Santos University Hospital, UFBA; 91 children with cancer and 30 HIV-seropositive children. Genomic DNA extracted from *Cryptosporidium*-positive samples was subjected to a nested PCR amplification of the gene COWP and restriction fragment length polymorphism analysis (PCR-RFLP) using the enzyme *RsaI*. **Results:** The analysis of 1,267 stool samples showed a *Cryptosporidium* total frequency of 1.7% (n=22). *Cryptosporidium* positive samples were most identified in unhealthy subjects, such as, children with diarrhea (4,4%, n=8), malnourished (3,8%, n=3), children with HIV (3,3%, n=1) and oncology patients (2.2%, n=2). *Cryptosporidium* was also detected in children of day care centers (0.3%, n=2) and in those from an interior town of Bahia, 1.9% (n=6). Out of the 22 *Cryptosporidium* positive samples, 16 were subjected to amplification by nested PCR of the COWP gene. From these, 14 showed the expected amplicon with molecular weight of 553 bp. The PCR-RFLP revealed 13 (92.8%) isolates with *C. hominis* genotypic pattern, and only one child of the interior town, had infection by *C. parvum*. **Main Conclusions:** *Cryptosporidium* is one of the important agents of intestinal infections in children, showing different prevalences according to the group of subjects studied, being more frequent in symptomatic and/or immunocompromised individuals. The species *C. hominis* was more frequent in all groups, suggesting that anthroponotic transmission of *Cryptosporidium* is the most important in our area. **Financial support:** FAPESB. **E-mail:** flathami@hotmail.com

Crypto014- Frequency of *Cryptosporidium* sp., *Giardia duodenalis* and *Entamoeba histolytica* in different groups of children using the enzyme-linked immunosorbent assay (ELISA)

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Introduction: The laboratory diagnosis of intestinal protozoa by microscopy requires trained technicians and has low sensitivity when compared to parasite antigen detection methods, such as ELISA. Additionally, ELISA can confirm the infection by *Entamoeba histolytica* in patients diagnosed with *E. histolytica*/*E. dispar* by parasitological routine examination. This study aimed to evaluate the frequency of infection by *Cryptosporidium* sp., *Giardia duodenalis* and *Entamoeba histolytica* in different groups of children using an ELISA for detection of coproantigens. **Materials and Methods:** stool specimens were analyzed by commercially produced ELISA kits (TechLab) to detect antigens of *Cryptosporidium* sp., *G. duodenalis* and *E. histolytica*. The following groups of pediatrics patients were examined: 181 with diarrhea and 79 with severe malnutrition, hospitalized in the Pediatric Center of the Prof. Edgar Santos University Hospital, UFBA; 91 children with cancer and 16 HIV-seropositive children. Moreover, 312 stool samples of healthy children from Salvador public day care centers and from Conde, a small town on the coast, Linha Verde road, were evaluated only for the presence of *Cryptosporidium* sp. and *G. duodenalis* coproantigens. **Results:** The 679 stool samples evaluated for the presence of *Cryptosporidium* and *G. duodenalis* coproantigens, showed a global frequency of 2.6% (n=18) of positive samples for *Cryptosporidium* and 13.4% (n=91) for *G. duodenalis*. When analyzing the groups individually *Cryptosporidium* was detected more often in children with HIV (6.2%), followed by children with diarrhea (3.9%), malnourished children (2.5%) and those with cancer (2.1%). Conversely, the frequency of *G. duodenalis* was higher in healthy children (21.8%), followed by HIV-positive children (12.5%), patients with cancer (9.9%), children with malnutrition (5.1%) and those with diarrhea (4.4%). Of the 367 stools analyzed for the presence of *E. histolytica* coproantigen, 6 (1.6%) were positive, all from children with diarrhea (n=5) or diarrhea and malnutrition (n=1). **Main Conclusions:** Intestinal protozoa are quite frequent in childhood, with differences in prevalence according to the parasite examined and health status of children. *G. duodenalis* is more frequent in asymptomatic carriers, such as day care centers children, while *Cryptosporidium* and *E. histolytica* are more associated with groups with diarrheal disease or other pathologies. **Financial support:** FAPESB. **E-mail:** flathami@hotmail.com

Crypto015- *Cryptosporidium* and other intestinal parasites in children with severe malnutrition

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Introduction: Seven out of every 10 deaths in children under 5 years are due to malnutrition, diarrhea, pneumonia, measles or malaria. Malnutrition by itself is considered as a major global health problem, being related to high morbidity and mortality in poor countries. The malnutrition associated to parasite infections can further aggravate this scenario. This study aimed to evaluate the prevalence of intestinal parasites, including opportunistic agents, such as *Cryptosporidium*, in children with severe malnutrition. **Materials and methods:** Fecal samples from 80 children with severe protein-energy malnutrition admitted to the Professor Hosannah de Oliveira Pediatric Center, Federal University of Bahia (CPPHO-UFBA) were examined by sedimentation-centrifugation, Faust and Baerman-Moraes methods for search of protozoan cysts and eggs/larvae of helminthes, and by modified Ziehl-Neelsen for identification of coccidian oocysts. Samples were also submitted to *Entamoeba histolytica*, *Giardia duodenalis* and *Cryptosporidium* coproantigen analysis using ELISA kits (*Cryptosporidium* II / *Giardia* / *E. histolytica*, TECHLAB, USA). **Results:** Out of the 80 samples tested, 13 (16.25%) were positive for intestinal parasites. The most common parasite observed was *Giardia duodenalis* (6.25%; n=5). Other parasite

infections included *Ascaris lumbricoides* (1.25%; n=1), *Trichuris trichiura* (1.25%; n=1), *E. histolytica* (1.25%; n=1) and *Blastocystis hominis* (1.25%; n=1). Malnourished children had also 5% of opportunistic infections by the protozoa *Cryptosporidium* and *Isospora belli*, with a frequency of 3.75% (n=3) and 1.25% (n=1), respectively. Two of the three cryptosporidiosis cases were only identified by ELISA. Because of malnutrition and opportunistic infections, including the isosporiasis diagnosed in this study, there was requested latter the serology for HIV infection of the children with *Isospora*, being positive. **Main conclusions:** The occurrence of opportunistic parasite infections in hospitalized malnourished patients, observed in this study, associated or not to other pathologies, indicates the obligation of using different parasitological methods and/or specific immunological analysis of fecal samples, in order to avoid medical complications in such important risk group. Financial support: FAPESB. **E-mail:** rknrs7@gmail.com

GIARDIASIS

Giard001- Prevalence of *giardia intestinalis* in faeces from children, pet and stray dogs in Lages, Santa Catarina, Brazil

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The growing number of pet animals, especially in urban areas, increases the contact between human beings and these one, especially children, exposing them to the risk of transmission of zoonotic agents. The study aimed to determine the prevalence of *G. intestinalis* in fecal samples from children enrolled in the first five years of elementary schools and in their attempting to correlate this data. We also determined the prevalence of *G. intestinalis* in stray dogs collected at the Center for Zoonosis Control (CCZ) in Lages-SC. We analyzed 92 stool samples from children, 101 from dogs in 75 house visited and 357 stool samples of stray dogs. The study was conducted during the months of August 2010 to December 2011. For parasitological examination flotation with zinc Sulphate technique was used. The study was approved by the Ethics Committee of the University of Santa Catarina Plateau (UNIPLAC 005-2009). In children fecal samples, were from 49 girls (53.26%) and 43 boys (46.74%), with positivity of 11 cases (11.95%) for *G. intestinalis* cysts. In dogs, 10 samples (9.9%) of domiciled and 17 (5.35%) in stray were positive for *G. intestinalis* cysts. Regarding to gender 6 (12.24%) girls were infected and 5 (11.63%) boys, while for canine 7/57 (12.28%) of males were infected and 3 / 44 (6.82%) of females had the protozoa. 80% of infected children were aged six to eight years, and 60% of positives domiciled dogs were aged two to three years. For stray dogs the prevalence of *Giardia* was mainly in six years old dogs, 80% of positive samples were within this range. With this study it was found that the transmission of *G. intestinalis* may be present between dogs and children in Lages-SC. **Keywords:** *Giardia intestinalis*, children, dogs, zoonosis. **E-mail:** rosileia@uniplac.net

Giard002- *Giardia duodenalis*: Molecular epidemiology in a Tietê riverside community, São Paulo State, Brazil

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Giardia duodenalis, a zoonotic intestinal protozoan of humans and a range of other mammals, is one of the most common nonviral causes of diarrhea in human living in developing countries. Currently, one of the major questions concerning *Giardia* is to achieve a better understanding of the dynamics of parasite transmission and for this purpose, genotyping of fecal isolates from different hosts has shed light on this

aspect. Thus, in 2011, we initiated a study in order to assess the occurrence and frequency of *G. duodenalis* genotypes circulating in a socially deprived environment where the close proximity to each other and the lack of sanitary conditions favor giardiasis transmission. This study is being conducted in a fishermen community of the Tietê River in São Paulo State, Brazil. Fecal samples obtained from 86 dwellers distributed in 35 families were examined for the presence of *Giardia* using zinc sulfate flotation and, cysts were found in 7% (6/86) of the samples. Total DNA was extracted from each *Giardia*-positive sample as well as from the negative samples obtained from household members of the infected individuals. The eluted DNA was submitted to PCR-based methods for amplification of β -giardin, *gdh*, *tpi* genes and, the products were be sequenced for molecular typing of each isolate. All microscopy-positive samples were successfully amplified and 79% (15/19) of the household members' negative samples produced an amplicon by at least one gene loci tested. Among the 21 PCR products, clear sequences were obtained for 11 isolates and the analysis revealed the occurrence of infections with genotypes AI (2; 18%), AII (4; 36%) and BIV (5; 45%). We can confirm in a preliminary way that in this population, only genotypes A and B were associated with human infection. Furthermore, it is important to highlight that the predominance of genotypes AII and BIV, the groups more commonly involved in human infections, reinforces the fact that the person-to-person transmission probably guarantees the most human infections, especially in collective environments where sanitary conditions are poor. Molecular analysis goes on in this community and in the next step; *Giardia* isolates obtained from dogs and from samples of raw surface water of the river will be genotyped for assessing the main genotypes circulating among these hosts and in the environment. **Supported by:** FAPESP (2011/52100-3; 2011/09963-0) **E-mail:** sgviaana@ibb.unesp.br; ericaboarato@yahoo.com.br

Giard003- Identification and functional assessment of the key enzyme in NAD Metabolism in *Giardia lamblia* (GINMNAT)

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Introduction: The protozoan *Giardia lamblia* is the causal agent of an acute intestinal disease called Giardiasis, which is not fatal, but could turn dangerous depending on the host's socio-economic situation and immunological state. Biologically, *Giardia* is a key organism, considered one of the ancient eukaryotes, because of its cellular reduction machinery and its particular ancient metabolic pathways. A central metabolic molecule is Nicotinamide Adenine Dinucleotide (NAD), an essential cofactor in redox processes and cellular events like gene expression regulation, DNA damage, calcium mobilization and lifespan regulation, from yeast to mammals. NAD biosynthesis is accomplished by two main pathways: the de novo and salvage pathways. These use different precursors and intermediates but share a central enzymatic step catalyzed by Nicotinamide Mononucleotide Adenylyltransferase (NMNAT; EC 2.7.7.1). Therefore, this enzyme constitutes the critical step of NAD synthesis, being fundamental for biochemistry and physiology of *Giardia*. The study of NAD metabolism in *Giardia* is important, because NAD-dependent epigenetic mechanisms contribute to the state differentiation in the parasite which is crucial to infect new hosts. **Materials & Methods:** We have built a bioinformatics structural model based on two putative GINMNATs sequences. Two isoforms NMNAT was cloned, overexpressed and purified. The enzymatic activity was obtained in vitro using both coupled and direct assays from a recombinant of *G. lamblia* NMNAT (His-GINMNAT). In order to investigate the sub-cellular distribution of the NMNAT in the parasite, we used the His-GINMNAT protein for polyclonal antibody production in murine and avian models. The obtained antibodies have been used in Western blot and immunofluorescence protocols of cell extracts and trophozoites. Additional we cloned NMNAT into an HA fusion vector, and transfected into *G. lamblia* trophozoites to determine the intracellular location of NMNAT with immunofluorescence microscopy. **Results:** NMNAT shows a 3D structural conservation despite its amino acid divergence. Two isoenzymes were identified and modeled. Both show structural elements congruent with the NMNAT function. NMNAT was expressed, purified and NAD synthase activity was found. The subcellular location was predicted preferentially cytosolic. **Conclusion:** There is at least one functional and active NMNAT in *Giardia*. NAD Synthesis is fundamental to *Giardia* development and this study constitutes the first

experimental approach to NAD metabolism in *Giardia*. **E-mail:** pamorenog@unal.edu.co, mhramirez@unal.edu.co

Giard004- In vitro Activity of Cysteine Proteases Inhibitors on *Giardia duodenalis* Trophozoites

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The high incidence of treatment failure, relapses and undesirable effects by the currently available drugs to giardiasis has led to the search for new therapeutic agents. As cysteine proteases directly act in physiological microorganisms processes and in different steps of the parasite-host interplay, these molecules are among the most promising targets for the development of antiparasitic drugs. Whereas the proteases are regulated by specific inhibitors, these substances have been assessed for their therapeutic potential in parasite infections, including by *Giardia*. Thus, the aim of this study was to evaluate the *in vitro* effects of cysteine proteases inhibitors on the growth, adherence and viability of *Giardia* trophozoites of a strain isolated and axenized in Brazil (BTU-11). Trophozoites (10^5), harvested in exponential growth phase, were resuspended in fresh medium TYI-S-33 containing the cysteine protease inhibitors E-64 and iodacetamide (IAA) at different final concentrations (10, 50 and 100 μ M), for 24, 48 and 72 h at 37°C, and in triplicate. In the growth and adherence inhibition assays, trophozoites number was determined using haemocytometer and, cell viability was analyzed by a dye-reduction assay using MTT. Controls were included in all assays (cultures containing only the parasites and cultures treated with metronidazole). Both E-64 and IAA exerted a significant inhibitory effect on parasite growth, viability and adherence, and the level of inhibition varied according to the assayed concentrations and incubation times. However, the highest rates of growth and adhesion inhibition and the lowest rates of viability were observed in cultures exposed to IAA, when the activity of this inhibitor was similar to that observed after exposure to metronidazole. It is likely that IAA show higher toxic effect on the trophozoites because of its lipophilic character, which makes this inhibitor more permeable to cell membranes. Considering that there is little research assessing the *in vitro* effect of specific cysteine proteases inhibitors on *Giardia*, the present results show that micromolar concentrations of these substances interfere on multiplication, adhesion ability and viability of trophozoites, which are essential processes for parasite survival and establishment of infection in the small intestine of the host. **Supported by:** CAPES. **E-mail:** sgviaana@ibb.unesp.br; thais_bc@yahoo.com.br

Giard005- Limit of detection and typing of *Giardia duodenalis* in stool samples by two molecular markers

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Introduction: *Giardia duodenalis* (Synonymy *G. lamblia*, *G. intestinalis*) is a flagellate protozoan that parasitizes the small intestine of humans and other domestic and wild animals in different regions of the world. Molecular tools as β giardin and Heat Shock Protein (HSP) have been widely used to detect genetic differences between strains of *Giardia* morphologically identical. In this work the limit of detection and typing of *G. duodenalis* in stool samples was tested using these two molecular markers. **Material and Methods:** The threshold for detection and typing by amplification of markers HSP and β giardin was determined by DNA concentration of trophozoite and cysts and by the number of cysts and tested in 26 clinical samples. The DNA extracted from trophozoites and cysts was serially diluted in 1:2 ratios and similarly a cyst suspension was diluted for subsequent DNA extraction. **Results:** To HSP marker the detection limit was 0.0002ng for trophozoite, 0.004ng for cysts and 16 cysts/reaction. To the β giardin marker the minimum concentration of DNA detected was of 0.0003ng for trophozoite, 0.008 ng for cyst and 40cysts/reaction. Concomitant use of these two markers detected DNA of *Giardia* in 75% (18/24) of the samples with positive parasitological test compared to that obtained for each marker (45.8% and 58.3%), respectively. Both markers also detected the parasite DNA in samples (1/2) with parasitological negative results. To the marker HSP in relation to β giardin was necessary to lower DNA amount of

trophozoite and cyst in the typing of the parasite. **Main Conclusions:** We conclude that the marker HSP has limit of detection and typing higher than marker β *giardin*, but the combination of these two markers increases this potential and allows the correct genotyping of the parasite. **E-mail:** criscolli@yahoo.com.br

Giard006- Molecular identification of zoonotic genotypes of *Giardia intestinalis* isolates in humans, dogs, cats, sheep, goats and cattle in Araçatuba, São Paulo State, Brazil

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Objective: we evaluated the prevalence of genotypes, which are considered zoonotic and the possibility of zoonotic transmission of giardiasis in a city in the Southeastern Brazil. **Material and Methods:** in the period from July 2009 to October 2010, fecal samples from 61 animals and 154 humans from the municipality of Araçatuba, São Paulo State were studied. Fecal samples from animals were collected in the Municipal Animal Shelter and the Veterinary Hospital of the Universidade Estadual Paulista. Human fecal specimens were collected in playschools in the outskirts of the city and from the private network of clinical analysis laboratories of the municipal. Diagnosis was by optical microscopy using the Faust and Hoffmann, Pons & Janer techniques. The genotypes of *G. intestinalis* were characterized by PCR-RFLP and confirmed by sequencing the β -*giardin* gene. **Results:** human specimens were positive in 25.3% (39/154) of the cases with 26.8% (36/134) of the specimens from children and 15% (3/20) from adults being positive. The frequency of *G. intestinalis* among the animals was 23.0% (14/61). A total of 32 isolates of *G. intestinalis* obtained from human feces and six from dogs and cats were characteristic of the A genotype (AI and AII/AIII). The results of this study in respect to frequency of giardiasis are similar to the percentages reported for children and adults in most publications from Brazil. The prevalence observed in animal populations conforms to worldwide infection rates. **Conclusions:** *G. intestinalis* genotypes considered zoonotic were detected in both pets and humans from the city of Araçatuba, suggesting a possible zoonotic transmission of the parasite in the northwestern region of São Paulo State. However, the absence of these genotypes in farm animals implies that they are not involved in the chain of transmission to humans in this region. **Funding:** Famerp and CNPq. **E-mail:** elenirmacedo@yahoo.com.br

Giard007- Overexpression and Inhibition of Two Sm proteins in *Giardia intestinalis*: advances in the study of the spliceosomal machinery

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Introduction: The protist *Giardia intestinalis* (*G.intestinalis*) is a human parasite which causes diarrheal disease throughout the world. It is frequently described as an ancient protist and it is an excellent model for studying complex cellular process due to its early divergence and genomic reduction. Few years ago, it was thought that *G.intestinalis* did not have introns, but in 2002 one was identified; now there are four introns known in the whole genome. Moreover, many proteins of the spliceosomal have been identified through bioinformatics tools. Despite it is assumed that introns are removed through spliceosomal, this big molecular machinery has not been characterized. In the present research we wanted to study central proteins of the ribonucleic particles which are part of the spliceosomal: the proteins Sm. **Material and Methods:** Firstly we cloned genes of proteins SmB and SmD3 of *G.intestinalis* into the vector pTubHApac N terminus; this vector allows the constitutive and stable expression of genes in *G.intestinalis* trophozoites by the presence of the α -tubulin promoter and selection with puromycin. In addition, each Sm gene was inserted inside the pTubHApac N terminus inversely, thus generating each Sm antisense vector in order to study the effect of inhibition of these genes in the parasite. Subsequently, trophozoites

were transfected with the constructs by electroporation and selected with puromycin. After 14 days, transfected parasites that overexpress SmB and SmD3 proteins were collected for their analysis in western blot and immunofluorescence assays using antibody anti-HA. On the other hand, we isolated total RNA from transfected parasites with SmB and SmD3 antisense vector, and knockdown was confirmed by RT-PCR using gene-specific primers. Results: We obtained viable clones transfected with all constructs, cell proliferation was not affected when compared with untransfected trophozoites. In western blot we detected the proteins SmB and SmD3 in the trophozoites. Immunofluorescence assays showed detection of proteins SmB and SmD3 in trophozoites of *G.intestinalis*, both of them have discrete localization inside and near the nuclei. The RT-PCR assays, in knockdown parasites, showed increase in the level of specific mRNA, both in sense and antisense strands. Conclusions: We overexpressed the proteins SmB and SmD3 in *G.intestinalis*, they localized inside and outside the nuclei, suggesting that these proteins may not only be involved in spliceosomal formation but that they have also different functions in this parasite. The RT-PCR assays showed that although there was an increase in antisense RNAs, it was balanced by a similar increase in sense RNAs, suggesting that these genes can suffer auto regulation at the posttranscriptional level, in a similar way to their homolog in bacteria, the Hfq protein. **E-mail:** mvgomezr@unal.edu.co

Giard008- Palmitoylation and its role in the protozoan parasite *Giardia lamblia*

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Introduction: Palmitoylation refers to the addition of palmitate to a cysteine residue of proteins. Despite palmitoylation has been involved in protein trafficking, cell signaling and localization to lipid rafts, its biological function has not been completely elucidated. Previous work by our lab showed that variant-specific surface proteins from *G. lamblia* are palmitoylated and revealed the site of palmitoylation. Three palmitoyl transferases (PATs) of *G. lamblia* have been identified and some of the possible biological consequences of palmitoylation in this parasite have been determined. The goals of the present work are to characterize other PATs and the target proteins. Proteins that promote palmitoylation have been identified in *S. cerevisiae* including effector of Ras function (Erf2) and the SNARE protein Ykt6 which have a common Asp-His-His-Cys-cysteine-rich domain (DHHC-CRD) motif that likely confers PAT activity. **Materials and Methods:** WB 1267 *G. lamblia* trophozoites were axenically grown in modified TYI-S-33 medium enriched with 10% heat-inactivated fetal bovine serum supplemented with 0.1% bovine bile at 37°C. Induction of encystation was performed as previously described. DNA from trophozoites was isolated and PCR was performed using specific primers. Plasmidic DNA was sequenced and trophozoites were then transfected. Inhibition of palmitoylation was carried out by adding 2-Fluoropalmitic acid. **Results:** Looking at *Giardia* genome database, we found other possible PATs. These proteins display the typical DHHC-CRD. By PCR we determined that four possible enzymes are present in the *G. lamblia* genome. PCR products were then cloned in pTub-ApaH7HApac or pINDG-V5 vectors. Immunofluorescence analysis of the epitope-tagged proteins showed that they localize mainly in the cytoplasm or around the nuclei in trophozoites transfected with each PAT. To know the substrates of palmitoylation, we are carrying out experiments with palmitic acid labeled with tritium. When palmitoylation was inhibited during encystation, the amount of cysts produced decreased. Moreover, we observed an increase of Cyst wall protein 1+ parasites in encysting *Giardia* trophozoites that overexpressed a PAT compared to wild type *G. lamblia* trophozoites. **Main Conclusions:** Due to the many intracellular signals involved in encystation it is possible that palmitoylation participates in that process. Further studies are needed to find out the molecular mechanisms in which palmitoylation may be involved. The characterization of proteins which may act as PATs and target proteins of palmitoylation in *Giardia* would contribute to clarify the mechanisms used by the parasite in key biological events such as antigenic variation and/or encystation. **E-mail:** mcmerino@immf.uncor.edu

Giard009- Genetic characterization of a refractory giardia duodenalis strain in a whole family returning from India

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Introduction: Persistence of *Giardia duodenalis* after treatment with nitroimidazoles represents a common problem in travel clinics and it is a cause of concern. In these cases, nitroimidazole resistant strains or the possibility of reinfection should be both considered. The genetic characterization of the parasite in pre and post-treatment samples may be a high-quality tool to evaluate the cause of the parasite persistence. **Methods:** We are conducting a prospective study to evaluate the prevalence and genotypic characterization of persistent *G.duodenalis* in returning travellers. In the context of this study, we describe a family that presented refractory giardiasis after a trip to India. Stool samples were analyzed for microscopy following standard formalin-ether concentration, and by rapid immunochromatographic. DNA of all samples was extracted using DNA stool kit (IBIAN® DNA Stool Kit) following the manufacturer's instructions and stored at -20°C until their processing. *G. duodenalis* assemblage was determined by a PCR of the triosephosphate isomerase (*tpi*) gene. Additionally, PCR products were purified with GFXTM PCR DNA Gel Band Purification Kit and direct sequenced in both directions. **Results:** In July 2011, a Spanish family (59, 57, 16 and 14 years) returned from India with gastrointestinal symptoms. All of them presented *G.duodenalis* cysts in the stool examination and received a single dose of tinidazole 2g. The 14 year old son improved significantly, being the stool examination and the faecal antigen test negatives one month later. The rest remained symptomatic after treatment and control stool samples revealed persistence of *G.duodenalis*. HIV infection and the presence of an Immunoglobulin A deficiency were ruled out. They received quinacrine (100mg t.i.d for 5 days) and symptoms improved. Follow-up microscopy examinations were repeatedly negative. From each member of the family one sample was collected before the treatment with tinidazol and 3 times monthly after treatment, analyzing a total of 16 samples of which 12 samples yield positive amplification for a 140 bp fragment of the *tpi* gene corresponding to Assemblage B, and none of them amplified for Assemblage A. Samples of all family members were positive for amplification of *tpi* gene up to 2 months after the negativation of the stool examination. All *tpi* sequences were almost identical with similarities ranged between 99.2% and 100%. **Main conclusions:** In our family cases, although an apparently identical genotype was successfully treated with tinidazole in one of the sons, the nitroimidazole therapy was ineffective for the rest of the family. Other unknown factors, including host-parasite interactions or the inoculum amount are probably of importance. Surprisingly, for all family members PCR remained positive long time after treatment without being able to detect cysts by microscopy or antigen by rapid immunochromatographic. It is possible that damaged and no recognizable cysts are eliminated in the faeces during long periods after therapy. Other explanation could be that patients respond partially to the infection leading to a situation where patients remain asymptomatic and the parasitic load is too low to be detectable for the conventional techniques but measurable by the more sensible PCR. If an alternative therapy might be offered remains unclear and further research is required to understand the value of PCR in the evaluation of refractory giardiasis. **E-mail:** ana.requena@cresib.cat

Giard010- Removal of *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts by a combined anaerobic/aerobic sewage treatment plant

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Giardia spp. and *Cryptosporidium* spp. are important waterborne pathogens that were responsible for several outbreaks related to consumption of contaminated water. Due to its long-term survival and peculiar behavior during sewage treatment, changes in system performance may result in decreased removal of these protozoa. The aim of this study was to evaluate the removal of cysts and oocysts in a

Sewage Treatment Plant, whose treatment system consists of up flow anaerobic sludge blanket (UASB) followed by conventional activated sludge. Samples of raw sewage, UASB effluent, UASB sludge, recirculated sludge of aeration tank, treated effluent and conditioned sludge were collected monthly from November 2010 to November 2011, with the hydraulic detention time of 22 hours. All samples were processed by centrifugal-concentration technique and protozoa were visualized by direct immunofluorescence assay (Kit Merifluor® - Meridian Bioscience, Cincinnati, Ohio) and confirmed by the incorporation of a vital dye DAPI (1:2500). High concentrations of *Giardia* spp. Cysts were detected in the affluent with an average of 116,923 cysts/L. Large removals of cysts by UASB were noted, the effluent from this point presented an average of 167 cysts/L and the sludge presented an average of 347,692 cysts/L. The effluent of UASB feeds the aeration tank, but an average concentration of 221,818 cysts/L was noted in the sludge of aerobic treatment, demonstrating that a buildup of cysts also occurs at this point. Lower average concentration of cysts (77 cysts/L) was observed in the final effluent and the sludge conditioning presented average of 221,818 cysts/L. *Cryptosporidium* spp. oocysts were detected in March, August and September 2011. In March, effluent of UASB presented 100 oocysts/L and the sludge of UASB presented 80,000 oocysts/L; in August, conditioned sludge presented 40,000 oocysts/L and in September, the sludge of UASB presented 80,000 oocysts/L. *Giardia* cysts removal efficiency rate ranged from 99.8 to 100%. The study of removing these parasites pathogen underscores the importance of sewage treatment to reduce the impact of effluents generated in the environment and in public health and the need for greater control regarding the treatment of conditioned sludge generated in sewage treatment plants. **Funding:** Propex/FURB (Doctoral Scholarship-announcement 05/2008) MCT and CNPq/14/2010-Edital Universal. **E-mail:** julianeag@gmail.com

AMEBIASIS

Amoeba001- A novel class of transporters that mediate lysosomal trafficking in *Entamoeba histolytica*

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The transport of lysosomal proteins is, in general, mediated by mannose-6-phosphate receptors, which recognize carbohydrate modifications of the cargo proteins. Here, we identified a novel class of receptors that regulate the transport of lysosomal hydrolases in *Entamoeba histolytica*. A 110-kDa cysteine protease (CP) receptor (CP-binding protein family I, CPBF1) was initially discovered by affinity co-precipitation of the major CP (EhCP-A5), which plays a pivotal role in the pathogenesis of *E. histolytica*. We demonstrated that CPBF1 regulates EhCP-A5 transport from the endoplasmic reticulum to lysosomes and its binding to EhCP-A5 is independent of carbohydrate modifications. Repression of CPBF1 by gene silencing led to the accumulation of the unprocessed form of EhCP-A5 in the non-acidic compartment and the mis-secretion of EhCP-A5, suggesting that CPBF1 is involved in the trafficking and processing of EhCP-A5. We also demonstrated that other predominant CPBF proteins, CPBF6 and CPBF8, bind to amylases, and [3-hexosaminidase and lysozymes, respectively. These results suggest that the CPBF represents a new class of transporters that regulate the trafficking, processing, and activation of lysosomal enzymes and, thus, regulate the physiology and pathogenesis of *E. histolytica*. References: Nakada-Tsukui, K., Tsuboi, K., Furukawa, A., Yamada, Y., and Nozaki, T. A novel class of cysteine protease receptors that mediate lysosomal transport. Cell. Microbiol. In press, 2012. Furukawa, A., Nakada-Tsukui, K., and Nozaki, T. Novel transmembrane receptor involved in phagosome transport of lysozymes and β -hexosaminidase in the enteric protozoan *Entamoeba histolytica*. PLoS Pathogens 8: e1002539.

Amoeba002- Characterization of the protein tyrosine phosphatase EhPRL of *Entamoeba histolytica*

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Introduction: Cell invasion is a process that involves cell signaling systems with phosphorylation and dephosphorylation reactions. We are engaged on the study of the protein tyrosine phosphatase (PTP) PRL (Phosphatase of Regenerating Liver) of *E. histolytica*, the causative agent of Amebiasis. The PRLs are a unique family of plasma membrane-associated PTPs that have been suggested to be involved in metastatic cancer, promoting cell migration and invasion. *E. histolytica* has a single gene encoding for PRL (EhPRL) compared with the 3 PRL isoforms identified in humans. For these reasons, it is our goal to characterize biochemically and biologically EhPRL phosphatase of *E. histolytica* and assess their possible participation in events of invasion and pathogenicity. **Methods:** *E. histolytica* HM1-IMSS trophozoites were cultured in axenic conditions and harvested at the logarithmic phase of growth. Amoebic liver abscesses in hamsters were induced by direct inoculation of 1.5×10^6 axenic trophozoites into the liver to get virulent amoebae. The amoebae were harvested and soluble extract and/or RNA was prepared. To get the recombinant protein ehprl was obtained by RT-PCR assays using specific oligonucleotides designed according to the sequence reported in GeneBank (Acc. No. NW_001914862). The amplicon was cloned in both pBluescript SK (+) vector and expression vector pRSET-A to obtain 6His::EhPRL (rEhPRL), this was purified by affinity chromatography column. A Phosphatase Activity Assays was conducted using 3-O-methylfluorescein phosphate (OMFP) as a substrate. In addition, we conducted an in gel-phosphatase activity assay by the Fast Garnet method. To determinate the cell localization, an IFI was made with trophozoites cultured on glass coverslips covered with either bovine type I collagen (COL) or fibronectin (FN) at different interaction times. To measure the level expression of mRNA and protein EhPRL a RT-PCR and total protein extract was get of axenic trophozoites or recovered from ALA, incubated with FN or COL. **Results and Conclusions:** *E. histolytica* genome contains one single copy of the EhPRL gene. EhPRL gene codifies for a 22 kDa protein containing the motif HCX5R present in the PTPs, at the C-terminal end it contains a CAAX box which may be post-translationally palmitoylated. rEhPRL protein showed low enzymatic activity with OMFP as a substrate at pH 7.0. However, a positive reaction was detected when the phosphatase activity was measured in native polyacrylamide gels; this activity was increased in gels incubated with $MnCl_2$ and $MgCl_2$, and no activity was detected with $CaCl_2$. Native EhPRL is located on the plasma membrane and cytoplasm. Trophozoites interacting with COL showed an increase of mRNA levels from 15 min until 60-90 min as compared with trophozoites cultured without COL. However, FN does not induce changes at the mRNA level but an increase was detected at protein level. EhPRL expression is regulated by trophozoite interaction whit extracellular matrix components. Trophozoites recovered from ALA showed higher EhPRL expression (mRNA) than trophozoites maintained only in axenic culture, suggesting that EhPRL could participate in virulence mechanisms and development of ALA. **E-mail:** ana_lili278@yahoo.com.mx*

Amoeba003- Luminex xTAG GPP panel: a useful tool for *E. histolytica* infection diagnosis?

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Objectives: *E. histolytica* infection is an intestinal parasitic disease endemic in tropical areas. This pathology may worsen, in some cases, into a severe illness with a poor prognosis. Diagnosis of *E. histolytica* is very difficult; requiring much time and it is operator dependent. A sensitive and specific method for identifying easily and quickly these protozoa could simplify and lighten the microbiology lab workload. **Material and Methods:** Luminex xTAG Gastrointestinal Pathogens Panel (GPP) is a diagnostic kit for a rapid identification of 15 gastrointestinal pathogens using Luminex technology. This panel includes the identification of *E. histolytica* and two other protozoas (*Giardia intestinalis* and *Cryptosporidium* spp).

22 non fixed stool samples of patients positives for intestinal pathogens by traditional methods (cultural assays for bacteria, concentration methods for stool protozoa, immunochromatographic methods for gastroenteric viruses and Clostridium difficult toxins were charged with Luminex xTAG GPP . The samples were assayed as manufacturer's indications. **Results:** Of the 22 positive patient samples, 3 samples resulted positive for *E.histolytica*; three were confirmed by concentration stool method as *E. histolytica*/dispar (2) and *E.hartmanni* (1). Any of these patients resulted positive to specific antibodies. One sample resulted positive for *E.histolytica*/dispar by concentration method but negative with XMAP luminex method. This sample result was revised as misdiagnosed afterward (leucocytes were exchanged as cysts of *E. histolytica*/dispar). **Conclusions:** Even though Luminex xTAG GPP could have been cross reactions with *E. dispar* or *E. hartmanni*, this assay could be a useful method for heavy suspect samples, and could be worthily introducing XMAP GPP panel in new specific diagnostic protocols for identifying this pathogen protozoa. **E-mail:** romualdo.grande@policlinico.mi.it

Amoeba004- Novel chemotherapeutic drugs on proliferation of *Entamoeba histolytica* in axenic cultures

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Introduction: Amebiasis is characterized as a chronic, no inflammatory, afebrile disease which involves the colon wall and can invade the intestinal mucosae and disseminable to other organs, such as the liver. In humans, the infection is treated with metronidazole, which presents several side effects. So several studies has been done to find drugs, and did not present adverse effects to the patient. **Material and Methods:** *Entamoeba histolytica* from the HM1:IMSS strain were used in all assays. Drugs were dissolved to concentrations of 5 and 10 μ M in 0.01% DMSO or in a 1:1 mixture of ethanol and DMSO (for TC95). Culture medium, vehicle, or a drug was added in the log period, determined by counting cells between 4 and 192 hours after the initial inoculum. Samples were collected by incubating test tubes on ice for 15 min, in order to detach adhered cells. Tubes were then delicately turned 10 times for complete detachment and then cells which were counted on Neubauer chambers under 0.025% eosin stain. Differences between treatments were analyzed using two-way ANOVAs, followed by Tukey's post-hoc tests when $p < 0.05$. **Results:** Metronidazol produced a concentration ($F_{[3, 136]} = 15.86$, $p < 0.0001$) and time-dependent ($F_{[4, 136]} = 98.62$, $p < 0.0001$) effect on proliferation. Post-hoc test revealed that the 5 μ M concentration inhibited growth at 36 and 48 h ($p < 0.05$ vs. DMSO and culture medium), and the 10 μ M concentration produced a larger effect from 24 h onwards ($p < 0.05$ vs. DMSO and culture medium). In the last two time windows (48 and 60 h), both concentrations decreased cell count ($p < 0.05$), with the highest concentration producing the largest effect. Miltefosine showed concentration-response ($F_{[3, 104]} = 11.28$, $p < 0.0001$) and time-response ($F_{[4, 104]} = 119.2$, $p < 0.0001$) profiles similar to metronidazole. Both concentrations inhibited growth at 48 and 60 h ($p < 0.05$ vs. DMSO and culture medium), and the higher concentration inhibited growth at 36 h ($p < 0.05$ vs. DMSO and culture medium). Oryzalin did not inhibit growth at both concentrations ($F_{[3, 104]} = 0.7105$, $p = 0.5545$). TC95 presented concentration ($F_{[3, 48]} = 50.24$, $p < 0.0001$) and time-dependent effects on trophozoite growth ($F_{[4, 28]} = 208.9$, $p < 0.0001$). At 5 μ M, the drug inhibited proliferation at 36 and 48 h ($p < 0.01$ vs. DMSO:Ethanol, $p < 0.001$ vs. culture medium); the higher concentration inhibited proliferation at the same intervals. **Main conclusions:** After analyzing the growth curves, it was found that the TC95 was the most effective drug of the three treatments, miltefosine had little inhibition and oryzalin was not effective. This could reflect the probable susceptibility of the parasite to membrane damage. **Keywords:** Antiparasitic chemotherapy. Microtubules. Cell division. Phospholipids. **E-mail:** betania.alvarenga@gmail.com

Amoeba005- *Acanthamoeba* spp. (Sarcomastigophora: Acanthamoebidae) found in wild populations of *Aedes aegypti* (Diptera: Culicidae)

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Introduction: Symbiotic, commensal and parasitic associations are widely reported in arthropods. Several studies have identified these kinds of associations between fungi, bacteria and protozoans, especially in insect vectors. By the fact of mosquito larvae and free-living amoebae (FLA) occupy the similar aquatic sites; the aim of this study was to determine the prevalence of *Acanthamoeba* spp. in *Aedes aegypti* larvae collected in the environment. **Material and Methods:** The amoebae were investigated in 60 pools, each containing 10 larvae of *A. aegypti* which were collected by using larvitrap installed in peridomestic areas in different neighborhoods of Porto Alegre, Rio Grande do Sul, Brazil, during 2010 and 2011. The larvae were previously washed three times in cold distilled water, and then in PBS solution. Next, the larvae were macerated, centrifuged and 50 µL of the sediment was deposited in the center of a sterilized plate containing 1.5% non-nutrient agar (NNA) with an over layer of *Escherichia coli* suspension (ATCC 25922), previously killed by heating. The bioassay was performed in triplicate, and pools of laboratory-reared larvae treated under the same conditions were used as negative control. *Acanthamoeba* isolates were morphologically characterized and submitted to Polymerase Chain Reaction technique to confirm the genus. In addition, genotype analyses as well as presumptive tests for pathogenicity in some samples were performed. **Results:** Among the pools, 54 (90%) were positive for FLA. From those isolates, 47 (87%) belong to the genus *Acanthamoeba*. The genotype groups T4, T3 and T5 have been identified corresponding to 14 (53.8%), 10 (38.5%) and two (7.7%) isolates respectively. The physiological tests performed in 14 strains showed that 12 (85.7%) were non pathogenic, while two (14.3%) were considered with low pathogenic potential. **Conclusions:** These results provide a basis for a better understanding between these protozoan and mosquitoes interaction in their natural habitat. Moreover, this study is the first to report isolation of *Acanthamoeba* spp. from mosquitoes collected in the environment. **E-mail:** dayaneotta@gmail.com

Amoeba006- Determining the *Acanthamoeba* genus in free-living amoebae isolated from portable and stationary eye-wash station

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Acanthamoeba is a free-living amoeba (FLA) genus widely distributed in the natural and artificial environments, presenting a great medical and environmental importance. These amoebae are opportunistic pathogens, causing keratitis in healthy people or encephalitis, mainly in immunosuppressed people. The portable and stationary eye-wash stations are equipments required for work environments that may expose employees to harmful chemicals. The purpose of this study is to determine the *Acanthamoeba* genus in FLA isolated from portable and stationary eye wash station, using the PCR technique. A total of 74 samples were collected from biofilm (37) and water (37), using sterile swabs and flask, respectively. After processing, the samples were inoculated in non-nutrient agar 1.5%, covered by *Escherichia coli* as substrate, and incubated at 30°C up to 10 days. Of the 74 collected samples, 43 (58.1%) were positive for FLA, and seven of these were already submitted to molecular identification of the *Acanthamoeba* genus, using PCR to amplification of the 18S rDNA gene. The FLA trophozoites cultivated were suspended in PBS 1x buffer, and submitted to DNA extraction, according to SALAH & ICIAR (1997). Then, PCR was performed with JDP primers, according to SCHROEDER *et al.* (2001). The PCR products were analyzed and six samples were confirmed as belonged to *Acanthamoeba* genus (one from biofilm, and 5 from water), indicating 85.7% of positivity. The *Acanthamoeba* prevalence is reported in several sources of treated water, such as swimming pools (Caumo *et al.*, 2009), drinking water (Winck *et al.*, 2011) and biofilms (Carlesso *et al.*, 2010). The presence of this protozoa in eye-wash stations provides risk to their users, since these equipments are used in case of ocular accident, which can cause

injury allowing the input of microorganism from water, or biofilm. Further studies are needed to make the genotypic characterization (using 18S rDNA sequencing) of *Acanthamoeba* isolates obtained and to evaluate the sanitary quality of these equipments. **E-mail:** lua.ferpan@gmail.com

Amoeba007- Distribution of Free-Living Amoeba (FLA) in swimming pools in Uberaba, Minas Gerais, Brazil

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Introduction: Free-living amoeba (FLA) are ubiquitous protozoa found in a wide variety of natural habitats, including water, soil, and air. Some genotypes of *Acanthamoeba* spp., *Naegleria fowleri*, *Balamuthia mandrillaris* and *Sappinia diploidea* can cause severe damage in central nervous system, skin, internal organs or eyes of humans and animals, according to the specie. Furthermore, this FLA and other genera as *Hartmannella* spp., *Vahlkampfia* spp. and *Willaertia* spp. can also establish symbiotic relationships and carry pathogenic microorganisms. **Material and methods:** In this study, the frequency of *Acanthamoeba* spp., *Naegleria* spp., *Willaertia* spp., *Vahlkampfia* spp. and *Hartmannella* spp. was evaluated in water samples of surface and bottom of 34 swimming pools of recreation center in Uberaba, Minas Gerais. These samples were analyzed by light microscopy, culture in soy agar 1,5% and PCR assay of the 18S region of rDNA of pathogenic and non-pathogenic genotypes of *Acanthamoeba* spp., and ribosomal internal transcribed spacers region of other genera of FLA and *N. fowleri*. Influence of pH, temperature, depth, water disinfection, coverage and heating system were evaluated too. **Results:** FLA was detected in 97% of water samples with major distribution of *Naegleria* spp. (65%), *Acanthamoeba* spp. (47%), all associated with pathogenic genotypes, and *Hartmannella* spp. (44%), followed by *Willaertia* spp. (26%) e *Vahlkampfia* spp. (23%) ($p=0,0033$). *N. fowleri* was not identified. Associations between different genera were observed in the same swimming pool and physical parameters evaluated did not influence the positivity of the samples. **Main Conclusions:** The high frequency of FLA, particularly *Naegleria* spp. and pathogenic genotypes of *Acanthamoeba* spp., in swimming pools in Uberaba, Minas Gerais, suggest that clinical and epidemiological implications of genera found should be investigated. **E-mail:** lucena_poli@yahoo.com.br

Amoeba008- Free-Living Amoebae in nasal mucosa and skin Lesion of Dogs in Porto Alegre City, RS, Brazil

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The free-living amoebae that cause human infections include *Acanthamoeba*, *Naegleria*, *Balamuthia mandrillaris*, and *Sappinia diploidea*. All 4 genera cause CNS infections that are frequently fatal. These amoebae are distinct from other pathogenic protozoa. They all have a free-living existence, have no human carrier state and a limited relationship with the spread of infection and poor sanitation. *Acanthamoeba* are ubiquitous organisms and have been isolated from soil, water (including natural and treated water), air, and dust. *The protozoa* has caused disease worldwide, including United States, Europe, Australia, Africa and South America. *Acanthamoeba* was first established as a cause of human disease in the 1970s. This genus causes 3 clinical syndromes: granulomatous amebic encephalitis (GAE), disseminated granulomatous amebic disease (eg, skin, sinus, and pulmonary infections), and amebic keratitis. Individuals who develop GAE or disseminated disease are usually immunocompromised, whereas those with amebic keratitis are usually immunocompetent. Despite the widespread existence of *Acanthamoeba*, GAE usually occurs among immunocompromised persons. In unusual cases, disseminated disease develops in immunocompetent children and adults. The incidence of GAE and disseminated disease appears to be rising, likely mirroring the increased number of persons worldwide who are living with immunocompromising conditions. To date, more than 100 cases of GAE have been described. Data on the incidence rates of these infections internationally are not available since it is not a reportable disease. GAE was also describing in non-human primates, horses and dogs. The aim of the

present study was to search and isolate free-living amoebae from dogs in Porto Alegre city in order to verify the occurrence of this organism in these animal group.. Until now it was collected 88 samples of nasal mucosa and skin lesion of 44 dogs. The samples were collected with sterilized swab and processed in the Laboratório de Parasitologia - Departamento de Microbiologia - Universidade Federal do Rio Grande do Sul. The samples were cultivated in non-nutrient agar (1,5%) plates coated with *Escherichia coli* over layer at 30°C. Among the samples collected, 21 were positive for AVL: 10 from nasal mucosa and 11 from skin lesion. The genus *Acanthamoeba* was confirmed by Polymerase Chain Reaction (PCR) in nine positive samples. The results of this study provide an important baseline for future investigations of the protozoan for the development of preventive and therapeutic treatment in susceptible hosts. **E-mail:** anacarlesso@yahoo.com.br

Amoeba009- Isolated *Acanthamoeba* of bottled mineral water

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Acanthamoeba is one of the free-living amoebae (FLA) in greater prevalence in the environment. This protozoan has already been isolated from drinking water, river, sea, swimming pool, contact lens case, soil and dust. *Acanthamoeba* cause serious diseases like keratitis, granulomatous amebic encephalitis (GAE) and cutaneous lesions in immunocompetent and immunocompromised people. The aim of this paper was to isolate and characterize FLA belonging to different bottled mineral water brands. 14 mineral water brands were investigated, in which one liter was filtrated using a nitrocellulose membrane with a pore of 3µm and after it was placed upside in non-nutrient agar Page plates coated with *Escherichia coli* over layer 30°C. After a maximum of 30 days of observation, the negative samples were neglected and the positives for FLA had the genus confirmed by Polymerase Chain Reaction (PCR). Among 14 samples analyzed, 09 were positive for the *Acanthamoeba* genus. The positive samples will have the DNA fragment sequenced to identify the genotype. These results indicate the necessity of proper hygiene and storage of the bottled mineral water. **E-mail:** anacarlesso@yahoo.co.br

Amoeba010- Isolation and characterization of free-living amoebae of *Acanthamoeba* genus from the phylloplane of bromeliads in Southern Brazil

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Free-living amoebae (FLA) of *Acanthamoeba* genus are among the most abundant and widely distributed protozoa in the environment. They have been isolated from soil, saltwater, freshwater, air samples and from all over the world, which add to the genus a great biological and ecological importance. Furthermore, it can be considered an important vehicle of human pathogens like *Legionella pneumophila*, *Mycobacterium avium*, and other “amoeba-resistant microorganisms” like viruses and fungi. The phylloplane is considered an important habitat for a wide variety of microorganisms, but the ecological relationships that occur there still need to be investigated. The main aim of the current study was to identify the presence of FLA in the phylloplane of bromeliads in Southern Brazil. *Acanthamoeba* samples were collected with swabs of 10 samples of bromeliads leaves. The swabs were placed in sterile tubes containing 50 ml of sterile distilled water, gently shaken, squeezed and discarded. The material was under sedimentation process for 2 hours. Then, centrifugation was performed at 250 x g for 10 minutes. The supernatant was discarded and the sediment resuspended in 0.5 ml of saline Page. Then, 100 ml of this suspension was used as inoculum on the plate containing a non-nutrient agar 1.5% covered with a suspension of *E. coli* and incubated at 30 °C for 10 days. Nine (90%) of the leaves samples investigated were found positive for free-living amoebae, all identified as belonging to *Acanthamoeba* genus. All nine isolates were positive in the *Acanthamoeba*-specific PCR that amplify the ASA.S1 region of 18S rDNA gene. All isolates are in the process of molecular identification and genotyping. The results of this first

study about the identification and characterization of *Acanthamoeba* in the phylloplane of bromeliads leaves in Southern Brazil confirm the presence of potentially pathogenic types that may present a risk to human health and ubiquity of the *Acanthamoeba* genus, which are distributed in the natural and artificial environment sources. **E-mail:** julianasalton@yahoo.com.br

Amoeba011- Relationship between the secretion of proteases and pathogenicity in vivo of clinical and environmental isolates of Acanthamoeba

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Acanthamoeba is a genus of free-living amoeba (FLA) widely distributed in the environment that under specific conditions can cause Acanthamoebic Keratitis and Granulomatous Amoebic Encephalitis in humans. The knowledge about the factors that actually determine the pathogenicity of *Acanthamoeba* isolates is limited, and the proteolytic activity has been associated with important functions related to pathogenicity of *Acanthamoeba*. This study evaluated the profiles of proteolytic activity of conditioned medium of *Acanthamoeba* isolated from clinical cases and environment in zymogram gel, in order to characterize the proteases secreted and associate them with pathogenicity *in vivo* and *in vitro* of the isolate. We evidence that the isolates secrete amounts and types of different proteases, although some isolates have identical proteases profile in zymograms. The proteases profile was not associated with the origin, genotype or species of the isolate, as well as with the *in vivo* *Acanthamoeba* pathogenicity. The *in vivo* model used was efficient for establish the disease and to differentiate the isolates into pathogenicity degree. Furthermore, we demonstrated that environmental origin strains can cause infections in animals, and determined that amoebas maintained for long time in laboratory are less virulent than those freshly isolated. Although the secreted proteases profile not showed influence in the virulence of the studied isolates, it is clear that proteases have a key role in this protozoan pathogenesis. **E-mail:** caroldmv@yahoo.com.br

Amoeba012- Vectorial implications of Acanthamoeba sp in the propagation of Legionella sp in natural pools from Madrid (Spain)

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Introduction: Free-living amoebae (FLA) are frequent carriers of amoebae-resisting bacteria and studies on the interaction of FLA and *Legionella* have shown that FLA are necessary for *Legionella* multiplication in water biofilms. In a previous study, *Acanthamoeba* T4 was identified in approximately 90% of water samples from drinking water treatment plants, wastewater treatment plants and surface waters from Madrid (Spain). Due to the high percentage of *Acanthamoeba* previously detected, we investigated the presence of *Legionella* and *Acanthamoeba* in natural pools from Madrid in order to evaluate its vectorial role in *Legionella* sp propagation. **Methods:** *Sample collection:* Five natural pools from Madrid (Spain) were sampled during 1 year, 2 times per season. Up to 50 L were collected following the US-EPA Method 1623, and concentrated by the IDEXX[®] Filta Max system. *Acanthamoeba culture:* 80 µl of the concentrated samples were inoculated into non-nutritive agar (NEFF) seeded with inactivated *Escherichia coli*. When positive, *Acanthamoeba* was isolated by dilution and lysed to detect *Legionella* inside them. *Legionella culture:* 100 µl of the concentrated water were inoculated into MWY medium after different treatments (heat shock, pH shock and both at the same time). Suspicious colonies were confirmed by culturing in selective medium BCYE with and without cysteine. *Legionella co-culture:* 200 µl of concentrated water were inoculated onto an *Acanthamoeba* monolayer in order to grow *Legionella*. *Acanthamoeba PCR:* A real time PCR was performed in the concentrated water. For genotyping we amplified the ASA.S1 region of the 18S subunit rRNA from the culture isolated amoebae. *Legionella PCR:* A seminested PCR was done in concentrated water, in co-cultures and *Acanthamoeba* isolates in order to confirm their vectorial role. **Results:** *Acanthamoeba* was found throughout the year in the 5 pools (100%) studied. We isolated *Acanthamoeba* T16 from one of the pools but the most prevalent genotype found

was T4 (94%). It is important to highlight that T4 genotype is the most common in environmental samples but it is also the most prevalent in human pathologies such as *Acanthamoeba* keratitis or encephalitis. *Legionella* cultures were all negative. However, by PCR we found more than 40% of *Legionella* positive samples in concentrated water. We also detected that a high percentage of the isolated amoebae were infected with *Legionella*. Co-cultures showed *Legionella* in 40% of the samples and *L. feeleeii* was identified in 3 pools and *L. fairfieldensis* in one. It should be noted that *L. feeleeii* is the causative agent of around 2% of *Legionella* pneumonia. **Conclusions:** To our knowledge this is the first study carried out on natural pools in Spain on the presence of *Acanthamoeba* sp and *Legionella* sp and their interaction, confirming the vectorial role of FLA. The high presence shown by both *Acanthamoeba* and *Legionella* suggests the potential role of this kind of water on the transmission of these pathogens. **E-mail:** capupue@ceu.es

DISEASES BY *BLASTOCYSTIS* SP.

Blasto001- Occurrence of *Blastocystis* spp in Uberaba, Brazil

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Blastocystis spp is an intestinal protozoan parasite found in humans and several other animal species. Although little is known about the clinical significance of this parasite in public health, it is considered the most common of the protozoa found in human stool samples from both immunocompetent and immunocompromised individuals. Some authors associate the presence of *Blastocystis* spp infection with clinical symptoms such as diarrhea, cramps, nausea, fever, vomiting and abdominal, however the subject is controversial. In Brazil, there are few studies on *Blastocystis* spp. The aim of this work is to investigate the presence of *Blastocystis* spp in individuals assisted at the Clinical Hospital from the Federal University of Triângulo Mineiro (CH-UFTM), MG, and Brazil. 1653 stool samples from patients attended at CH-UFTM were analyzed during the period from April/2011 to January/2012. Of these, 46,8% were male and 53,1% female, and 43,4% of the individuals were between 1-10 years old. Stool sample analysis was performed by both direct and Ritchie methods and showed that 25.2% (417/1653) of the samples tested positive for intestinal parasites. The most frequent parasites found were *Blastocystis* spp 16,9%, *Giardia* spp 5,4%, *Escherichia coli* 4,1%, *Endolimax nana* 2,4%, *Entamoeba histolytica* 1,7%, and *Taenia* sp 0,5%. Parasitism was 1.35 times higher (IC95%=1,08-1,68) in male patients (28,2%) than female patients (22,6%) (p=0,009). Analysis by Ritchie method was more sensitive (91.8%; 257/280) than the one by the direct method (89.0%; 249/280), with a discordance proportion of 2.2% (36/1653). However, the association of both methods increases the proportion of positive samples. Association of *Blastocystis* spp with other parasites occurred in 30.7% (86/280) of cases, being 6.1% with *Giardia* spp, 5.0% with *E. histolytica*, 1.4% with *Taenia* sp, 0.7% with *Ascaris lumbricoides*, 0.4% with *Strongyloides stercoralis* and 17.1% with non pathogenic protozoa. Consistency of stool samples analyzed were classified as solid (65,0%; 1074/1653), pasty (18.7%; 309/1653) and liquid (6.0%; 99/1653). There was no difference in positivity regarding consistency of feces. *Blastocystis* spp was detected in 21.4% of pasty feces and in 17.9% of solid samples, percentiles that are higher than the positivity observed in liquid samples (8.1%) (p=0,011). These data do not rule out the possibility that the parasite can induce intestinal disorders, as pasty and liquid stools were observed in individual infected only with *Blastocystis* spp. Anyway, further studies on the determination of the parasite subtype found in the three types of feces can bring new information about the pathogenic potential of this parasite. **Supported by:** FAPEMIG. **E-mail:** marlenecabrine@yahoo.com.br

Blasto002- Implantation of *Blastocystis* sp and degree of pathogenicity in the gastrointestinal tract of mice according to different inocula and time of infection

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Introduction: *Blastocystis* sp is an enteric protozoan reported worldwide, but still raises questions about its pathogenicity. Severe cases of blastocystosis, *Blastocystis* infection, are giving pathogenic character to the parasite and studies in animal models have identified the protozoan in the intestinal tract of many hosts. The aim of this work was report the implantation of *Blastocystis* sp and degree of pathogenicity in the intestinal tract of mice in accord to different inocula and time of infection. **Material and Methods:** Swiss mice were infected by intragastric route with a suspension of 100, 500, 1,000, 5,000 and 10,000 cysts of *Blastocystis* sp. These mice were examined before and after infection for confirmation. They were euthanized 7, 14, 21, 28 and 60 days post-infection and portions of the small intestine, large intestine and cecum were collected for preparation of histological sections stained with hematoxylin-eosin for subsequent microscopic examination. **Results:** All animals had forms vacuolar eliminated in the feces from the second day of infection, regardless of inoculum used. Vacuolar forms of *Blastocystis* sp were found throughout the intestinal tract of mice; however most of the parasites were visualized in the cecum. Concomitantly with the presence of vacuolar forms were also identified changes in the inflammatory response in tissue. In the first week of infection many cysts implanted in the intestinal tract (37%) were observed in relation to 60 days post-infection, when none (0%) cysts was detected. At the midpoint of the infection (14, 21 and 28 days) there were no significant differences in the amount of implanted cysts. Animals infected with 10,000 cysts were those with greater tissue inflammation and increased lymphoid hyperplasia, without differences related to implantation of cysts. The invasive character and pathogenicity of *Blastocystis* sp can be confirmed by the presence of the parasite in the muscular layer of the intestine of mice infected with 5000 cysts and submitted to necropsy 7 days after infection. **Main Conclusions:** These results suggest that *Blastocystis* sp has predilection for the cecum and has pathogenic potential by invasion of mucosal. Implantation of cysts occurs in the acute period of infection and inflammation of tissue has relation with inoculum. **E-mail:** pavanelli.mari@gmail.com

Blasto003- Diagnosis of *Blastocystis* sp, use of modified BOECK & DRBOHLAV 'S medium

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Introduction: *Blastocystis* sp. is an intestinal protozoan controversial although it is easy to diagnose with routine laboratory techniques. But for morphological, biochemical and molecular studies we must cultivate the parasite. *Blastocystis* sp. has a wide genetic variability and attempt to link this with the biochemical, molecular and pathogenicity of the organism. **Objective:** To compare of culture with direct examination and spontaneous sedimentation in the diagnosis of *Blastocystis* sp. **Materials and Methods:** We used samples of the indigenous inhabitants from Itopoicon and patients treated in the coproparasitological laboratory of Universidad de Oriente. For the cultivation of *Blastocystis* we used a modification of Boeck and Drbohlav medium. Initially there were several trials to develop, modify and standardization, using positive fecal samples (positive controls) and negative (negative controls) for *Blastocystis* sp from patients seen at the Laboratory during the months of June and July 2010. Then we used 100 samples of Itopoicon community residents for the comparative study of techniques. **Results:** Of the 100 samples cultured and subjected to direct examination and spontaneous sedimentation in 90 were diagnosed with *Blastocystis* sp. Of these, 83 were positive in culture, while 60 were positive on direct examination and 57 in spontaneous sedimentation. **Conclusion:** Culture had a better diagnostic yield (83%) than the direct examination (60%) and spontaneous sedimentation (57%) in the diagnosis of *Blastocystis*. **Keywords:** *Blastocystis* sp., Diagnosis, culture. **E-mail:** rodolfodevera@hotmail.com

Blasto004- Nitazoxanide in the treatment of children infected with *Blastocystis* spp. ciudad Bolivar, Bolivar state, Venezuela

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Introduction: The Nitazoxanide was shown to be useful in treating various infectious agents. A study was conducted to determine the usefulness of the Nitazoxanide in the treatment of children infected with *Blastocystis* spp. **Materials and Methods:** Initially we conducted a cross-sectional study of prevalence in order to diagnosis of *Blastocystis* spp cases by direct examination, Kato and spontaneous sedimentation techniques. 530 fecal samples were evaluated from the same number of children under 15 years of both genders living in Zanjonote community (101) or enrolled in Schools U.E.E. "José Antonio Páez" (60), U.E.E. "19 de abril" (72), U.E.B. "Las Flores" (107) and E.B.N. "Los Próceres II" (190) in Ciudad Bolivar, Bolivar State. All children with *Blastocystis* spp were summoned to be treated with Nitazoxanide at a dose of 100 (2-4 years) or 200 mg (5-14) every 12 hours for 3 consecutive days. Then again underwent clinical examination and parasitological examinations three controls at days 7, 14 and 21 (± 1 day) post-treatment using the same diagnostic techniques. **Results:** A total of 92 children attended and were given the drug, but were subsequently excluded 25 who did not make the three post-treatment controls. There was parasitological cure in 43.9% (29/66) of cases. There was a low frequency of adverse reactions following the ingestion of the drug. **Conclusion:** In the sample studied, Nitazoxanide was not useful in the treatment of children infected with *Blastocystis* spp at the dose recommended by the manufacturer because it presented a low parasitological cure rate (43.9%). **Key words:** *Blastocystis* spp., Nitazoxanide, treatment. **Funding:** Research Council of Universidad de Oriente, Project: No.CI-5-040606-1349/08. **E-mail:** rodolfodevera@hotmail.com

Blasto005- *Blastocystis hominis* in immunocompetent: Case Report

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Introduction: *Blastocystis hominis* previously considered harmless yeast, is now classified as a protozoan inhabiting the human and animal digestive tracts. The pathogenicity of *B. hominis* remains being controversial for human beings. Its classification shows nine subtypes, some of which can have a role in symptomatic cases. Recent reports have indicated that *B. hominis* infections are common in residents of tropical, subtropical, and developing countries. The occurrence of *B. hominis* infections has been related to weather conditions, with the suggestion that infections are more common during hot weather or during the premonsoonal months, which is currently the subject of extensive debate. As a result of the uncertainty surrounding the pathogenic role of *B. hominis*, large-scale treatment trials of *B. hominis* infection have so far been lacking. **Case Report:** 73-old year man presented with complaints of abdominal pain, episodes of liquid pasty stool with foul odor, tiredness, drowsiness, all of one year duration. He also reported abdominal distension. He informed he had undergone several stool examinations one year before and that the results had been always positive for *Blastocystis hominis* and *Endolimax nana*. He looked for medical advice and the recommendation was to not take any treatment. Due to persistence and worsening of symptoms, he looked for medical counsel again and then analysis of three fecal samples, collected in alternate days in MIF solution, was performed. **Material and Methods:** The search for evolutive forms of enteroparasites was performed with light microscopy, using direct and spontaneous sedimentation methods. Immunoenzymatic methods for the search of *G. Lamblia*, *Cryptosporidium* sp (RIDA@QUICK *Cryptosporidium*/*Giardia lamblia*) and *E. histolytica* (*E. histolytica* Test II TechLab) were also employed. **Results:** Using the pasitological method, vacuolar and amoeboid forms of *B. hominis* (5-7 per field) and *Endolimax nana* were observed. The sample was negative for the three immunoenzymatic methods employed. Metronidazol (1,0 g/day for 10 days) was prescribed, resulting in total elimination of the symptoms with good tolerance by the patient. After treatment, a new sample of feces was performed which result was negative. **Main Conclusions:** In the absence of other identified

causes of symptoms, patients presenting with diarrhea or other gastrointestinal symptoms should be assessed for the presence of *B. hominis*. Our patient presented the symptoms and positive diagnosis for *B. hominis*, both for one year. It's imperative to highlight that the treatment for symptomatic patients positive for *B. hominis* is necessary, in the view several case reports have suggested that *B. hominis* may be the causative agent of a variety of diseases including enteritis, colitis, and terminal ileitis and may complicate ulcerative colitis, especially in elderly and children patients. **E-mail:** taniachaves2iecpa.gov.br

OTHER PROTOZOAN

SARCOCYSTOSIS

Sarco001- Microscopical and serological studies on Sarcocystis infection with first report of *S. cruzi* in buffaloes (*Bubalus bubalis*) in Assiut, Egypt

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Abstract: This study was performed for the purpose of investigating the prevalence and the species composition of Sarcocystis spp. in buffaloes in Assiut province, Egypt. Macroscopically we reported the infection of buffaloes with (*Sarcocystis fusiformis*) while Microscopically 3 *Sarcocystis* species (*Sarcocystis cruzi*, *Sarcocystis levinei* and *Sarcocystis hominis*) cysts were recognized, and were differentiated by their morphological features using both histopathological sections and electron microscope scanning. Regarding the prevalence of Sarcocystis species among buffaloes in Assiut province, we reported that, using gross examination of 90 buffaloes' esophagus, only 23 samples out of 90 (25.5%) were found to be infected; on the other hand, by using microscopical examination, the prevalence was 27.7% (25 samples out of 90 samples were found to be infected).. Using ELISA, 85 samples out of 90 (94.4%) were found positive, an overall prevalence of 94.4%. In this work we conclude that customary meat inspection methods in abattoirs in Egypt are insufficient for detecting Sarcocystis infection. Due to the presence of hidden or microscopic cysts, we strongly recommend the use of combined microscopical examination and ELISA for Sarcocystis diagnosis, to avoid human infection of such zoonotic parasite and to control the consequent disease. In addition we introduce the first report of *S. cruzi* in buffaloes in Egypt; our findings prove the hypothesis that *S. cruzi* is able to use animals such as water buffalo as intermediate hosts. **Keywords:** Buffaloes; Sarcocystis; ELISA, Assiut, *S. cruzi*. **E-mail:** dr_mosab2081@yahoo.com

TRICHOMONIASIS

Tricho001- The prevalence of Trichomoniasis in women's assisted in the sector of gynecology of the University Hospital of Rio Grande, RS

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Introduction: Trichomoniasis is a non-viral sexually transmitted disease (STD) most frequent in the world, caused by a flagellate protozoan, *Trichomonas vaginalis*. It is estimated that the number of infected people per day is approximately 685.000 and the number of new cases each year reaches 250 to 350 million. The incidence depends on factors such as age, number of sexual partners, sexual frequency, presence of other STD'S and diagnostic methods used. The Trichomoniasis has been associated with breast cancer, pelvic inflammatory disease, infertility, premature birth, low birth weight newborns and infection with human immunodeficiency virus (HIV). Due to similar symptoms to those of others STD'S the clinical diagnosis of Trichomoniasis is difficult, making necessary laboratory investigation. This study aimed to determinate the prevalence of Trichomoniasis in women's assisted in the sector of gynecology of the University Hospital of Rio Grande, RS. **Material and Methods:** The study of prevalence was cross-sectional of positivity to *T. vaginalis* in women of different ages. Was evaluated a sample of 73 women. The sample of vaginal discharge was collected during the prenatal examination, by an obstetrician participant of this project. In the laboratory, was carried out cultivation of these samples in Diamond's medium, used for the isolation and growth of *T. vaginalis*. **Results:** Of the 73 samples examined 2,7 % of these samples was positive. Querying to the medical records, was verified that was not reported any suspicion and solicitation for diagnosis of Trichomoniasis. **Main Conclusions:** The results showed that is necessary to use specific laboratory methods in the routine, in order to avoid the underdiagnosis of Trichomoniasis in the population. **E-mail:** nessadalben@hotmail.com

BABESIOSIS

Babes001- Case report, human babesiosis

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Babesiosis is a serious animal health problem in Brazil, affecting especially cattle. It is now considered an emerging cosmopolitan disease and its transmission from infected animals to humans has been documented in many countries. It is caused by the protozoan *Babesia microti*, and transmitted by ticks of the genus *Ixodes*, discovered in 1957, in Yugoslavia, and first identified in Brazil in 1983, in the state of Pernambuco. The wild rodent *Oligoryzomys nigripes* is the newest known *Babesia* protozoa host of the genus in Brazil. The scientists also observed a 50% prevalence of *Babesia* infection in specimens of *Rattus norvegicus*, commonly known as rat. The index is similar to that found in endemic regions such as the northwestern United States. The aim of this study is to report a case of human babesiosis, a statement was made by researchers, signed by the patient, who agrees about the disclosure of the case in favor of science. Patient, female, mulatto, 39, divorced, born and raised in Feira de Santana (Bahia -Brazil), two high school degree, unemployed, evangelical, was admitted to hospital on September 22th, 2011 complaining of bloody urine for 02 weeks. At the hospital, the hematuria persisted for 02 weeks; patients also said that she presented sporadic episodes of epistaxis, without precipitating factors, gingival and myalgia. Denies making the prior use of medication. Denies blood transfusions prior to this admission. He lived in the Amazon for 09 years, and lives in his current address for 05 months. Physical examination showed petechiae on upper limbs and face. On admission, he presented $2,000 \times 10^3$ platelets and Hb 9.6 g / dL was transfused with packed red blood cells and platelets. Myelogram was done to rule out leukemia and leishmaniasis, and thick smear test to ward malaria off, reaching the diagnosis by the morphology of peripheral blood, being treated with quinine and clindamycin for 10 days. A month ago the patient was admitted again to hospital complaining of hypermenorrhea and myalgia, and is under treatment. Once a person is bitten by infected tick, symptoms appear approximately within seven to 28 days. The patient presents a clinical picture similar to malaria, with interspersed fever, anemia, intestinal problems, fatigue, body aches, headache, and

chills; similar clinical picture was presented by the patient. But not everyone develops the most severe forms of the disease; many do not have any symptoms of infection. If not diagnosed and treated properly, human babesiosis can cause death, especially in individuals with low immunological or patients using immunomodulations to control autoimmune diseases. Because it is a poorly studied disease and relatively rare, it is not possible to measure the extent of human Babesiosis, once it is not a notifiable disease for the Brazilian Ministry of Health. So the tick has to have greater visibility, since it is the largest vector of pathogens to animals and the second largest one for humans. **E-mail:** sumultidao@hotmail.com