Dear Colleagues,

It is a great privilege to host and organize the XVIII International Congress for Tropical Medicine and Malaria – ICTMM together with the LXVIII Congress of the Brazilian Society of Tropical Medicine (SBMT) and the Annual Meeting of Applied Research on Chagas Disease (XXVIII) and on Leishmaniasis (XVI) in Rio de Janeiro. We are especially proud and pleased because the ICTMM returns to our wonderful city, 49 years after the SBMT hosted and organized in Rio the VII ICTMM (September 1ST -11TH, 1963) when we are commemorating the 50th anniversary of our vigorous and vibrant SBMT.

The XVIII ICTMM features 63 round tables, 52 conferences and 11 plenary lectures, with a total of 405 invited speakers among chairpersons round-table participants, conference panelists, and plenary lecturers. The scientific program also includes 197 abstracts selected for oral presentations, divided into 27 parallel sessions. In addition, 1,620 abstracts will be presented, for the first time, in an ICTMM and a Congress of the SBTMT, as electronic posters, which will be available both in electronic and printed versions for registered participants. The program also contains several Satellite *Symposia,* workshops and a course.

The main theme of the Congress is "Neglected Tropical Diseases: a New Challenge for the XXI Century". We do hope that the conference will provide a forum for the exchange of information, where scientific collaborations can be hatched, and where new information is at the forefront of tropical medicine and malaria.

We are very thankful to all conference sponsors for their contribution towards the organization of ICTMM 2012, and to you for your active participation.

We hope you have a pleasant and fruitful stay in Rio!

Pierre Ambroise-Thomas
President of the IFTM

José Rodrigues Coura
President of the XVIII ICTMM

Cláudio Tadeu Daniel-Ribeiro
President Elect of the IFTM and
of the XVIII ICTMM Scientific Committee

Carlos Henrique Nery Costa
President of the SBMT

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MAJOR CONFERENCES

MajConf1- **Paleoparasitology: human evolution, the origin and dispersion of parasitic diseases.**

Felipe Guhl[a] and Arthur Aufderheide[b]
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Introduction: Living creatures arose nearly 3,500 million years ago (mya) and life on Earth unfolded in a slow, but spectacular panorama of events. Microorganisms have dominated the planet for much of its history and adapted to terrestrial environments prior to the appearance of modern humans. Since the beginning of the evolutionary process, living organisms adapted successfully too many different environments including the living environment itself. Such a long interval accommodated the adaptation of various infectious agents to their new hosts. The ancestors of present–day man (*Homo sapiens sapiens*) appeared in East Africa some 3.5 mya (*Australopithecs*) and then migrated to Europe, Asia, and later to the Americas. Reconstruction of the behavior of a modern disease during antiquity is a formidable challenge. However, success in such an endeavour would allow for the creation of a new database, and this new information could then spawn new hypotheses. Their results could then be blended with our present knowledge to produce an unbroken history of infectious diseases from deep antiquity to the present. Paleoecological integration of such data could help explain chronological changes whose causes could be exploited for novel modern therapeutic or preventive control of the condition. However, there are currently only three methodological tools that can be used in such searches: genetic variation, archaeology, and biochemistry. **Materials and methods:** Tissue specimens from 283 naturally dissecated human mummies from coastal and low valley sites in northern Chile and southern Perú were tested with a DNA probe directed at the kDNA of *Trypanosoma cruzi*. The time interval spanned by the eleven major cultural groups represented in the sample ranged from 9 000 years B.P. to 450 B.P. **Results and main conclusions:** Forty-one percent of the tissue extracts amplified by the PCR reacted positively. Prevalence patterns demonstrated no statistically significant differences among the individual cultural groups .The results suggest that the zoonotic cycle of Chagas disease was probably well established at the time that the earliest humans first peopled this segment of the Andean coast and accidentally joined the many other mammal species acting as host of the parasite. **E-mail:** fguhl@uniandes.edu.co

MajConf2- **The Origins of Human *Plasmodia***

Richard Culleton
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In very recent times our understanding of the natural history of the two most prevalent human malaria parasites, *Plasmodium falciparum* and *Plasmodium vivax*, has changed dramatically. This is due, to a large extent, on the recent discovery of a plethora *Plasmodia* species parasitic on African great apes. Such research has produced evidence suggests that that *P. falciparum* originally infected humans as a result of a host switch from gorillas, which, today, harbor its most closely related sister-species. In the case of *P. vivax*, the picture is less clear, but mounting evidence suggests that the long-held belief that it originated as a human parasite in South East Asia may not be true. Mitochondrial genome sequencing of large numbers of *P. vivax* parasites collected worldwide, along with evidence of the presence of closely related parasites infecting African great apes raises the possibility of an African origin for this parasite too.

MajConf3- **Neglected Diseases, or diseases of neglected people?: mapping a way forward for preventable NTDs in Africa**

Donald Bundy
Coordinator of the African Program for Onchocerciasis Control, The World Bank

The term Neglected Tropical Disease is typically understood to include prevalent diseases that are important to health and development and which have traditionally been overlooked by public health programs. A key group within this category is the highly prevalent infectious diseases which can be controlled by regular but infrequent chemotherapy. The infections in this group – which includes lymphatic filariasis, onchocerciasis, schistosomiasis, soil transmitted helminthiasis and trachoma – are exceptionally prevalent, including some of the most common chronic infections of humans, and have all proven at least partially controllable with simple, safe and effective semiannual or annual treatment. Because the treatment regimens are similar this may permit a common, community-based delivery system, thus allowing the potential to deliver these interventions at low cost within a common community health system, including school-based health systems, alongside the delivery of other important interventions, such as malaria bednets and micronutrient supplements. In Africa, which has the largest share of the global burden of these diseases, there seems little justification for these diseases remaining neglected. Regional disease specific programs, such as the African Program for Onchocerciasis, have already demonstrated that tens of millions of people across the continent can be treated regularly and effectively by community directed delivery systems, while some national programs, especially with the support of key bilaterals USAID and DfID, have demonstrated the feasibility of integrated programs for several preventable NTDs. A movement to end the neglect has emerged: African Health Ministers have decided to invest more in controlling these diseases; WHO is setting milestones for eliminating some of these diseases by 2020 and helping countries across Africa to incorporate NTD control into national health plans; and some 13 pharmaceutical companies across the world have committed to the London Declaration to donate treatment for these diseases.

MajConf4- **Iron and heme acquisition by *Leishmania amazonensis***

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Iron and heme are essential for several cellular pathways, but until recently nothing was known about the pathways by which the protozoan parasite *Leishmania* acquires these critical co-factors. Trypanosomatids are not capable of synthesizing heme, so must acquire it from the environment. Studying *Leishmania amazonensis* we recently identified the Fe^{2+} transporter LIT1 (1), the Fe^{3+} reductase LFR1 (2), and the heme transporter LHR1 (3). These surface molecules are up regulated when *L. amazonensis* promastigotes are grown in iron and heme-deficient medium, and mutant parasite strains lacking one or two copies of these proteins have distinct phenotypes in iron or heme acquisition, differentiation into infective forms, and/or infectivity for macrophages and mice. Characterization of LIT1 null strains of *L. amazonensis* also revealed that iron uptake acts as a sensor for the differentiation of promastigotes into amastigotes. (1) Huynh, C, Sacks, D.L. and Andrews, N.W. A *Leishmania amazonensis* ZIP family iron transporter is essential for parasite replication within macrophage phagolysosomes. J. Exp. Med. 203: 2363-2375, 2006. (2) Flannery, A.R., Huynh, C., Mittra, B., Mortara R.A. and Andrews, N.W. The LFR1 ferric iron reductase of *Leishmania amazonensis* is essential for the generation of infective parasite forms. J. Biol. Chem. 286:23266-23279, 2011. (3) Huynh, C, Yuan, X, Miguel, D.C., Renberg, R.L., Protchenko, O, Philpott, C.C., Hamza I. and Andrews, N.W. Heme uptake by Leishmania amazonensis is mediated by the transmembrane protein LHR1. PLoS Pathogens in press, 2012.

MajConf5- **Eliminating NTDs in the Americas through R&D for new 'antipoverty vaccines'**

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The neglected tropical diseases (NTDs) represent the most common infections affecting the estimated 100 million people living below the poverty line in the Latin American and Caribbean (LAC) region. An additional 5 million people live in poverty in the state of Texas, especially in South Texas. Almost all of the "bottom 100 million" (as well as many of the 5 million Texans living in poverty) are affected by one or more NTD led by hookworm and other intestinal helminth infections, lymphatic filariasis, schistosomiasis, fascioliasis, Chagas disease, leishmaniasis, and neglected bacterial and viral infections such as leptospirosis, yellow fever, and dengue. Through mass drug administration (MDA) using donated and low-cost generic NTD drugs, great strides have been made in eliminating selected NTDs in specific LAC countries, such as schistosomiasis in the Caribbean region; LF in Costa Rica, Suriname, and Trinidad; Trachoma in Mexico; onchocerciasis in the six remaining endemic LAC countries; and leprosy throughout the LAC except for Brazil. Moreover great strides have been made in eliminating Chagas disease in the Southern Cone of LAC. There is an urgent need to expand MDA efforts through support of government and agencies such as USAID, and a LAC NTD fund sponsored jointly by the Interamerican Development Bank, PAHO, and the Global Network for NTDs. However, in parallel it will be necessary to develop and test new control tools such as new drugs, vaccines, and diagnostics for many of the NTDs. Several "antipoverty vaccines" targeting NTDs are currently under development and undergoing clinical testing through the activities of academic institutions and product development partnerships (PDPs) in collaboration with developing country manufacturers and partners in Brazil (FIOCRUZ, FIOCRUZ-BioManguinhos, Instituto Butantan), Mexico (CINVESTAV, Birmex), Cuba, and elsewhere. Together these organizations are leading a path for innovation to produce new vaccines for Chagas disease, cysticercosis, dengue, fascioliasis, hookworm, leishmaniasis, leprosy, leptospirosis, onchocerciasis, schistosomiasis, and yellow fever. Beyond direct vaccine development it will be essential to implement global access strategies for these new potentially lifesaving products, which ultimately could facilitate the elimination of several key NTDs as public health problems in the LAC region.

CONFERENCES

Conf1- **The *Schistosomiasis Consortium* for Operational Research and Evaluation: How can operational research assist control program managers gain control, sustain control and eventually eliminate *schistosomiasis*?**

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The World Health Assembly has approved a resolution to intensify morbidity control of schistosomiasis and move to elimination (the interruption of transmission) wherever possible. Achieving these goals with current tools and approaches will be challenging. The Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) is funded by the Bill and Melinda Gates Foundation and involves over 50 investigators in 26 different institutions in 16 countries. Its goal is to provide tools and data to help schistosomiasis control program managers better accomplish their objectives to gain control, sustain control and eliminate schistosomiasis in their countries. SCORE's major field studies, being done in 5 African countries, examine if Mass Drug Administration (MDA) with praziquantel is best done, in terms of

lowering prevalence and intensity of infection, through community-wide treatment or school-based programs, and if outcomes depend on starting prevalence and frequency of MDA. The studies have 25 villages/study arms and are in their 2nd of 5 years. Layered on these studies are studies on changes in morbidity and the population structure of schistosomes under different levels of drug pressure, and the role of snail infections on the outcome of MDA. Another major field study is focused on schistosomiasis haematobium elimination from Zanzibar and determination of how best to stop its transmission. This SCORE study is embedded in a major collaboration of partners called ZEST (Zanzibar Elimination of Schistosomiasis Transmission). It has 3 arms, which are: the National Program (twice a year MDA and standard health education); the National Program plus snail control; and the National Program plus behavioral change interventions. The other main SCORE efforts are on the development and evaluation of a better tool for mapping *Schistosoma mansoni* for MDA and a better diagnostic to assist in the elimination and surveillance of schistosomiasis. The former evaluated a commercially available Point-of-Contact/Circulating Cathodic Antigen urine assay for *S. mansoni* in 5 African countries. The preliminary analyses, which indicate that this assay can, and perhaps should, be used for prevalence mapping, will be presented. SCORE has also funded the optimization of an Up-converting Phosphor-Circulating Anodic Antigen assay which can now detect 1 pg of CAA/ml of plasma or 4 ml of urine, which approximates the best estimates for the level of CAA produced per one worm pair. These two assays, as well as the progress in the field studies, will be presented and discussed.

Conf2- **Molecular epidemiology of the *Cryptococcus neofarmans/C. gattii* species complex and its clinical implications**

Prof. Wieland Meyer and countless collaborators world-wide
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The emergence of specific genotypes as causes of on-going disease outbreaks and increasing numbers of infections has let to great efforts to gain insides in the population genetics of the *C. neoformans/C. gattii* species complex. Several genotyping methods including PCR fingerprinting AFLP and MLST (ISHAM consensus scheme: *CAP59, GPD1,* IGS1*, LAC1, PLB1, SOD1 & URA5*) have identified seven haploid monophyletic lineages/major molecular types: *C. neoformans* (VNI [VNB], VNII and VNIV) and *C. gattii* (VGI, VGII, VGIII, VGIV) and a number of hybrids, indicating a continuing speciation process. Applying the molecular clock *C. gattii* diverged from *C. neoformans* 49; *C. neoformans* var. *grubii* (VNI/VNII) separated from *C. neoformans* var. *neoformans* (VNIV) 24, VNI and VNII 4.7 and the *C. gattii* major molecular types VGIII and VGI 8.5, VGIV 11.7 and VGII 12.5 million years ago. The genetic variation found between the haploid monophyletic lineages indicates that they all warrant variety or species status. Global molecular epidemiology and associated studies of fungal virulence in two different animal models (mice and *Galleria mellonella* larvae's) and of antifungal susceptibilities have shown distinct correlations between the different major molecular types and or even specific genotypes within these major molecular types. To enable global surveillance, early outbreak detection and a better treatment choice two new web based dynamic MLST databases were established for *C. neoformans* and *C. gattii* (accessible at http://mlst.mycologylab.org/) as the result of an international collaboration. High genetic variation and the presence of recombination indicate South Africa and South America as origins for *C. neoformans* and *C. gattii*, respectively. In addition, population genetic analysis suggests South America as the origin of outbreaks due to and emergence of certain VGII strains on Vancouver Island, Australia and other parts of the world. Similar trends were observed for VGIII strains, which emerged as an important pathogen in the USA (California), Mexico and Colombia, with sporadic cases in other parts of the world.

Conf3- **Pathogenesis and Treatment of Severe Malaria**

Mats Wahlgren

The ability of *P. falciparum* parasitized RBC (pRBC) to form rosettes with normal RBC is linked to the virulence of the parasite and RBC polymorphisms that weaken rosetting confer protection against severe

malaria. The adhesin PfEMP1 mediates the binding to heparan sulfate (HS), the ABO-blood group antigens or CR1 on the RBC and specific antibodies prevent sequestration in the micro-vasculature. Binding to HS is mediated by the N-terminal NTS-DBL1α domain of PfEMP1 in which high affinity binding requires 12-mers of HS and the presence of 2-N- and 6-O-sulfate groups. Our recent work employing the rosetting NTS-DBL1a of the heparan sulfate-dependent rosetting FCR3S1.2 argues that sub-domain 3 (SD3) is critical for binding (Angeletti *et al*, submitted). Surface-reactive Abs was found to bind directly to SD3-loop sequences in linear peptide-arrays or indirectly in competition experiments with pRBC. By depletion of pIgG on a loop peptide we could eliminate ≈ 70% of the anti-rosetting activity and immunizations with an isolated SD3 domain of PfEMP1-DBL1a generated surface-reactive antibodies that disrupted rosettes. In contrast, antibodies to subdomains 1 and 2 (SD1, SD2) stained the live pRBC in 50% of parasites tested but the antibodies did not disrupt rosettes. Further, a depolymerized heparin-derived molecule has been developed with a drastically decreased effect on coagulation (sevuparin) that binds PfEMP1-DBL1α but still carries a robust inhibitory effect on sequestration *in vivo* in animal models and inhibits merozoite invasion *in vitro*. Successful pre-clinical studies and a clinical Phase I study performed during 2009 lead to the formation of a consortium to test the low anti-coagulant heparin, sevuparin, in malaria patients. A clinical phase I/IIa trial were conceived and the first patient was admitted in September 2011. The complex cell-to-cell interactions that give rise to severe malaria in the human micro-vasculature will be discussed in view on the ongoing activities

Conf4- **Chagas disease: a worldwide dispersal**

Gabriel A Schmunis

Chagas disease is a protozoa zoonotic disease caused by *Trypanosoma cruzi.* It is transmitted to man by Triatomine vectors, blood transfusion, from mother to newborn, and by the oral route when ingesting food or beverages contaminated with *T. cruzi.* The number of infected individuals in the Continental Western Hemisphere was estimated to be 7.5 million and 11,000 deaths in 2005. One way that *T. cruzi* has the potential to be exported from the Western Hemisphere to Europe, Africa, Asia or Oceania is by an infected American triatomine travelling as an unwanted passenger in a cargo hold of a plane or a cargo vessel. Another possible mean of acquiring *T. cruzi* at *a* destination country*,* is by an infected host, human or animal, being hit by a potentially susceptible destination triatomine, like *T. rubrofasciatus,* which lives in most of the *T. cruzi* non endemic destination countries. Although both ways are possible, assuming that a potential vertebrate host is found, the first route was considered more possible. On the other hand, political repression and/or economic stagnation stimulated the flow of migration from the 17 Latin American countries endemic for Chagas disease to developed countries since the 1980s. Because of this migration, Chagas disease is becoming a global health problem. In 2006, 3.8% of the 80,522 immigrants from those 17 countries to Australia were likely to be infected with *Trypanosoma cruzi*. In Canada, also in 2006, 3.5% of the 156,960 immigrants from Latin America whose country of origin was identified were estimated to be infected. In Japan, there were 370,000 immigrants from Latin America in 2006. Eighty-four percent were from Brazil, and several of them have been reported to be infected with *T. cruzi* or presented symptoms of Chagas disease. In 15 countries of Europe in 2005, excluding Spain, 2.9% of the 483,074 legal Latin American immigrants were estimated to be infected with *T. cruzi*. By 2008, Spain has received 1,678,711 immigrants from Latin American endemic countries; of these, 5.2% were potentially infected with *T. cruzi* and 17,389 may develop Chagas disease. Furthermore, it was estimated that 24–92 newborns delivered by South American *T. cruzi* infected mothers in Spain may have been congenitally infected by *T. cruzi* in 2007. In fact, several clinical cases of vertical transmission have been diagnosed in Spain as well as in Sweden and in Switzerland. Organ transplants and blood transfusions have also been identified as a cause of *T. cruzi* infection in the two previously mentioned countries. Because current economic problems in Spain may decrease the number of Latin American immigrants to this country, the migration to northern European countries where the economic situation is better may increase. In the United States, it was estimated that 2% (340,000) of 17 million immigrants in 2007, were potentially infected with *T. cruzi*. Of these, 65,134, may have developed or may develop symptoms and signs of chronic Chagas disease. Cases of infection through blood transfusion have been reported from the Unites States and Canada, as well as 2 cases of transplant of solid organs, and also 2 cases of vertical transmission from the United States. Governments should implement policies to prevent

donations of blood and organs from *T. cruzi* infected donors. In addition, an infrastructure that assures detection and treatment of acute and chronic cases as well as congenital infection should be developed.

Conf5- **Worldwide emergence of human *Fascioliasis*: a multidisciplinary challenge**

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Fasciola hepatica and *F. gigantica* are two large-sized fasciolid trematode parasite especies that are transmitted by freshwater lymnaeid snail vectors. Fascioliasis is a disease which affects humans and livestock especies almost everywhere. This highly pathogenic liver parasitosis is emerging in many countries of Latin America, Europe, Africa and Asia in the last two decades. This emergence phenomenon has partly been related to climate change, given the high dependence of both lymnaeid snails and fasciolid larval stages on climatic and environmental characteristics. Throughout its large geographical distribution, fascioliasis is a well-known veterinary problem. Moreover, studies carried out in recent years have shown it to be an important public health problem as well. Human cases have been increasing in the five continents. Recent articles estimate human infection up to 17 million people, or even higher depending from the hitherto unknown situations in many countries, mainly of Asia and Africa. From 51 countries with human infection counted in 1990, the present situation already includes 81 countries in which human infection has been described. A global analysis of the geographical distribution of human cases shows that the expected correlation between animal and human fascioliasis only appears at a basic level. High prevalences in humans are not related to areas where fascioliasis is a great veterinary problem. Major health problems are known in Andean countries (Bolivia, Peru, Chile, Ecuador), the Caribbean area (Cuba), northern Africa (Egypt), the Near East (Iran and neighboring countries) and western Europe (Portugal, France and Spain). When comparing different human endemic areas, a large diversity of situations and environments appear, including different human endemic/epidemic situations, different human demographies, races, diets, habits, traditions and religions, different domestic and wild mammal reservoir species, different lymnaeid transmitting species, zones in both the Northern and Southern hemispheres, altitudes from –27 m (as besides the Caspian Sea) up to 4,200 m (as in Andean countries), hot and cold weathers, seasonal and yearly constant temperatures, scarce to pronounced annual rainfall, low and high mean annual potential evapotranspiration, and from lack of dry period to lack of wet period through different dryness/humidity rates. Moreover, from the landscape point of view, these areas include from altiplanos to valleys, from islands to mainlands, from natural to artificial irrigations, from lakes to lagoons, from large rivers to small streams, and from permanent to temporal water bodies. When translating all this to disease terms, fascioliasis in human hypo- to hyperendemic areas appear to present, in the different continents, a very wide spectrum of transmission and epidemiological patterns related to the very wide diversity of environments. **Funded by** Project No SAF2010-20805 of the Ministry of Economy, Madrid, and by Red de Investigación Cooperativa en Enfermedades Tropicales – RICET (Project Nº RD06/0021/0017 of RETICS/FEDER), FIS, Ministry of Health, Madrid, Spain

Conf6- **Neglected mycobacteria as emerging pathogens in the last decades**

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Nontuberculous mycobacteria (NTM) are common in the natural environment, especially in water sources, and exposure to these environmental microorganisms is unavoidable. Consequently, NTM may contaminate medical solutions and equipment in practices in which enough care is not performed adequately. By the other way, lung infections by NTM, although less frequent than tuberculosis, have also been reported as an emerging disease. Rapidly growing mycobacterial (RGM) have been described as important human pathogens responsible for an increasing number of health-care-associated infections, mainly in tropical countries such as Brazil, where a recent significant epidemic caused by *Mycobacterium abscessus* subsp. *bolletii* (previously named *Mycobacterium massiliense*) belonging to the clone BRA100 was observed. This clone has been responsible for several outbreaks of wound infections related to

medical invasive procedures (mainly laparoscopy) in many states of Brazil from 2004 to 2011. The largest epidemiological event associated with RGM in Brazil occurred between 2006 and 2007, when 1051 notified possible cases of postsurgical infections were spread around 63 hospitals in the state of Rio de Janeiro. Other important RGM infections outbreaks have also been reported worldwide related to common esthetic procedures such as piercing, mammaplasty, “mesotherapy”, pedicures and others. Slowly growing mycobacterial (SGM) infections, mainly lung ones in immunocompetent patients and associated to *Mycobacterium avium* complex and *Mycobacterium kansasii*, have also been reported as a critical health problem to be treated in several regions in the world in the last decades. Family outbreaks, *in vivo* inducible resistance, refractory or relapsing NTM lung diseases are some important points that have recently challenged physicians and microbiologists. Further, there are few therapeutic choices for treating most NTM diseases additionally to the inefficacy of anti-tuberculosis drugs. Combining these aspects with drug intolerance and high medication costs have led to unsolved therapeutic compromises. New mycobacterial threats have emerged in previously non-successful scenario where tuberculosis still remains.

Conf7- **The FML-vaccine Leishmune® and the Nucleoside hydrolase synthetic vaccine in vaccination and therapy of visceral leishmaniasis**

CB Palatnik-de-Sousa

The FML-saponin dog vaccine (Leishmune®) is composed of the Fucose–Mannose ligand antigen of *L. donovani* promastigotes and the QS21 and deacylated saponins of *Q. saponaria.* The vaccine was considered safe and well tolerated. After two years of vaccination, 1% of 550 vaccinated and 39% of 588 untreated dogs died of ZVL. The vaccine reduced the parasite burden for sand flies, as disclosed by the negative results of PCR, immunohistochemistry and xenodiagnosis. The generated antibodies block the transmission of the disease by sand flies. When formulated with double saponin concentration it promotes the parasitological and clinical cure while sterile cure required immunochemotherapy. Leishmune® induced enhanced levels of IFN-γ, NO and anti-*L. chagasi* IgG2, the early and persistent activation of neutrophils and monocytes, and increased the IFN-γ-CD8+ T-cells. Dogs vaccinated with Leishmune® did not become seroreactive in the official control test. Only 1.3% of vaccinated dogs were detected in a dog enquire. The districts of greater vaccine coverage exhibited declined or incidence and those with less vaccine coverage, rising curves. The main antigen of the FML complex is the Nucleoside hydrolase of *L. donovani* (NH36). Protection against *L. chagasi* in mice is related to its C-terminal domain (F3= amino-acids 199-314) and is mediated by a CD4+ T cell driven response with a lower contribution of CD8 + T cells. Immunization with this peptide exceeds in 36 % the protective response induced by the cognate NH36 protein. Increases in IgM, IgG2a, IgG1 and IgG2b antibodies, CD4+ T cell proportions, IFN-γ secretion, ratios of IFN-γ/IL-10 producing CD4+ and CD8+ T cells and percents of antibody binding inhibition by synthetic predicted epitopes were detected in F3 vaccinated mice. The increases in DTH and in ratios of TNFα/IL-10 CD4+ producing cells were strong correlates of protection which was confirmed by *in vivo* depletion with monoclonal antibodies and a pronounced parasite load decrease that was long-lasting. No decrease in parasite load was detected after vaccination with the N-domain of NH36 (Nico *et al*., 2010). Both peptides reduced the parasite load and lesion sizes by *L amazonensis* (prophylaxis and immunotherapy) and by *L. chagasi* (immunotherapy). Nucleoside hydrolases are involved in the purine salvage pathway and are not found in mammal cells. The *L. donovani* nucleoside hydrolase accepts inosine, guanosine, adenosine, uridine and cytidine as substrates with a slight preference for adenosine and inosine. Guanosine is not a good substrate. In collaboration with Prof. VL Schramm (A Einstein College of Medicine NY, USA) we have analyzed potential nucleoside hydrolase inhibitors, as an approach to block purine salvage. Immucillin-H and Immucillin-A gave K_i values of 19 and 80 nanomolar respectively. Immucillin-H (Forodesine) is currently in human clinical trials against lymphatic cancers. Our results may be useful as leads for anti-leishmania agents.

Conf8- **Molecular markers for *Fasciola* differentiation**

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Fascioliasis is a zoonotic disease caused by the liver fluke species *Fasciola hepatica* and *F. gigantica*, whose geographical distribution differs. *F. hepatica* is present in Europe, Africa, Asia, the Americas and Oceania, whereas *F. gigantica* is only found in Africa and Asia. Despite having always been recognised by its great veterinary importance throughout the world, fascioliasis has also proved to be a widespread human health problem only recently. This emergence phenomenon has partly been related to climate change, given the high dependence of both fasciolid larval stages and their freshwater lymnaeid snail hosts on climatic and environmental characteristics. When analysing how better to define control measures for endemic areas, it would be useful to have genetic markers which could distinguish each type of transmission pattern and epidemiological situation. Genetic techniques applied to liver flukes allow us to ascertain intraspecific and interspecific variabilities of "pure" *F. hepatica* and "pure" *F. gigantica* by means of complete sequences of rDNA ITS-2 and ITS-1 spacers and mtDNA *cox*1 and *nad*1 genes from areas with only one fasciolid species. Similarly as in other vector-borne infectious diseases, the vectors are crucial in establishing the transmission patterns and epidemiological scenarios of the disease. In the case of controversial groups, such as *Galba*/*Fossaria* and *Radix* groups which include the main vector species of *F. hepatica* and *F. gigantica* respectively, classification of specimens is only affordable by means of DNA marker sequencing. The definitive haplotypes established appear to fit the proposed global evolutionary scenario. Problems posed by fasciolid crossbreeding, introgression and hybridisation in overlap areas are analyzed. Nuclear rDNA appears to correlate with adult fluke characteristics and fasciolid/lymnaeid specificity, whereas mtDNA does not. However, flukes sometimes appear so intermediate that they cannot be ascribed to either *F. hepatica*-like or *F. gigantica*-like forms and snail specificity may be opposite to the one deduced from the adult morphotype. Recent sequencing results suggest that present assumptions on fasciolid-lymnaeid specificity might be wrong. The sequence analysis of mitochondrial markers allow us to define, for the first time, the nucleotide positions providing specific differentiation between both species. In the comparative analysis of cox1 and nad1 of *F. hepatica* and *F. gigantica*, we have defined a total of 113 and 70 polymorphic sites distributed throughout these genes, representing a specific differentiation 7.37% and 7.75%, respectively in both markers. Results obtained with lymnaeid snails in American countries shows that almost all vector species proved to belong to the *Galba*/*Fossaria* group of small-sized lymnaeids, and, with a few exceptions, endemic areas showed to present more than one vector species involved in disease transmission. **Funded by** Project Nº SAF2010-20805 of the Ministry of Economy, Madrid, and by Red de Investigación Cooperativa en Enfermedades Tropicales – RICET (Project Nº RD06/0021/0017 of RETICS/FEDER), FIS, Ministry of Health, Madrid, Spain

Conf9- **The Outstanding Features of Human Leptospirosis in Latin America and Caribbean Countries**

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Background: Leptospirosis is a zoonosis widely distributed worldwide. The human disease has been mentioned as an emerging, re-emerging or neglected disease according to the geographical area and time. The gaps in knowledge may cross the major areas of the actual biological research. There are challenges in the diagnosis and treatment of the severe forms characterized by clinical signs and symptoms of hemorrhage and renal failure. Surveillance and control measures are also challenging due to the gaps in technical and scientific knowledge. It leads to a circular movement despite of the emerging situations caused by climatic changes and recent epidemic outbreaks in Latin America and Caribbean countries (LAC). **Methods:** The meta-analysis of the available information from scientific literature and complementary official documents is showed. It was commissioned by WHO in order to identify the

knowledge gaps that prevent the surveillance and control measures. **Results:** The estimation of the burden of leptospirosis was considered to be high in LAC with an incidence of 12.5 per 100000 population compared to the global incidence of 5.1 per 100000 population. A total of 14.212 cases were reported from 2007 to 2010 in Brazil with 1.378 deaths, corresponding to a case fatality rate of 9.7%. Recent epidemic outbreaks have been reported in Brazil, Mexico and Nicaragua. The impact of epidemic outbreaks due to environmental changes with heavy rains and flooding are noteworthy leading to the need of focusing risk areas e population groups to the proper surveillance and control measures. The main challenges to be faced are described including: misdiagnosis, needs of therapeutic alternatives, and vaccines to prevent the disease. **Conclusions:** There are gaps in scientific knowledge that leads to gaps in information regarding the burden of the human Leptospirosis. This is true both as a global or regional perspective. There are promising and relevant information in areas of great scientific development as genomic and proteomic. It may lead to new findings and products such as simple tests for diagnosis and vaccines. However, despite of the large amount of information and impressive new findings the ultimate goals seem have not been reached. Further studies are needed to get new validated diagnostic tests, therapeutic alternatives and a vaccine for human use. Fields such as pathology, immunology and pathogenesis need more attention since these are of utmost importance to therapeutic alternatives. **Keywords:** *Leptospira*, Leptospirosis, Epidemiology.

Conf10- **Elimination of *Onchocerciasis* in Ecuador, a dream made reality.**

Maurício Espinel

In 1982, communities with a high prevalence for onchocerciasis were found in the Cayapas-Santiago river basin with secondary foci in the rivers Tululvi, Canandé, Sucio, Verde, Vilsa, Viche, Cojimies. All were located in the province of Esmeraldas, in the northern coastal region of Ecuador. The disease affected more than 20,000 people of the approximate 50,000 inhabitants of 190 communities located along the banks of the rivers. This tropical forest area is considered one of the poorest of the country. An increasing incidence of disease, especially in the upper part of the rivers, was verified by successive prevalence studies. Many papers were published between the 80´s and 90´s describing in detail the clinical, immunological, ophthalmological, dermatological, and epidemiological aspects of the disease. This work reflects the immense effort of research and health care attention provided during that time. Entomological studies incriminated the very efficient black fly *Simulium exiguum* as the main vector, with parasite prevalence of more than 1%. As many as 89% of the infected people presented different kinds of lesions caused by microfilariae (punctuate keratitis, chorioretinitis, optical nerve atrophy) In 1990 a distribution program of ivermectin was begun with the aim of onchocerciasis elimination by achieving a semi-annual distribution coverage greater than 85% in the affected communities. In this regard community participation and health promoter organization and training were key factors. All the affected population was progressively incorporated in the program in the first decade. By 2010 pre certification surveillance was begun and now studies are in progress to verify the interruption of transmission. The program is an outstanding example of how public heath can fight neglected diseases in a tropical area with poor access to health services, with a very poor population, and with multiple difficulties relating to transportation, human resources and work conditions. Key factors to achieve this goal have been the leadership of Dr. Ronald Guderian, who put together and trained an untiring team of research interested young professionals, along with a dedicated team of health care providers. Research always supported health care and vice versa. Preventive and curative health care services were always implemented in the visited communities to accompany the ivermectin distribution. In all the activities, social participation was always taken into account. The leader implemented a variety of strategies to fund all the activities. Involvement of local authorities was permanently present, despite their high rate of rotation. This is a model example of how research and public health care can work together and be successfully in controlling endemic neglected diseases. Some 50 000 people thank this reality

Conf11- **Network epidemiology and the transmission of infectious diseases**

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The vast majority of both statistical analyses and mathematical models in the field of infectious diseases do not take in consideration the very structure of the social fabric and/or the complex interdependence of ecological elements, comprising both the unpredictability of abiotic parameters over long time periods and the interactions and energy exchanges between biological organisms living in a given community. The analysis of social networks represents a key contribution towards a better understanding of such phenomena, fostering conceptual development and substantial improvements in terms of data collection, management, and analysis. The presentation will briefly summarize the history of social network analysis, starting with the first insights advanced by Euler on graph theory and its 20 century developments by Erdös and Renyi. From such breakthroughs, social network analysis became a booming field of knowledge in recent decades, with essential applications in the field of ecology, the dynamics of infectious diseases in context, and the monitoring of current epidemics and forecasting of potential threats to the sustainability of ecosystems, urban areas and human populations in the years to come. Practical examples will include the prevention of influenza, worldwide efforts to curb HIV spread, as well as some preliminary analysis on vector-borne diseases, such as malaria and dengue fever.

Conf12- **Molecular mechanisms controlling antigenic variation in the intestinal parasite *Giardia lamblia***

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Giardia is one of the most common parasites of humans and a major cause of diarrhea worldwide. To evade the host's immune response, *Giardia* undergoes antigenic variation; a process that allows the parasite to develop chronic and recurrent infections. From a repertoire of ~190 variant-specific surface proteins (VSPs) coding genes, *Giardia* expresses only one VSP on the surface of each trophozoite at any given time, but switches to the expression of a different VSP once every 6-22 generations. Here we show that regulation of VSP expression involves a system comprising homologues of RNA-dependent RNA-polymerase (RdRP), Dicer, and Argonaute (Ago), known components of the RNA interference (RNAi) machinery. In *Giardia,* clones expressing a single surface antigen efficiently transcribe several other *vsp*s but only accumulate transcripts encoding the actual VSP. Detection of antisense RNAs corresponding to the silenced *vsp*s and cleavage of dsRNAs into 22-25 nt siRNA from the silenced but not for the expressed *vsp* implicate the RNAi pathway on *Giardia*'s antigenic variation. Remarkably, knocking-down of either Dicer or RdRP leads to a change from single to multiple VSP expression in individual trophozoites, while loss of Ago shows more drastic effect on *Giardia* viability. Our results suggest the involvement of a PTGS mechanism in regulating the expression of surface antigens in this important human pathogen. **E-mail:** hlujan@ucc.edu.ar

Conf13- **New drugs for Chagas disease treatment**

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Chagas disease, a systemic parasitosis caused by the Kinetoplastid protozoon *Trypanosoma cruzi,* remains the largest parasitic disease burden in the American continent and is now spreading to non-endemic areas due to international migrations. Specific chemotherapy of this complex condition is unsatisfactory due to limited efficacy, particularly in the prevalent chronic stage, and common side effects of currently available drugs (nifurtimox and benznidazole), as well as controversies on the pathogenesis of the disease in the chronic stage. Recent studies have concluded, in contrast to long held views on the autoimmune origin of the pathological manifestations of the chronic stage of the disease, that the

persistence of parasites is the key factor underlying the sustained inflammatory responses that lead to the characteristic lesions of chronic Chagas disease. As a consequence, there is growing consensus that this condition should be treated as an infectious, not autoimmune, disease and that specific treatment should be offered to all seropositive patients, independently of the stage of the disease. Among the most promising approaches to new treatments is (a) ergosterol biosynthesis inhibitors such as posaconazole and ravuconazole, which are currently undergoing Phase 2, poof-of-concept, clinical trials in Latin America and Spain, (b) amiodarone, an antiarrhythmic drug recently shown to have also potent anti-*T. cruzi* activity, (c) inhibitors of cruzipain, the main cysteine protease of *T. cruzi* and (d) combination therapies such as benznidazole, nifurtimox or amiodarone associated with ergosterol biosynthesis inhibitors or combinations of ergosterol biosynthesis inhibitors acting at different steps of the pathway. Finally, a major stumbling block for the evaluation of new drugs, or the true efficacy of currently available drugs, is the lack of reliable biomarkers for parasitological cure or the modification of parasite loads in chronically infected individuals, but several novel approaches to this problem are being advanced, including non-conventional serology and specific anti-*T. cruzi* T cell responses.

Conf14- **Nutrition and neglected tropical diseases: a great challenge for developing countries Integrating strategies and public policies to fight extreme poverty, hunger and neglected tropical diseases in Brazil**

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The concept of neglected diseases emerged 40 years ago and evolved to the present TDR/WHO concept of infectious diseases of poverty (IDP). Their essential social determinants are low income & education, poor housing, sanitation and water supply. Hunger and malnutrition come out as strong vulnerability factors. We study the complex interplay between nutrition/selenium (Se) status and Chagas disease (CD) to inform clinical practice. Low Se levels correlated positively with cardiac insufficiency in advanced chronic patient. A phase III clinical trial is under way to test if Se treatment can reduce the rate of progression of heart dysfunction in chronic CD (clinical trial NCT00875173). Mice fed with Se deficient chow are more susceptible, and that Se supplementation alleviated heart damage, restored intestinal motility, reduced parasitaemia and mortality. Our intent is to refine dietary recommendations and to develop a public policy to reduce disease progression. Brazil has rescued from poverty and extreme poverty a number of people equivalent to the size of the France population. In 2012 Brazil is the 6th largest economy in the world. Mass vaccination campaigns are extremely efficient, smallpox and polio were eliminated, measles and rubella are virtually controlled; the number of cases of tuberculosis and AIDS decreases slowly but dengue and syphilis has doubled over the past five years. Malaria, schistosomiasis, leishmaniasis, leprosy, CD and other ancient parasitic diseases still impact in health indicators. More than 100 million Brazilians still live with IDP, which is increasingly assumed to be diseases “promoters of poverty”. IDP affect people in the most productive years of their lives, taking their strength and working time, leading to physical disabilities and learning capacity, and stealing their chances of human development, scoring a negative goal for public policies against poverty. Brazilian scientists advocate that fighting extreme poverty will only be feasible in concert with a national priority of IDP control. The good news is that the program “Brasil sem Miséria” (BSM, Brazil without Misery), launched in 2011, aims to eradicate extreme poverty including the fight of IDP as one of the main health axis. The program associates income transfer & increasing access to public services & job access (formal/informal). Health problems of people in extreme poverty include higher exposure to environmental risk factors and to infectious diseases and chronic non-communicable diseases; poor nutritional status; difficulty on access to health services and medicines; high fertility rate; high prevalence of tooth loss, impairing social interaction and working; difficulty of access to an ophthalmological exam and to get glasses contributing to school dropout and illiteracy. In BSM, nutrition, health and education will be particularly followed, since they are conditions to get access to the program that transfer income to people living with < US$35/person/month (Programa Bolsa Familia). To control the old diseases that promote poverty is a prerequisite for Brazil to eradicate poverty, in pursuit of greater equity and social justice. Our work on Se supplementation for altering the natural course of chronic CD, preventing and

reverting heart and gut effects, has now gotten its social dimension, for which dietary recommendations and special supplementation with trace elements as selenium, zinc and iron will be specially welcome. **E-mail:** taniaaj@ioc.fiocruz.br

Conf15- **Forging ahead with malaria control and elimination**

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Over the past decade, malaria control has been reinstated as a global development priority. International funding commitments have risen from <$100 million (2003) to ~$2 billion (2011), allowing for rapid scale-up of proven interventions, especially long-lasting insecticidal nets (LLINs), indoor residual spraying, universal diagnostic testing of suspected malaria, and treatment of confirmed cases with artemisinin-based combination therapies (ACTs). As a result, malaria cases and/or deaths have fallen by >50% in 43 countries since 2001, and malaria mortality rates have declined by >25% globally. In the Americas, malaria transmission occurs in 21 countries; nearly 30% of the population in the region is at some risk of malaria transmission. There has been a 40% decline in the number of confirmed cases reported in the Americas, from 1.18 million (2000) to 669,000 (2010). Argentina, El Salvador, Mexico and Paraguay are now classified as pre-elimination countries. Despite these successes, malaria remains an enormous global health problem, responsible for an estimated 216 million cases (uncertainty range 149-274 million) and 655,000 deaths (uncertainty range 537,000-907,000) annually. Four countries, all in Africa, account for >50% of these deaths. In Asia and the Americas, *Plasmodium vivax* remains a major programmatic challenge. The greatest immediate threat to the continued success in the control and elimination of malaria is inadequate funding. Malaria resurgences have been documented where coverage with LLINs was not sustained in Rwanda, Zambia, and Zanzibar. The Global Fund has not categorized malaria prevention and control interventions as eligible for Continuity of Services funding, as is the case for anti-retroviral and anti-tuberculosis medicines, risking a reversal in malaria control gains when funding gaps occur. Perhaps the greatest biological challenge to the continued success of malaria control is *P. falciparum* resistance to artemisinins, which is likely to spread beyond the Mekong Region without aggressive, timely, and coordinated action. The WHO Global Malaria Programme (WHO-GMP), working with Roll Back Malaria (RBM) partners and stakeholders, has developed the *Global Plan for Artemisinin Resistance Containment*, which aims to protect ACTs as effective treatment for falciparum malaria. Anopheline resistance to insecticides represents another major challenge to forging ahead with malaria control and elimination. Vector control has been a pillar in the success of the past decade. While current tools remain effective, vector resistance to at least one insecticide has been documented in 64 countries. Therefore, WHO-GMP, working with RBM, has developed the *Global Plan for Insecticide Resistance Management* to ensure the continued effectiveness of current and future malaria vector control tools At the same time, tremendous opportunities exist for enhancing malaria control efforts, notably universal diagnostic testing for suspected malaria, which when coupled with effective treatment and timely surveillance, will allow for the acceleration of gains already achieved through vector control. To galvanize these efforts, WHO launched the *"T3: Test, Treat, Track"* initiative on World Malaria Day 2012. If political will and financial commitments can be sustained to fully fund global malaria efforts, including research and development for new tools, then malaria control can be a driving force towards achieving the health-related Millennium Development Goals by 2015.

Conf16- **"The simultaneous multidiagnosis of infectious agents as a routine laboratory screening test for the near future"**

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Introduction: From the clinical and epidemiological point of view, the diagnosis of co-infections (the simultaneous infection of a host by multiple pathogen species) or the overlapping of infectious agents in the same region are frequent events worldwide. However, in most cases the search is done disease by

disease, increasing costs and delaying the treatment and control. Three published diagnostic techniques comply with the concept of simultaneous multidiagnosis: the antigen/antibody microarrays, the multiple laser detection beads (Multiplex ®) and the Multiple Antigen Blot Assay (MABA). The first two have been used for the detection of antigens, antibodies or nucleic acids, while the third one only for antibodies, so far. With this purpose, MABA can be implemented for the most frequent infectious diseases of each region or adapted for particular selected populations and institutions, such as blood banks, pediatric diseases, hemorrhagic fevers, immunocompromised patients, etc. It is also a practical, rapid, reproducible, sensitive and cheap technique for the identification and evaluation of different type of antigens (crude, chemically synthesized and recombinant antigens) and sera. One of the main advantages of this technique is that a single MABA assay allows the simultaneous evaluation of at least 26 different sera against 26 different antigens being equivalent to seven conventional ELISA plates. The goal is to develop an initial screening test for health institutions that would provide a general serological profile against the main regional infectious agent that must be confirmed with their respective gold standards. **Material and Methods:** This technique is based on the use of an acrylic device (Miniblotter^{R}) that allows the sensitization of different antigens in a single nitrocellulose strip, which reacts when exposed to an immune serum and thereafter with the appropriate peroxidase conjugate and the corresponding substrate. Signals in those reactive spots are recorded as black squares in a negative photographic film using a chemiluminiscent substrate or as blue spots when a precipitable colorimetric substrate (TMB) is used. Semi quantitative results ("Cross MABA) can be obtained using an image analyzer (Chemidoc, BioRAD^{R}). In the present case, emphasis is given to infectious and parasitic diseases such as: schistosomiasis, Chagas Disease, leishmaniasis, toxocariasis, toxoplasmosis, viral hepatitis C, HIV, histoplasmosis and leptospirosis. Type of antigens used: 7 crude homogenates (CA=7), fractions (FA=3) and synthetic peptides (SP=4). Sensitivity and specificity is calculated individually in relation to the "Gold Standard" technique for each disease. **Results and conclusions:** Overall sensitivity is above 75% but varies depending of each antigen. Low specificity has been observed with *Toxocara canis, Histoplasma capsulatum and Leptospira sp*. The highest sensitivities and specificities have been obtained with *Entamoeba histolytica, Toxoplasma gondii, Fasciola hepatica, Trypanosoma cruzi, Schistosoma mansoni*, ***cisticercus*** from *Taenia solium* and synthetic peptides from HIV and HCV. Other infectious agents (*Plasmodium falciparum*, HBV, HAV, VPH and *Helicobacter pylori*) are under study. In conclusion, this technique has confirmed its great versatility with different types of antigens and its potential use for clinicians and epidemiologists. **Acknowledgments:** Proyecto Multidiagnóstico (Proyecto No. UCV-G-2005000387), Misión Ciencia del FONACIT (Subproyecto 1-Proyecto No. 2007001425), Venezuela **E-mail:** noyaoo@yahoo.com

Conf17. **Environmental Changes and Future Scenarios of Geographic Spreading of American Cutaneous Leishmaniasis in Brazil**

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The focal transmission of American Cutaneous Leishmaniasis (ACL) provides a close relationship among micro-ecological conditions, parasites, vectors and reservoirs, in which environmental changes influence the incidence and growth, by the contact of man with the wild zoonotic cycles, and the adaptation of vector and hosts to new habitats. Depending on the eco-epidemiology of a particular focus, environmental changes, natural or by human action, could result in an increase or decrease in the incidence of the disease. There are hypotheses suggesting that climatic fluctuations produce an effect on the transmission dynamics of leishmaniasis. Lutzomyia (Nyssomyia) whitmani, vector of Leishmania (Viannia) shawi in Amazon and Leishmania (V.) braziliensis in the Midwest, Northeast, Southeast and South, adapted to different climate and vegetation and associated with most epidemiological circuits of ACL, significantly participates in the transmission profile related to environmental changes (destruction of vegetation and formation of settlements). On the other hand, a sylvatic cycle of ACL related to a severe clinical form of disease associated with Leishmania (Leishmania) amazonensis also extends to the human dwelling environment with the participation of Lutzomyia (Nyssomyia) flaviscutellata, vector found in most geographical regions except South and adapted to different types of climate and vegetation. The Cerrado biome has been suffering environmental changes resulting from extractive activities and developmental projects, which have contributed to the establishment of groups in rural and peri-urban areas without

infrastructure, allowing close contact with vectors of human pathogens. In this context, in the state of Tocantins the epidemiology of ACL is associated with deforestation, for construction of highways, railroads and hydroelectric dams favoring the establishment of settlements and villages. Previous studies developed in Tocantins suggested Lu. (N). whitmani as putative vector of Le. (V.) braziliensis in areas impacted by the construction of hydroelectric and agricultural activities, where this sand fly species was found inside and outside houses, near the animal's shelters. In this state, Lu. (N.) flaviscutellata has been collected in periurban areas and new cases by Le. (L.) amazonensis would be the result of the spreading of this sand fly vector. Possibly, the correlation between environmental and epidemiological information associated with Lu. (N.) whitmani and Lu. (N.) flaviscutellata may define determinants of their geographic expansion and occurrence of transmission cycles of ACL associated with these vectors, as well as anticipating future scenarios and a better planning of surveillance and prevention actions.

Conf18- **Research priorities for the control of vectors of leishmaniasis in the Old World**

Paul Ready

In the short term, leishmaniasis control could be much improved by the better application of existing techniques (Control of the leishmaniasis: report of a meeting of the WHO Expert Committee on the Control of Leishmaniasis, Geneva, 22-26 March 2010. WHO Tech. Rep. Ser. 949:1-186). The biting rates of sand fly vectors can be modified locally by control methods that will be outlined. The importance of controlling the nuisance biting of sand flies should not be underestimated. However, transmission modeling is required in more leishmaniasis foci, in order to predict better where and when vector control might reduce disease incidence. Vaccination of humans and some reservoir hosts (e.g. the domestic dog) is likely to provide the only long term solution for co-existing with leishmaniasis. Vaccine development will benefit from a better knowledge of the roles and micro-evolution of the salivary peptides and other molecules that sand flies inject into their mammalian hosts. Priorities will be proposed for this basic research, which also includes the possibility of anti-vectorial prophylaxis.

ROUND TABLES

RT1- **Experiences and achievements in the control of Chagas disease in Honduras: Interruption of the *Trypanosoma cruzi* transmission by the vector *Rhodnius prolixus***

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Introduction: Chagas disease was reported for the first time in Honduras in 1960. Studies established that the disease constitutes a serious problem for health in the country, with high indexes of infection in the general population and blood donors, and a large population at risk. The vector transmission of *Trypanosoma cruzi* occurs through two species of domiciliary triatomines: *Rhodnius prolixus* main vector and introduced to Central America and *Triatoma dimidiata* native species of Mesoamerica. The main clinical manifestation in the country has been chronic cardiopathy with a high medical and social cost. In 1997, the Initiative of the Central American Countries (IPCA) was launched for the "Interruption of the Vector and Transfusion Transmission and Medical Care of Chagas Disease". Within this framework, the National Program for Prevention and Control, with the support of the international and national cooperation, the systematic interventions begin, introducing innovations to traditional methodology for the vector control with an integral approach, which lead to significant achievements, until reaching the Interruption Certification of the transmission by *Rhodnius prolixus* in 2010. **Material and Methods:** The operation model for the vector control interventions in Honduras followed the traditional pattern in the

work phases, but introduced innovations in the methodology which made possible, with the available resources, the control of Chagas disease in a broad scale. From the beginning, carrying out a “serological exploration” in school children with rapid tests at the field level, allowing to get fast and safe information to: a) level and assign priorities of endemic areas; b) identify transmission focuses; c) have a base line of seroprevalence in schools. With the information obtained the priority areas are intervened integrally: serological survey of adolescents under 15 years of age; entomological spraying of dwelling with residual action insecticides; etiological treatment of cases with recent infection and surveillance activities with community participation. **Results and Main Conclusions:** Before beginning the control interventions, the seroprevalence in adolescents under 15 years of age was of 4.9%, in 2011 after the control it was 0.5%; there was infestation by *R. prolixus* in 11 departments of 18 in the country; currently the vector is eliminated. For *T. dimidiata* the current infestation index is of 3%, compared to the previous years where the national average index was 25%; the number of dwellings intervened with insecticide from 2004 to 2010 was of 221,259 and in 2011 it was of 88,104 dwellings. 1,388 infants under 15 years were treated etiologically for recent infection, and the seroprevalence of blood donors fell from 11.6% to 1.3% in 2011. The conclusions of this control experience are: *i*) the used methodology allows for the integral and rational approach, with the reduction of operative costs and time saving in the execution of the interventions. *ii*) The objectives and goals of the National Program allowed for the support from the national and international cooperation. *iii*) The experiences and achievements in Honduras are a contribution to achieving the goal of the interruption of the transmission of Chagas disease. **E-mail:** concepcionzuniga@gmail.com

RT2- **Snakebite Envenomings in Latin America**

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Snakebite envenomings constitute a relevant neglected tropical disease in Latin America. This pathology affects predominantly the rural population and has a high impact in impoverished regions where the provision of health services is insufficient. The total number of snakebite cases per year in Latin America has been estimated to be in the range of 80,000 to 130,000, although this pathology is often underreported. The majority of envenomings are inflicted by species of the genera *Bothrops* and *Crotalus*, classified in the family Viperidae, whereas few cases are provoked by coral snakes (genus *Micrurus*, family Elapidae). Parenteral administration of antivenom constitutes the mainstay in the therapy of this pathology. There are several laboratories which manufacture antivenom in the region. A significant body of knowledge has been gained in the taxonomy of the snakes, the biochemistry, toxicology and immunology of venoms, and the clinical manifestations of envenomings, and the preclinical and clinical performance of antivenom. In addition, important advances have been made in the prevention and treatment of this neglected tropical disease in Latin America through concerted national and regional efforts. An effective control of this disease in the region should be based on inter-sectorial and inter-programmatic interventions aimed at: (a) Improving the knowledge on snakes and their venoms; (b) assessing the actual incidence and mortality of snakebite envenomings; (c) increasing the volume of antivenom produced and, in some cases, in the quality of antivenoms; (d) strengthening of national quality control laboratories; (e) developing more effective strategies of distribution of antivenoms, especially to remote rural areas where snakebites are frequent; (f) fostering permanent education programs for the health staff in charge of the treatment of these envenomings; (g) providing support to people that suffer physical or psychological sequelae as a consequence of snakebites; and (h) strengthening community programs aimed at improving the prevention and adequate management of these accidents. These complex set of tasks demands the participation of many actors working in a coordinated fashion under a philosophy of cooperation and solidarity. **E mail:** jose.gutierrez@ucr.ac.cr

RT3- **Molecular epidemiology and diagnosis of Coccidioidomycosis**

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Coccidioidomycosis (CDM) is a deep mycosis caused by any of two dimorphic fungi, *Coccidioides immitis* (endemic in California, USA) and *C. posadasii* (endemic in the rest of the Americas). **Diagnosis:** Conventional microscopic examination of clinical specimens, culture, and serology are still very reliable tools for clinical diagnosis. Molecular methods have great potential in the diagnosis of CDM, especially in immunosuppressed patients. Real-time PCR has showed high diagnostic accuracy in respiratory specimens and fresh biopsy material but lacked sensitivity in paraffin-embedded tissues. A PCR technique based on amplification of the specific gene *Ag2/PRA* was successfully applied in these latter specimens. **Epidemiology:** In USA, more than 100,000 new *Coccidioides* spp. infections are estimated to occur each year. There is no precise data for the rest of the American continent, and in some countries the incidence of the disease appears to be increasing. In 1992, the emergence of CDM in California led to a seminal epidemiological study on patient and environmental sources where strains were classified as California (CA) genotype (now *C. immitis*), and non-Californian (non-CA) genotype (now *C. posadasii*). Allegedly, the CA genotype is geographically restricted to California. However, other authors have found the CA genotype in Venezuela, Colombia, and Argentina. So, is it correct the proposed geographical distribution of species? The total number of isolates identified to the species level is still too low in South America. Further molecular epidemiology studies are necessary to accurately identify the species are circulating in the region. Increasing CDM rates in Arizona have been attributed to a dominant, hyper virulent strain. However, the analysis of microsatellite polymorphisms did not support this hypothesis. Molecular methods are also used to determine the distribution of *Coccidioides* spp. in soil of endemic areas. However, its isolation in soil does not necessarily imply that the fungus is an environmental saprophyte. It has been recently suggested that *Coccidioides* spp. have evolved to associate specifically with animal hosts, probably rodents. Other animal species might also play a role. Next-generation sequencing is the most recent molecular tool applied to answer a particular epidemiological question. Whole genome sequence typing of C. immitis has been used to confirm that a single donor was the source of organs transplanted to three different recipients. Molecular methods are evolving fast and, in the near future, are expected to solve current diagnostic troubles and answer key epidemiological questions**. E-mail:** ccanteros@anlis.gov.ar

RT4- ***Cryptococcus gattii*** **in Latin America and Amazonia: emerging patterns and endemic behavior**

Elizabeth Castañeda
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Cryptococcus gattii is common in Latin American as agent of cryptococcosis and its natural occurrence in the environment. In the study published by Kwon Chung J and Bennett J in 1984 (Am J Epidemiol 1984; 120:123-130) containing 628 clinical isolates, 43 were from Latin America and 14 were *C. gattii*, 11 from Brazil, and one from Argentina, Mexico and Paraguay, each. Due to these findings it was proposed at that time, that *C. gattii* is mainly associated with tropical and subtropical regions of the world. However, this paradigm has been challenged since the Vancouver Island outbreak, which was due to this species. Clinical cases have been reported from Latin America since 1989 with data from Venezuela. In 1994 the first Brazilian cases were published, which at present have been documented in 27 papers. One of the most important observations in Brazil is the high proportion of cryptococcosis due to *C. gattii* in the immunocompetent host, HIV negative patients, especially in children from the Eastern Amazon region. As such *C. gattii* should be considered as endemic in this region. Publications from Argentina and Peru are from 1997 and 1998. In Colombia data on cryptococcosis due to *C. gattii* started to be published in 2000. However, the most relevant C*. gattii* studies done in Latin America, due to the great impact in the knowledge of cryptococcosis have been the ecological studies, starting as early as 1993 in Uruguay. Important and relevant data especially from Brazil were published since 1998 and from Colombia since 1994 (11 papers on the subject each). As a complement to the phenotypical data, molecular studies have revealed the presence of the four genotypes VGI, VGII, VGIII and VGIV in the region and have made the observation of an overall high genetic diversity among those isolates. The high genetic variations as well

as the presence of recombination indicate South America as origin for *C. gattii*. Other studies suggest South America as the origin of the VGII outbreaks recently described worldwide.

RT5- **The impact of next-generation sequencing in the understanding of drug resistance in malaria parasites**

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Human malaria is caused by parasites of the genus *Plasmodium* and is one of the most devastating infectious diseases worldwide, claiming over one million lives every year. Malaria control and eradication attempts through chemotherapy have been fraught by the outstanding capability of the parasite to evolve drug resistance. The complete genome sequence of human malaria parasites has been available for 10 years, but the recent development of Next Generation high-throughput DNA Sequencing (NGS) has conveyed a huge boost in our capacity to use and interpret malaria genomics. NGS allow generating and analyzing large amounts of genomic and transcriptomic sequence data rapidly, and at a relatively low cost. Thus it can hugely assist efforts to prevent and/or minimize the negative consequences of antimalarial drug resistance. Population genomics studies, for instance, can vastly increase the amount of genetic data available, facilitating the identification of drug-adaptive molecular variation and improving the estimation of important parameters such as the origins of drug-resistant parasites and their geographical migration. NGS can also work towards improved identification of mutations involved in drug resistance, by using broad comprehensive sequence data, that integrates genomic data with transcription and proteomic profiling, allowing to further our knowledge on drug evasion pathways and identification of new alternative leads. Presently, NGS has already been used by a number of malaria research groups resulting in a number of recently published reports that represent a significant advance in our knowledge about the mechanisms of drug resistance in malaria.

RT6- **Phenotypic and genotypic study of artemisinins derivatives susceptibility in *Plasmodium falciparum* strains**

Areas, A. L. L; Batista, C. N; Bustamante, C; Zalona, A. C. J.; Brasil, L. W; Vera, O; Sá, M. S; Soares, M. B. P; Zalis, M. G.

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 was 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.

RT7- **Platelets in Dengue Infection**

Fernando Bozza

Dengue is the most common vector-borne viral disease in the world. Although mitigating and controlling dengue outbreaks remain a high priority, an increased understanding of the disease is of paramount importance. One area still obscure in dengue pathogenesis involves the mechanisms by which dengue alters platelet behavior and, as a result, induces thrombocytopenia in the clinic. In this presentation we review the classical mechanisms of thrombocytopenia in dengue. Additionally, we present new evidences of enhanced platelet activation in patients infected with dengue, and we demonstrate the molecular mechanism responsible for this phenotype. We also show that DENV can directly activate platelets in-vitro, reproducing the phenotype observed in dengue-infected patients, and we discuss the potential receptors and signaling involved in this process.

RT8- **Dengue in Rio de Janeiro: epidemiological and laboratorial aspects in 25 years**

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Dengue is currently considered the most important mosquito-borne viral disease in humans who live in the tropical and subtropical areas of the world. In Brazil, social conditions as environmental determinants such as temperature, latitude, longitude, and disorganized urbanization, favored the expansion of the vector *Aedes aegypti.* High dengue activity in Brazil during the past 25 years is evidenced by the large number of cases, in almost all states of the country. DENV-1, DENV-2 and DENV-3 were introduced in Rio de Janeiro, in 1986, 1990 and 2000, respectively. DENV-4 has recently been isolated after 28 years of its first isolation. The introduction of DENV-2 in 1990 caused the first cases of dengue haemorrhagic fever and dengue shock syndrome. The introduction of DENV-3 led to severe epidemics in 2002 with the largest number of cases, DHF cases and deaths. In 2007–2008, the country experienced the most severe dengue epidemic in terms of morbidity and mortality and a higher incidence of severe cases in children. Genomic analysis performed on DENV-2 identified the introduction of a distinct lineage of the Asian/American genotype. In 2009 and 2010, DENV-1 re-emerged and, this serotype was prevalent in many States of the country. In Rio de Janeiro, this serotype caused the largest epidemic in terms of numbers in 2011. The phylogeny has also demonstrated the introduction of distinct lineages of DENV-1. Since 1986, laboratorial diagnosis has played an important role in the disease surveillance and epidemiology. Virus isolation and IgM ELISA were first used in 1986. With the introduction with DENV-2 in 1990, immune response characterization was performed by the hemaglutination inhibition test (HI), replaced by an IgG-ELISA. In the 90´s, molecular techniques such as RT-PCR and sequencing were used for nucleic acid detection and characterization. Real –time PCR and immunohistochemistry showed to be essential for dengue fatal cases confirmation and studies. More recently, NS1 protein capture tests have been used for the early diagnosis of dengue infections. Our experience has shown that the implementation of new laboratorial diagnosis techniques over the years constituted an important and reliable tool for the disease surveillance in the State of Rio de Janeiro, Brazil. **E-mail:** flaviab@ioc.fiocruz.br

RT9- **Parasites in extinct animals: where did our diseases come from?**

Adauto Araujo

Some infectious agents of human disease have coevolved with species in the *Homo* lineage for over 400 000 years. It is clear that our most common parasites had a concomitant origin with earlier species in the human evolutionary line and these co-evolutionary events certainly date to an early common ancestor of

humans and apes. These long-term, coevolved parasites are sometimes called ‘heirloom parasites’ or, in the parlance of evolutionary biology, parasites that are biological symplesiomorphies. As humans evolved, their heirloom parasites evolved with them. This appears to be the case for both *Trichuris trichiura* (whipworm) and *Enterobius vermicularis* (pinworm). As pointed out by Cameron in 1958, *Ascaris lumbricoides* (intestinal roundworm), and *Ancylostoma duodenale* (hookworm) are also human heirloom parasites. Humans have acquired a myriad of other parasites during their long biological and social history. Sometimes called souvenir parasites, these include species that occur in humans commonly, arriving through host- switching from other animal species. Animal domestication might have provided opportunities for parasites to colonize humans. This was considered the origin of two species of taeniid tapeworms in humans that use cattle and pigs as intermediate hosts. However, phylogenetic systematic studies show that association of beef tapeworm in humans (*Taenia saginata*) pre-dates the development of the domestication of cattle (*Bos* spp.) or swine (*Sus scrofa*). Tracing the origin of some human parasites has been possible associating phylogeny of both host and parasite, together with paleoparasitology data. This is the case of *Ascaris lumbricoides* and *Ascaris suum* in humans and pigs, respectively. Combining epidemiology, molecular biology, and paleoparasitology data, it was possible to conclude that both hosts are infected by only one species, *Ascaris lumbricoides*. Going back to an oldest scenario, we started to look for parasites in extinct animals. Oxyurids and ascarids are nematode parasites of a broad spectrum of present-day hosts. They are considered highly adapted parasites, and phylogenetic lines of parasite-host co-evolution can be studied for the known species. We found ascarid and oxyurid eggs in cynodont coprolites from the Upper Triassic in southern Brazil (about 230 million years ago). That find shows the antiquity of these parasites in vertebrates, especially given that these reptiles are considered predecessors of mammals. This coprolite find underlines the potential of parasite finds in animal fossils, which contribute to the study of parasite-host co-evolution. Oxyurids are found in invertebrates (arthropods and annelids), fish, birds, reptiles, amphibians, and mammals. Parasite-host co-evolution has drawn researchers’ attention, particularly as regards parasitism in rodents and primates. Vertebrate hosts and their oxyurid parasites belong to phylogenetic groups that diverge in the distant past. It is remarkable how highly host-specific most of the species are. A striking number of species infect rodents and primates. These orders of mammals, which have probably inherited oxyurids from ancestral species, have been the focus of a great deal of research. Ascarids are another group of parasites widespread in present-day hosts. Ascarids eggs were found in iguanodonts in Belgium, showing that these parasites infected reptiles of the Lower Cretaceous. The diagnosis of oxyurid and ascarids in cynodont coprolites opens up new prospects for evolutionary studies. This is a significant find, because the cynodonts are considered a sister-group to the mammals. They emerged in the late Permian and diversified during the Mesozoic, especially the Triassic, after the mass extinction in the Permian. The find of oxyurid and ascarid eggs in Cynodontia shows the antiquity of parasitism at the reptile-mammal transition point, some 230 million years ago. These finds can contribute to new studies of the origin of parasitism in the remote past, in hosts that became ancestors of present-day mammals. The morphology of the eggs of the two species of parasite was preserved for millions of years, enabling them to be recognized using routine microscopy techniques. This shows that, contrary to what was thought previously, there are fossil records of nematodes. These results also show the ability of paleoparasitology to find evidence of parasitism in extinct animals, enhancing the potential of studies in fossils dating from millions of years ago. Lastly, this is a find of parasites in a group of hosts that were ancestors of mammals, showing the scope for comparative studies, which for the moment are limited to morphology.

RT10- **Parasite infections in the Old World and the prehistoric period**

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Summary: Paleoparasitology is a bioarchaeological research field that aims in identifying ancient parasites and their distribution throughout time and space. At the cross between biology, archaeology, anthropology and health sciences, it uses coprology principles, but also immunology and molecular biology tools to recover parasite markers in archaeological sites. Since hundred years, collaboration between human science specialists and biologists led to the analysis of various archaeological materials like natural mummies, coprolites, embalming materials, cesspit sediments, or organic soils, and allows in

completing the knowledge about the presence and the frequency of ancient pathogens in the Old World. Among the periods in which parasite eggs were identified, the Neolithic seems to be one of the most interesting. Human populations settled and surrounded with vegetal and animal resources, probably increasing the risk of parasite exchanges between potential hosts. From this period to the Middle-Age, parasite nosology evolves in the Old World. The presence and the absence, but also the appearance and disappearance, as well as re-emergence of parasite species are linked to animal biodiversity evolutions, climate changes or ethnological aspects, and implicate host-parasite relationship modifications. Some examples concerning history of helminth worms and protozoan will be presented and discussed here.

RT11- **What can we provide through molecular and immunological approaches?**

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Background: Cysticercosis caused by the metacestodes of Taenia solium has well been known as one of the NTDs endemic in developing countries where people eat undercooked pork. However, through globalization in business, tourisms, the increase in number of refugees and immigrants of global scale, cysticercosis has become important NTD not only in developing but also in developed countries. It may be one of the typical borderless NTD in 21st century and still a potentially one of the most lethal NTDs. **Purpose:** It is to 1) develop highly reliable tools for detection of people and pigs suffering from cysticercosis, and that of taeniasis carriers who contaminate our living environment, 2) evaluate these tools in endemic areas, and 3) apply these tools for towards control of this zoonotic cestode infection. **Results:** We have developed 1) highly reliable serology for humans, pigs and dogs (Ito et al. 1998. Am J Trop Med Hyg 59, 291; Sako et al. 2000. J Clin Microbiol 38, 4439; Ito et al. 2002. J Helminthol 76, 311; Sato et al. 2003. Vet Parasitol 111, 309; Sato et al. 2006. J Infect Dis 194, 1783) and have established an immunochromatographic rapid kit (Sako et al. in prep), 2) highly reliable molecular tools to identify Taenia species (mitochondrial genes) and new tools for analysis of the cestode's strategy in reproduction biology (mitochondrial vs nuclear genes). In Asia, we have three human Taenia species: *T. solium*, *T. saginata* and *T. asiatica* (Ito et al. 2003. Lancet 362, 1918). In T. solium: there are two genotypes: one is Asian; the other is Afro/American (Nakao et al. 2002. Parasitology 124, 657). Multiplex PCR (Yamasaki et al. 2004. J Clin Microbiol 42, 548) and LAMP (Nkouawa et al. 2010. J Clin Microbiol 48, 3350) have been developed to identify these species. Analysis of both mitochondrial and nuclear genes, we are getting new information suitable for evaluation of *T. asiatica*. Due to our most recent studies, it is not an independent species, since we have found hybrid and/or hybrid derived worms of *T. saginata* and *T. asiatica* from areas where these two species have been confirmed to be distributed sympatrically (Okamoto et al. 2010. Parasitol Int 59, 70; Yamane et al. 2012 Parasitol Int 61, 351) **Discussion and Perspectives:** Haplotype network studies can reveal or suggest where cysticercosis patients were exposed to eggs of T. solium (Yanagida et al. 2010. J Travel Med 17, 206; Jongwutiwes et al. 2011. J Travel Med 18, 284). Comparative studies of mitochondrial and nuclear genes can provide that although platyhelminth, cestode is hermaphroditic and selfing is the basic reproductive strategy, outcrossing may occur between these two species. There is additional evidence of outcrossing between Asian and Afro/American genotypes of *T. solium* in Madagascar (Yanagida et al. in prep.). Based on these recent topics, we overview what we have been doing in several endemic areas in Asia, Indonesia, Thailand and China. The importance of real-time analysis through the field work is essential for treatment and education of local people and health workers including veterinarians. Taeniasis carriers are easily escaping from remote areas to local or central capital cities. It is the real situation of taeniasis/cysticercosis in Asia (Yanagida et al. 2012. Parasit Vectors 5, 18) and may be in the world. Recent activity for international collaborations including transfer of technology with sharing the philosophy "work together for living together" has been summarized (Ito et al. 2011. Parasit Vectors 4, 114).

RT12- **Vaccine development to prevent neurocysticercosis**

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Introduction: *Taenia solium* cysticercosis is a major parasitic disease that seriously and frequently affects human health and the economy of undeveloped countries. Since pigs are indispensable intermediate hosts, it is conceivable to curb transmission by reducing pig cysticercosis through effective vaccination. Several reports indicate the effectiveness of different subunit vaccines against cysticercosis. Among them, the anti-cysticercotic vaccine S3Pvac stands out. S3Pvac is composed of three peptides: KETc12 (8-amino acid long), KETc1 (12-amino acid long) and GK1, an 18-amino acid long peptide derived from the KETc7 peptide from *T. crassiceps,* also present in *T. solium*. To improve its effectiveness and reduce its cost, S3Pvac was recombinantly expressed in filamentous phages (S3Pvac-phage). S3Pvac, either synthetic or recombinant, induces high level of protection against pig cysticercosis on the field and reduced the viability of established cysticerci. S3Pvac-phage also significantly reduced porcine hydatidosis due to the homology in the vaccine peptides between both cestodes. The widening of the protective capacity of S3Pvac-phage to include hydatidosis is a convenient feature that allows in a single action reducing the prevalence of two frequent zoonoses in rustic pig rearing. S3Pvac-phage is being applied in a control program against taeniasis/cysticercosis since 2009 with the support of the Secretary of Animal Health in highly endemic states in Central Mexico. In spite of the effectiveness and low cost of the vaccine, it would be desirable to design a low-cost oral vaccine to be administered by the pig owners themselves when feeding the pigs. An oral version of the vaccine will allow us to elude the costly logistic difficulties to apply an injectable vaccine to the five millions of pigs bred under non-technical conditions each year. S3Pvac expression in transgenic plants could cope with both limitations. **Materials and Methods:** S3Pvac was expressed in the genome of papaya embryogenic cells and in tobacco chloroplasts using a synthetic operon. **Results:** Promising results were obtained by oral immunization with both vaccine versions in the murine and rabbit experimental models of cysticercosis. These oral vaccines are now being tested against porcine cysticercosis to eventually replace the injectable version by the more efficient oral one. **Main conclusions:** Overall, these advances offer new insights in cysticercosis prevention and in the development of new antigen delivery systems useful to design more effective and affordable oral subunit vaccines. **Supported by** CONACyT CB 152793 and DGAPA IT214311

RT13- **Control of *Cysticercosis***

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Diagnosis: 1. Detection of DNA with species specific PCR tests to identify all stages of the parasite in tissues, feces and CSF. 2. Detection of antibodies using recombinant parasite antigens and synthetic peptides. 3. Detection of secreted HP10 antigen to identify viable metacestodes.4. Detection of “coproantigens” to identify carriers. **Protection:** Surface/secreted oncosphere adhesion molecule HP6, a molecule mediating protection that may facilitate oncosphere tissue invasion and stimulate concomitant immunity.

RT14- **Dengue´s spread in Southern Brazil: Social and environmental/climate influences**

Mendonça, Francisco.

The outbreak of dengue’s fever, a human illness caused by a virus and that has the *Aedes* mosquito as its vector, involves aspects of the natural environment (climate) and social environment (economic, political and cultural). For its study and the development of policies and actions for prevention and control, it is necessary to treat it with a multifactorial approach. Dengue’s fever affects more than 100 million people per year, around 20 thousand of which die. According to data from WHO (2009), the average annual number of dengue cases has jumped all over the world. Last 5 decades the number of cases of

DHF killed approximately from 0 to over 60 people by year in all continents. An important part of the continents are located in the tropical zone of the planet, than the risks of an epidemic of dengue are high due to the vulnerability of the majority of people in these areas. Temperature and rainfall are the climatic elements that explicit more connection with the occurrence of dengue's fever and associated epidemics. Among the populations that inhabit the countryside, it seems to be little correlation between the habits of the inhabitants and the incidence of dengue's fever. As for cities, however, although the whole population may be at risk of action of the vector and therefore of developing the disease, it has been observed that the main victims are among the poorest. Dengue's fever was reintroduced in Brazil in 1986, until 1994 a low number of cases dengue's fever were recorded. After 1995, at which time the records of the cases became more systematic, the annual mean rose, reaching in some years higher rates than 500 000, as in the years of 1998, 2002, 2007 and 2008, when it was recorded important epidemics; around 1 000 000 in 2010. Southern Region of Brazil has made important records of dengue cases after 1995 ($190/10^5$hab in 2007). Trends in regional climate show a slight warming of about 1°C after the 1960s, accompanied by elevation of total annual mean rainfall (approximately 50mm). However, significant heterogeneity in the region was observed because some localities have a tendency to reduce heat and rainfall. Dengue's epidemics occur in Southern Brazil at the end of summer season and early autumn. The best performance of the vector occurs when the air temperature is between 25ºC and 30°C and under intermittent rainfall. These studies also showed that social conditions and lifestyle of the population play an important role in the occurrence of the epidemic, because an important portion of registered cases was related to low-income population (slums and semi-slums). In the neighborhoods of greater incidence of cases, it was observed the predominance of "informal solid waste pickers", which use the spaces of yards and streets as deposits of various kinds of plastic, glass, rubber and metals. These materials exposed in open air, become, with the fall of rain in summer, excellent breeding spot for the *Aedes aegypti* mosquito. Considering that the IPCC predictions about the global climate changes (variability!) indicate a warming of the planet's atmosphere, it is estimated that dengue's fever will show an expansion in latitude and altitude. Considering also that the urban areas of tropical countries have a tendency to expand, and that dengue's fever has shown very good adaptation to these urban environments, one can speculate that the incidence will show an important increase in coming decades.

RT15- **Malaria prophylaxis: from the opinion of experts to the evolution of guidelines.**

Guido Calleri

Malaria is still endemic in vast areas of Africa, Asia, Oceania and Latin America, which are frequent destinations for travelers from all over the world. Travelers from non-endemic countries are at substantial risk for acquiring this severe and possibly complicated disease, and are thus normally recommended chemoprophylaxis with antimalarial drugs. Nonetheless these drugs may have frequent and often severe side effects, so that a precise balance of risk and benefits of chemoprophylaxis must be done in the individual traveler. A number of factors are involved in the evaluation of risk, such as area of travel, precise itinerary, duration of stay, season, personal characteristics, adherence to protection from mosquito bites etc. Due to the inhomogeneity of this picture and to the individual low risk of the disease, conducting efficacy trials is very difficult, and evidence based data are lacking. Within TropNet, a European network for imported infectious diseases, we conducted a study using the Delphi method: a structured series of questionnaires was administered to European experts working in this field and routinely providing recommendations to travelers. The study provided interesting data, showing a significant diversity of behavior in different European countries, and started a discussion on this topic within the network. On these bases several statements have been produced and published by TropNet, and some guidelines have been modified. National guidelines in Europe still show significant diversity, also in key aspects, like chemoprophylaxis for *Plasmodium vivax*, or key areas, like the Indian Subcontinent or the Amazonas, or key drugs, like mefloquine or proguanil, and many other points. The same questionnaire re-administered five years later showed substantial modifications in responses, suggesting changing habits among professionals, but still an incomplete adherence to current local guidelines. The discussion is ongoing with the goal of increasing the consensus among opinion leaders, homogenizing recommendations and facing new challenges like elderly travelers, children, visiting friends

and relatives (VFRs), long and repeated travels. However, new and evidence based data about the epidemiology of the disease and the effects of prophylactic drugs are required.

RT16- **Diagnosis of sleeping sickness: update and perspectives**

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Important progress in the development and evaluation of diagnostic tests for sleeping sickness (human African trypanosomiasis) has been made in recent years. Antibody detection is still the method of choice for screening of populations at risk for *gambiense* sleeping sickness, and improved rapid antibody detection tests are under evaluation. Individual lateral flow tests recently entered phase I and II evaluation trials, and recombinant antigens and synthetic mimotopes have been generated and demonstrated clear diagnostic potential. As gold standard laboratory test for *gambiense* specific antibodies, the immune trypanolysis test has been re-introduced. The mAECT test is the most sensitive parasitological test that is applicable in the field and is produced as a ready-to-use kit in Kinshasa. Molecular diagnostics such as PCR and LAMP are highly sensitive and specific and several formats have been developed. Despite their potential for diagnostic and epidemiological purposes, these next-generation diagnostics remain restricted to laboratory settings due to their infrastructural requirements. A single-tube LAMP kit for application in a heating block with integrated illumination source has been recently introduced and is currently under evaluation. PCR has been evaluated for stage determination and as a test of cure for *gambiense* sleeping sickness patients but its value for assessment of treatment outcome was poor. The potential role of the different diagnostic tests in patient management and disease control will be discussed. **E-mail:** sdeborggraeve@itg.be

RT17- **New approaches for assessing drug efficacy on HAT**

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Human African trypanosomiasis (HAT) or sleeping sickness is a lethal disease caused by infection with *Trypanosoma brucei (T.b.) gambiense* or *T.b. rhodesiense*. The management of HAT patients is difficult since the treatment depends on the disease stage and two years post-treatment follow-up visits at regular intervals are required to evaluate clinical outcome. This long period of follow-up is hardly ever observed in practice for diverse *reasons. Often*, the patient comes back to the treatment clinic only in case of patent treatment failure when the disease has already reached an advanced stage. In such cases, the patient is at risk for serious lifelong disabling sequelae. A novel follow-up algorithm, based on WBC CSF count and detection of the parasite with definition of relapse and cure at each control visit, was developed. The duration of post-treatment follow-up for *gambiense* HAT patients can be reduced from the currently recommended 24 months to 12 months. Several biomarkers have been proposed to improve but have not yet been evaluated. Molecular tests show some limits. Noninvasive methods, such as actigraphy, are being evaluated.

RT18- **Vivax malaria – no more benign!**

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Introduction: *Plasmodium vivax* (*Pv*) infection is now well recognized for causing severe manifestations, which are commonly reported with *P. falciparum* (Pf) infection. The newer diagnostic methods including

PCR have challenged the earlier myth of missing co-infection in these cases. There are many reports from India, Papua New Guinea, Indonesia and Brazil describing severe vivax malaria in both adults and children. **Material & Methods and Results:** India contributes substantially to the global burden of vivax malaria. This observational prospective study was conducted on admitted patients at a tertiary care hospital, Bikaner (Northwest India), which is low endemic region experiencing predominantly seasonal malaria in post rainy period. *Pv* malaria accounts for about 90% of total cases of malaria in this region. There is no evidence of asymptomatic carriers as well as chloroquine resistance in this area. In an analysis of 351 (273 adults and 78 children) cases of severe vivax malaria diagnosed by PBF, RDT and confirmed by PCR, the complications observed were hepatic dysfunction and jaundice in 124 (45.42%) adults and 19 (24.36%) children (odd ratio [OR] = 1.86 [95% confidence interval (CI) = 1. 08-3.21], P = 0.01), renal failure in 43 (15.75%) adults and 12 (15.38%) children (OR = 1.02, CI = 0.514 – 2.03], P = 1), severe anemia in 93 (34.07%) adults and 56 (71.79%) children (OR = 0.47, CI = 0.31 – 0.71, P = 0.0003), cerebral malaria in 30 (10.99%) adults and 22 (28.21%) children (OR = 0.38, CI = 0.21 – 0.71, P = 0.001), Acute respiratory distress syndrome (ARDS) in 10 (3.66%) adults and 9 (11.54%) children (OR = 0.31, CI = 0.124 – 0.80, P = 0.13), shock in 5 (1.83%) adults and 1 (1.28%) children (OR = 1.42, CI = 0.164 – 12.40, P = 0.6), hypoglycemia in 1 (0.37%) adult and multi organ dysfunction (MODS) in 101 (37%) adults and 39 (50%) children (OR = 0.73, CI = 0.47 – 1.15, P = 0.185). In another study on 840 patients (460 adults and 380 children) of *Pv* malaria, thrombocytopenia (platelet <1.5 lac) was found in 143 adult and 278 children (OR = 0.42, CI = 0.33 – 0.54, P = 0.0001). Severe thrombocytopenia (platelet <20,000) was observed in 26/143 adults and 60/278 children (OR = 0.36, CI = 0.221 – 0.578, P = 0.0001) associated with or without bleeding tendency. **Conclusion:** There is definite evidence of severe manifestation in both adults and children. Hepatic dysfunction was more common in adults whereas severe anemia, cerebral malaria and severe thrombocytopenia were more common in children. These observations are against the long considered description of this disease as benign infection. It requires adequate projection to attract the attention of policy makers and funding agency to enhance more research activities for this important but neglected disease.

RT19- **Epidemiology and control of leishmaniasis in Argentina**

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In Argentina tegumentary leishmaniasis (TL) is mainly rural and endemic from the northern border to the 38° SL, while urban visceral leishmaniasis (VL) is a recent epidemic event reported since 2006. The report of both leishmaniasis is mandatory. The National Program of Argentina was created in 1999. Autochthonous TL cases were recorded from 9 provinces. Since the outbreaks of 1985, 1998 and 2002 TL the human incidence remains in a low endemic channel (7300 cases since 1985, 1120 cases from 2002 to 2011). The decrease of cutaneous cases (CL) generates a high ratio of cutaneous/mucosal leishmaniasis (up to 18%), mainly from former infections during the outbreaks. The incidence rates in youngsters and females since the first outbreak suggested transmission in domestic environments. *Leishmania braziliensis* is the main parasite species isolated from cases related to outbreaks, domestic mammals and vectors, *L. amazonensis* was isolated just from a NW restricted area, and two reports of *L. guyanensis* require further studies to define its epidemiological importance. Phlebotomine captures are performed in 14 provinces, 29 species were reported, *Nyssomyia neivai, Ny whitmani, Evandromyia cortelezzii, Micropygomyia quinquefer* were found naturally infected by PCR-RFLP – sequencing, *Migonemyia migonei* was also incriminated as vector due to epidemiological evidence. From 1925 to 1989 14 cases of VL were reported from Argentina. These cases were scattered through the area of CL without records of *Lutzomyia longipalpis*. *Lu. longipalpis* (few individuals in non-populated areas) were reported in 1953 (Candelaria) and 2000 (Corpus) in the province of Misiones (NE), without cases of VL during this period. Phlebotomine surveillance was intensified in border areas since 2000 due to the southern reports of VL in Brazil and Paraguay. *Lu. longipalpis* was found spatially clustered in Clorinda (Formosa) in 2004, 2007, 2011, but the canine cases (VLc) were dispersed related with the migration through the border and within the city. The first autochthonous case with concurrent VLc and vectors were recorded during 2006 in Posadas (Misiones). Since then human cases were reported in several localities of Misiones and Virasoro, Santo Tome and Corrientes city (province of Corrientes) all in urban environments. There are up to now 102 cases reported with 11% of fatality related mainly with co-

morbidity in adults, while the proportion in youngsters is rising. Social and commercial pet-related networks dispersed VLc in almost all the country. *Lutzomyia longipalpis* dispersion is described from year to year reaching the northern border of Misiones, Corrientes, Chaco (Resistencia), Entre Ríos (Chajari), and in the Republic of Uruguay (Salto, Bella Unión). In Santiago del Estero (Center) and Salta (NW) VL human and dog cases were related with a different epidemiological scenario, with *Mg migonei* as putative vector. The localities are stratified according to the risk associated to each leishmaniasis. The eco-epidemiological studies in different spatial and temporal scales are contributing to propose control strategies appropriate to the different risk scenarios. The control measures proposed are consistent with the WHO recommendations, and the regional agreements, and there are currently evaluated in the field.

RT20- **(Re-) Emerging visceral leishmaniasis in Europe and an outbreak in Spain**

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Endemic visceral leishmaniasis in Europe (particularly in southwestern countries) is caused by *L. infantum* and is associated to a zoonotic cycle with domestic dogs as the main reservoir host and vector sandflies of the subgenera *Larroussius* and *Adlerius*. Although traditionally considered a disease of childhood it affects adults in varying proportion. Its incidence is low with an approximate 700 new human cases each year but a high ratio of asymptomatic/symptomatic infection is estimated (30-100/1); this together to a roughly 25% seroprevalence of infection in dogs indicates a latent public health problem, demonstrated in the past with the advent of HIV infection. Europe accounts for more than 2000 cases of *Leishmania*/HIV co-infection since its first report in 1986 with a dramatic peak of 717 cases in 1996-98 (the highest proportion reported from Spain). Fortunately, the introduction of HAART therapy favored a sharp decrease of co-infection cases. Nevertheless there is still a risk or (re-)emergence. And examples are the recent propagation of leishmaniasis to northern Italy, autochthonous canine and human cases in Hungary and Germany respectively, and in bovids and equids in Switzerland, increasing reports of imported human and canine infections from Mediterranean countries to the north, and also of exotic species in different European countries. In addition, studies identifying infection in domestic and wild animals different to dogs are on the raise. Immunosuppression as a risk factor for spreading is still present due to the growing number of both transplant recipients and treatment regimens using immunosuppressive pharmacological agents. To this scenario adds the GIS framework models combining entomological and environmental variables, which indicate that global warming, could lead to the spread of sandfly populations to previous non-colonized areas. And last but not least: an urban outbreak of visceral and cutaneous leishmaniasis is taking place in the southern region of Madrid autonomous community, capital city of Spain. Since 1997 leishmaniasis is a compulsory notifiable disease in this community with an annual incidence of 12-25 human cases. However, the outbreak which started in 2009 affecting also previously non-endemic municipalities has caused more than 300 human cases up to now. All age groups are affected, without significant association to immunosuppression, 41% of the cases are visceral forms while 59% are tegumentary. Presence of dogs around the cases has been identified as a possible risk factor; but search on alternative reservoirs identified a high prevalence of infection (30%) in hares indicating their possible role as secondary reservoirs, recently their ability to transmit the infection to sandflies has been proved through xenodiagnosis studies. The origin of the outbreak has been attributed to environmental changes. Control measures are taking place and include identification of risk areas, desinsectation, environmental sanitation, capture of stray dogs and hares, establishment of an entomological surveillance system, campaigns to raise public awareness and strengthen of communication between veterinary and human health professionals. **E-mail:** cruzi@isciii.es

RT21- **Progressing NCEs through clinical development for the treatment of neglected tropical diseases (DNDi)**

Introduction: Effective treatment of neglected diseases, especially those resulting from kinetoplastid infections (Chagas disease, sleeping sickness and leishmaniasis) is hampered by outdated, often toxic drugs that impose heavy burdens of health systems. As a result, these diseases continue to affect millions across the world, often the poorest among the poor. New treatments are sorely needed to

support sustainable control or elimination of these diseases. **Materials & Methods:** Building the future of novel and effective treatments for neglected diseases includes progressing promising New Chemical Entities (NCEs) through the development pipeline, by accessing new chemical libraries or compounds and developing strong lead optimization consortia. Successful advancement of new leads and optimized leads in the discovery and pre-clinical phases is a key to building a robust pipeline for the coming years, whilst taking into account the realities of the field is essential when progressing candidates from the pre-clinical to the clinical phase. **Results:** Since its inception in 2003, DNDi and partners have delivered six new treatments for malaria, sleeping sickness, visceral leishmaniasis, and Chagas disease. At the same time, by establishing fruitful collaborations with a variety of partners from the pharmaceutical industry, biotechs, academia and other PDPs, as well as disease-endemic countries, DNDi has established a strong portfolio, with 11 NCEs at various stages of development, to treat kinetoplastid diseases, as well as specific helminth infections and paediatric HIV. **Main Conclusions:** This session aims to give an overview of the realities of developing new drugs for the treatment of neglected tropical diseases, and to present DNDi's model of progressing NCEs through the development pipeline.

RT22- **Malaria, pregnancy and HIV: immunological cross-talks in the context of *Plasmodium falciparum* infection**

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Antibodies against VAR2CSA, the *Plasmodium falciparum* variant surface antigen that binds placental chondroitin sulfate A, have been suggested to be markers of protection against malaria in pregnancy. However, other studies have suggested that they are markers of infection in pregnancy. These discrepancies may be partially explained by difficulties in distinguishing unexposed women from those truly protected by anti-parasite immunity in areas of heterogeneous exposure to the parasite, as well as by differences in malaria transmission intensities and different overlapping distributions of malaria and HIV providing varying opportunities for interactions. Our studies have shown that antibody responses in pregnant women reflect temporal and geographical variations in the exposure to *P. falciparum*, and that these heterogeneities need to be taken into account to assess the clinical impact of antibody responses against malaria during pregnancy. The use of intermittent preventive treatment during pregnancy is associated with a reduction of antibodies in HIV-infected women, but not in HIV-uninfected women. Moreover, HIV-infection attenuates the parity-dependent increase of antibodies against the parasite observed in HIV-negative women, suggesting its impact on immunological memory. HIV-associated impairment of antimalarial immunity in pregnant women may contribute to a higher transmission of *P. falciparum* to their infants and to a higher carriage of resistant parasites in women receiving IPTp. These results highlight a) the potential of anti-parasite antibodies in pregnant women as markers of cumulative exposure to *P. falciparum* during pregnancy; b) the importance of developing additional methods to adjust for heterogeneity in parasite exposure when assessing immune responses that contribute to protection against malaria infection in pregnancy and c) the need of further studies to understand the basis for the impact of HIV infection on antimalarial immunity. **E-mail:** agmayor@clinic.ub.es.

RT23- **A reliable ex-vivo invasion assay of human reticulocytes by *Plasmodium vivax* for the development antibody-based vaccines**

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Currently, there are no reliable red blood cells invasion assays to guide the discovery of vaccine against *Plasmodium vivax*, the most prevalent malaria parasite in Asia and South America. Here we describe a protocol for an *ex vivo* invasion assay that can be easily deployed in laboratories located in endemic countries. The assay is based on mixing enriched cord blood reticulocytes with matured, trypsin treated *P. vivax* schizonts concentrated from clinical isolates. Validation of the assay and demonstration of its reliability was achieved using a large panel of *P. vivax* isolates freshly collected from patients in Thailand. The assay was also validated to assay the effect of antibodies against the Duffy antigen, a receptor for *P. vivax* merozoite invasion of reticulocytes or its parasite ligand, the Duffy binding protein.

RT24- **New serological biomarker of *Anopheles* mosquito bites and indicator of malaria vector control effectiveness**

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Abstract: Mosquito-borne diseases (such as malaria) represent a major public health problem, especially in developing countries of tropical and subtropical areas. The transmission of malaria pathogens is closely linked to the exposure level to infected female *Anopheles* mosquito bites. Preventive methods are currently directed against malaria vectors (insecticide-based control). However, tools measuring their impact are still desperately lacking. Much effort is therefore being devoted to develop new indicators evaluating reliably their effectiveness. The quantification, in human, of the antibody response specific to immunogenic salivary components of the *Anopheles* vector appears to be a promising way. The present talk contributes to a better understanding of the human-vector immunological relationship in malaria. It resumes the majority of studies eliciting the roles of *Anopheles* mosquito saliva in the human host physiology and immunology, approaches and techniques used to develop specific candidate-biomarkers of exposure to *Anopheles* bites. A specific protein in *Anopheles* saliva was then identified and validated as a pertinent biomarker and applied for evaluating malaria control effectiveness in several epidemiological settings in Angola, Senegal and Benin. Effects of various explanatory variables (age, sex, and seasonality, movements of individuals and differential use of vector control measures…) on the antibody response to *Anopheles* salivary antigens are also discussed in the aim to optimize its utility in epidemiological surveillance of malaria vector control programs

RT25- "**Pertussis: diagnosis tools for a re-emerging disease"**

Maria Lucia Tondella
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Pertussis, also known as whooping cough, is the most poorly controlled bacterial vaccine preventable disease in the U.S. The etiologic agent, *Bordetella pertussis* is a fastidious organism and difficulties associated with diagnosis by culture make public health surveillance challenging. Polymerase chain reaction (PCR) has become the most used laboratory test for pertussis diagnosis. The majority of clinical laboratories use a highly-sensitive, single-target PCR assay (IS*481*). However, assay specificity is compromised because the same target sequence occurs in other *Bordetella* species such as *B. holmesii*, which can also cause pertussis syndrome. Reported pertussis cases are based on clinical presentation along with laboratory confirmation by either isolation of the bacterium or PCR testing. Serology is not currently included in the U.S. pertussis case definition as a confirmatory test. Regardless, serology is increasingly being used in the U.S. to report case. Serodiagnosis can be a useful tool for adolescents and adults, and in the later phases of the disease, when performed by a well standardized, validated test. The Centers for Disease Control and Prevention (CDC) has been actively engaged in a comprehensive approach to improve diagnostic testing practices for pertussis, including developing, validating, standardizing and transferring the technology of diagnostic assays, establishing criteria for result interpretation, developing guidance of best practices for clinicians to optimize use of diagnostics and

increasing reference capacity at public health laboratories. The concept of complementary testing as the best approach to improve confidence in the accuracy of diagnostics was established. Current challenges will be discussed.

RT26- **Molecular characterization of *Cryptosporidium* spp: implication for infection source**

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Genotyping and subtyping tools have been widely used in characterizing the transmission of human cryptosporidiosis. Thus far, five *Cryptosporidium* spp., including *C. hominis*, *C. parvum*, *C. meleagridis*, *C. canis,* and *C. felis*, are responsible for most *Cryptosporidium* infections in both immunocompetent and immunocompromised persons. Differences have been observed among endemic areas in the proportion of infections caused by each species, with *C. hominis* most prevalent in developing countries and some industrialized nations, *C. parvum* most prevalent in Mideast countries, *C. parvum* and *C. hominis* both prevalent in Europe and New Zealand, and *C. meleagridis* prevalent in focal areas. Results of subtyping based on sequence analysis of the gp60 (gp40/15) gene suggest that there is a high genetic heterogeneity of *C. hominis* in developing countries and low heterogeneity in most industrialized nations. In developing countries, human infections with *C. parvum* are probably largely results of anthroponotic rather than zoonotic transmission, whereas in industrialized nations, both routes play a role in cryptosporidiosis epidemiology. Multilocus subtyping analysis further indicates the existence of geographic segregation in *C. hominis* subtypes in developing countries. Mixed and sequential infections with different *Cryptosporidium* species/genotypes and subtypes are common in developing countries. Differences in clinical presentations and outbreak potential have been observed among *Cryptosporidium* species and *C. hominis* subtypes. These findings reveal the complexity of human cryptosporidiosis transmission in endemics areas and highlight the need for comprehensive molecular epidemiologic studies of cryptosporidiosis transmission in areas with a wide spectrum of socioeconomic and environmental conditions. Our understanding of cryptosporidiosis transmission can be improved significantly through systematic use of genotyping, gp60 subtyping, multilocus sequence typing, and newly developed comparative genomics tools in well-designed case-control and longitudinal cohort studies.

RT27- **Amebiasis: Diagnosis and immunity to *E. histolytica* infection**

Dr. Rashidul Haque, MB, PhD
Senior Scientist & Head of Parasitology Laboratory

Entamoeba histolytica is an invasive enteric protozoan parasite that is the cause of amebiasis. *Entamoeba dispar* and *Entamoeba moshkovskii* are non-pathogenic parasites that are identical morphologically to *E. histolytica*. *E. histolytica, E. dispar* and *E. moshkovskii* therefore cannot be distinguished by a stool ova and parasite (O&P) test, which has been the traditional diagnostic method. Because *E. dispar* and E*. moshkovskii* are often as prevalent as *E. histolytica,* it is important clinically to use *E. histolytica*-specific diagnostic tests. Several molecular diagnostic tests for diagnosis of amebiasis have been developed and have been used in the epidemiological studies in Bangladesh. Real-time PCR assays that we have developed for detection of *E. histolytica* infection in stool, liver abscess pus, saliva and urine would be extremely useful for diagnosis of amebic liver abscess and amebic colitis patients. During the last twelve years our research was focused to understand the acquired immunity to *E. histolytica* infection in a cohort of children in Dhaka. This is the only cohort study in children to understand human immunity to *E. histolytica* so far we know. This community-based prospective study of E*. histolytica* infection in children in Mirpur has yielded important findings: 1) acquired immunity to *E. histolytica* infection exists. Children with an intestinal IgA directed against the *E. histolytica* Gal/GalNac lectin carbohydrate recognition domain (CRD) have an 86% reduction in new infections at one year of follow-up; 2) Amebiasis is an important cause of preschool childhood morbidity; 3) Increased

susceptibility to new infection in individuals that are positive for anti-lectin antibody ; 4) A potential protective association was observed with the class II allele DQB1*0601 and the heterozygous haplotype DQB1*0601/DRB1*1501. 5) We have shown that there is an association between IFN-γ and *E. histolytica* associated diarrhea/dysentery ; and 6) Demonstration of the association between a genetic polymorphism in an adipocytokine receptor with susceptibility to *E. histolytica* infection in humans.

RT28- **Genotype variability of the Microsporidian parasites**

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Overview: Microsporidia are ubiquitous pathogens with striking biological characteristics. They have the most compacted eukaryotic genome, combining eukaryotic and prokaryotic features, although Microsporidia have recently been related to fungi. This phylum includes more than 140 genera, 8 of which have been associated with human infections (*Enterocytozoon bieneusi* and the species of *Encephalitozoon* are the most frequently reported) and, among insect parasites, *Nosema ceranae* seems to be the major cause for the alarming worldwide losses of honey-bee colonies. The availability of genome sequences variable at the interspecific level is important in two main fields. One is their practical use in the characterization of isolates, providing information about their clinical features; also, the comparison with other isolates is epidemiologically relevant. The second field concerns the evaluation of the species-wide diversity and the selective forces shaping it; not only is a basic knowledge always necessary, but it will ultimately reinforce the possibility for the control of these parasites. However, finding variable loci in microsporidia is not an easy task. The genomes are extremely reduced and, accordingly, so are the number of non-coding sequences prone to show allelic variants. The scarce number of Short Tandem Repeat (STR) loci has become clear after the genome projects which have already been carried out in a few microsporidia species. **Variable genetic markers:** The Internal Transcribed Spacer (ITS) between the two ribosomal genes has proved to be a useful genotypic marker for both *Encephalitozoon cuniculi* and *E. hellem*; in *Enterocytozoon bieneusi* the high variability in the ITS has allowed the establishment of pathways in the zoonotic transmission of this parasite. By contrast, in *Encephalitozoon intestinalis* the ITS is obstinately uniform, which is not so surprising given its short length. The ribosomal DNA genotyping may also be confusing since, like in the other eukaryotes, it is repeated in the genomes of microsporidia. In the species above, rDNA repeats, including RNA genes and spacers, are uniform within a given strain. However, concerted evolution is not necessarily a general rule and, in fact, in the honey-bee parasite *Nosema ceranae* the strong polymorphism in the rDNA is also extended to individuals (spores) and different rDNA sequences exist in the same genome. A second group of markers includes the genes for surface proteins – such as the Polar Tube Proteins and the Spore Wall Proteins which have repeated domains. The number and organization of such repeats tends to vary among strains and, moreover, the different alleles may be related to different biological properties. A number of point polymorphisms in coding and non-coding sequences have been described but additional efforts are clearly needed, particularly in species where the above markers are not informative. **Conclusions:** The analyses of the ribosomal ITS and the surface protein genes have produced encouraging results concerning the interspecies variability in Microsporidia. Nevertheless, those markers also have a number of limitations that emphasize the importance of additional genotyping based on other markers, preferably unique sequences. Understanding how the diversity is modulated will not only offer new opportunities for the control of microsporidiosis but also provide an approach to the biology and evolution of these peculiar and minimal forms of eukaryotic life.

RT29- **Molecular epidemiology of giardiasis in Rio de Janeiro, Brazil**

Octávio Fernandes

Giardia duodenalis is one of the major diarrhea agents in human and animals distributed worldwide, and present high levels of genetic diversity, showing seven genotypes: A, B, C, D, E, F, and G. Only Assemblages A and B have been detected in humans and in a wide range of other mammalians hosts, whereas the remaining Assemblages (C–G) are host-specific. Molecular characterization of cysts of

human and animal origin are useful to address the co-circulate isolates between these host, and represents an objective means to evaluate zoonotic infection hypothesis. This presentation shows the findings of the molecular characterization of *G. duodenalis* genotypes from human and animal samples from Rio de Janeiro, Brazil. Several molecular methods were applied to characterize two genes: β giardin and malic enzyme. The major findings were: (i) a higher prevalence of genotype A1 being present in both humans and pets showing its zoonotic potential, (ii) several point mutations were found in the β giardin gene revealing that the population heterogeneity is higher than the current described, (iii) the same phenomenon regarding the presence of point mutations was also found when using the malic enzyme gene, (iv) a direct PCR based method to amplify this latter gene from feces was developed, (v) a real time PCR for this same gene characterizing samples belonging to genotype A1 revealed to be more sensitive than the traditional PCR. This finding could also be evidenced when the β giardin gene was used. In conclusion, Giardiasis can be considered a zoonosis in Rio de Janeiro, Brazil and the molecular characterization of the isolates is mandatory to a better understanding of the epidemiology of the disease.
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RT30- **Rotavirus disease mechanisms: the gut-nerve cross talk**

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Rotavirus can cause severe dehydration and is a leading cause of childhood deaths worldwide. While most deaths occur due to excessive loss of fluids and electrolytes through vomiting and diarrhea, the pathophysiological mechanisms that underlie this life-threatening disease remain to be clarified. Our previous studies revealed that drugs that inhibit the function of the enteric nervous system could reduce rotavirus diarrhea. The fact that oral rehydration corrects electrolyte and water loss, indicates that enterocytes in the small intestine have a functional sodium-glucose co-transporter. Moreover, rotavirus infection delays gastric emptying and loperamide appears to attenuate rotavirus diarrhea, thereby suggesting activation of the enteric nervous system. The pathophysiological basis of virus-induced emesis, a hallmark of illnesses caused by rotavirus is poorly understood. We have recently addressed (Hagbom et al, PloS Path, 2011) the hypothesis that rotavirus infection triggers the release of serotonin (5-hydroxytryptamine, 5-HT) from enterochromaffin cells in the intestine leading to activation of vagal afferent nerves connected to brain stem structures associated with vomiting and activation of Fos expression in the nucleus of the solitary tract of CNS, the main target for incoming fibers from the vagal nerve. Moreover, 5-HT_3 receptor antagonists are today commonly used to treat vomiting associated with acute gastroenteritis in children, all suggesting nerve involvement also in rotavirus-induced vomiting.

RT31- **Molecular Diagnostics for the Point-of-Care**

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Background: Sensitive, affordable, and rapid diagnosis of malaria is critical for patient care and to support eradication strategies. Our objective is to develop a modified blood capillary tube that enables direct testing for malaria by real-time PCR. **Methods:** Reagents for real-time PCR are combined with an acrylamide solution within a capillary to form a 'hydrogel'. The hydrogel is dried for long-term storage and rehydrated by dipping the capillary into a diluted clinical sample. Capillaries are run on a small real-time PCR instrument that was developed in our lab. **Results:** The capillary-based assay detects the four major species of *Plasmodium* directly from unprocessed clinical samples. Samples can be screened for *Plasmodium* and identified at the species-level. The limit of detection is 10 parasites/μL of blood. Positive and negative controls were also designed to provide quality control for molecular testing in capillaries. **Conclusions:** This innovative platform combines sample collection and testing within a single, low-cost,

disposable capillary for malaria diagnosis at the point-of-care. Blood can be added to capillaries directly, without requiring sample processing. Capillaries are also adapted for long-term storage in the field obviating the need for a cold chain to preserve molecular reagents. The sensitivity, affordability and user-friendliness of this technology make it an excellent platform for a variety of clinical applications within resource-limited settings.

RT32- **Malaria and quinine resistance: the history and circulation of a medical and scientific issue**

Jaime Benchimol

This research note addresses the discussion surrounding quinine-resistant *Plasmodium falciparum* malaria in the early decades of the twentieth century. Observed by Arthur Neiva during an anti-malaria campaign in the Baixada Fluminense region of Rio de Janeiro in 1907, the biological and social resistance of malaria sufferers to preventive and curative treatment with quinine was corroborated three years later by Oswaldo Cruz during construction of the Madeira Mamoré Railway in the Brazilian Amazon. Likewise in 1910, ailing German workers were transferred from Brazil to Hamburg's Institute for Maritime and Tropical Diseases, where quinine resistance was confirmed by the facility's director, Bernard Nocht, and by military physician Henrich Werner. The researchers hurried to publish a paper on the topic shortly after an article by Arthur Neiva came out in *Memórias do Instituto Oswaldo Cruz*, where the Brazilian scientist advanced his first hypotheses about the resistance. When World War I saw failures in treating and preventing malaria with quinine along with violent outbreaks of the disease on the Turkish and Balkan fronts, resistance to this alkaloid became the topic of the day within the field of experimental medicine in Germany. New attempts were made to account for the resistance, especially by physician Ernst Rodenwaldt, who explored the topic by applying modern heredity research and relying on the copious information and experiences amassed in German colonies. The present note offers a preliminary survey and analysis of pronouncements about quinine resistance, shedding new light on the circulation of knowledge in the field of tropical medicine. The directions taken by investigators' statements about malaria parasite resistance to quinine show that research into the topic held sway over individual trajectories and collective thought. Rather than sharing any kind of international research program, investigators adhered to their own "national styles" in researching and fighting this disease, deemed prototypical of tropical illness over a large range of latitudes.

RT33- **Tropical medicine – A new scientific field in the Portuguese context**

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Abstract: Tropical medicine becomes an independent scientific field, in the transition from the nineteenth to the twentieth century, with the institutionalization of its teaching and research, at specialized schools of tropical medicine, created in the context of European imperialism. As a scientifically recognized research area, tropical medicine developed its own language and methodology, in order to fight effectively against diseases existing in the tropics, since immemorial times. In Portugal, tropical medicine emerged as an independent field of enquiry with the foundation of the School of Tropical Medicine of Lisbon, and the Colonial Hospital, in 1902. With these two institutions devoted to teaching, research and clinical practice, the Portuguese State acknowledged the importance of this medical discipline as a fundamental tool to its imperial policies in Africa. This paper aims to characterize and analyze the main trends followed by Portuguese tropical medicine in Africa, between 1902 and 1972, by taking trypanosomiasis as a case-study. In this context, it will highlight the contributions of generations of prestigious researchers in the international scene ─Ayres Kopke, Fraga de Azevedo, Francisco Cambournac and Cruz Ferreira ─ in particular their interpretations of the etiology and therapeutics of this disease and their views on public health and hygiene. Tropical medicine as a twentieth-century field of scientific, political and medical action faces new challenges in the present. Although, great epidemics have diminished, diseases which for long

have been neglected pose new questions urging creative solutions. Will tropical medicine become a new field of medical intervention in Europe? **E-mail:** ima@fct.unl.pt

RT34- **Between Tropical Medicine and International Health: Francisco Cambournac and the African Regional Office of the World Health Organization, c. 1950s**

Marcos Cueto
Casa Oswaldo Cruz, FIOCRUZ

The links between the Cold War and international 'tropical' medicine during the 1950s have not been studied. There are still gaps on what happened in the decades previous to the founding of new centers devoted to tropical medicine such as the World Health Organization's (WHO) Special Program in Research and Training in Tropical Diseases, TDR (1967). Important developments took place during the Cold War in areas of the world where the legacy of European imperialism and extended infectious were paramount such as Africa. The study will focus on the work of the Portuguese malariologist Francisco Cambournac; head of the African regional office of the new multilateral agency created in 1948: WHO. After World War II European colonial nations, such as France, Belgium and U.K, wanted to control their colonial possessions and disliked "regionalization" approved by the 1948 WHO Constitution. By 1951, regional offices with varying degrees of autonomy operated in all six regions of the globe. The Africa office (AFRO) comprised countries and territories in vague terms that could be identified mostly with colonies in sub-saharian Africa. It was preceded by the establishment of an office in Geneva in 1950 and later relocated its headquarters in French Equatorial Africa in 1952. The Lieutenant General François Daubenton was chosen to lead the regional office. A year later a new director, trained in tropical medicine and malariology, was appointed: Cambournac. He would remain as head of the AFRO until 1964. The regional office produced important studies on yellow fever, malaria and onchocerciasis, among others. The office carried out yellow fever surveys between 1951 and 1953, to determine the southern limit of the infection. By the mid-1960s about ten regional committees have taken place and a new AFRO building existed in Brazaville. He also organized the little-studied Third African Malaria Conference held in Yaoundé, Cameroon on 1962, which consolidated the notion of 'pre-eradication' programs to build the basic foundations of the public health infrastructure; a precedent to Primary Health Care. In the mid-1960s independence and important political changes led to the appointment of more Africans in the office such as the first African Regional Director, Alfred Comlan Quenum, from Benin, appointed in 1965. The following year Cambournac returned to Lisbon to direct the School of Tropical Medicine. Change in the African region was reflected in the fact that from only 3 member states and the same number of associate members that existed in 1957; ten years later the regional office recognized 29 members and two associate members. The new developments of the 1960s made appear changes of the 1950s as cosmetic. A mistake addressed in this paper.

RT35- **Prevention of congenital toxoplasmosis**

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In several European countries, national plans have been implemented for the prevention of congenital toxoplasmosis. In France, this program is based on a serological assay for anti-*Toxoplasma* antibodies performed during the first medical examination of the pregnant woman. In the event of a negative serological assay, regular antibody monitoring (every 3-4 weeks) and strict hygienic rules are prescribed (avoid eating raw or rare meat, raw vegetables and fruits particularly those grown on soil and avoid contact with ground contaminated by cat feces). The serologic monitoring is continued throughout pregnancy. In the event of an infection (appearance of antibodies), spiramycin treatment (3x 3M IU per day) is prescribed and a prenatal diagnosis will be performed, at least 4 weeks after the infection and beyond the 18-20th week of pregnancy. Prenatal diagnosis consists of amniotic liquid puncture in which *Toxoplasma* will be searched for by PCR and mice inoculation. PCR will give a result in a few hours

whereas mice inoculation results will be obtained in a few weeks. If the prenatal diagnosis is positive, a maternal-fetal treatment by sulphadiazine-pyrimethamine or sulfadoxine-pyrimethamine is continued until childbirth. Moreover, monthly ultrasonography is carried out until end of pregnancy and if severe fetal lesions are seen, a pregnancy medical interruption can be considered. After birth, thorough clinical (neurological and ophthalmologic), radiological and biological examinations are performed. *Toxoplasma* can be searched by inoculating a piece of placenta to mice (not necessary if PCR has been performed on the amniotic fluid). If the prenatal and post natal assessments are negative, a follow-up of the child is done at regular intervals until a one year age. Repeated serological assays will check mother transmitted antibodies disappearance. In the event of a positive diagnosis, sulphamide & pyrimethamine treatment is continued during 12 to 18 months. Recent techniques have allowed progresses in infection dating (antibodies avidity), in early detection of the own antibodies of the new-born (mother-child comparative western-blots) and in parasitic load quantification (real time PCR). The implementation of a prevention program similar to the French program is likely to be very expensive. A serological assay at the beginning of the pregnancy, regular ultrasonography follow-up and IgM testing of the child at birth could allow an early treatment of possible cases of congenital toxoplasmosis. But this program will not prevent the occurrence of serious debilitating complications which could have benefited from an in utero treatment. Cost-effectiveness and different options for the prevention of congenital toxoplasmosis will be discussed.

RT36- **Polymorphisms in *CXCL9* and *CXCL10* may control myocardial chemokine expression and intensity of myocarditis in Chagas disease cardiomyopathy**

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Background: Chronic Chagas' disease cardiomyopathy (CCC), a life-threatening inflammatory dilated cardiomyopathy, affects a proportion of the ca. 8 million patients infected by *Trypanosoma cruzi.* Even though the Th1 T cell-rich myocarditis plays an obvious role in CCC pathogenesis, little is known about the factors controlling inflammatory cell migration to CCC myocardial tissue. **Methods and Results:** Using confocal immunofluorescence and quantitative PCR, we studied cell surface staining and gene expression of chemokines and receptors of the CXCR3, CCR4, CCR5 and CCR7 axes in end-stage samples of CCC myocardium. CCR5+, CXCR3+, CCR4+, CCL5+ and CXCL9+ mononuclear cells were observed in CCC myocardium. mRNA expression of the chemokines CCL5, CXCL9, CXCL10, CCL17, CCL19 and to a lesser extent CCL3, CCL4, CCL21, as well as their receptors, was up regulated in CCC myocardium. CXCL9 mRNA expression directly correlated with the intensity of myocarditis, as well as with mRNA expression of chemokines and receptors of the CXCR3, CCR4, CCR5 and CCR7 axes. We also analyzed single-nucleotide polymorphisms for genes encoding such the most highly expressed chemokines and receptors in a cohort of Chagas disease patients. CC C patients with ventricular dysfunction displayed reduced genotypic frequencies of CXCL9rs10336GG, CXCL103921GG, and increased CCR5rs1799988CC as compared to those without dysfunction. Significantly, myocardial samples from CCC patients displaying the "protective" CXCL9/CXCL10 genotypes displayed a 2-6 fold reduction in mRNA expression of CXCL9, CXCL10, and other chemokines and receptors, along with reduced intensity of myocarditis, as compared to those with other CXCL9/CXCL10 genotypes. **Conclusions:** Our results indicate that protective genotypes in the closely linked CXCL9 and CXCL10 genes may modulate local expression of the chemokines themselves, and simultaneously affect myocardial expression of other key chemokines. Together with the correlation of CXCL9 expression with intensity of myocarditis, our results may indicate that CXCL9 and CXCL10 are master regulators of myocardial inflammatory cell migration, perhaps affecting clinical progression to the life-threatening form of CCC.

RT37- **Molecular Mimicry as a Mechanism for the Initiation of Autoimmunity in Chagas Disease**

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Trypanosoma cruzi is the protozoan parasite that causes Chagas disease, a complex illness in which infected individuals may have no symptoms of infection or may develop cardiomyopathy or mega disease of the esophagus or colon. Several mechanisms may contribute to the development of cardiomyopathy, including (1) parasite-mediated myocytolysis, (2) microvasculopathy, (3) dysautonomia, (4) immunity to persistent parasites, (5) parasite-derived toxin and (6) autoimmunity. There are two major questions about autoimmune pathogenesis: What is its origin? And what is its role in pathogenesis? We have investigated both using a variety of combinations of parasite and mouse strains giving the varied cardiac outcomes observed in human infection: (1) parasitosis with myocarditis, (2) neither parasitosis nor myocarditis (resistance), and (3) myocarditis without parasitosis. Initially, we focused on the *T. cruzi* Brazil -A/J mouse combination, which has the first outcome above, and came to believe that, for this combination, parasite myocytolysis leading to host repair was the primary mechanism of inflammation. However, these mice also developed massive autoimmunity as well with cardiac myosin as the dominant autoantigen, of a magnitude similar to that observed in mice with myosin-induced experimental autoimmune myocarditis (no *T. cruzi*). This implied that myosin autoimmunity contributed to inflammation. We employed a powerful mechanism of antigen-specific peripheral immune tolerance to shut down myosin-specific autoimmune responses and found that infected mice still developed myocarditis, indicating either that additional mechanisms of inflammation (listed above) were operative as well. It was also possible that cardiac autoimmunity with additional specificity developed as well, either through epitope spreading after the myosin response or through bystander activation from the initial myocytolysis event. It also occurred to us that an additional mechanism—molecular mimicry—might underlie the development of autoimmunity. To test this, we immunized mice with a *T. cruzi* protein powder or heat-killed *T. cruzi* and, to our initial surprise, mice developed strong autoimmunity in addition to the expected parasite-specific immunity. Again, myosin was the dominant autoantigen, although many other cardiac autoimmune responses developed as well. Importantly, parasite-specific immunity developed in myosin-immunized mice as well (bidirectional crossreactivity) and these responses were cross-tolerizable as well (myosin autoimmunity could be prevented by tolerization to *T. cruzi*). Further, injection of heat-killed *T. cruzi* led to cardiac damage and release of serum cardiac troponin I. The basis for the difference in whether the myosin autoimmunity is relatively benign (heat-killed *T. cruzi* immunization) or pathogenic (myosin immunization) is under investigation. Finally, we investigated the role of parasite persistence in autoimmunity. Elimination of *T. cruzi* with benznidazole treatment caused a resolution of autoimmunity as well, which returned rapidly upon exposure to the parasite. Thus, although autoimmunity may be pathogenic, it is also dependent on the presence of the parasite and is not self-propagating. **E-mail:** d-engman@northwestern.edu.

RT38- **The *Trypanosoma cruzi* Infection in Humans and Experimental animals: Parasite-free Chagas Heart Disease in the chicken model.**

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Abstract: The *Trypanosoma cruzi* autochthonous in America is now present in all Continents. The human acute *T. cruzi* infections can be asymptomatic but chronically infected individuals die of Chagas disease. The parasite mitochondrial kDNA minicircle transfer to the genome of chagasics can explain the pathogenesis of the disease; in Chagas cases with evident cardiomyopathy the kDNA minicircles integrate mainly in retrotransposons at several chromosomes, but the minicircles are detected also in coding regions of genes that regulate cell growth, differentiation, and immune responses. An accurate evaluation of the role played by the genotype alterations in the autoimmune rejection of self-tissues in Chagas disease is achieved in the crosskingdom chicken model system refractory to the *T. cruzi*

infections. The inoculation of *T. cruzi* in embryonated eggs prior to incubation generates parasite-free chicks, which retain the kDNA minicircle sequence mainly in the macrochromosomes coding genes. The crossbreeding transfers the kDNA mutations to the chicken progeny. The kDNA-mutated chickens develop severe cardiomyopathy in adult life and die of heart failure. The phenotyping of the lesions reveals cytotoxic CD45, CD8γδ, CD8α T-lymphocytes carry out rejection of the chicken heart. These results suggest that the inflammatory cardiomyopathy of Chagas disease is a genetically driven autoimmune disease. **E-mail:** antonioteixeirarl@gmail.com

RT39- **Much to Learn from Geographic Studies of Sandflies and Leishmaniasis: Transmission Dynamics, Climate Change, and Vector Distributions.**

Peterson, A. T., S. Brandao Filho, and J. Shaw

Studies of leishmaniasis transmission have been challenged by several gaps in current knowledge of the transmission cycle of this disease system. One particularly important gap regarding this study includes the complex interactions among spatial distributions of host vertebrates, phlebotomine vectors, *Leishmania* species, and human cases: rarely is information comprehensive regarding entire geographic distributions, and never has information been assembled for multiple dimensions of this transmission cycle. We document how such comprehensive information can provide useful, novel, and detailed information regarding leishmaniasis transmission, informing about the suite of vector species necessary to explain transmission of a given *Leishmania* species, likely effects of climate change on transmission risk, etc. We also document knowledge gaps, which limit current ability to take advantage of these novel inferences.

RT40- **New criteria for incriminating Leishmania vectors**

Elizabeth Rangel, Paul Ready

Leishmaniasis transmission cycles have long been associated with distinctive landscapes, and consequently landscape epidemiology has often preoccupied “leishmaniacs”. Reports frequently try to identify one primary vector, and for zoonotic leishmaniasis one primary reservoir host, associated with a single transmission cycle that is assumed to predominate in each landscape. However, there is increasing evidence that each *Leishmania* species is often transmitted by more than one species of phlebotomine sand fly, even within a single ecotope and geographical region in the Old World. It is difficult to incriminate a sand fly species as a vector, and a consistent approach is required. Proposals have been made for updating existing criteria for incrimination and adding new criteria (Ready, P.D. 2013. Biology of phlebotomine sandflies as vectors of disease agents. *Annual Review of Entomology*, in press). Some sand fly species can transmit a *Leishmania* species but might not be able to help maintain a transmission cycle. Therefore, epidemiological criteria are required to incriminate vectors that are biomedically important, and this involves transmission modelling. Approaches to such modelling will be presented for discussion. This level of thoroughness may not always be possible or cost effective, but the modelling of transmission in more leishmaniasis foci would help us to understand better the significance for disease control of “identifying” a vector.

RT41- **Developing safe and efficacious drugs to treat Chagas disease**

DNDI Session

Introduction: Of all the neglected diseases, Chagas is among the diseases that receive the least investment for R&D. It is caused by the *Trypanosoma cruzi* parasite, transmitted by the bites of insects known as “kissing bugs”. A silent killer, it affects millions across the globe and takes approximately 12,000 lives every year. The disease occurs in two phases: the first phase is often asymptomatic or unrecognized due to non-specific symptoms. The second phase is a chronic one that can affect the heart and the gastrointestinal tract and, if left untreated, can lead to death. **Materials and Methods:** Existing

drugs, benznidazole and nifurtimox, have been used for decades, but because their efficacy against the chronic phase of the infection is poorly documented, they are of limited use in disease control strategies. In addition, long treatment periods (60-90 days) make patient compliance challenging, with increased risk of drug resistance development. In addition, until recently, there was no adapted paediatric formulation for either of the existing drugs. **Results:** As part of a short term strategy to address urgent patient needs, DNDi and its partners have recently developed a pediatric formulation of benznidazole, manufactured in Brazil by LAFEPE. In the long term, new treatments that are safe, efficacious, and effective against the chronic phase of the disease – which is when most patients are diagnosed – are sorely needed to effectively fight the disease. In addition, a better understanding of biomarkers is essential to gain understanding of the disease progression and ease the development of test-of-cure diagnosis tools that support drug development. **Main conclusions:** The session will explore the current state of the Chagas disease portfolio and give an overview of what is needed to provide patients with a safe, efficacious, easy-to-use and affordable treatment, with a focus on early drug discovery and assessment of treatment efficacy.

RT42- **Mycoses of Implantation: How neglected they are**

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The neglected diseases constitute a group of tropical or subtropical infections, which are especially endemic in low-income populations in developing regions of Africa, Asia, and Latin America. The World Health Organization (WHO) as a symptom of poverty and disadvantage acknowledges them. A list of endemic diseases, including helminthic, protozoan, bacterial and viral infections but not fungal diseases. The endemic mycoses encompass the systemic and the implantation mycoses. Both groups are associated to substantial morbidity and mortality in the endemic areas, so they are orphan and they should be included among the neglected diseases. The **Implantation mycoses** encompass a heterogeneous group of fungal diseases that have in common the fact that they develop at the site of a penetrating transcutaneous trauma. The majority of causative agents of the implantation mycoses are found in soil, vegetation, and decaying matter in tropical, subtropical, and humid environments, and infection is usually the result of penetrating injury. Infections especially occur in low socioeconomic groups; in those living in rural areas or involved in farming, hunting, or other outdoor activities; and particularly in adult men. The list of implantation mycoses includes sporotrichosis, eumycetoma, chromoblastomycosis, phaeohyphomycosis, entomophtoramycosis (subcutaneous zygomycosis), and lacaziosis (lobomycosis). They are also known as “subcutaneous mycoses” but this term seems to be inadequate because some of these infections may also involve other sites like muscles, fascia, cartilage and bones, beyond the skin and the subcutaneous tissues. Mycoses of implantation are a frequent health problem in tropical and subtropical areas around the world. They are chronic diseases with frequent delays in the diagnosis, may be caused by a variety of fungi and are difficult to treat. Treatment success depends on an early diagnosis and the choice of the appropriate treatment that may combine medical and/or surgical therapies. Most agents that cause these infections respond to triazole especially itraconazole. For refractory cases, the second-generation triazoles (voriconazole, posaconazole and isavuconazole) are good options. Mycoses of implantation are challenging orphan diseases and relatively neglected by researchers. Diseases linked to poverty likewise offer little incentive to industry to invest in developing new or better products for a market that cannot pay.

RT43- **Chromoblastomycosis in Mainland China**

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As a chronic, cutaneous and subcutaneous infection, more than 589 cases of chromoblastomycosis have been reported in China since 1950s. Several dematiaceous fungi are involved with the disease etiology in China, but *Cladophialophora carrionii* is the most common agents in the north, *F. monophora and Fonsecaea pedrosoi* are most common agents in the southern part of the China. Infection commonly

initiated after the etiologic agents gain entrance through puncture wounds and more common involved extremities of the males. Clinically, skin lesions are found in different sites, like the extremities, buttocks, trunk and face, and presenting a diversity morphology including erythematous cutaneous-subcutaneous nodules, vegetating plaques, scaling cauliflower-like tumors, verrucose lesions, ulcers and crusts, scar. There are seven different clinical types found in Mainland China: plaque type, tumoral type, cicatricial type, verrucous type, pseudo-vacuole type, eczymatous type and mixed type of lesions. The success of treatment for chromoblastomycosis is related to the causative agent, the clinical form and severity of the lesions. Though several new antifungal agents can be the choice, chromoblastomycosis still is a therapeutic challenge for clinicians due to the recalcitrant nature of the disease. Most of the patients can be treated successfully with the physical treatment, chemotherapy and combination therapy. The itraconazole, terbinafine or a combination of both, or voriconazole are commonly medication for these patients. Photodynamic therapy (PDT) primarily developed as a treatment for cancer has interest been proposed as a therapy for a large variety of localized infections in recent years, but very rare in fungal infection. We applied it on some recalcitrant cases of chromoblastomycosis and found its good clinical response, and hopeful to be a promising therapy in near future. **E-mail:** xiliyan@mail.sysu.edu.cn

RT44- **ACTs for the treatment of *P. vivax* malaria**

Professor Ric N Price
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Early parasitological diagnosis and treatment with artemisinin-based combination therapies (ACT) are seen as key components of global malaria control programs, although the evidence for this comes almost entirely from studies of *P. falciparum.* Blood-stage *P. vivax* infections are mostly still treated with chloroquine. A separate treatment scenario maybe justifiable where *P. vivax* remains sensitive to chloroquine, providing that diagnostic tests reliably distinguish *P. vivax* from *P. falciparum*. However, the high frequency of misdiagnosis in routine practice and the rise and spread of chloroquine-resistant *P. vivax* provides a compelling rationale for a unified ACT-based strategy for vivax and falciparum malaria in co-endemic areas. This presentation will review the evidence for the role of ACT in the treatment of *P. vivax* and the implications for the impact on morbidity and mortality of deploying a unified policy for uncomplicated malaria.

RT45- **New products in research for the treatment of *P. vivax* malaria**

Dr Didier Leroy
Director Drug Discovery, Medicines for Malaria Venture

The only approved medicine able to eliminate hypnozoites and thus provide a radical cure for *P. vivax* is primaquine – an 8-aminoquinoline that triggers hemolysis in G6PD-deficient patients. Today, Medicines for Malaria Venture (MMV) is actively looking for new molecules that will provide a radical cure for *P. vivax* malaria and therefore put an end to the relapse. Historically, research to discover such new classes of medicines has been hampered by the lack of a suitable model to mimic the hypnozoite, on which we could test new compounds. The absence of long term *in vitro* blood stage culture systems of *P. vivax* is still the roadblock that impedes to assess the effect of new molecules against liver stages *in vitro*. To overcome this gap, surrogate *in vitro* assays with *P. yoelii* a rodent parasite and *P. cynomolgi*, a non-human primate parasite are now part of MMV's drug discovery strategy and have started to deliver few promising lead molecules with potential anti-hypnozoite properties. Emerging *in vivo* models in rodents are the next key components to be integrated into this strategy. Recently, in collaboration with MMV, various research teams have developed drug testing assays covering the entire life cycle of *Plasmodium* and marketed antimalarials, as well as molecules in development, have been tested in these assays. This presentation will show the main outcomes from these studies and reveal the test cascade that is currently in place to identify potential new antimalarials targeting the liver stages of *P. vivax*.

RT46- **Radical cure of *P. vivax* malaria (8-aminoquinolines and tafenoquine) Symposium: Challenges of *P. vivax* treatment**

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Universidad Peruana Cayetano Heredia

Researchers are actively looking for new molecules that will provide a radical cure for *P. vivax* malaria, replace the only licensed anti-hypnozoite primaquine, and therefore put an end to the relapse threat. A next-generation 8-aminoquinoline, tafenoquine, is in clinical development for this indication. A phase IIb dose ranging study being conducted in partnership between Medicines for Malaria Venture and GlaxoSmithKline is on-going in several countries of Asia and South America. Earlier studies showed that tafenoquine could be taken with chloroquine as a 1 to 3-day treatment course to remove this liver stage, which could be seen as a significant improvement on primaquine's 14-day course by treatment non-compliers and prescribers currently resistant to use of radical cure. As tafenoquine is of the same chemical family as primaquine, G6PD related haemolysis remains a significant safety risk. Therefore an additional study on the safety of tafenoquine in G6PD-deficient patients is also currently on-going in Thailand. This session will provide an overview of these and other study data related to use of tafenoquine as a radical cure for *P. vivax* malaria.

RT47- **Early diagnosis of Chagas disease reactivation and T.cruzi genotyping by PCR analyses directly in tissues of patients submitted to heart transplantation**

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Chagas disease has a variable clinical course, ranging from symptomless infection to severe chronic disease with cardiovascular or gastrointestinal involvement. The factors influencing this clinical variability have not been elucidated, but genetic aspects of both the host and the parasite has been evoked. Chagas heart disease (CHD) is the most serious and frequent manifestation, affecting 25-30% of infected individuals and representing the leading cause of myocarditis worldwide. Heart transplantation (HTx) is a useful therapy for end-stage of CHD, although Chagas reactivation remains as a major complication. In the last six years, 112 HTx were carried out at the Clinical Hospital of the UFMG, from which 50 were from patients suffering from CHD. After HTx, patients are submitted to periodic endomyocardial biopsies to monitor transplant rejection and Chagas reactivation. On average 50% of the transplanted patients developed infection reactivation within a period varying from 3 to 24 months post-transplant. Since amastigotes are rarely found in histopathological analyses of the biopsies, it is very difficult to achieve differential diagnosis between inflammatory process resulting from allograft rejection and from infection reactivation. The aim of this study was to investigate the usefulness of PCR strategies for early identification of *Trypanosoma cruzi* DNA in the follow up endomyocardial biopsies and also to genotype the parasites presented in explanted tissues obtained from the transplanted patients. Diagnoses were conducted by PCR targeted to the kDNA and qPCR to the 24Sα rRNA gene. The tissues samples encompassed heart explants, blood, and endomyocardial biopsies. From 35 patients and 250 samples so far analyzed, *T. cruzi* DNA was detected in 70 samples of 23 patients. For further parasite genotyping we used a three-based PCR protocol developed by our group, which includes polymorphism analyses of the cytochrome oxidase subunit II (COII), spliced leader intergenic region (SL-IR) and ribosomal DNA (rDNA 24Sα) genes. From 70 positive tissues genotyped up to now, we identified TcII in 66 samples, and one case of each: TcVI, TcV or TcVI (it was not possible yet to distinguish between these two possibilities), a mixed infection of TcII and TcVI, and a case of TcI. This last may represent the first reported case of TcI detected directly in heart of a CHD patient in Brazil. In a retrospective study with 4 patients who presented reactivation of infection, positive results were detected in the firsts held endomyocardial biopsies, 1–18 months earlier than the clinical reactivation. These results indicate that PCR is a good

strategy to the early diagnosis of Chagas disease reactivation with potential to assist physicians in treatment decisions before onset of reactivation. **E-mail:** andrea@icb.ufmg.br

RT48- **Chronicles of Malaria and HIV: a dance of antibodies.**

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Plasmodium falciparum and HIV are two of the major pathogens in terms of morbidity and mortality in Africa. Among those affected by *Plasmodium falciparum*, pregnant women represent a special group as they are more susceptible than their adult non-pregnant counterparts. Among these, primigravidae (women experiencing their first pregnancy) and secundigravidae (women on their second pregnancy) are the most susceptible. This susceptibility decreases with increasing number of pregnancies and is generally accompanied by an increase in the levels of antibodies specific for a parasite phenotype that binds in the placenta. It has been established that co-infection of *Plasmodium falciparum* and HIV exacerbates the effects of both pathogens. In line with this, HIV seems to decrease the levels of antibodies to pregnancy-specific variants of *Plasmodium falciparum* in pregnant women. We measured the levels of antibodies to pregnancy-specific variants of *Plasmodium falciparum* in the serum of a cohort of HIV-infected and HIV-uninfected primigravidae and secundigravidae. Total levels of parasite-specific IgG, levels of parasite-specific phagocytic antibodies and levels of total and antigen specific IgE were evaluated. Levels of total IgG and phagocytic antibodies were decreased in HIV-infected women when compared to their non-infected counterparts. Levels of phagocytic antibodies were associated with higher birth weight in secundigravidae only. Parasite-specific IgE were shown to be associated with the phagocytic activity of the serum of HIV-infected primigravidae. These results suggest that, in HIV-positive women with low levels of parasite-specific IgG, IgE might have a role in the response to *Plasmodium falciparum*.

RT49- **Chagas disease and HIV infection**

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From June 1889 until April 2012, 60 HIV/*T. cruzi* co-infected patients were followed up in the Faculdade de Medicina, USP, 13 presented reactivation of Chagas disease. Sex distribution showed 37 men and 23 women, from 23 to 59 years old; CD4/mm^3 levels were < 200/mm^3 in 43.1%, between 200 and 350/ mm^3 in 15.7% and > 350/ mm^3 in 41.2 %. The distribution of chronic Chagas disease form at the first assessment was: 15% Typical cardiopathy; 11.6% Non-Typical Cardiopathy; 3.3% Non-classified Cardiopathy; 5.0% Typical Cardiopathy + Digestive form; 5.0% Non-Typical Cardiopathy + Digestive form; 6.7% Digestive form; 36.7% Indeterminate form; 15.0% Reactivation; 1.7% not reported. Considering 60 patients, 4 were presented reactivation after 12-84 months of follow-up and 9 were diagnosed as reactivation at the first assessment. The rate of reactivation of co-infected HIV/*T. cruzi* patients after 6 months of follow-up was 9.8% The clinical forms of 13 patients with reactivation were: Myocarditis 30.7%; Meningoencefalitis 30.7%; Meningomyelitis 15.4%; Oligosymptomatic 15.4% (1 Oligosymptomatic mother with indeterminate form had a baby with congenital Chagas disease); Erythema nodosum 7.7%. The relationship between increased parasitaemia and simultaneous increased HIV viral load was registered in one patient during the reactivation period. Multicenter study and one study in the USP showed higher rate of parasitaemia by xenodiagnosis and/or blood culture in HIV infected patients in comparison with HIV non infected patients. Higher rate of positive nymphs per exam (> 10% + nymphs was observed in 44, 8% HIV infected patients and in 3, 7% HIV non infected patients). HIV infection is the only factor associated with increased parasitaemia. In a comparative study with 29 HIV/*T. cruzi* co-infected patients and 57 Chagas disease patients, significant higher levels of parasite DNA both by competitive PCR and qPCR were reported in co-infected patients, and the highest levels were registered in those with reactivation of

Chagas disease. The authors also compared qualitative PCR with KDNA primers and showed its higher sensitivity in comparison to parasitological methods. Positive correlation between *T. cruzi* parasitaemia and HIV viral load as well as negative correlation between parasitaemia and CD4+ cells/mm^3 was demonstrated. Benznidazole was recommended (5-10 mg/kg/d) during 60 days for the reactivation of Chagas disease. In our series, 8 patients without reactivation but with a high parasitaemia (> 20% of nymphs positive by *in vitro* xenodiagnosis test) also received treatment. Successfully outcome was registered in patients that finish 60 days of the treatment; 8 of the 13 patients died by Chagas disease, five by reactivation (2 without treatment). Adverse effects such as exanthema, fever, leucopenia, thrombocytopenia, polyneuropathy and ocular neuritis were observed in 6 out of 13 patients. No secondary prophylaxis has been prescribed and no recidives were seen. The high incidence of congenital Chagas disease in co-infected mothers' patients emphasizes the need of monitoring this gestational age group for early treatment aiming to avoid reactivation in this age group and congenital disease transmission, as recommended by "Network for care and Studies on HIV/*Trypanosoma cruzi* infection and other immunosuppressive conditions".

RT50- **The new consensus for *Trypanosoma cruzi* classification: epidemiological relevance.**

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The diversity of the *T. cruzi* genotypes and phenotypes is well recognized along with its eco-epidemiological complexity. Designation of relevant groups for *T. cruzi* has oscillated between a few discrete groups and many. In 2009, in an effort to unify the nomenclature of *T. cruzi*, an expert committee recommended that *T. cruzi* strains should be referred to by six discrete typing units (DTUs *T. cruzi* I-VI) (Zingales et al., 2009). The goal of a unified nomenclature is to improve communication within the scientific community involved in *T. cruzi* research and guide future investigation on comparative epidemiology and pathology. To achieve this aim a straightforward and reproducible genotyping strategy is required for DTU assignment. At present some typing approaches are available for widespread use in endemic areas, and research is in progress to optimize sensitivity so that they may be applied directly to clinical and biological samples. The ecological history of *T. cruzi* DTUs has been traced, although for some DTUs the level of sampling is insufficient (reviewed in Zingales et al., 2012). In terms of propensity to cause Chagas disease, all six DTUs are infective to humans. Great regional diversity of disease severity and the nature of the chronic infection have been reported, attributed to a set of complex interactions including parasite genetics, host genetics, and environmental factors. TcI is implicated with human disease in Amazonia, the Andean region, Central America, and Mexico. Clinical presentations of this DTU include chagasic cardiomyopathy. In the Southern Cone region, TcII, TcV and TcVI are the main causes of Chagas disease. TcII predominates in eastern and central Brazil, TcV in Argentina, Bolivia, and Paraguay, and TcVI in the Gran Chaco. Throughout the Southern Cone chagasic cardiomyopathy can be severe, and a proportion of cases may develop digestive syndromes. TcIII and TcIV are rare in chronic infections. A major goal of *T. cruzi* taxonomic studies is to identify links between the infecting DTUs and the clinical presentation of the disease. Conclusions of clinical manifestations and parasite genotype are complicated for several reasons, among which is the fact that isolates from blood do not necessarily reveal the full complement of infecting parasites and mixed infections are common. A DTU-specific serology could provide a historical profile of all DTUs infecting a patient. Natural resistance of *T. cruzi* strains to the available drugs for Chagas disease treatment (benznidazole and nifurtimox) is recognized but no association to a specific DTU has been found. Nevertheless, evaluations of serological conversion carried out to measure treatment effectiveness indicate relatively high seroconversion rates in Central America as compared to Southern Cone countries. One primary explanation may be the presence of different DTUs circulating in these geographic regions. The DTU nomenclature is certainly a dynamic structure as research progresses and more discoveries are made. Additionally, sequence data reveal that considerable genetic variation is hidden at the sub-DTU level. For the immediate future multilocus sequence typing is likely to be the gold standard for population studies. It is expected that greater advances in our knowledge on pathogenic and epidemiological features of these parasites will occur in

the coming decade through the comparative analysis of the genomes from isolates of various DTUs. **E-mail:** zingales@iq.usp.br

RT51- **"*Trypanosoma rangeli* and *T. cruzi* strains' interactions with triatomines and the resulting effects on transmission cycles"**

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The complex interactions between trypanosome strains and the immune system of triatomines or between trypanosomatids and symbionts microorganisms in insects' intestines are determinant factors in parasite survival or elimination in a particular vector. There has been growing interest in understanding the determinant factors concerning *T. cruzi* and *T. rangeli* genotypes' geographical distribution and their association with domestic or sylvatic transmission cycles. The Tropical Parasitology Research Laboratory (Laboratorio de Investigaciones en Parasitología Tropical - LIPT), in collaboration with several Colombian and foreign research groups, has been working on the trypanosome-vector interaction during the last few years, finding a close association between trypanosome genotypes and the vector's phylogenetic lineages, suggesting a parasite-vector co-evolutionary relationship. **Interactions between *Trypanosoma rangeli* strains and *Rhodnius* species** it has been observed that *Rhodnius* species are susceptible to *T. rangeli* strains having the same geographical origin (Cuba-Cuba, 1998; D'Alessandro and Saravia*,* 1992). Several *T. rangeli* strains have been shown to have variable behaviour in different *Rhodnius* species; for example, strains from Colombia and Costa Rica do not produce metacyclic trypomastigotes in *R. pallescens* or in *R. ecuadoriensis.* On the other hand, the salivary glands of *R. neglectus* do not become invaded following infection by strains from Panamá or Costa Rica (D'Alessandro, 1976). Additionally, it has been observed that *T. rangeli* strains isolated from *R. colombiensis* have not developed in *R. prolixus* salivary glands (Vallejo *et al*., 2002). All such findings have shown the extensive biological variability of *T. rangeli* strains from different geographical origins, suggesting the existence of different genotypes. Two molecularly different groups have been described, called *T. rangeli* KP1+ and *T. rangeli* KP1-, based on kDNA amplification products (Vallejo *et al*., 2002, 2003; Urrea *et al*., 2005; Salazar-Anton *et al*., 2009). Close molecular characterisation of more than 100 *T. rangeli* strains isolated from the salivary glands of different vector species' from several geographical regions in Latin-America has confirmed the existence of these two groups. Other molecular polymorphisms have been detected in the Spliced leader intergenic region (SL-IR) and SSU rRNA sequences, revealing five genotypes (A, B, C, D, E) (Maia da Silva *et al*., 2004a, 2004b, 2007, 2009). Comparative analysis of these genotyping systems (kDNA, SL-IR and SSU rRNA) have indicated that genotype A corresponds to *T. rangeli* KP1(+) strains whilst B, C, D and E genotypes are polymorphic subgroups within *T. rangeli* KP1(-) (Carranza *et al*., 2012, unpublished data). Urrea *et al*., (2011) have sequenced the spliced leader (SL-IR) intergenic region from 24 *Trypanosoma rangeli* strains isolated from *Rhodnius colombiensis*, *R. ecuadoriensis*, *R. pallescens* and *R. prolixus* salivary glands. This work revealed the existence of 4 genotypes having microsatellite repeats CA, GT, TA, ATT and GTAT and the presence of characteristic indel and SNPs for each genotype. Such genotypes have been called "robustus", "pallescens", "colombiensis" and "ecuadoriensis", showing that the strains isolated from the same vector species or the same phylogenetic line of vectors have the same genotypes, even in cases where the strains were isolated from vectors captured in geographically distant regions or where strains were isolated from vectors from the same evolutionary line from the *Rhodnius* genus*.* The dendrograms constructed from analysing the miniexon gene's intergene spacer sequences and RAPD analysis had the same topology, suggesting ancestral co-evolutionary association between *T. rangeli* genotypes and its vectors. Recent observations have indicated that triatomines selectively transmit different parasite subpopulations, thereby explaining determined genotypes' predominance in different regions of Latin-America. Pulido *et al*., (2008) and Zabala *et al*., (2011) have demonstrated a trypanolytic factor in *R. prolixus* haemolymph; such trypanolytic factor selectively lyses *T. rangeli* KP1 (-) populations, but not KP1

(+) populations, so that only KP1 (+) strains are isolated in vectors and vertebrates but not KP1 (-) strains in regions where *R. prolixus* is found (Vallejo *et al*., 2009, Urrea *et al*., 2011). *T. rangeli* genotypes are thus found to be separately associated with vectors living in some cases in sympathy with a lack of genetic flow between parasites. Additional molecular studies involving many more *T. rangeli* isolates from other *Rhodnius* species or subpopulations (e.g. from *R. pallescens* I from Panamá and *R. ecuadoriensis* I from Ecuador) will be most useful for understanding *T. rangeli* epidemiology and genetic structure and its role regarding the epidemiology of Chagas' disease. **Interactions between *Trypanosoma cruzi* strains and triatomine species** the nomenclature for six *T. cruzi* genotypes (I-VI) has been unified recently (Zingales *et al*., 2009; 2012); however, few studies have been made concerning the differential transmission of *T. cruzi* subpopulations in different triatomine species. Previous work has shown that the vector's immune response could be determinant in the transmission of different *T. cruzi* genotypes (García *et al*., 2010). Our research group has thus examined *R. prolixus* haemolymph trypanolytic factors' activity against *T. cruzi* I-VI, finding evidence of trypanolytic activity against *T. cruzi* II, V and VI, agglutination against *T. cruzi* IV, but no activity against *T. cruzi* I and III (Zabala *et al*., 2011). Studies are currently being carried out aimed at detecting lytic factors and agglutinins present in the haemolymph and in the intestinal compartments of different triatomine species in Colombia. The *R. prolixus* haemolymph has lytic activity against genotypes which are absent from areas where this vector is distributed, but lacks lytic activity against *T. cruzi* I which is precisely the predominant genotype in areas of *R. prolixus* distribution. The latter observation strengthens the hypothesis that *T. cruzi* genotypes' (I-VI) geographical distribution would be determined by local triatomine species' vector ability to transmit determined genotypes in particular and impede the transmission of other genotypes. Even though the co-evolution of trypanosomes and their vectors continues being a working hypothesis in Latin-American countries, to date this hypothesis has not been adopted by vigorous phylogenetic studies using an important number of genes from vectors and from parasites transmitting them. However, the results presented at this conference about *Trypanosoma rangeli* and *T. cruzi* genotypes' interaction with different triatomine species suggests a certain degree of evolutionary association and invites the exploration of new methodologies for strengthening the parasite-vector co-evolution hypothesis regarding American trypanosomiasis and very precisely identifying genes from parasites or genes from vectors implicated in the transmission of determined trypanosome genotypes. **Supported by:** COLCIENCIAS and the Universidad Del Tolima's research fund

RT52- **Accurate diagnosis of schistosomiasis: how do current tools measure up?**

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Accurate diagnosis of schistosomiasis, especially in the context of disease control in sub-Saharan Africa, poses a significant challenge owing to the varied needs, settings and expenses in which present diagnostic tools are applied. Even though the present repertoire of tools is rather limited, optimization of their use has not been fully explored which has led to the confusion, for example, if a geographical area is to be designated disease endemic or if an individual is shown to be diseased or not. Existing assays are typically focused upon visualization of schistosome eggs in host excreta by microscopy or by capture of excreted worm antigens in immuno-chromatographic dipsticks or by measurement of host antibody titers to egg antigens. The complex lifecycle and aquatic infection dynamic of this dioeciously parasite, makes sensitive detection of worms within the human host problematic. For instance, detection of infections when adult female worms are not yet egg-patent is particularly contentious or when the adult worm burden is 'light' and so egg output is at best sporadic. Similarly, each of the diagnostic tools have differing levels of sensitivity and precision during the evolution of disease within a given individual as the infection progresses through the pre-patent, acute and then chronic stages. In addition, there is also a limited set of morbidity markers which can track the severity of disease and its putative reversion after periodic treatment(s) with praziquantel. In this review I hope to highlight application of several of these methods with particular reference to the discovery of two 'new' burdens of schistosomiasis, the detection of infections within infants and preschool-age children, and anthropozoonotic transmission in chimpanzees. **E-mail:** jrstoth@liv.ac.uk

RT53- **Characterization of placental malaria by *Plasmodium Falciparum* infection in Luanda - Angola**

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Introduction: Malaria during pregnancy remains a serious public health problem. The aim of this study was to determine the prevalence and risk factors of *P. falciparum* infection in Angolan pregnant women living in Luanda province. **Methods**: We conducted a cross-sectional study involving 866 women when they presented for delivery at the Augusto Ngangula and Lucrécia Paim Maternity Hospitals between April 2006 and February 2008. *P. falciparum* was diagnosed by light microscopy and PCR assay. Maternal peripheral blood was collected by finger prick and after delivery cord blood and four sub-samples per placenta were collected onto filter paper. Microsatellites were used to genotype *P. falciparum* from peripheral, placental and umbilical cord blood from 143 pregnant women who were infected at least in one of the 3 compartments. **Results**: The average women age was 24.1 years, 39.6% were primiparous and 60.4% were multiparous. One in six women had *P. falciparum* at delivery. The prevalence of *P. falciparum* infection in pregnant women studied was 15.6%. Age, residence and history of malaria during pregnancy were significantly associated with *P. falciparum infection,* but gravidity and use of anti-malarial drugs were not. Moreover, infections in placenta were significantly higher in women ≤ 18 years old and primigravidae, but we could not correlate placental infections with poor pregnancy outcomes. The 9 loci studied were highly polymorphic. **Conclusions**: The presence of parasites in cord blood suggests congenital malaria. All these findings are relevant to policies in relation to malaria control in pregnant women in Luanda, Angola. **Keywords:** Malaria, Pregnancy, *Plasmodium falciparum*, Angola.

RT54- **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-g, TNF, IL1-b, IL-6, IL-10, TGF-b, 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

RT55- ***Plasmodium vivax*** **and placental injuries: searching for clues.**

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It is well established that pregnant women who become infected with *Plasmodium falciparum* during their pregnancy are at an increased risk of developing adverse outcomes such as severe anaemia, delivery of low birth weight babies or even miscarriage. These outcomes are often associated to a specific parasite phenotype that involves the adherence to chondroitin sulphate A (CSA) in the placenta. Histological analysis of placental tissue from these women has shown that parasite products such as hemozoin inside monocytes and within fibrin deposits, as well as a high number of immune cells within the intervillous space of the placenta, are associated with the severity of these outcomes.
A CSA-binding phenotype for *Plasmodium vivax* has yet to be clearly shown but similarly to *Plasmodium falciparum*, women who become infected with *Plasmodium vivax* have an increased risk of miscarriage, anaemia and low birth weight babies. However, the associated placental lesions have not been systematically evaluated. In this study we have evaluated by histology the presence of parasite product and placental lesions in a cohort of women who became infected with *Plasmodium vivax* during pregnancy. Despite none or very few parasites in the placenta observed in the great majority of women infected with *Plasmodium vivax* during pregnancy, there was an increase in syncytial knots and fibrinoid necrosis when compared to non-infected controls. These results call for larger studies to be performed so that the pathological mechanisms of *Plasmodium vivax* during pregnancy are elucidated.

RT56- **The Pertussis Problem: Do We Need New Vaccines?**

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Whole cell pertussis vaccines (wcP) were highly effective if well made, although some whole cell vaccines were poorly immunogenic before booster immunization. In addition, they were reactogenic and accused by some of causing neurologic adverse reactions. In the 1990s and later, many countries substituted acellular pertussis vaccines (acP) for the whole cell type, after large trials demonstrated efficacy almost as good as that of wcP. Recently, the importance of pertussis in adolescents and adults has become apparent, partly due to better diagnosis but also possibly due to shorter duration of efficacy after acP. Moreover, outbreaks have occurred in highly vaccinated populations. To complicate interpretation, the acP being used have varying components and concentrations of antigens. However, we will consider what current epidemiology tells us and what might be done to improve immune responses to acP.

RT57- **Functional genomics in sandflies, a tool for study vector parasite interaction**

Marcelo Ramalho-Ortigão

Transcriptome analyses of several sand flies species have provided new insights into the midgut proteins that are associated with the development of *Leishmania* within the sand fly vector, and the salivary proteins inoculated into the skin of the vertebrate host and which influence the establishment and outcome of infection. These studies coupled with the current ongoing sand fly genome sequencing project

will likely pave the way towards new vector-based strategies to prevent transmission of leishmaniasis. Functional or reverse genomics, through the use of molecular tools such as RNAi can be used in the characterization of the biological role of sand fly molecules, and contribute to the further understanding of the complex interactions between *Leishmania* and sand flies. I plan to discuss the development of an RNAi platform for sand fly studies in my laboratory, and present data related to the characterization of sand fly molecules and how they are involved in *Leishmania major* development within its natural vector *Phlebotomus papatasi*.

RT58- **Colonization resistance in the bloodsucking sand fly *Lutzomyia longipalpis*: *Leishmania* protects its host from bacterial pathogenesis.**

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Lu. longipalpis males and females are plant feeders but only females feed on blood for egg maturation. During their feeding visits *Lu. longipalpis* have ample opportunity to ingest bacteria, yeasts, viruses and protozoans from plants and animals but little is known about the impact of these microbes on *Leishmania* growth and development within the sand fly gut. Gram-positive and gram-negative bacteria, as well as yeasts were isolated from the midguts of urban *Lu. longipalpis* in Teresina, a region endemic for VL in Northeast Brazil. Prior midgut colonization with different indigenous bacteria and yeast species significantly reduced numbers and infection rates of *Leishmania mexicana* promastigotes within the sand fly midgut. The antileishmanial effect increased proportionally with increase in bacterial load. Conversely, previous *Lu. longipalpis* infections with *Leishmania* mexicana extended the longevity of the sand fly when insects were challenged with the insect pathogen *Serratia marcescens*, when compared to bloodfed uninfected sand flies, suggesting that colonization resistance might be triggered by *Leishmania* towards sand fly gut bacteria. One of the bacteria able to promote CR towards *Leishmania* in the females is an acetic acid bacterium of the genus *Asaia* isolated for the first time from wild caught *Lu. longipalpis* in Teresina. *Asaia* stably colonizes *Lu. longipalpis* male and female midguts; transformed *Asaia* expressing GFP were observed within the sand fly gut up to 10 days after inoculation. *Asaia* is insect symbionts that are easy to transform, its ability to be venereally and vertically transmitted in sand flies is currently being assessed. This bacterial species is a potential candidate for studying sand fly paratransgenesis. Is a ROS-mediated response involved in sand fly colonization resistance towards *Leishmania*? *Leishmania* parasites reside in the sand fly gut but the nature of the immune response to the presence of Leishmania is unknown. Reactive oxygen species (ROS) are a major component of insect innate immune pathways regulating gut-microbe homeostasis. ROS concentration increased in sand fly midguts after they fed on the insect pathogen *Serratia marcescens* but not after feeding on the *Leishmania* that uses the sand fly as a vector. Moreover, the *Leishmania* is sensitive to ROS either by oral administration of ROS to the infected fly or by silencing a gene that expresses a sand fly ROS-scavenging enzyme. The treatment of sand flies with an exogenous ROS scavenger (urate) altered the gut microbial homeostasis, led to an increased commensal gut microbiota, and reduced insect survival after oral infection with S. marcescens. Our study demonstrates a differential response of the sand fly ROS system to gut microbiota, an insect pathogen, and the *Leishmania* that utilize the sand fly as a vehicle for transmission between mammalian hosts.

RT59- **Sandfly immunity**

Andre Pitaluga

Sandflies are the principal vector of leishmaniasis in the Americas and can also transmit bartonellosis and virus. The role of sand fly immune system in defense against those pathogens is poorly understood. We are currently investigating several aspects of the sand fly *Lutzomyia longipalpis* innate immunity in the

context of microbiota homeostasis, bacterial challenges and Leishmania and West Nile virus infections. The integrity and composition of the microbiota is very important to the innate immunity effectively. We characterized the events that might regulate the abundance of the sand fly microbiota after sugar fed and antibiotic treatments. Regarding to bacterial challenges, we identified a modulation in the antimicrobial peptide defensing in response to different bacterial infections. The sand fly response to a Leishmania infection was also evaluated. Besides of differences in defensing expression at later time points, there is a relationship between IMD pathway and the Leishmania establishment success. The silence of the IMD repressor gene caspar expression showed for the first time the importance and the relationship of the innate immunity for Leishmania infection establishment success. The depletion of caspar led to a reduction of Leishmania survival in infected sand flies. The molecular mechanisms underlying viral resistance are unknown. Recently, working in an RNAi silencing system for *L. longipalpis* we identified an antiviral response to a non-specific double-strand RNA transfection. After infect the *L. longipalpis* LL5 cell line with a West Nile Virus-Like particle carrying a luciferase reporter gene several different dsRNAs were transfected to the infected cells. Surprisingly, we found that dsRNAs unrelated to the virus, diminished replication of the VLP-encoded genome. More recently the role of RNA interference as an antiviral response was evaluated as well. The role of this pathway in antiviral defense is known in other insects but had no information on sand flies. Since we demonstrate the activation of a non-specific antiviral response by dsRNA we silenced key genes from RNAi pathway (Ago2 and Dicer2) using siRNA. The silencing of selected genes increased the viral infection in LL5 cells. These results are the first indication for a nucleic acid-induced, non-specific antiviral response in this important insect vector and also show the role of RNAi pathway as a functional antiviral strategy. The innate immunity of insects is mainly deduced from the drosophila or mosquitoes models. Both insects have a large set of genetic, transcriptomics, immunity and genomic information that support them as good models. Although they are quite precise and have some small differences among them, it would be naïve to assume that is valid for all insects. In this aspect, we are showing strategies used by the innate immunity of sandflies to deal with different pathogens challenges, some are similar with current models others are quite different.

RT60- **Down-modulation of the inflammatory response by *Schistosoma mansoni* antigens**

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Evidence has accumulated suggesting that helminth infections or their products protect against the development of allergic and autoimmune diseases. The mechanisms underlying this protection may include regulatory cells and cytokines. We have demonstrated that *S. mansoni* infections are associated with a poor response to allergy skin-prick tests and with low asthma pathology. We have tested in the *in vitro* and in experimental studies the ability of the recombinant *S. mansoni* antigens Sm22.6, Sm29 and PIII in modulating the inflammatory response in atopic asthma. The Sm22.6 and Sm29, are membrane-bound glycoprotein found mainly in the tegument of *S. mansoni* adult worm, while PIII is a fraction of *S. mansoni* soluble adult worm antigen (SWAP). These antigens have been tested as vaccine to prevent schistosomiasis and/or liver pathology associated with the disease in mice. We found, in a murine model of OVA induced airway inflammation, that immunization with Sm22.6, PIII and Sm29 lead to a reduction in the number of inflammatory cells, eosinophils and OVA-specific IgE, compared to non-immunized mice. The *S. mansoni* antigens are also capable to suppress the *in vitro* Th2-inflammatory response in uninfected asthmatics. Additionally, the ability of *S. mansoni* antigens in down-modulate the *in vitro* Th1-inflammatory response in cutaneous leishmaniasis patients have been tested. Leishmaniasis is one of the most severe infectious diseases, affecting 12 million people worldwide. Cutaneous leishmaniasis (CL) is the most common clinical manifestation of the disease, characterized by one to several skin lesions in exposed areas. Control of *Leishmania spp* infection depends on the immune response mediated by Th1 cells, which produce interferon-gamma (IFN-γ) and are able to activate tissue macrophages. However, high production of IFN-γ and tumor necrosis factor-α (TNF-α) can lead to intense tissue damage and

development of cutaneous and mucosal leishmaniasis. Considering the potential of *S. mansoni* antigens to prevent some Th1-mediated diseases, we have evaluated the effects of the addition of *S. mansoni* antigens to the immune response induced by soluble *Leishmania* sp antigen (SLA) in cutaneous leishmaniasis patients. We examined IFN-γ, TNF-α and IL-10 production by peripheral blood mononuclear cell (PBMC) of CL patients stimulated *in vitro* with SLA in the presence or absence of rSm29, tetraspanin 2 (rSmTSP-2) and PIII. The addition of Sm29, SmTSP-2 and PIII to the cell cultures reduced the levels of IFN-γ in high number of patients, independently of the number and size of lesions. Although to a lesser extent, the three antigens used in the study were also able to decrease the production of TNF-α by cell from CL patients in response to SLA. The modulation of IFN-γ and TNF-α production in cultures stimulated with SLA in the presence of *S. mansoni* antigens was generally followed by an increase in IL-10 production. The *S. mansoni* antigens evaluated in these studies induced the production of regulatory cytokine and down-modulated the Th2 and Th1 immune response which participates, respectively in the pathology of asthma and cutaneous leishmaniasis.

RT61- **Asymptomatic plasmodial infection: evidence from in population-based studies in the Brazilian Amazon**

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The increasing interest in understanding the epidemiology of malaria in Brazil is mainly due to its high morbidity in populations continuously exposed to the risk of infection. The surveillance system of malaria in Brazil is based on active and passive detection of symptomatic cases, and asymptomatic infections can go undiagnosed. It is not clear what the clinical course of these asymptomatic infections is and what roles they play in the maintenance of transmission of the parasite. Population-based studies conducted in the Brazilian Amazon have been detecting asymptomatic cases in riverside, agricultural and urban areas, and these studies attempt to provide scientific evidence that could answer these questions about the role of asymptomatic plasmodial infections. Studies conducted at a rural locality in Acre showed a prevalence of asymptomatic infection of 29%, and only 10% of these cases developed symptoms over a period of 30 days. In another recent agricultural settlement in the Amazon, the prevalence of asymptomatic infections was 27% over 12 months of study, and the residence time at the site was associated with a decrease in symptom intensity. Ongoing studies in the urban area of Mâncio Lima, in Acre, will detect the prevalence of asymptomatic infections in the urban environment and the clinical outcome of these infections. The detection of asymptomatic infections can contribute to a new perspective for malaria control.

RT62- **"Asymptomatic carriers of *Plasmodium*" - Asymptomatic infections and malaria control in Africa**

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A characteristic of *Plasmodium falciparum* infections is the acquisition of clinical immunity resulting from exposure to the parasite and its maintenance through boosting by frequent reexposure. Despite intense research efforts, the molecular bases of protection against complicated forms of malaria remain unresolved. Hypotheses can be generated and tested by applying mathematical models to suitable data collections. This presentation consists of two parts. First, clinical data from eight endemic regions in sub-Saharan Africa is used to inform a model that links the age profiles of clinical malaria with the underlying transmission dynamics. The model reproduces previous observations in malaria epidemiology, namely that the cumulative burden of clinical malaria during childhood decreases as transmission increases from mesoendemic to holoendemic. Moreover, we detect a positive feedback that enhances transmission when endemicities are established while inhibiting transmission once the levels of infection fall below a certain threshold, leading to bistability and suggesting a reinterpretation of measured indices of transmission. The results have their most significance in regions of mesoendemic transmission, with important implications for the design of sustainable control programs. Second, serological data specific to

P. falciparum is used to inform a model of seroconversion and seroreversion as an intervention to reduce mosquitos densities is taking place in a West African island. **E-mail:** ggomes@igc.gulbenkian.pt

RT63 - **Selective Intermittent Preventive Treatment of Vivax Malaria: Reduction of Malaria Incidence in an Open Cohort Study in Brazilian Amazon.**

Tony Hiroshi Katsuragawa

In children the intermittent preventive treatment (IPTc) was considered effective on malaria control due the reduce malaria incidence in Papua New Guinea and in some Africa areas with seasonal malaria. However, the IPT has not been indicated due your association with drug resistance and hinder development of natural immunity. Thus, we evaluated the IPT impact on malaria cases in three riverside communities from Madeira river, Porto Velho-RO, Brazil. The work was realized from January 2008 to June 2012 in Vila Amazonas (VA), Cachoeira do Teotônio (CT) e São Sebastião (SS). In 2008, the asymptomatic *Plasmodium* carriers (APC) were identified by laboratory tests (thick blood smear and PCR), and a systematic surveillance to early identify symptomatic carriers was performed. The symptomatic carriers received complete treatment supervised. In June 2009, APC from VA and CT received the complete treatment supervised. Furthermore, to avoid relapses and recurrence of malaria in VA, the selective intermittent preventive treatment (SIPT) was performed. In August 2010, the SIPT was implemented in CT. SS not received SIPT. The SIPT consist in a weekly use of two chloroquine tablets of 150mg for 12 week in adults, and an equivalent dose to children, after complete treatment supervised. The clinical parasitology and epidemiology surveillance showed a statistical reduction on falciparum malaria incidence with the APC treatment. Our data also showed a reduction on relapses and recurrence of vivax malaria after SIPT implementation. The SIPT can be effective on malaria vivax control in the study areas.

RT64- **Performance of Rapid Serological Diagnostic Tests for Infection with *Trypanosoma cruzi* / Chagas Disease**

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Chagas disease is one of the main Latin American public health problems. Around 10 million people are estimated to be infected with *Trypanosoma cruzi* and more than 40 million are at risk of developing the disease. In the last decades, mainly due to population movements, *T. cruzi* infection has increasingly spread in the world. As a typical neglected disease, the underdiagnoses index of this infection is around 90%. This study aimed to evaluate the performance of commercialized rapid serological diagnostic tests (RDT) for *T. cruzi* infection/Chagas disease with serum/plasma through a multicentre study, in eleven national reference laboratories (NRL) representing the regions of the Americas, Europe and Western Pacific. **Methods:** We evaluated the 11 commercialized RDTs for *T. cruzi* infection comparing their performances on over 400 samples obtained from the serum banks of NRLs, previously tested for Chagas disease. All RDTs were bought and distribute via MSF Logistique ensuring same batch numbers, adequate package and transport conditions. The sensitivity and specificity of each RDT was measured, as well as the concordance between the RDTs results, using the kappa test. Simultaneously, one additional reference laboratory was selected to measure potential cross reactions. A comprehensive set of serums from patients with infectious and non-infectious diseases was selected for this phase (i.e: Leishmaniasis, malaria, HIV) **Results:** Test results were divided into positive, negative, and invalid/indeterminate, strictly following the definition provided by manufacturers. For each test, sensitivity and specificity were analyzed for the whole pool of results obtained. Beside this, additional analysis was also performed for the easy-to-use, cross-reaction, performance related to different geographical areas and degree of agreement between laboratories. With all results, the commercialized RDTs could, therefore, be classified in three categories: high, medium and low performance. Among the evaluated

RDTs for Chagas disease, the majority of them were classified in the medium performance category, three were classify as low performance and 2 as high performance. **Conclusions:** In line with the promotion of the access to diagnosis and treatment of the chronic phase of Chagas disease, starting at primary health care level, results found in this study showed that, alternative serological RDTs can be useful and reliable instruments to achieve this objective. Complementary studies will be needed, especially to evaluate the selected RDTs under "field conditions", using whole blood, and assessing the financial, time and human-resource cost implied. These will be also fundamental to propose new diagnostic algorithms for Chagas disease screening and diagnosis based on RDTs.

RT65- **Ecology of Amazonian triatomines: lessons for surveillance and control**

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Here I summarize past knowledge and recent advances in triatomine bug ecology in Amazonia. I distinguish three levels of scrutiny relevant to understanding the relationships between triatomine distribution patterns and Chagas disease risk. I argue that both disease-risk analyses and the design of rational surveillance/control schemes must include the consideration of vector ecology at all these levels. **Macro-scale (eco region-level)** analyses help us identify higher-risk areas. Thus, *Triatoma maculata* is strongly domiciliated in the savannas of Roraima; *Rhodnius brethesi* attacks *piaçava* workers of the middle-upper Rio Negro; *R. stali* forms peridomestic foci in the Bolivian Amazon; and larger colonies of *Rhodnius* spp. occur in palms of the rich-soil regions of western Amazonia. These 'focal' patterns are however overlaid onto a background of widespread occurrence of both *Rhodnius* and *Panstrongylus* species. By regularly invading houses and food-processing premises, they maintain continuous transmission and cause acute-disease outbreaks linked to oral contamination. Attraction to artificial light is probably an important driver of such behavior. Therefore, surveillance must be region-wide, albeit with increased efforts directed towards some well-identified, higher-risk sub-regions. **Meso-scale (landscape-level)** heterogeneity can also modify risk patterns. However, recent evidence suggests that, within a given region, variation in triatomine occurrence among distinct landscape classes is modest. Thus, the rates of palm infestation by *Rhodnius* spp. are similar in forested, rural, and urban landscapes. In cities, forest-fragment palms are often infested by *Rhodnius* spp., and other triatomines (*Panstrongylus*, *Eratyrus*) can also be collected. This suggests that some triatomine species are fairly resilient to landscape-level ecological disturbance, underscoring the need for surveillance not only in rural areas, but also in densely-populated urban-periurban environments. Finally, **micro-scale (ecotope-level)** ecological traits have emerged as key drivers of triatomine occurrence in the Amazon. Thus, domiciliated *T. maculata* prefer mud-walled houses with abundant food resources (mainly fowl and rodents), and Amazonian *Rhodnius* spp. preferentially occupy tall palms with large amounts of decaying vegetable debris on their crowns/stems. These traits can be used to single out "high-risk ecotopes" for targeted surveillance/control interventions. Domestic infestation by *T. maculata* will require traditional insecticide-spraying campaigns followed by sustained, participatory surveillance and housing improvement. Where large, 'dirty' palms occur near households, environmental interventions, combining palm management with insect-screening of houses and food-processing equipment/premises, could significantly reduce transmission probabilities. In **conclusion**, current knowledge of triatomine bug ecology allows for the tentative design of flexible surveillance/control strategies for the greater Amazon, but much remains to be learned. In particular, innovative operational research will be required to establish which among those strategies will work best in each of the highly diverse ecological and epidemiological scenarios of this vast region. **Funding:** TDR-WHO, Fiocruz-CNPq and Fiocruz-Fapeam agreements **E-mail:** fernando@amazonia.fiocruz.br

RT66- **Clinical and laboratory follow-up of the two largest oral outbreaks of Chagas disease**

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Two large school oral transmission outbreaks have occurred in Venezuela. The first one in December 2007 in a medium class neighborhood of Chacao in Caracas, and the second outbreak occurred in March 2009 in a rural school in Chichiriviche, a small touristic seashore town at 60 Km at the north west of Caracas. Once the cases were confirmed by parasitological finding of *Trypanosoma cruzi,* by serology and PCR, treatment was immediately given to minimized morbidity and prevents mortality. Nifurtimox, the only drug available in the first outbreak, was given for 90 days in Chacao School and Benznidazol for 60 days was indicated to Chichiriviche patients. Herein we report the overall clinical and laboratory features of three years follow-up of these study populations. **Materials and Methods:** Parasitological screening was achieved by direct visualization on blood smears, blood culture and inoculation of mice. For conventional serology we used an "in house" immunoenzymatic assay (ELISA) (IgG and IgM) performed with delipidized epimastigote antigen of *T. cruzi* and Indirect Haemagglutination (IHA) using the same antigen adsorbed to fresh sheep red cells. Complement-mediated antibody dependent lysis (CoML) test using fresh suspensions of *T. cruzi* 1593 strain was done to detect lytic antibodies against *T. cruzi*. For DNA preservation, blood were mixed with an equal volume of 6M guanidine and the amplification reactions for the PCR were targeted to the 330–base pair minicircle fragment of the *T. cruzi* kinetoplastid DNA **Results:** In Chacao group, 1000 persons were evaluated and 103 were registered as confirmed cases attending WHO guidelines. A hundred persons have been followed-up. By culture, 4 individuals were positive six months later and 2 resulted positive in the blood culture eighteen months thereafter. CoML test was 98% sensitive and 100% specific in the baseline diagnosis. It was positive in 78/100 persons 30 months after treatment, and in 76 /100 persons at 48 months. PCR was also positive in 69% treated personas sometime during the period march 2008 - January 2011. The actual symptomatology is mainly related to the consequences of side effects of the drugs. In Chichiriviche, 441 persons were evaluated and 79 children and 9 adults were confirmed as Chagas disease cases. These were the results: 86/88 (97, 7%) were ELISA IgG (+); 87/88 (98, 9%) were ELISA IgM (+) and 85/88 (96, 6%) were positive by IHA. Blood culture was done in 67 persons and *T. cruzi* was seen in 51 (76, 1%). PCR was carried out in 50 individuals demonstrating *T. cruzi* DNA in 40 of them (80 %). During three years, 83 persons have been followed-up and only 26, 5% have sero-converted by conventional serology (ELISA IgG/IHA), being only 8 persons negative by lytic antibodies. Parasites were seen in the blood culture of 16 persons during the period may 2010 – May 2011. All persons with positive PCR or blood culture, usually complained of mild symptoms (headache, fatigue), were treated by second time. **Discussion and conclusions:** These two outbreaks are similar because they occurred in schools in which the source of infection seems to be the same ie. guava juice prepared the night before and accidentally contaminated by infected triatomines. Children were the main affected population and all persons were somehow related with the school (teachers, cookers, food-handlers). In the first outbreak the available drug was Nifurtimox which was given for 90 days and certainly more persons developed side effects. Few parasites isolates (9) were recovered from the first episode in contrast to 51 in Chichiriviche. Although Chichiriviche was investigated early on the base of the frequency of IgM, the mortality was higher (4 persons) in comparison to Chacao (one child). Specific IgM and IgG detection by ELISA is the most rapid and best test to cover large exposed populations in order to identify infected persons. In parallel, a second sample should be taken in order to isolate parasites and give early treatment. Cultures are cumbersome, time consuming and of low sensitivity but can be used early to isolate parasites. CoML test and PCR are ideal tests to achieve cure in the control of massive outbreaks. Factors such as the parasite isolate, the parasitic load, the route of infection or the immunological status of the patients, may be playing some role that interfere with the process of cure in a population constituted mainly by children and treated early during infection which is supposed to respond faster to treatment that it has been shown. **Partial finantial support**: Proyecto Misión Ciencia G-2007001442

RT67- **Planning and production of new synthetic antimalarials: artesunate hybrids for clinical trials**

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Most antimalarials have its origin in natural products, although the semi-synthetics and synthetics have assumed great importance in current clinical. Nowadays is widely recommended the use of antimalarial drug combinations with artemisinin or with one of its derivatives, mainly, through a fixed dose combination (FDC). While the artemisinin derivative can rapidly kill parasites, but is also promptly excreted, another antimalarial with longer half-life time can achieve full eradication of parasites and prevent the recrudescence. In this paper we present some antimalarial synthetic hybrids, having the planning and production of both MEFAS (artesunate mefloquine) and PRIMAS (primaquine artesunate) as case studies.

RT68- **African plant-derived compounds as inhibitors of erythrocytic and liver stages of *Plasmodium* infections**

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Malaria is oone of the most severe public health problems in Africa. The increasing prevalence of drug-resistant *Plasmodium falciparum* strains is one of the greatest challenges in malaria control. In order to overcome drug-resistance, new antimalarial drugs are urgently needed. Natural product-derived compounds have played a major role in drug discovery and development. In case of malaria drug discovery, the great significance of plant-derived drugs for the treatment of the disease is highlighted by quinine, artemisinin and their derivatives, which are currently the mainstay of the antimalarial therapy. As part of our search for bioactive compounds from medicinal African plants, we have carried out a preliminary screening of different plants species for their antimalarial activity. *Momordica balsamina* L. (Cucurbitaceae) was found to be the most active plant. *M. balsamina,* also referred to as the balsam apple, or African pumpkin, is an extensively cultivated vegetable consumed in many tropical and subtropical regions of the world. It has also been widely used in traditional medicine in Africa to treat various diseases, mostly diabetes, and malaria symptoms. Bioassay-guided fractionation of the methanol extract of the aerial parts of *Momordica balsamina* led to the isolation of several cucurbitane-type triterpenoids. These compounds and acylated derivatives were evaluated for their antimalarial activity against the erythrocytic stages of the *Plasmodium falciparum* chloroquine-sensitive strain 3D7 and the chloroquine-resistant clone Dd2. Evaluation of the activity of some compounds against the liver stage of *P. berghei* was also carried out, measuring the luminescence intensity in Huh-7 cells infected with a firefly luciferase-expressing *P. berghei* line, *Pb*GFP-Luc_{con}. Toxicity of compounds was assessed on the same cell line through the fluorescence measurement of cell confluence. Moreover, toxicity towards human cells of compounds was also investigated in the MCF-7 breast cancer cell line, showing that most of them were not toxic or exhibited weak toxicity. In blood stages of *P. falciparum,* several compounds displayed antimalarial activity, revealing some alkanoyl ester derivatives the highest antiplasmodial effects, with IC_{50} values in the nanomolar range. The highest antiplasmodial activity against the liver stages of *P. berghei* was also displayed by ester derivatives**,** with high inhibitory activity and no toxicity.

RT69- **Aspidosperma species (*Apocynaceae*) as sources of antimalarials: from an ethnomedicinal survey to active indole alkaloids and chromatographic profiles of potential phytomedicines**

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For thousands of years, plants have formed the basis of traditional medicine systems. The first drug to be used against malaria was quinine which has been isolated from *Cinchona* bark, used by Peruvian Indians, and that was isolated in 1820. More recently, artemisinin, isolated from the Traditional Chinese

Medicine (TCM) plant *Artemisia annua* L., and its semi-synthetic derivatives (artemether, arteether, artesunate) have been used successfully against malaria that has become resistant to chloroquine. Plant derived remedies are still used as antimalarials and antipyretics in different countries. A survey on the traditional use of *Aspidosperma* species to treat malaria and/or fevers in Brazil disclosed a total 24 species. We have carried on a screening of extracts of six out of those, by inhibition of *Plasmodium falciparum* growth (trityated hypoxanthine method). Bio guided fractionation of an ethanol extract from *A. parvifolium* bark (IC_{50} < 50 µg/ml) afforded uleine, an indolomonoterpenoid alkaloid which has shown a good antiplasmodial activity (IC_{50} = 0. 98 µg/ml). Possible targets for uleine have been studied by confocal microscopy using a proton fluorescent probe (BCECF-AM) in *P. falciparum* synchronous culture of W2-infected red blood cells by comparison with mefloquine (MQ) and bafilomycin A1 (BAF). Dynamic images have shown that uleine was able to mobilize protons altering the pH gradient in the digestive vacuole (DV), like MQ, a weak-base antimalarial quinoline at 5 ng/ml. This work shows that uleine is able to act on the DV, probably due to its alkaloidal structure. Uleine was shown to be present in other *Aspidosperma* species whose extracts have also shown to be active against *P. falciparum.* Antimalarial extracts and fractions were characterized by different chromatographic analyses. These data disclose *Aspidosperma* species as sources of potential antimalarial drugs and phytomedicines. **Financial support:** FAPEMIG, CNPq, MS, Brazil. **E-mail:** alaide.braga@pq.cnpq.br

RT70- **Facing the complexity of transmissible diseases: Multiscale analysis of leptospirosis in Rio de Janeiro**

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Disease transmission involves multiple and intricate factors acting from genes to ecosystems. The recognition of this complexity does not mean perplexity. On the contrary, the identification of these factors along the different scales they occur is a crucial task for epidemiological studies and planning of control measures. Complex systems are characterized by the interaction of its parts, which promotes emergent proprieties. In this sense, disease can be considered as a tension between one level and the immediate upward level, such as cells into an organism, and individuals into a community. A methodological alternative to deal with complexity is the study of disease incidence along the different scales in which it is manifested. Leptospirosis presents a broad diversity of exposure routes, reservoirs, etiological agents and clinical features. Therefore, the studies' findings on leptospirosis distribution are restricted to the context in which they were produced, comprising the scale, unity of analysis and employed indicators. In this study we review common indicators used to characterize risks of leptospirosis transmission and analyzed environmental and socioeconomic factors acting in the regional, municipal and local scales. We verified that the existence of poor sanitation areas may represent a risk at regional scale, but can be considered as a protective factor in the local scale. These areas present low incidence during the epidemic periods due to acquired immunity, but contributes to the increase of incidence of adjacent areas. This apparent contradiction of results reveals the complementarities and interactions between processes taking place in different scales.

RT71- **Eco-epidemiology of infectious and parasitic diseases - Eco-epidemiology of schistosomiasis and malaria in coastal Kenya**

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In the south coast of Kenya, we analyzed temporal changes in: 1) spatial patterns of active *Schistosoma haematobium* infection in different age groups and their association with ponds infested with *Bulinus* snails; 2) Anopheline vector populations composition and abundance. A major drought between 2001 and 2009 resulted in drying of ponds that were known sources of infection, and reduction in number of

mosquito larval development sites. Mass distribution of bednets further reduced exposure of the human population to anopheline malaria vectors, and distribution of praziquantel reduced the duration of human infection with schistosomiasis. We detected very few or no snails in ponds that were infested in the past, and found few mosquito larvae in and around studied communities. The household-level spatial pattern of schistosomiasis infection for children of various age groups in 2009 was contrasted with historical data from 2000, and the number, composition, human biting rate and entomological inoculation rate (EIR) of Anopheline mosquitoes in 2009-11 was compared to data from 1997-1998. The significant local clustering of high and low schistosomiasis infection levels among school age children that occurred in 2000 was absent in 2009, and we attribute its disappearance to the decade-long drought in our study area. Compared to 1997-1998, a significant decline in the relative proportion of *An. gambiae* s.s. among collected mosquitoes was noted, coupled with a proportionate increase of *An. arabiensis*. Following > 5 years of 60-86% coverage with bed nets, the density, human biting rate and EIR of indoor resting mosquitoes were reduced by more than 92% for *An. funestus* and by 75% for *An. gambiae* s.l. In addition, the host feeding choice of both vectors shifted more toward nonhuman vertebrates. The implications of extreme weather and climate conditions and of mass interventions on risk and transmission of *S. haematobium*, and plasmodia, the persistence of parasite transmission and the relevance to control strategies are discussed.

RT72- **Eco-Epidemiology**

Fernando Dias de Avila-Pires

In its original Greek sense, *epidemia* is the equivalent to the Latin *visitatio*, meaning transient. It is generally admitted that modern epidemiology dates from John Gaunt's analysis of the Bills of Mortality in England, published in 1662. Two centuries later, William Farr took where he had left, in order to delineate the field as we understand it nowadays. The key concepts of causality and risk have been the source of pursuit, enlightenment, and disagreement, among present day epidemiologists. Causality was masterly discussed by Mervyn Susser in *Causal thinking in the health sciences* (Oxford University Press, New York, 1973) and risk was the recent subject of a book by Castiel, Guilan, and Ferreira *Correndo o risco* (FIOCRUZ, Rio de Janeiro, 1910). In 1996, Susser and Susser suggested to avoid the *risk factors* as central to epidemiological thinking by turning to ecology, and a multi-level *eco-epidemiology*. In this paper, I tried to point out that the reduction of epidemiology to an analysis of risk or to the search of simple causal factors is *de facto*, reductionism. The main difference between epidemiology and disease ecology lies in the position of mankind in the ecosphere. In ecology, man is seen as occupying a high level in Elton's trophic pyramid, and the focus lies in the *relationships* of the members of an ecosystem. In epidemiology, man is the central object of the study, and the ecological relationships among its components are analyzed in order to explain the origin and distribution of a disease or condition affecting health

RT73- **Optimizing Vector-Control against Dengue**

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Dengue is considered the most important vector-borne infection, affecting almost half the world population with 50 to 100 million cases every year. In this work, we present one of the simplest models that can encapsulate all the important variables related to vector control in dengue fever. The model considers the human population, the adult mosquito population and the population of immature stages, which includes eggs, larvae and pupae. The model also considers the vertical transmission of dengue in the mosquitoes and the seasonal variation in the mosquito population. From this basic model describing the dynamics of dengue infection, we deduce thresholds for avoiding the introduction of the disease and for the elimination of the disease. In particular, we deduce a Basic Reproduction Number for dengue that includes parameters related to the immature stages of the mosquito. By neglecting seasonal variation, we calculate the equilibrium values of the model's variables. We also present a sensitivity analysis of the impact of four vector-control strategies on the Basic Reproduction Number and on the Force of Infection

of dengue. Each of the strategies was studied separately from the others. The analysis presented allows us to conclude that of the available vector control strategies, adulticide application is the most effective, followed by the reduction of the exposure to mosquito bites, locating and destroying breeding places and, finally, larvicides. Current vector-control methods are concentrated in mechanical destruction of mosquitoes' breeding places. Our results suggest that reducing the contact between vector and hosts (biting rates) are as efficient as the logistically difficult but very efficient adult mosquito's control.

RT74- **Entomology as a major tool in malaria vector control in the Americas. "Updates on vector control in malaria"**

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Malaria vector control in the Americas presents a unique set of challenges. A diverse array of *Anopheles* species inhabiting a variety of ecological settings are competent vectors of both *Plasmodium falciparum* and *Plasmodium vivax.* Patterns and intensity of malaria transmission can be remarkably diverse, requiring a variety of approaches for both entomological surveillance and vector control. The best developed malaria vector surveillance indicators and techniques are mainly applicable to areas with intense malaria transmission. However, in the Americas, malaria endemic areas are generally considered moderate to low transmission settings. The Amazon Malaria Initiative (AMI) recently proposed linking entomological surveillance techniques and their periodicity to certain eco-epidemiological characteristics, including malaria transmission intensity. Doing so should provide a more practical framework in which to assess entomological risk and design suitable vector control strategies. Globally, malaria vector control programs rely primarily on the indoor residual spraying of insecticides (IRS) and the distribution of insecticide-treated bed nets (ITNs). However, there are significant knowledge gaps regarding their efficacy in the Americas, where vector species can vary substantially in host-seeking behaviors and respond differently to interventions. The emergence of insecticide resistance in malaria vectors in the region highlights the urgent need for greater understanding of how interventions can be optimized and resistance can be prevented and/or managed to ensure sustained vector control efficacy. **E-mail:** AJL8@cdc.gov

RT75- **Human antibody response to *Anopheles* saliva as indicator of the effectiveness of malaria vector interventions**

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Abstract: For improving malaria vector control, immunological marker based on human antibody responses to *Anopheles* saliva has been investigated as a new indicator to compare and evaluate the efficacy of different vector control methods, such as insecticide-treated nets (ITNs), impregnated wall lining (WL) and insecticide residual spraying (IRS). Parasitological, entomological, and immunological assessments were carried out in children from 2 to 9 years old from a malaria-endemic region, Balombo (Angola) before and after the introduction of vector controls. Immunoglobulin G (IgG) levels to *An. gambiae* saliva were positively associated with the intensity of *An. gambiae* exposure and malaria infection. A significant decrease in the anti-saliva IgG response was observed after the introduction of ITNs, and this was associated with a drop in parasite load and density of *Anopheles* vectors. This study confirms the efficacy of such immunological marker for tailor-made vector control strategies.

RT76- **Larviciding experiences in Africa as a preventive method for the malaria control.**

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Malaria control and/or elimination in Africa remain a challenge for humanity. According to reports from WHO-AFRO at the end of 2010, 86% of malaria cases as well as the 91% of mortality and 85% of children fewer than 5 years in the world belongs to this continent. Vector control strategies play an increasing role in front of the development of resistance to major drugs used, the low level of diagnosis and the difficult access to the effective treatments by the population. Using biolarvicides within the actions for control of larval source gains an important place in the integrated control strategies; reports of resistance to pyrethroids and other insecticides used as the unique control measures (LLINs and IRS) and still controversial use of DDT confirm this. In Ghana, Angola and Nigeria has been implemented within the NMCP the use of biolarvicides (Bactivec- Bti and Griselesf-Bs) for the control of larvae of *Anopheles sp*.; had been established an entomological and epidemiological database that allows a correct identification and location of main breeding sites, and following the selection criteria for the treatment of breeding sites positive to *Anopheles sp* close to the big communities enabling to establish a primary structure for spraying, aerial application over large or difficult access areas and indoor applications involving community participation. Implementing an impact assessment methodology in sentinel breeding sites had showed a reduction in RLD by more than 90%, decrease of female *Anopheles sp* in more than 80% and reducing the prevalence in over 50% in intervened areas, similar results were obtained in studies of LSM in Sudan, Mauritania and Kenya. Demonstrated that the use of biolarvicides, as part of the Integrated Vector Management strengthen and potentiate malaria elimination in Africa.

RT77- **Current practices in the management of lymphatic filariasis**

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Introduction: Current practices in the management of lymphatic filariasis (LF) have been influenced by the push for integrated control of neglected tropical diseases amenable to mass drug administration (MDA) using diethylecarbamazine citrate (DEC) or ivermectin, in combination with albendazole. This treatment regime affects other helminth infections including onchocerciasis and soil transmitted helminths (STH). In Africa, South America, and the Caribbean islands, where LF is often co-endemic with onchocerciasis, ivermectin 200–400 µg/kg with albendazole 400 mg is the recommended regimen. In regions without coendemnicity, DEC 6 mg/kg with albendazole 400 mg is recommended. Building on the pro-poor community directed intervention strategy used by the African Programme for Onchocerciasis Control (APOC); the World Health Organization has developed a preventive chemotherapy strategy for integrated control of LF, onchocerciasis and STH. To scale up mass drug distribution, many LF endemic countries in Africa have adopted preventive chemotherapy for control of multiple helminths on an integrated platform. The strategy ensures that stakeholders advocating for different diseases have a collective responsibility for increased treatment coverage, resulting in increased community compliance. In India, distribution by the health service was less cumbersome and proved more acceptable than community-directed treatment. **Methods and results:** By adopting preventive chemotherapy, the Global Programme to Eliminate Lymphatic Filariasis (GPELF) has expanded MDA coverage from three million people treated in 12 countries in 2000, to more than 450 million in 53 countries in 2010 . During that period, the disease was eliminated in China and Korea. Nine countries no longer require MDA because of a natural decline in transmission intensity that is connected to a history of intense vector control. Nevertheless, many LF endemic countries are yet to initiate MDA including 20 in Africa. The majority of the untreated countries are post-conflict or otherwise fragile states. Great progress has been made in the Americas and Asia-Pacific regions, where many countries have achieved 100% MDA coverage of all their endemic districts and reduced microfilaria prevalence to below 1%. During 2000–2010, greater than 3.4 billion doses of medicine were delivered to a targeted population of 897 million people. However, in limited areas in Africa where LF may be coendemic with Loaiasis (a filarial disease of the skin and eye

caused by the nematode worm Loa loa) treatment with ivermectin has been associated with serious adverse events presented as progressive neurologic decline and encephalopathy. Nevertheless, confidence in the preventive chemotherapy strategy has increased the momentum to eliminate lymphatic filariasis over the past 10 years and funding to control it and other neglected tropical diseases will increase substantially, from $100 million in 2010 to over $600 million in 2012. The use of treated mosquito nets for malaria control has also been associated with accelerated decrease in LF transmission intensity in Papua New Guinea and other countries in Africa and where Anopheles mosquitoes are the main vectors. Anti-wolbachia treatment is proving effective in loa loa coendemic areas. **Conclusion:** The main challenge for interrupting the transmission of LF globally by 2020 will be organising and coordinating the increasing financial support for integrated control of NTDs, rather than focusing on, new drugs, vaccines, treatment regimens or intervention strategies against the disease.

RT78- **Current status of Chagas disease control in the Paraguayan Chaco**

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The Gran Chaco extends over the territories of Bolivia, Paraguay and Argentina with a population of 4 million people, and is a hyperendemic zone for Chagas disease. The main control strategy in the area is domestic spraying to eliminate the vector; however the elimination of *Triatoma infestans*, the main and intradomiciliar vector of the disease, has failed for reasons not yet well defined. In this Eco region, it has been routinely observed that reinfestation by the vector occurs rapidly after spraying with residual insecticides, so there is an urgent need for a sustainable monitoring system for poor, rural populations that are difficult to reach. The initial seroprevalence data in the Paraguayan Chaco ranges between 12 to 83% and the infestation of households with *T. infestans* ranges from 26 to 100% (Rojas de Arias 1996, 2003; Rojas de Arias et al., 1993). More recently, serological evidence of infection with *T. cruzi* was demonstrated in 43% of 519 Amerindians and in only 2% of 161 Mennonites of German descent and Paraguayans of Spanish descent living in the western Chaco (Ferrer et al., 2003, 2004). In the Central Chaco in 2003, the National Chagas Program estimated a mean prevalence of infestation pre-spray of 17%, reaching 45% in some locations. By 2005 the prevalence of reinfestation was 5 to 10% in indigenous communities (SENEPA, 2005). During an evaluation conducted in 2009, a total of 7,902 housing units corresponding to 248 sites found a total of 358 households infested with *T. infestans* (4.6%). Following this spraying was done to protect 30,050 people in the Chaco region. The big challenge is the establishment of entomological surveillance in indigenous communities dependent on the Family Health Units established in the region. Among the main difficulties which may be mentioned as relevant to the surveillance system for Chagas disease and including installing the way to eliminate *T. infestans* from homes it should be noted that (Rojas de Arias, 2003): a. Language families and characteristics of different groups: Linguistic diversity and the complexity of the worldview of these ethnic groups make the dissemination of information about Chagas disease difficult. b. Housing characteristics and peridomestic surroundings: The materials used for the construction of indigenous households hinder the removal of vinchucas (kissing bug vectors). The Nivaclé and Angaité groups use stucco, while wood is more often used by the Ayoreos and the Guarani Ñandeva. The Guarayos most frequently use adobe walls. Accumulations of household goods, where the vinchucas persist after the chemical spraying of the house, make their removal difficult. The peridomestic surroundings are minimal, with the presence of outdoor kitchens and bathrooms which are precarious or nonexistent. c. The residual insecticides and potential resistance to them: In the Chaco, the residual effect of the insecticide is poor on different substrates, so the initial impact of insecticides on triatomine populations is essential for the elimination of T. *infestans* from the home. d. The excessive heat, water salinity, and constant winds in the Chaco region are impediments to the residual insecticide. From different studies in the area and in consultation with residents it is known that the vinchucas come from flying from the bush to the house during the dry season. e. Migration and the distances between communities: Historically the indigenous groups were hunters and gatherers; however, in recent decades, several groups have sedentarized, which has facilitated the installation of vinchucas in the houses. The greatest impact of this factor is linked to the dispersion of vinchucas and the difficulties that may cause for the control and surveillance of Chagas disease. f. The recent discovery of the presence of wild *T. infestans* around the houses opens a new

research perspective that will support the control program in the area. Studies have shown a potential gene flow from the populations in the houses and peridomestic area with wild foci, which opens the perspective of dynamic displacement between populations strengthening the hypothesis about processes of reinfestation by both domestic and wild populations (Rolon et al., 2011). The communities covered by the National Program did not know of Chagas disease, they only knew of: the vinchuca, but it was never associated with disease. Chagas disease is silent and does not draw attention by its signs and symptoms; it passed unnoticed in these communities. During the first actions of the program for the start of captures, identification of vinchucas and spraying of homes, officials distributed information on the presence of the disease and its impact on people accompanied by an informational brochure which was given to the inhabitants of the houses (Russomando et al, 2007). The initiative to provide information to the communities has been the basis for subsequent actions such as entomological surveillance and the training of leaders. These have been well-received by the population, as well as the incorporation of activities with an important role in the local schools (Rojas de Arias et al, 1993). The school activities achieve a double purpose, by increasing the knowledge and participation of children and young people, and the creation of a school curriculum about a public health problem, which is repeated annually in endemic areas of disease during Chagas week (Rojas de Arias & Russomando, 2001). Since 2009 the program conducted a series of workshops with the purpose of changing the community evaluation tool during the surveillance process in order to use risk indicators identified at the local level. These indicators for housing conditions include both the inside of the house and its external environment. This risk approach methodology has permitted the Program to focus its actions in the areas of permanent reinfestation, gaining effectiveness, and saving time and money. The Project decided to address the Chaco with a multicultural approach and provide comprehensive care to the indigenous peoples in the zone. This integrated model has had significant achievements: 85% of health professionals provide comprehensive support to the communities of a targeted 96%; furthermore a system for recording data was established in the 13 reporting centers and more than 180 notifying posts that participate in the project. In addition, the National Chagas Program has established three community surveillance centers where they receive reports of infestations with the collaboration of the municipalities and health organizations. During 2012 prenatal diagnosis of congenital Chagas disease is being implemented. For the multiethnic perspective of the Chaco region, the Program fights to form bridges between the different ethnic cultural codes and the school which brings knowledge to these communities. Therefore, there are a number of factors embodied in the knowledge, values, and languages shared by the different ethnic groups, which interact in spectrum of the social networks that exist in the Paraguayan Chaco.

RT79- **Schistosomiasis control in Africa: challenges, opportunities and way forward. Problem statement**

Narcis B Kabateriene

Six hundred million people are at risk of schistosomiasis infection globally and 85% of these live in Africa. On this continent, about 134.6 million children and 451.7 million adults are targeted for regular treatment with praziquantel, but only 25% of the children and just 9.5% of the adults were treated in 2010. Of the 40 endemic countries, just 32 have initiated schistosomiasis control programmes while only 8 of the 32 have scaled up the control to the national level. Mapping to determine the magnitude of the problem is slow thus vast areas of the continent are either unmapped or partially mapped. The challenges for scaling up control include limited funding, inadequate skilled personnel, lack of effective communication strategy in an area where appropriate policy and behavior change to promote preventive measures is rather difficult in the absence of alternative sources of safe water. Sanitation on the continent is appalling and resources to implement drastic improvement are lacking. Equally challenging is snail control which requires highly skilled personnel to identify where? When? And how? To apply snail control measures. Molluscicides are expensive, snails re-populate quickly and modifying the environment through draining of wetlands is currently unpopular. Despite the above problems, elimination of schistosomiasis on the continent is more promising today than ever before. WHO is highly committed to eliminate the disease and has made the necessary resolutions and produced the necessary guidelines to achieve the target. Schistosomiasis has been included in the health sector strategic plans of endemic countries most of which have comprehensive plans of action to eliminate the disease. Above all, the required amount of praziquantel,

the drug of choice is usually donated to endemic countries and the drug is highly efficacious. As a way forward, it is vital to integrate control efforts into existing health systems. Continuous partnership building is vital to raise the necessary resources for sustainability of control measures. It is important that future water development projects engage Health Impact Assessment and implement its resolutions. The endemic communities must be fully involved in control efforts to achieve transmission control. Equally important is to strengthen surveillance, monitoring and evaluation and to invest in safe water supply and sanitation improvement. Obviously, there is a need to review the existing guidelines and tools to align them with transmission control rather than morbidity control. The under-fives who are currently excluded in preventive chemotherapy must be part of the future target for mass treatment campaigns. Equally important is to ensure that the donated praziquantel is of good quality through regular assessment of the drug efficacy and its impact on the parasite gene pool.

RT80- **Diagnosing and treating schistosomiasis aimed at its elimination in Brazil**

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In May 2012 the Brazilian Government adopted Resolution WHA65.19 of the World Health Assembly aimed at intensifying schistosomiasis control towards its elimination. Guidelines set up by the Schistosomiasis Control Program of the Ministry of Health (PCE/MS) in the late-1990's comprised active search of infected persons through periodic stool surveys followed by selective treatment at the primary health care level together with auxiliary, preventive measures. The control efforts have contributed to a progressive reduction of prevalence in the endemic areas. Of the 699 endemic municipalities for which there are minimally reliable data in both the 1999-2004 and the 2005-2010 periods, the number of municipalities with prevalence lower than 5% (low risk) increased from 274 (39.2%) to 514 (59.3%), whereas the number of those with prevalence of 25% or more (high risk) decreased from 27 (3.9%) to only seven (1.0%). Overall prevalence dropped significantly ($p < 0.05$) from 8.3% (688,342 egg-positives out of 8,334,390 exams) to 6.4% (627,878 egg-positives out of 9,760,458 exams) between the two periods; a reduction in prevalence was observed in 80.1% of the municipalities (560 out of 699). Interruption of transmission, defined as no new cases in children and no infection in host snails in a circumscribed area for a specified time period, may not be achievable in the short term in Brazil. Thus, an Active Surveillance Phase (ASP) is recommended for municipalities with less than 5% prevalence, aimed at reducing transmission to the lowest feasible level as a preliminary step towards elimination. The ASP should include a quadrennial school-based stool survey followed by a community-based survey whenever an egg-positive child is found, and treatment of all egg-positive cases. The ASP should also comprise preventive interventions such as health education, environmental sanitation, safe water supply and snail control where appropriate. Efforts needed for diagnosing and treating the ASP target population may be estimated on basis of the number of eligible persons from the low-risk endemic municipalities between the years 2005-2010. In Pernambuco State, for instance, there were 37 low-risk endemic municipalities totaling 945,154 inhabitants, of which 182,639 (19.3%) were school-aged children (6 – 15 years), according to the 2010 census. Egg-positives have been found in 670 (63.9%) of the 1,049 localities covered by the PCE/MS in those municipalities, with an estimated number of 866,204 residents targeted for community-based survey and 35,745 targeted for treatment based on an egg-positivity of 4.1% (9937 positives out of 240,802 exams). Current control strategy in Brazil is mainly focused in the moderate-risk areas (through active-search surveys and selective treatment) and high-risk areas (mass drug administration – MDA); the strategy for the low-risk areas comprises primarily passive case detection and investigation. It is clear that moving towards the interruption of transmission would require the implementation of increased, active surveillance measures in the low-risk areas while scaling up the control efforts in the more troubled areas. Improved diagnosis and treatment are mandatory to accomplish the goal of eliminating this devastating, poverty-related disease as a public health problem in Brazil.

RT81- **A Gold Standard for the diagnosis of active Schistosomiasis: detection of a single worm pair.**

Van Dam Govert

Introduction: The well-studied schistosome antigen detection ELISA (CAA-ELISA) has been transformed into an ultra-sensitive UCP lateral flow based assay (CAA-UCP). The objective of the study presented here was to improve robustness and sensitivity of this assay under lab and field conditions. Data showed: 1) the development of dry-reagent assays with extended shelf life allowing shipping and storage at ambient temperature; 2) usage of portable readers; 3) simplification and increase of sensitivity by modification of serum and urine sample handling; 4) large scale applications in various high and low resource settings; 5) detection of ultra-low infection levels, down to one worm-pair. The conclusion is that the assay is robust, suitable for use in low-resource settings, ultra-sensitive and specific, and has a significant potential to be developed into a Gold Standard diagnostics for schistosomiasis.

RT82- **Characterization of the *Anopheles aquasalis* immune response to *Plasmodium vivax***

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Malaria affects millions of people worldwide annually, and 450.000 only in Brazil. The interaction of malaria vectors and parasites has been extensively studied, but very little is known about the pair *Anopheles aquasalis-Plasmodium vivax*, of great importance in the malaria scenario in Brazil. One of the reasons for this lack of information relies on the virtual impossibility of *P. vivax* cultivation, and the recently challenged belief that disease caused by this parasite is not serious. We are characterizing the immune response of *A. aquasalis* to *P. vivax*. We constructed subtraction libraries, comparing infected and non-infected insects. Surprisingly, few immunity genes were identified 2 and 24 hours after infection (hAI). Among these were a serine proteinase with diminished expression, and a carboxipeptidase with increased expression. We also identified a GATA transcription factor, more expressed in males than females and induced (almost 15 times) 36 hAI. Infection increased 63% after GATA knock-down, confirming its importance in the immune response of *A. aquasalis* against *P. vivax.* Specific genes were amplified using degenerate primers and characterized. The immune response genes STAT, PIAS and NOS were induced by infection, demonstrating the importance of the JAK/STAT pathway in response against the parasite. Silencing of STAT caused an increase in oocysts count. In relation to the detoxification enzymes, we observed an increased expression of SOD and catalase 36 hAI and a decreased activity at 24 hAI. Fluorescence microscopy using a redox state probe showed a reduction of free radicals in both blood fed and infected insects when compared to sugar fed insects. RNAi-mediated silencing of catalase reduced enzyme activity in the midgut and resulted in increased *P. vivax* infection and prevalence. Our findings suggest that the interactions between *A. aquasalis* and *P. vivax* do not follow the model of ROS-induced parasite killing. It appears that *P. vivax* manipulates the mosquito detoxification system in order to allow its own development. These findings provide novel information on unique aspects of the main malaria parasite in the Americas interaction with one of its natural vectors.

RT83- **Epithelial nitration by a heme peroxidase/NADPH oxidase system as a response of mosquito midgut to *Plasmodium* infection**

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When *Plasmodium* ookinetes invade mosquito midgut epithelial cells, they inflict irreversible damage and trigger protein nitration. Invaded cells induce nitric oxide synthase (NOS) expression which catalysis the formation of nitric oxide (NO), a substrate for tyrosine nitration of cell proteins. It was observed that nitration appears to be a two-stage process in which production of NO is followed by the induction of epithelial peroxidase activity. We identified a heme peroxidase (HPX2) and a NADPH oxidase 5 (Nox5) as critical mediators of midgut epithelial nitration that enhances NO toxicity towards *Plasmodium* parasites in *Anopheles gambiae*. *Plasmodium* ookinetes traverse midgut epithelial cells before they come in contact with the complement system in the mosquito hemolymph. Our studies provide direct experimental evidence that two immune mechanisms targeting ookinetes, epithelial nitration by HPX2/Nox5 system and thioester containing protein 1 (Tep1)-mediated lysis, are part of the same immune response against *Plasmodium* and work sequentially. We propose that epithelial nitration is working as an opsonization-like system that promotes activation of the mosquito complement cascade.

RT84- **Bioinformatics in the control of vector populations**

Christos Louis

After the recently renewed effort to eliminate malaria (and, for that matter, other vector-borne diseases) control of arthropod disease vectors needs to start using novel strategies to achieve the goal in a most efficient way. My presentation will draw round the use of modern information-technology (IT) tools to this effect. In addition to the usage the now available genome sequences of several disease vectors, which provide starting blocks for the design of efficient molecular methodologies, novel IT-based approaches will include, most notably, intelligent databases and decision support systems, especially ones driven by ontologies. The community-wide adoption of ontologies will not only enable interoperability of the IT tools but also will widen their utilization across different diseases and their vectors.

RT85- **The utility of the *Leishmania*-macaque infection model to assess different prime-boost vaccination approaches**

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Leishmaniasis is a vector-borne disease caused by the *Leishmania* genus of protozoan parasites, constituting an important global health problem for which there are few effective drugs. Given the urgent need to identify a safe and effective *Leishmania* vaccine to help prevent the two million new cases of human leishmaniasis worldwide each year, all reasonable efforts to achieve this goal should be made. This includes the use of animal models that are as close to leishmanial infection in humans as is practical and feasible. Divergent evolution (~ 210 million year divergence between rodents and humans) limits the relevance of murine models in vaccine development. In this regard, the Asian rhesus macaques (*Macaca mulatta*), which diverged from humans approximately 25 million years ago (*Science 316*: 222-234, 2007), are emerging as invaluable *in vivo* model of pathogenesis and immunity to infectious diseases requiring cellular immunity (*Semin Immunol 19*: 310-317, 2007), but are also a key tool in the final stages of evaluation of vaccine candidates that have already shown consistent induction of significant protective immunity in mice (*Mol Med Today 6*: 267-270, 2000). It has also been reported (*Mem Inst Oswaldo Cruz 103*:629-644, 2008) that outbred macaques are quite susceptible to leishmanial infection, develop a human-like disease, exhibit antibodies to *Leishmania* and parasite-specific T-cell mediated immune responses both *in vivo* and *in vitro*, and can be protected effectively by vaccination. Nonetheless, depending on the particular vaccine approach used, varying degrees of protective immunity have been achieved, as determined by the level of parasite burden in infected sites and/or lesion size following infectious challenge (*Infect Immun 69*: 4103-4108, 2001; *Mem Inst Oswaldo Cruz 97*: 1041-1048, 2002). Data will be presented on *Leishmania* antigen candidates that are efficacious as subunit protein or DNA-based vaccines against leishmaniasis in macaques. Further development of the *Leishmania*-macaque

model should prove useful in guiding the design of human vaccine trials, but it will also be necessary to move from the laboratory to the field to validate if an effective vaccine can have an impact on the prevention of the human disease. **Funding:** Fiocruz and INCT/CNPq-420067/2005-1, Brazil **E-mail:** grimaldi@bahia.fiocruz.br

RT86- **Hepatitis delta in South American countries**

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Hepatitis Delta infection is considered to be the most severe form of viral hepatitis, often leading to the rapid development of liver cirrhosis. Furthermore HDV infection has also been linked with a higher risk for the development of hepatocellular carcinoma. HDV is found throughout the world, but its prevalence, incidence, clinical features, and epidemiological characteristics vary by geographic region. There are eight genotypes (1 to 8) of HDV distributed over different geographic areas. HDV/1 is distributed worldwide, whereas HDV/2 to HDV/8 is seen locally. HDV/1 is prevalent in Europe, Middle East, North America and North Africa; HDV/2 prevails in Japan, Taiwan and Russia; HDV/3 in the Amazon region in South America; HDV/4 in Japan and Taiwan; and HDV/5 to HDV/8 prevails in Africa. HDV is endemic in many populations with a high prevalence of HBV, ranging from 70% among chronic HBV carriers in the Amazon basin to 20% in Africa to <1% in North America. In some regions of the Mediterranean, Africa and the Middle East, more than 24% of HBV carriers have markers for HDV. However, this infection is uncommon in countries like the United States, where this infection is largely confined to risk groups like drug users and hemophiliacs. Latin America is an area of intermediate HBV endemicity, where HDV is not restricted to groups at risk, and has been associated with severe and fatal fulminant hepatitis. In Brazil, HDV has been reported in the western Amazon region, where a large number of cases of acute and chronic infections by this virus have been described. The interpretations of epidemiological studies on infection should take into account the fact that it requires the presence of HBV. Several studies performed in the 1980s showed the presence of HDV infection in South America. HDV usually induces a severe disease but its clinical manifestations are very broad, ranging from asymptomatic cases to fulminant hepatitis. HDV-3 has been isolated in the northern area of South America only (Amazon Basin of Brazil, Peru, Colombia and Venezuela). For HDV/3, studies in the Amazon region on prevalence of HBV and HDV showed that family members are reservoirs for transmission of infection by HDV. In this way, the chances of contamination from an extra-familial source are expressed by highly divergent isolates and the sequence similarity in most families units indicate a single source of infection providing evidence that HDV infection is probably mostly transmitted within the families. Recently, it was determined that HDV/3 spread exponentially from early 1950s to the 1970s in South America. It was suggest that the measures implemented to control HBV transmission resulted in the control of HDV/3 spreading in South America, especially after the important raise in this infection associated with a huge mortality during the 1950s up to the 1970s. Novel strategies to increase HBV immunization in the Latin American population are necessary to coverage the rural region to control the HDV infection. FAPESP 2001/50562-0, FFM and HCFMUSP **E-mail:** monica.viviana@usp.br

RT87- **Open access and the NTDs**

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PLoS Neglected Tropical Diseases was established in 2007 as an open-access journal with a multipurpose mission to 1) serve the community of expanding NTD scientists, physicians and other health care practitioners, and public health workers; 2) build scientific and editorial capacity in disease-endemic countries, especially in low- and middle-income countries (LMICs); and 3) help shape a new public policy around the emerging concept of the NTDs. Today PLoS NTDs has an editorial board heavily represented by investigators from disease endemic countries – 30% of our associate editors are from LMICs. After the U.S., Brazil now represents the second most frequent country from where the journal receives papers,

followed by the U.K., France, India, and China. Innovative approaches to measuring the impact of PLoS papers, such as article-specific metrics allow us to assess the areas of research and policy of greatest interest to the NTD and global health community.

RT88- **Molecular epidemiology of hepatitis B and C virus in Latin America**

Flor Pujol

Around 11 million persons are infected with hepatitis B virus (HBV) and 7 million with hepatitis C virus (HCV) in Latin America. The Southern regions of South America and Central America exhibit lower prevalence of HBV infection, compared to the higher prevalence found in the Amazonian and the Caribbean regions. A very high prevalence of infection is found among Amerindians. Eight HBV genotypes (A–H) have been described. Genotypes A and D are predominant in the Old World but are also widely distributed in all the continents. Genotype A is also predominant in Brazil Genotypes B and C are found mainly in South East Asia and the Far East, while genotype E circulates in sub-Saharan West Africa. Genotype G has been reported in the US, Mexico and Europe, but its distribution is not fully known. HBV genotype F is the most divergent of the HBV genotypes, autochthonous to South America and highly predominant in the region. The recently described HBV genotype H is closely related to genotype F and seems to be restricted to Central and North America. The relative frequency of HBV genotype F in Latin America is in close correlation with the degree of admixture of the general population with Amerindians. The origin of HBV is still an open question. Human HBV genotypes might also have emerged through several zoonotic introductions, as proposed for human immunodeficiency viruses. HCV has been classified in 7 genotypes, and in several subtypes inside each genotype. HCV genotypes 1, 2, and 3 have a worldwide distribution. HCV genotype 3a is frequent in intravenous drug abusers in Europe and the United States. HCV genotype 4 is prevalent in Africa and the Middle East, and genotypes 5 and 6 seem to be confined to South Africa and Asia, respectively. HCV genotype 7 was more recently identified in Canada, in an emigrant from the Democratic Republic of Congo. Changes in hepatitis C virus (HCV) genotype distribution with time have been reported in several countries. In Venezuela, for example, a significant reduction of the circulation of HCV genotype 1b was observed in the last decade, with the increase of circulation of genotype 2j. Several subtypes of HCV genotype 2 and 4 were introduced in some countries of the Americas during slave trade in Martinique and of HCV genotype 2 in Venezuela. While the introduction of genotype 1a and 1b has been estimated in several Latin American countries at the beginning of the last century, the introduction of some genotypes 2 and 4 might have occurred at the 18th century. It is difficult to estimate for how long HCV has been present in human populations. HCV may have been endemic in Asia and Africa for a considerably longer time than in Western countries. HCV subtypes might have diverged around 200-250 years ago and genotypes around 500-2000 years ago.

SATELLITE *SYMPOSIA*

SS1- ***P. vivax*** **epidemiology Symposium: Challenges of** ***P. vivax*** **treatment**

Professor Marcus VG Lacerda
Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Universidade do Estado do Amazonas, Universidade Nilton Lins

Malaria became the most relevant parasitic disease in tropical areas, being *Plasmodium vivax* the most widespread species. Since 2007, with the new wave of considerations about malaria eradication, this formerly considered ‘benign’ parasite became the major challenge for eradication. This much more evolved parasite is able to develop hypnozoites in the liver (responsible for continuous relapses), releases gametocytes in the peripheral circulation much earlier, is more resilient to climate changes and is transmitted by vectors with both indoor and outdoor biting capacity. Conjointly, these characteristics make *P. vivax* a more resistant type of malaria to control. To complicate matters, the disease triggered by

this parasite has been increasingly reported to be more virulent, and chloroquine-resistance in many endemic areas worldwide is not negligible. In South America, especially in Brazil, which concentrates ~50% of the malarial cases in this sub-continent, public health improvement in general, added to the integrated use of the available tools for the control, was responsible for a decrease in *P. falciparum* infection, however it could not change the profile of *P. vivax*, which still defies national control programs. In the Brazilian Amazon, in the last 20 years, *P. vivax* became responsible for 85% of the cases. In Manaus, in 2011, this species caused 99% of the cases. The major gaps of knowledge to control this infection are discussed.

SS2- **Evaluating cure for neglected parasitic diseases: a challenge for the success of drug efficacy trials.**

Faustino Torrico (Bolivia), José Muñoz (Spain), Zeno Bisoffi (Italy), Anis Rassi (Brazil), Marco Albonico (Italy)
COHEMI Network

Most neglected parasitic diseases share common challenges in the clinical practice: diagnosis can be difficult, cure assessment methods lack of enough sensitivity or specificity, and current therapies are few, old and sometimes not enough efficacious. The definition of cure used for different neglected parasitic diseases is diverse, depending on the disease and the context of study. When monitoring treatment efficacy at individual level and in randomised clinical trials (RCT), treatment must aim at completely eradicating the infection, and very sensitive tools are needed to avoid overestimating the efficacy of the drug tested. In the scenario of control/elimination programs based on preventive chemotherapy, cure has a different definition: it is aimed at decreasing morbidity and reducing transmission. “Elimination” of a disease in public health is a broader concept than “cure” in clinical medicine, as it implies interruption of transmission but it is reached after several rounds of treatment. In this context, monitoring drug efficacy is of paramount importance for the potential occurrence of drug resistance. Improving our methods to evaluate cure for neglected parasitic diseases is imperative to foster our capacity to detect treatment failures in clinical practice, to evaluate drug campaigns for public health purposes and to test new drugs in clinical trials. The case of *Trypanosoma cruzi* and *Strongyloides stercoralis* is paradigmatic: clinical symptoms do not usually help evaluating cure shortly after treatment, and microbiological cure can be difficult. In the case of Chagas disease, microbiological cure does not necessarily correlate with the clinical progression of the disease. One of the main concerns in Chagas disease is the lack of a diagnostic gold standard for the evaluation of drug efficacy in patients treated with benznidazole or nifurtimox. In acute, early congenital or reactivated *T. cruzi* infection, clearance of parasitaemia, and disappearance of antibodies are taken together as cure criteria by most authors. However, in the chronic phase, new methods for monitoring response to treatment are urgently needed. Negative seroconversion by conventional assays takes 1-3 decades to occur after successful treatment, and due to the subpatent parasitaemia, parasitological tests by blood culture or xenodiagnoses have limited sensitivity. This means that parasitological test results are meaningful only when they are positive, indicating a therapeutic failure. The variable sensitivity of PCR-based techniques also limits their usefulness in the evaluation of antitrypanosomal drug therapy in patients with chronic Chagas disease. *In the case of* Strongyloides stercoralis *infection, the traditional methods of larvae detection in stools lack sensitivity, and need to be complemented by other tools. This limitation is crucial for the monitoring of treatment efficacy at individual level and in randomised clinical trials (RCT), bearing in mind that the treatment must aim at completely eradicating the infection, and this cannot be demonstrated if insensitive diagnostic methods are used. On the other hand, indirect methods (serology) are certainly more sensitive, but also less specific. PCR as well as antigen detection methods have been developed but their role in the evaluation of cure is still uncertain. The treatment of choice is ivermectin, but the best drug regimen has yet to be determined, due to the only partial reliability of the RCT carried out so far.* **Supported by** the EC within the 7th Framework Program under grant agreement no. FP7 - GA-261495

SATELLITE WORKSHOP

SW1- **Scientific Writing in Travel Medicine Research**

Alfonso J. Rodriguez-Morales
Commission on Scientific Publications and Teaching, Latin American Society for Travel Medicine (Sociedad Latinoamericana de Medicina Del Viajero, SLAMVI) Faculty of Health Sciences, Universidad Tecnológica de Pereira and Office for Scientific Research, Cooperativa de Entidades de Salud de Risaralda (COODESURIS), Pereira, Risaralda, Colombia.

In the context of the activities of promotion of travel medicine practice and research of the Latin American Society for Travel Medicine (Sociedad Latinoamericana de Medicina Del Viajero, SLAMVI), a four-day workshop on scientific writing has been planned. This activity is oriented to take the participants step by step through getting published in Travel Medicine and related disciplines, introducing those novels in this process as well increasing the publication effectiveness of those involved in practice and research in Travel Medicine from Latin America, as well from other regions, particularly those less developed and with less scientific production of articles in the area. Programmed topics include: Introduction to Scientific Writing and Publication and Relevance for Travel Medicine at Global and Regional Levels; Beginning with Basics: Structure of a Scientific Paper; Writing a Methods section in a manuscript; Presenting Results: how to make effective communication of research findings; A necessary background of your paper: Introduction; Finding meanings: the Discussion; Case reports and Letters to the Editor: Importance in Travel Medicine Research; How to select the most appropriate journal for your manuscript; and Surviving peer-reviewing: how to do it.

WORKSHOP

Worksh1- **The Global Plan on Insecticide Resistance Management in Malaria Vectors (GPIRM) – Staying Ahead of the Curve**

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During the past decade, unprecedented progress has been achieved in malaria control. However, reports of insecticide resistance in a number of countries especially from sub Saharan Africa, threaten these fragile gains. Long-lasting insecticidal nets (LLINs) and indoor residual spraying are central pillars for malaria vector control and remain highly effective in most settings. However, urgent action is required to prevent resistance from emerging at new sites, and to maintain the effectiveness of vector control interventions in the short, medium and long-term. To address this, the WHO Global Malaria Programme coordinated the development of a Global Plan for Insecticide Resistance Management in malaria vectors (GPIRM). This was achieved by synthesizing input from over 130 stakeholders representing all the constituencies of the malaria control community. The result of this exercise is an urgent call to action, laying the foundations for integrated insecticide resistance management practices in all malaria-endemic countries. GPIRM consists of five major activities (pillars) spanning in the short, medium and long term. These include the planning and implementation of insecticide resistance management; ensuring proper, timely entomological and resistance management and effective data management; developing innovative vector control tools; filling the gaps in knowledge on mechanisms of resistance and impact; and ensuring that enabling mechanisms (advocacy, human and financial resources) are in place. The first two activities are country-driven. However, given the limited entomological capacity in many countries, support from the international community is critical to achieving these activities. The purpose of this presentation is to

provide awareness regarding the GPIRM and advocate for resources and commitment for its implementation at all levels. In this way we will stay ahead of the curve of insecticide resistance.

ORAL PRESENTATIONS

DISEASES BY PROTOZOAN

MALARIA

Malaria control

Mal1. **Controlling malaria using livestock-based interventions**

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Introduction: In areas where human malaria is transmitted by zoophilic vectors, control interventions have been proposed based on using animals to divert vector biting from people (zooprophylaxis), as well as baits to attract vectors to insecticide (insecticide-treated livestock). Contradictory results have been obtained on the effects of untreated livestock on malaria transmission and although the insecticide treatment of livestock appears to be a promising strategy, much of its potential remains to be uncovered. This study aims to clarify the different effects of livestock on malaria and to understand under which circumstances livestock-based interventions could play a role in malaria control programmes. **Material and Methods:** The impact of livestock abundance, availability and insecticide-treatment (ITL) were explored, by developing a deterministic mathematical model and integrating it with data from Pakistan, where an ITL trial for malaria control has been performed (Rowland et al. 2001, Lancet. 357:1837-41), and from Ethiopia, where a field study was conducted to parameterize the model. **Results and Main Conclusions:** The model allows explaining situations where livestock by itself can lead to a decrease, increase or no impact at all on malaria transmission to humans. The key factors affecting the effects of untreated livestock on malaria transmission are the relative density and availability of the livestock and human hosts, the vector density in relation to the carrying capacity of the ecological system previous to introduction of livestock, and the time elapsed since livestock introduction. The findings further indicate that ITL is likely to be more beneficial in settings with highly zoophilic vectors as in Asia, than in Sub-Saharan African settings with the more opportunistic vector *An. arabiensis.* Nevertheless, the intervention is still likely to substantially decrease malaria incidence in the African settings. This work also highlights the importance of accounting for potential excito-repellency effects of the insecticide applied on livestock, although the results suggest that only if the insecticide has very strong excito-repellency would ITL cause an increase in malaria cases, and thereby become prejudicial. It also highlights that when designing and implementing an ITL intervention for malaria control it is important to have a good understanding of the density-dependent regulation that is operating in the vector population, as that can have determinant effects upon the intervention outcome. It is hoped that this work may pave the way for the implementation of an ITL intervention trial in an African region with *An. arabiensis* where this strategy could contribute to the integrated control of malaria and livestock diseases.
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Mal2. **Assessment of intervention impact on immunity against *Plasmodium falciparum* antigens in Príncipe Island – a sero-epidemiological study and mathematical modeling insights**

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Introduction: In Sub-Saharan Africa, the place where malaria eradication seems more feasible is Príncipe Island, the smaller partner island in the archipelago of São Tomé and Príncipe due to its geographically isolated position, low number of inhabitants and historically lower parasite rates than neighbor São Tomé and rural areas of central Africa. Since the implementation of control measures in 2003, incidence has decreased 99% in 5 years and slide-positivity rate prevalence was 0.9% in 2009, with most cases being asymptomatic. These measures only assess the parasitological state of individuals and do not inform about the loss of antibodies against *P. falciparum* antigens, a fact that is a threat with intervention failure. **Methods:** Cross-sectional surveys were conducted in 11 communities of Príncipe Island in 2005, 2008 and 2010 to inform about the parasitological status of individuals and potential risk factors of transmission. A subset of individuals had their sera collected and analyzed for the presence of antibodies against antigenic variants of PfEMP1 protein, and against conserved antigens expressed by *P. falciparum.* Statistical analyzes were performed to determine significant factors that influence the parasitological and serological data. Mathematical models were developed to inform about seroconversion and seroreversion rates to conserved antigens to assess transmission intensity and cumulative exposure to malaria antigens, the evolution over time of parasitaemia and immunity to conserved antigens in the population and the evolution of acquired immunity to PfEMP1 variants as an estimate of the repertoire of protection. **Results and Main Conclusions:** The results are informative about the role of serological markers of malaria transmission and the usefulness of mathematical models to conclude about intervention effectiveness on malaria transmission and forecast risks of intervention disruption. **E-mail:** cbandeiras@igc.gulbenkian.pt

Mal3. **Coartem®: A 10 year experience of patient-centric approach to fighting malaria**

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Introduction: Over 480 million treatments of Coartem® Artemether-lumefantrine (AL, Novartis), the accepted gold standard artemisinin-based combination therapy (ACT) for malaria, have been deployed to endemic countries over the last 10 years. M**ethods:** From providing a quality medicine in public/private partnership with WHO, our focus shifted to a holistic, 'patient-centric' approach, addressing the unmet medical needs in children by developing dispersible tablets and involving multiple partnerships, with a focus on educating caregivers and patients to ensure that patients with uncomplicated *P.falciparum* infection receive timely treatment of malaria and adhere to full course of medication. **Results:** A dispersible formulation was developed jointly with Medicines for Malaria Venture (MMV) to meet the specific needs of children. AL dispersible tablets can be given dispersed in a small amount of liquid and are sweetened to mask the bitter taste which is typical of most antimalarials. To enhance treatment adherence in low literacy settings, innovative packaging was designed in partnership with WHO, using pictograms to remind the patient how many tablets to take at each time point and showing the importance of completing the full treatment course. Novartis has an ongoing commitment to train and educate health care workers and communities. Initiatives include best practice sharing workshops for Public Health

officials from National Malaria Control Programs in developing countries, development of patient information and training material. To increase affordability and availability, over 480 million treatments have been provided without profit to governments and nongovernmental organizations. New strategies to expand access to ACTs have also been implemented like the SMS for Life initiative, part of the Roll Back Malaria (RBM) program, a tool for supply chain management based on electronic mapping technology and short text messages sent via mobile phones. Currently, we are evaluating novel approaches that may be of use in malaria elimination strategies. A study assessing the utility of AL in reducing parasite transmission and disease burden by mass screening and targeted treatment of *P. falciparum* asymptomatic carriers in entire village populations is ongoing and first data is expected during the second half of this year. **Conclusion:** These initiatives go beyond a mere deployment of drugs, support maintaining and further evolving a patient-centric approach, and are essential for achieving a sustained health benefit in developing countries. **E-mail:** kamal.hamed@novartis.com

Mal4. **A descriptive study in acute uncomplicated Plasmodium falciparum malaria in infants <5 kg body weight in 4 Sub-Saharan African countries**

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Introduction: Published reports on malaria in younger infants are scanty, leaving a significant knowledge gap about the pattern and outcome of malaria in this sub-population. Artemisinin-based combination therapy (ACT) is recommended as first-line treatment for infants ≥5 kg of body weight (BW) with uncomplicated malaria caused by *P.falciparum*, but no ACTs are indicated in the population <4.5 kg. Epidemiological data to inform trial design in this age-group is a priority. **Methods:** Hospital charts from 4 countries in Sub-Saharan Africa (Bénin, Democratic Republic of Congo, Nigeria, and Togo) were retrospectively reviewed for the period between 2006-2010 for inpatient neonates and infants <5 kg BW with a confirmed diagnosis of uncomplicated *P. falciparum* malaria. Clinical features, age group, treatment, and outcome were collected. **Results:** The number of patients admitted per year ranged from <20 to >90 across hospitals and calendar years. The proportion of cases varied by age (≤28 days vs >28 days): the proportion of infants in the older group was generally higher, but the younger group represented from <2% at one hospital in the Democratic Republic of Congo to >70% at another in Togo. The most frequent clinical presentation was fever, followed by dyspnea, crying, and vomiting. Whenever results were available, parasite load was generally low; <10% of the infants presented with parasitaemia >5,000/µL. The majority of the infants were treated with oral quinine, except at two hospitals in Bénin and Togo, where artemether-lumefantrine and intramuscular artemether were administered, respectively. **Conclusions:** Although infrequent, malaria in neonates and infants <5 kg of BW does exist in certain endemic countries and calls for appropriate treatment. Further clinical evidence regarding the use of ACTs in this population is warranted. **E-mail:** kamal.hamed@novartis.com

Mal5. **Modeling the influence of local environmental factors on malaria transmission in Benin and its implications for cohort study**

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Introduction: Malaria remains endemic in tropical areas, especially in Africa. For the evaluation of new tools, knowing the environmental risk of transmission—even at a very local scale—is essential. The aim of this study was to assess how malaria transmission is influenced and can be predicted by local climatic and environmental factors. **Material and Methods:** As the entomological part of a cohort study of 650 newborn babies in nine villages in the Tori Bossito district of Southern Benin between June 2007 and February 2010, human landing catches were performed to assess the density of malaria vectors and transmission intensity. Climatic factors as well as household characteristics were recorded throughout the study. Statistical correlations between *Anopheles* density and environmental and climatic factors were tested using a three-level Poisson mixed regression model**. Results:** The results showed both temporal variations in vector density (related to season and rainfall), and spatial variations at the level of both village and house. These spatial variations could be largely explained by factors associated with the house's immediate surroundings, namely soil type, vegetation index and the proximity of a watercourse. Based on these results, a predictive regression model was developed using a leave-one-out method, to predict the spatiotemporal variability of malaria transmission in the nine villages**. Main Conclusions:** This study points up the importance of local environmental factors in malaria transmission and describes a model to predict the transmission risk of individual children, based on environmental and behavioral characteristics. **E-mail:** gilles.cottrell@ird.fr

Malaria vaccines and immune response

Mal6. **A variant of the autophagy-associated *IRGM* (immunity-related GTPase family M) gene associated with *falciparum* malaria incidence**

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Introduction: *Plasmodium falciparum* is exposed to various intracellular environments that may influence the growth of parasite stages and the development of malaria. Autophagy is involved in cellular recycling and elimination processes including removal of intracellular pathogens. The immunity-related GTPase family M gene (IRGM; 5q33.1; MIM *608212) contributes as an essential component for maturation of phagosomes to the defense against intracellular pathogenic agents by triggering autophagy. Based on recent evidence of the role of the *IRGM* gene in *Mycobacterium tuberculosis* infections of murine and human cells and the genetic association of the *IRGM*$^{-261T}$ promoter allele with protection from pulmonary tuberculosis through enhanced autophagy we hypothesized that this variant might also be relevant in malaria. Therefore, we expored the impact of the autophagy-enhancing *IRGM*$^{-261T}$ allele on human malaria and further explored autophagy-related cellular events. **Material and Methods:** The *IRGM* polymorphism at position -261 (rs9637876; allelic variants *IRGM*$^{-261C/T}$) was genotyped in 980 Ghanaian children who were enrolled into a longitudinal study with monthly active assessment of malaria parasites over the period from their 3rd to 24th month of age. **Results:** The total infection rate was 2.1 episodes/PYAR (CI 2.1-2.2). Poisson regression analyses revealed in individuals carrying the genotypes *IRGM*$^{-261CT}$ and *IRGM*$^{-261TT}$ an incidence rate (IR) of 22% higher (CI 13%-32%, P=6.2 x 10^{-7}) and 34% higher (CI 20%-50% P=3.2 x 10^{-7}), respectively, than in children with the genotype *IRGM*$^{-261CC}$. Compared to carriers of *IRGM*$^{-261CC}$, the IR ratio (IRR) of heterozygous carriers of *IRGM* alleles was 17% higher (CI 6%-30%, P=3.0 x 10^{-3}) and the IRR of homozygous carriers (*IRGM*$^{-261TT}$) was 40% higher (CI 21%-62%, P=6.1 x 10^{-6}). Such an additive effect was also seen for the risk of severe malaria manifestations in children with the allele *IRGM*$^{-261T}$ (78% increase in IR per mutation, CI 17%-270%, P=6.6 x 10^{-3}).**Main**

Conclusions: In summary, the results demonstrate a strong association between the incidence of human malaria episodes and a mutation of the autophagy enhancing variant of the *IRGM* gene varaint. Further experimental data supported the hypothesis that autophagy promotes the development of malaria parasites in hepatocytes. **E-mail:** may@bnitm.de

Mal7. **Sporozoite neutralizing antibodies elicited in mice and rhesus monkeys immunized with *P. falciparum* circumsporozoite repeat peptide conjugated to meningococcal outer membrane protein complex (OMPC)**

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Introduction: High titers of antibody specific for the NANP tetramer repeats of *P. falciparum* circumsporozoite (CS) protein are required to protect against malaria challenge. Peptide vaccines, while safe and scaleable, require delivery platforms and adjuvants for optimal immunogenicity. Detergent extracted OMPC from *Neisseria meningitidis* provides a unique carrier with a vesicle structure (100-200 nm) and large capacity for peptide chemical conjugation. OMPC is used as a carrier for *Hib* polysaccharides in a licensed pediatric vaccine and for recombinant malaria protein in an experimental transmission blocking vaccine (Wu et al, 2006). The current studies examine the immunogenicity of a malaria CS repeat peptide conjugate, $(NANP)_6$-OMPC, in mice and Rhesus monkeys. **Materials and Methods:** BALB/c and C57Bl mice were immunized intramuscularly (IM) with three doses of $(NANP)_6$-OMPC adsorbed to alum. Rhesus monkeys received three IM immunizations with alum-adsorbed conjugate with or without ISCOMS as co-adjuvant. Antibody titer was measured by ELISA using repeat peptide and by immunofluorescence (IFA) using air-dried *P. falciparum* sporozoites. Sporozoite neutralizing antibodies were measured *in vitro* using a transgenic PfPb rodent parasite that expresses the *P. falciparum* CS repeat region. Protective efficacy *in vivo* was determined by challenge of $(NANP)_6$-OMPC immunized C57Bl mice by exposure to bites of PfPb-infected mosquitoes. Parasites levels in hepatoma cultures or in the liver post challenge were measured by RT-PCR using parasite 18S rRNA as standard. **Results:** $(NANP)_6$-OMPC immunized mice and monkeys developed high anti-repeat ELISA and IFA titers. Pre-incubation of PfPb sporozoites with murine or Rhesus immune sera blocked $\geq$ 90% of sporozoite invasion into hepatoma cells *in vitro*. Rhesus immunized with two doses of $(NANP)_6$-OMPC/alum/ISCOM developed anti-repeat antibodies that persisted for 662 days and a third dose of conjugate at that time elicited strong anamnestic responses. Immunized mice challenged by exposure to the bites of PfPb infected mosquitoes had reduced liver parasites (>90% inhibition) that correlated with sterile immunity or delayed time to blood stage infection. **Conclusion:** High levels of anti-repeat antibodies that neutralized sporozoite infectivity *in vitro* and *in vivo* were elicited in inbred mice and non-human primates immunized with *P. falciparum* $(NANP)_6$-OMPC conjugates. These preclinical studies support OMPC conjugates as a safe, human acceptable carrier for development of highly immunogenic CS peptide vaccines that can be combined as multiantigen malaria vaccines to block invasion and transmission of *Plasmodium.* **E-mail:** elizabeth.nardin@nyumc.org

Mal8. **Strategies for Discovering Novel Pre-Erythrocytic Antigens for Malaria Vaccine Development**

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Introduction: The malaria vaccine antigen pipeline is inadequate, with only a handful of antigens from the 5,000 plus proteome currently undergoing clinical trials in human volunteers. Apart from the modest

protection afforded by CSP, no other antigen has induced substantial protection in humans. However an efficient vaccine should be achievable based on the sterile immunity induced in humans by immunization via mosquito bite with radiation-attenuated sporozoites (RAS). The protective immunity associated with RAS is mostly mediated by cytotoxic T cells (CTL) and/or interferon gamma (IFNγ) secretion in response to multiple as yet unidentified antigens expressed by pre-erythrocytic stages (PE) of the parasite. The objective of this research is to identify these antigens and thereby construct a protective PE sub-unit vaccine. We are currently employing four complementary screening strategies to identify novel protective PE antigens: (i) Putative Pf antigens from PE stages, expressed via wheat germ cell-free system, are being screening for their reactivity to sera and PBMC's from RAS-immunized volunteers. To date antibody screening has identified 25 and cellular screening 11 novel PE antigens, with stage-specific expression and subcellular localization within sporozoites and liver stages. Vaccine for several *P. yoelii* (Py) orthologs have shown efficacy in the Py murine challenge model. (ii) Collaboration with Genocea employs a high-throughput immuno-screening likewise based on PBMC's. A proprietary bacterial library expresses 900 PE antigens targeting antigens specifically to proteosomal or endosomal processing pathways for specific discovery of CD4 or CD8 T cell antigens. The CD8 T cell screening has identified several Pf antigens (iii-iv) The third and fourth screens Py antigens and splenocytes from RAS-immunized mice, with down-selected antigens tested alone or in combination for their ability to reduce or eliminate liver parasite burden measured by RT-PCR following Py sporozoite challenge. The third project has generated and screened DNA vaccines by IFNγ ELISpot assay prior to protection studies, while the fourth project, a collaboration with GenVec, Inc, has applied proprietary technology to create an adenovirus vector-array Py genes and screened as above. Results from the screening and protection experiments will be presented. Top antigens emerging from the four screening algorithms will be selected for clinical development. **E-mail:** ajanaus@gmail.com

Therapy and chemoresistance in malaria

Mal9. **Antiplasmodial activity of metallodrugs**

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One of the recent strategies to search for new antimalarials is to develop metal complexes from existing drugs which include old antimalarials. Metallodrugs are used as first line treatment against cancer and parasitic diseases. The following classes of metallodrugs have been synthesized and were presently evaluated for biological activities: (i) aryl hydrazones (AHR), with known pharmacological applications as antimicrobial, anticonvulsant, analgesic, anti-inflammatory and anticancer, complex with gallium (Ga); (ii) molecules of ferrocene, a promising class of new antimalarials undergoing clinical screening for e.g. ferroquine; (iii) two chloroquine analogues (monoquinoline- MAQ and bisquinoline- BAQ), recently shown to be active against *P. falciparum* and *P. berghei* both of which significantly inhibited hemozoin formation, in a dose-dependent manner; and were weak PfLDH inhibitors (Aguiar et al., Plos one 2012 in press); MAQ and BAQ now complex with platinum, palladium and iron. All such compounds were tested for activity against *P. falciparum* chloroquine resistant W2 clone, before and after metal complex. The cytotoxicity of the metallocomplexes was determined against hepatoma cells (HepG2), and the selectivity index (SI) calculated (a ration between MDL_{50} and IC_{50}). Three AHR tested showed intense antiplasmodial activity (SI up to 5314). After a gallium complex they become more active, however more toxic (a lower SI thus, between 10 and 600). Six ferrocenes tested were active, but were toxic (SI <10). The chloroquine analogues, MAQ and BAQ, complex with Fe, Pt or Pd were more active than the original compounds (higher SI, 98 to > 4405). In conclusion the metal complex was an effective strategy for chloroquine analogues only, MAQ and BAQ did not become toxic, and MAQ had a higher selectivity index than BAQ, but yet similar to CQ. The most active compound will be assessed for chloroquine cross-resistance and against *P. vivax* and *P.falciparum* isolates *ex vivo*. Financial Support: CNPq and FAPEMIG. **E-mail:** akrettli@cpqrr.fiocruz.br

Mal10. **Novel peroxides and primaquine hybrids highly active against malaria gametocytes**

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Introduction: In the absence of a vaccine, malaria case management is based in antimalarial drugs. With the exception of quinine, resistance to all antimalarials has now been demonstrated clinically for *P. falciparum*. One of the most relevant anti-malarials is primaquine (PQ), active against the gametocytes of all Plasmodia strains. PQ also exhibits sporontocidal activity and is the only anti-malarial effective against hypnozoites, the hepatic latent forms responsible for relapsing *P. vivax* and *P. ovale*. But, PQ is far from being an ideal drug, due to three factors: metabolic inactivation, significant toxicity and low blood-schizontocidal activity. We have been working on the modification of PQ, these novel peroxides and primaquine hybrids are highly stable under physiological conditions. Malaria transmission is now recognized as a key target for intervention. **Material and Methods:** Four peroxides hybrids (DM12, DM16, RKH282 and FMG243) were tested for their transmission-blocking activity as follows. Nine Balb/C mice were infected IP inoculation of *P. berghei* ANKA-GFP. After observing gametocytes and microgamete exflagelation, mice were randomly separated into groups of three animals, one group was treated by IP injection with 10 µmol/kg of tested compound and the 2nd group with 25 µmol/kg a 3th group of mice received PBS. Two hours after treatment, mice were anesthetized and placed on top of individual cages containing ca. 50 glucose-starved *Anopheles gambiae* female mosquitoes, which were allowed to feed for 1h. Ten days after the blood meal, mosquitoes were dissected for microscopic detection of oocysts in midguts under UV microscopy. PQ was used as positive control. **Results:** Transmission-blocking activity was measured using two parameters: infection-rate and oocyst-burden. Infection-rate and oocyst-burden for the four compounds and PQ are presented in Table 1. Table1. Transmission-blocking activity of novel peroxides and primaquine hybrids. Blood-schizontocidal activity was also performed using W2 strain of *P. falciparum* labeled with YOYO-1 ,IC_{50}s were 21,1 ± 0,49 nM for DM12 and 45,2 ± 2,24 nM for DM16. **Main Conclusions:** The hybrids DM12 and DM16 exhibited the best transmission-blocking activity relatively to PQ, they were also very active against blood forms of *P. falciparum*. **E-mail:** fnogueira@ihmt.unl.pt

Mal11. **Current Status of New Antimalarial Endoperoxides**

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Introduction: We report the in vitro and in vivo antimalarial activities of 6-(1, 2, 6, 7-tetraoxa spiro [7.11] nonadec-4-yl) hexan-1-ol (N-251) against Plasmodium falciparum and P. berghei parasites. The N-251 showed high antimalarial potencies both in the in vitro and the in vivo tests (EC50: 2.3 x 10 −8 M; ED50: 15 mg/kg (per oral)). The potencies were similar to that of artemisinin in vitro and greater than artemisinin's activity in vivo (p.o.). In addition, N-251 has little toxicity: a single oral administration at 2000 mg/kg to a rat gave no health problems to it. Administration of N-251 to mice bearing 1% of parasitaemia (per oral 68 mg/kg, 3 times a day for 3 consecutive days) resulted in a dramatic decrease in the parasitaemia: all the 5 mice given N-251 were cured without any recurrence, with no diarrhea or weight loss occurring in the 60 days of experiment. N-251 deserves more extensive clinical evaluation, desirably including future trials in the human. I introduce our N-251 research for control of malaria includes new data currently received. **Materials and Methods:** The antimalarial activity of reagents against the P. falciparum FCR-3 strain in vitro, and the cytotoxicity to mouse mammary FM3A cells in culture were determined as described earlier [1]. For the measurement of cytotoxicity, mouse mammary cell FM3A was cultured as described before [2]: The in vivo antimalarial activity was assessed using ICR mice infected with P. berghei (strain NK65). We used the protocols described previously for drug candidates

[1]. Two tests were performed; one, administration of drugs at the time of the start of parasite infection, and another, a curative test, i.e., administration of drugs after the parasitaemia has been established.
Results and conclusions: The EC50 against the parasite was 2.3 x 10−8 M, while the EC50 to FM3A was a higher concentration 8.0 x 10−6 M; the selective toxicity was thus 348 fold. Similar high selectivity was observed for N-251 against other strains of malaria parasites, the chloroquine-resistant K1 and the mefloquine-resistant strain that has been established in our laboratory (data not shown). For the in vivo effect of N-251 for mice show that orally administered N-251 possesses an antimalarial effect with an ED50 value of 15 mg/kg using in 4-day suppressive test. It should be noted that artemisinin showed an ED50 value at 30 mg/kg in a comparable experiment we performed. We then proceeded to explore curing mice of malaria by oral administration of N-251. The mice (5 in each group) of 1% of parasitaemia, and then given N-251 orally at 68 mg/kg, three times a day for 3 consecutive days. At 8 h after N-251 administration, changed into a decrease at 16 h, and a complete disappeared at 48 h in all treated mice. In sharp contrast, all the 5mice given N-251 continued to live at the 60th day, and no parasites were detected in the blood of these mice. In concluded that a 100% cure for the 1% parasites-infected mice was achieved without any recurrence. It is remarkable that no side effects, such as diarrhea and weight loss, occurred. It should be noted that artemisinin is known to be unable to completely kill the parasite in the blood, and its effect has a short life [3]. In this regard, N-251 seems to be superior to artemisinin. It should also be remarked that a single-drug therapy was shown to be possible with N-251. It is hopeful that N-251 could be effective in curing malaria in the human. We are now going to attempt preclinical trials of N-251 that would involve pharmacokinetics studies and safety evaluations. **References:** [1] Kim HS, Shibata Y, Wataya Y, Tsuchiya K, Masuyama A, Nojima M. Synthesis and antimalarial activity of cyclic peroxides, 1,2,4,5,7-pentoxocanes and 1,2,4,5- tetroxanes. J Med Chem., 1999; 42: 2604–9. [2] Kim HS, Nagai Y, Ono K, Begum K, Wataya Y, Hamada Y, et al. Synthesis and antimalarial activity of novel medium-sized 1,2,4,5-tetraoxacycloalkanes. J Med Chem., 2001; 44: 2357–61. [3] Mita T, Tanabe K, Kita K. Spread and evolution of Plasmodium falciparum drug resistance. Parasitol Int., 2009; 58:201–9.
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Mal12. **A comprehensive study of genes encoding the enzymes of DNA Mismatch repair in *Plasmodium spp***

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Drug resistance has been described in several strains of *Plasmodium*, which has hampered the control of the disease worldwide. Since drug-resistance has been associated to defective mismatch repair systems (MMR) in different organisms, we hypothesize that MMR defects are associated to development of drug-resistance in *Plasmodium* strains. The MMR recognizes and repairs incorrectly paired bases, caused primarily by DNA polymerase errors during DNA replication. Reduction or loss of activity of these enzymes have been linked to some types of cancer, microsatellite instability and drug resistance. Given the importance of these enzymes and the scarcity of studies concerning them in *Plasmodium*, the aim of this study is the identification and in silico analysis of enzymes involved in the *Plasmodium* MMR system. First, we searched for *Plasmodium falciparum* genes encoding MMR enzymes using Blast methodology to find homologous enzymes related to generation of microsatellite instability in other organisms. Afterwards, we looked for orthologous proteins in other *Plasmodium* species (*P. vivax, P. berguei, P. chabaudii, P. knowlesi* and *P. yoelli*) using OrthoMCL and aligned orthologs using ClustalW to identify the conservation of functional domains (Pfam database). The major MMR enzymes from *P. falciparum* [PF14_0254; MAL7P1.206; PFE0270c; PF11-0184; MAL7P1.145] as well as their orthologs had their sequences retrieved from PlasmoDB. In silico analysis showed that the major functional domains of MMR enzymes are preserved in the *Plasmodium* species analyzed. We found a *P. falciparum* MutS homologous protein previously described in other Apicomplexa species, which showed only the domains I and V and lacking the other domains (II-IV). No homologous MSH3 was found in *Plasmodium* species neither in other Apicomplexa in OrthoMCL database. As this enzyme is involved in the recognition of 2-12 indel loops, maybe these loops are not efficiently repaired in these species or Apicomplexa has an alternative pathway to repair this kind of error. At this time, we are comparing the MMR gene sequences from *P. falciparum* strains with different drug susceptibility and also from *P. vivax* isolates associated with

high number of recurrence episodes. Financial support: FAPEMIG, CPqRR/FIOCRUZ. **E-mail:** cristiana@cpqrr.fiocruz.br

Mal13. **A Common treatment for *Plasmodium vivax* and *Plasmodium falciparum*: therapeutic efficacy of *Artemether-Lumefantrine* + *Primaquine* in a prospective multicenter study in Guyana**

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Introduction: *Plasmodium vivax* and *Plasmodium falciparum* both circulate in Guyana and increasing reports of chemo-resistance from other parts of the world led to question treatment regimens, which are currently *Chloroquine* + *Primaquine* for *vivax* malaria and *Artemether-Lumefantrine* for *falciparum* malaria. Therefore we conducted a therapeutic efficacy study for *Artemether-Lumefantrine* and *Artemether-Lumefantrine* + *Primaquine* against *P. falciparum* and *P. vivax* respectively, to re-evaluate the treatment regimen against *falciparum* malaria and assess an alternative for *Chloroquine* as a treatment option against *vivax* malaria. **Material and Methods:** From 2007 to 2010, we conducted a non-controlled study among patients 5 years of age or older in two hospitals in Guyana. We included 90 microscopically confirmed *P. falciparum* case-patients who received *Artemether-Lumefantrine* as a 6-dose regimen twice a day for 3 days, as currently used according to national treatment guidelines and 74 microscopically confirmed *P. vivax* case-patients treated in the same way, but with additional 0.25 mg/kg/d *Primaquine* for 14 days. We assessed patients clinically and checked parasitaemia on days 0,1,2,3,7,14 and 28. The pfmdr1 copy number assessing for parasitic resistance was determined in all *P. falciparum* samples. A subset of 8 *P. vivax* patients was analyzed for pvmdr1 mutation, reflecting *Chloroquine* resistance. We used msp1, msp2 and glurp genotyping among *P. falciparum* patients with suspected therapeutic failures to distinguish between recrudescence and reinfection. **Results:** For *P. falciparum*, *Artemether-Lumefantrine* induced a rapid clearance of parasites (52%, 78% and 100% on day 2, 3 and 7, respectively) and the 28-day cure rate was 100%. Two patients had a reinfection with *P. falciparum*, as indicated by different genotyping patterns. For *P. vivax*, 100% of parasites were cleared on day 1, but two patients (3%) had reoccurrence of parasites on day 28, suggesting relapse. No pvmdr1 mutation or raised pfmdr1 copy number was detected. The treatment regimen was well tolerated. **Main Conclusions:** In Guyana, *Artemether-Lumefantrine* remains efficacious against P. falciparum and represents an adequate option against P. vivax when combined with *Primaquine*. We can recommend *Artemether-Lumefantrine* + *Primaquine* as an alternative treatment option for *P. vivax* infections instead of the currently used *Chloroquine*. Having this alternative on hand will be of great importance in case of emerging *Chloroquine* resistance against *P. vivax*. **E-mail:** daniel.eibach@chu-lyon.fr

Mal14. **Progress towards the development of next generation 8-Aminoquinoline-like compounds**

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The 8-aminoquinolines is the only known drug class to exhibit both causal prophylactic and curative efficacy against relapsing malaria. However, these compounds have been shown to cause hemolytic anemia in G6PD deficient individuals through mechanisms long thought to involve unstable metabolic by products. We sought to characterize the metabolic profile of PQ, as a representative compound, with the objective of developing the next generation of 8AQ-like compounds or drug combinations with enhanced

therapeutic index compared to existing 8AQs. Using *in vitro* metabolism and mass spectrometry, we determined that CYPs 2D6, 3A4, and 2C19, and Monoamine Oxidase-A (MAO-A) are major contributors to primaquine metabolism into carboxyprimaquine, the major yet non-efficacious, metabolite *in vivo*. RAF Clint showed the relative role of each enzyme to be MAO-A, 2C19, 3A4, and 2D6, with 76.1, 17.0, 5.2, and 1.7 % contributions to PQ metabolism, respectively. A metabolite at m/z 261, consistent with the primaquine alcohol, was observed after incubation with MAO-A. Other metabolites, m/z 276, consistent with the hydroxylated species largely implicated in primaquine's efficacy/toxicity profile, were mediated predominantly by CYP 2D6. While CQ exhibited drug interaction potential *in vitro*, it potentiated the PQ in rodents, based on parasite clearance, half-life, AUC and Vd. Interestingly, the Cmax and Cl were reduced, suggesting potentiation through multiple mechanisms of action, which was further supported by the carboxyprimaquine data. Selective CYP and MAO inhibitors block hemolytic toxicity *in vivo* and their effect on efficacy is being determined. *In vivo* efficacy testing of selected 8AQ analogs and corresponding enantiomers appear 3-5 times more potent than PQ and are being tested in our G6PD deficient models. Likewise, the mechanism of action of drugs shown to enhance TI of 8AQs *in vivo* is in progress. The data is being used to better understand SAR and to design the next generation 8AQ-like compounds. **E-mail:** victor.melendezsr@us.army.mil

Pathogenesis in malaria

Mal15. **Efficacy of different nitric oxide-based strategies in preventing experimental cerebral malaria by Plasmodium berghei ANKA**

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Background: Low nitric oxide (NO) bioavailability plays a role in the pathogenesis of human as well as of experimental cerebral malaria (ECM) caused by Plasmodium berghei ANKA (PbA). ECM is partially prevented by administration of the NO-donor dipropylenetriamine NONOate (DPTA-NO) at high concentration (1 mg/mouse), which also induces major side effects such as a sharp drop in blood pressure. We asked whether alternative strategies to improve NO bioavailability with minor side effects would also be effective in preventing ECM. **Methodology/Principal findings:** Mice were infected with PbA and prophylactically treated twice a day with bolus injections of L-arginine, Nω-hydroxy-nor-Arginine (nor-NOHA), tetrahydrobiopterin (BH4), separately or combined, sodium nitrite, sildenafil or sildenafil plus DPTA-NO starting on day 0 of infection. L-arginine and BH4 supplementation, with or without arginase inhibition by nor-NOHA, increased plasma nitrite levels but failed to protect against ECM development. Accordingly, prophylactic treatment with continuous delivery of L-arginine using osmotic pumps also did not improve survival. Similar outcomes were observed with sodium nitrite sildenafil (aimed at inhibiting phosphodiesterase-5) or with DPTA-NO. However, sildenafil (0.1 mg/mouse) in combination with a lower dose (0.1 mg/mouse) of DPTA-NO decreased ECM incidence (82 ± 7.4% mortality in the saline group and 38 ± 10.6% in the treated group; $p<0.05$). The combined prophylactic therapy did not aggravate anemia, had delayed effects in systolic, diastolic and mean arterial blood pressure and induced lower effects in pulse pressure when compared to DPTA-NO 1 mg/mouse. **Conclusions/Significance:** These data show that sildenafil lowers the amount of NO-donor needed to prevent ECM, resulting also in lesser side effects. Prophylactic L-arginine when given in bolus or continuous delivery and bolus BH4 supplementation, with or without arginase inhibition, were able to increase NO bioavailability in PbA-infected mice but failed to decrease ECM incidence in the doses and protocol used. **E-mail:** yuricmartins2004@yahoo.com.br

Mal16. ***In vivo*** **imaging experimental placental malaria: addressing iRBCs sequestration and uncovering a role for fetal macrophages in PAM pathogenesis**

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We have developed an experimental system to visualize interactions of iRBCs with murine placenta by intra-vital imaging. Time-lapse imaging revealed that normal maternal blood flow in the placenta is uneven with localized areas of unusually prolonged stasis. At contrast with non-pregnant uterus vascularization where iRBCs travel within the blood flow mass, we observed considerable number of iRBCs located in the margins of placental vascular spaces. Placental iRBCs frequently showed very prolonged times of contact with trophoblast layer. Quantification of parasite accumulation in the placenta reveals that areas of intermittent stasis in the placenta provide vascular niches for increased parasite accumulation. Combining mutant parasites and pixel analysis provides *in vivo* evidence that iRBC ability to adhere to the trophoblast and parasite accumulations in vascular niches are determinants of iRBC sequestration in the context of placenta hemodynamics. Furthermore, we have obtained *in vivo* unique evidence for involvement of fetal macrophages in response to iRBC accumulation in the placenta. Together, the data supports a PAM pathogenesis hypothesis linking placenta hemodynamics, iRBCs accumulation in vascular spaces, iRBC adhesion to the trophoblast and fetal macrophage activation response. **E-mail:** cpenha@igc.gulbenkian.pt

Mal17. **Splenic cytokine responses during and after recovery of *Plasmodium falciparum*** **infection in *Saimiri sciureus*** **monkeys**

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Introduction: New World monkeys of the genus *Saimiri* can be infected by plasmodial species that infect humans such as *Plasmodium falciparum* and *P. vivax,* but still limited knowledge is available regarding immune responses elicited by infection in this model. **Material and Methods:** we generated sets of primers for the cytokines IL2, IL6, IL10, IL12, IFN-γ and TNF-α based on sequences optimized for *Saimiri sciureus*. Groups of intact *Saimiris* were infected with *P. falciparum* FUP strain and the spleen removed on day 7 during ascending parasitaemia or 15 days after chloroquine treatment. Spleens were processed for histology and for splenocyte purification. Splenocytes were cultured *in vitro* and stimulated with *P. falciparum* parasitized red blood cells (pRBC). Total mRNA was obtained; cDNA generated and amplified using the cytokine primers previously developed. **Results:** the spleens of *Saimiri* monkeys at day 7 of infection showed several immunoblasts and plasmacytes. Limits between the red and white pulps were blurred, centroblasts were widespread, and mitosis and apoptosis foci were abundant. PRBC-stimulated splenocytes showed a marked increase in IFN-γ expression. IL6 and IL12 were also increased, whereas IL2, IL10 and TNF-α showed no major changes in expression. Fifteen days after chloroquine treatment, phagocytes were still heavily laden with malaria pigment; follicles were much enlarged and dominated by vast numbers of activated cells (centroblasts and mitotic cells). PRBC-stimulated splenocytes showed no changes in expression of any of the cytokines tested. However, and interestingly, non-stimulated cells showed increased expression of IL2, IL6, IL10, IL12 and TNF-α, but not IFN-γ. **Main conclusions:** histological features of splenic responses in *Saimiri* are similar to those described in human and murine malaria. Widespread polyclonal activation, disorganization of germinal centers, apoptosis and macrophages heavily laden with malarial pigment indicate that *P. falciparum* induces defective immune responses which may impact negatively on acquisition of immunity. Marked increases in IL12 and specially IFN-γ during the acute phase correlate well with the several immunoblasts found by histology. However, during recovery pRBC stimulation induce an anergic-type response, switching off cytokine

production. These findings may impact our understanding of how the host responds to *P. falciparum*.**E-mail:** falves@ufpa.br

Mal18. **Analysis of Apoptosis and vascular permeability in malaria associated acute lung injury/acute respiratory distress syndrome**

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Introduction: Malaria is a huge burden on global health. This parasitic disease is 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 in 2010 (Murray, et al, 2012). Acute Lung Injury (ALI)/Acute Respiratory Distress Syndrome (ARDS) are a type of severe malaria that is manifested in the lungs. We established a murine model that mimics various human ALI/ARDS aspects, such as pulmonary edema and hemorrhages, pleural effusion and hypoxemia. Our goal was to elucidate the role of apoptosis and vascular permeability in mice that may be involved in the pathogenesis of malaria associated ALI/ARDS. **Methods and Results:** We infected DBA/2 mice with 10^6 iRBC of *P. berghei* ANKA intraperitoneal via. Lungs were analyzed at different time-points during infection. To classify experimental animals euthanized at the different time-points as ALI/ARDS or HP (hyperparasitaemia), we made use of different parameters obtained from the control group (gold standard), such as breathing pattern (enhance pause and respiratory frequency) and parasitaemia to establish a cut-off using a ROC curve (Receiver Operating Characteristic). Experimental animals were euthanized at the different time-points and classified as ALI/ARDS or HP (hyperparasitaemia), according to the established parameters. Preliminary results demonstrated that cell death by apoptosis was involved in ALI/ARDS. Expression of pro-apoptotic (BAX and Bak) and anti-apoptotic (Bcl2) genes were analyzed by qRT-PCR. An increase in the expression of BAX and Bak ($p<0.05$) and a reduced level of Bcl2 ($p<0.05$) was observed in the ALI/ARDS group. We also found a higher number of apoptotic cells in the lungs of ALI/ARDS mice compared to the HP ones (TUNEL method). In addition, we observed significant increase of pulmonary vascular permeability (breakdown of the alveolar-capillary barrier) by Evans blue dye quantification in the ALI/ARDS group. **Conclusion:** Apoptosis could be involved in the pathogenesis of ALI/ARDS (apoptosis of parenchymal and endothelial cells) and/or early remodelling (with death of neutrophils), which are involved in tissue restoration. We hypothesize that if apoptosis occurs in endothelial and parenchymal cells, this may contribute to the breakdown of the alveolar-capillary barrier. In addition, we demonstrated that the pro-apoptotic genes (*BAX* and Bak) are involved in malaria associated to ALI/ARDS and that the anti-apoptotic gene (*Bcl2*) can help to protect mice from developing ALI/ARDS. Other genes related to apoptosis are been studying in our model of malaria associated to ALI/ARDS. **E-mail:** michelleklein@usp.br

Mal19. ***Plasmodium falciparum*** **infection increases ATP release in human erythrocytes**

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Introduction: In human erythrocytes (RBCs) various stimuli induce increases in cAMP which triggers the non-lytic release of ATP, with pannexin 1 being one of the main proteins mediating such a release. Previous reports showed that the resulting extracellular ATP (ATPe) can act on purinergic receptors of endothelial cells to promote the relaxation of the vascular caliber in the microcirculation. RBCs are one of

the main targets of the malaria parasite Plasmodium falciparum, and patients infected with these parasites exhibit altered microvascular responsiveness of unknown origin. Still it is not clear whether these circulatory problems of malaria patients are linked to an altered release of ATP by infected RBCs, and whether the potential changes in ATPe accumulation might affect the infectious process and the host response. **Materials and Methods:** Quantitative analysis of cAMP induced ATP release was done by luciferin-luciferase reaction. The kinetics of ATPe concentration from RBCs at various stages of infection (ring, trophozoite and schizont stages) with Plasmodium falciparum was determined. A mixture, here called 3V containing isoproterenol (β-adrenergic agonist), forskolin (adenylate kinase activator) and papaverine (phosphodiesterase inhibitor) was used to induce cAMP dependent ATP release. Results: Addition of 3V to healthy RBCs induced an acute increase of ATPe to a maximum value of 1.5 pmoles/106cells, which remained constant thereafter, as previously shown. Using infected RBCs (parasitaemias ranging from 2, 5 to 12, 5%) at different stages produced the release of ATP with similar kinetics as in healthy cells, but the amplitude of the response was significantly increased. All infected RBCs produced approximately 2-fold increase in ATPe release as compared with controls, with schizont cells showing the highest values (2.5 fold). In separate experiments, a positive correlation was found between ATPe concentration and parasitaemia in the ring and trophozoite stages ($p< 0,05$ and $p< 0,01$,respectively); but not in the case of schizont infected cells ($p>0,05$). To study the mechanism enabling ATP efflux in infected RBCs we used mefloquine and carbenoxolone two well-known blockers of pannexin 1. When a pure population of trophozoites was incubated with either mefloquine (100 nM) or carbenoxolone (100 µM) 10 min before 3V addition, ATP release was inhibited by 71 and 65% respectively. This is at variance with healthy RBCs, where carbenoxolone and mefloquine and the same doses are able to inhibit 100% of the 3V induced response. **Main Conclusions:** These preliminary data suggest that parasite invasion will enhance pannexin1-mediated ATP efflux of adrenergically stimulated RBCs, with potential consequences on microvascular resistance. We are currently evaluating potential changes in ATPe degradation by RBCs ectonucleotidases, which could partially counteract the observed enhancements of ATP release. With grants from CAPES-MINCYT (197/11), INCT-Instituto Nacional de Ciência e Tecnologia; CNPq; FAPERJ. **E-mail:** alvarezcora@hotmail.com / cora@biof.ufrj.br

Mal20. **Placental histopathological lesions associated with *Plasmodium vivax*: a pilot study**

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Introduction: It has been estimated that up to 125 million pregnant women worldwide are exposed to the risks of Malaria in Pregnancy (MiP) each year. Both *Plasmodium falciparum* (*P. falciparum*) and *Plasmodium vivax* (*P. vivax*) can be detected during MiP, nevertheless most of the research on the placental pathology associated with MiP has been directed at *P. falciparum* infections only. The presence of *Plasmodium* and/or its products (such as hemozoin in fibrin deposits or in leukocytes) in the placenta is a defining feature of MiP. After *P. falciparum* infections it is usual to find an accumulation of maternal immune cells (predominantly monocytes/macrophages and neutrophils) in the placenta as well as an increased presentation of other signs of injury including syncytial knotting, thickening of the basement membrane of the trophoblast layer and fibrinoid deposits. In this pilot study we aimed to evaluate the pattern, severity and prevalence of lesions associated with *P. vivax* infections during pregnancy. **Material and Methods:** We analyzed histological sections of 120 placentas delivered at the Cruzeiro do Sul Maternity, in Cruzeiro do Sul, State of Acre, Brazil (an area of known *P. vivax* transmission). **Results:** *Plasmodium vivax* infections were reported during pregnancy in 59 women while 19 had *P. falciparum* infections (these were considered our “positive-control”). Both syncytial knotting (Mann-Whitney: $p=0.038$) and thickness of the placental barrier ($p<0.001$) were increased in placentas from *P. vivax* infected women when compared to non-infected women. *Plasmodium vivax* infections were more likely to induce placental pathology if more than one infection had occurred during pregnancy or if the infections had occurred close to labor. **Conclusion:** Identifying the pattern and severity of *P. vivax*-induced placental

injuries is crucial to the understanding of the pathological processes involved during MiP. **E-mail:** marinho@usp.br

Molecular approaches in the study of malaria

Mal21. **Rapid and highly sensitive detection of malaria-infected erythrocytes using a cell microarray chip**

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Background: Malaria is one of the major human infectious diseases in over 100 endemic countries, there being almost 300 million cases and 2 million deaths per year. For prevention of the spread of malaria in the world, it is necessary to develop an early, sensitive, accurate and conventional diagnosis system. Methods and Findings: A cell microarray chip was used to develop a detection system for malaria-infected erythrocytes. The chip, with 20,944 microchambers (105 μm width and 50 μm depth), was made from polystyrene, and its surface was rendered hydrophilic by means of reactive-ion etching, which allowed the formation of mono-layers of erythrocytes in the microchambers. Cultured Plasmodium falciparum strain 3D7 was used to examine the potential of the cell microarray chip for malaria diagnosis. An erythrocyte suspension in a nuclear staining dye, SYTO 59, was dispersed on the chip surface, followed by 10 min standing to allow the erythrocytes to settle down into the microchambers. About 130 erythrocytes were accommodated in each microchamber, there being over 2,700,000 erythrocytes in total on a chip. A microarray scanner was employed to detect any fluorescence-positive erythrocytes within 5 min, and 0.0001% parasitaemia could be detected. Furthermore, the presence of malaria parasites in fluorescence-positive erythrocytes was confirmed by Giemsa staining after microarray scanning. To examine the possibility of contamination by leukocytes of purified erythrocytes from healthy human blood, 20 μl of whole blood was mixed with 10 ml of RPMI 1640 medium, and the mixture was passed through a leukocyte isolation filter. The eluted portion was centrifuged at 1,000 x g for 2min, and the pellet was dispersed in 1.0 ml of medium. SYTO 59 was added to the erythrocyte suspension, followed by analysis on a cell microarray chip. Similar accommodation of cells in the microchambers with a malaria culture was observed. The number of contaminating leukocytes was less than 1 on a cell microarray chip. Conclusion: The results indicated the potential of the cell microarray chip for the detection of malaria-infected erythrocytes, it offering 10-100 times higher sensitivity than that of light microscopy with Giemsa staining and easy operation in 15 min with purified erythrocytes. **E-mail:** yatsushiro.s@aist.go.jp

Mal22. **Molecular characterization of Plasmodium *simium/ Plasmodium vivax* from Atlantic Forest**

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The high genetic similarity between *P. vivax*, a human malaria parasite, and the non-human primate malaria, *P. simium*, suggests that recent host transfers have occurred. Furthermore, due to their morphological and genetic similarities, the specific differentiation between these two parasites has been challenging. The Brazilian Atlantic Forest has not be considered endemic for human malaria, thus, autochthonous malaria cases in this area have been associated with zoonotic transmission of simian parasites. The aim of the present study was to provide a more detailed knowledge about the relationship between human and simian plasmodia. For that, we have been investigating natural *Plasmodium* infection of the howler monkey, *Alouatta clamitans*, by using molecular and serological approaches. A

total of 46 blood samples from wild and captivity monkeys (Santa Catarina state) were collected. Using primer for *P. vivax*, we observed malaria infection rates of 50% in wild monkeys, and 5% in captive monkeys. Infections were confirmed using different PCR targets, including 18S (SSURNA), cytochrome oxidase 1 (cox1), merozoite surface protein 1 (msp1), duffy binding protein (dbp) and circumsporozoite protein (csp), followed by DNA sequencing of these PCR products. We also tested *P. vivax* microsatellites (MS) in an attempt to differentiate the simian plasmodia from *P. vivax*. Analysis of the genotypes from 4 MS loci, reveled 7 new alleles, not previously described for *P. vivax* populations. In ELISA, 18% (6/33) of monkey's sera showed specific antibodies against synthetic peptides of *P. vivax* dbp, while 21% (7/33) showed specific antibodies against *P. vivax* msp1. We also tested two blood samples from monkeys in a functional in vitro cytoadherence assay, where mammalian cells (COS-7) expressing the region II of PvDBP form rosettes in the presence of erythrocytes expressing its cognate surface receptor, protein DARC. We were able to verify a specific interaction between monkey erythrocytes and PvDBP using this assay, suggesting that *P. simium* shares a common invasion pathway with *P. vivax*. In order to investigate their phylogenetic relationships, the dbp gene of *P. simium* was sequenced and compared to those from other *Plasmodium* species already available in public databases. In our preliminary evolutionary analysis, it was not possible to resolve distinct clades using dbp gene. Overall, our results suggest a possible zoonotic cycle between human and simian malaria, where simians living in areas of the Atlantic Forest could play a role as a reservoir for human *P. vivax*. **Support:** FIOCRUZ, CNPq, CAPES, FAPEMIG. **E-mail:** dani@cpqrr.fiocruz.br

Mal23. ***Pfatp6*** **molecular profile of** ***Plasmodium falciparum*** **isolates in the Western Brazilian Amazon**

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Plasmodium falciparum resistance to antimalarial drugs has emerged as one of the biggest challenges to be faced in malaria control worldwide. In September 2006, the World Health Organization (WHO) recommended strategy for the avoidance of drug resistance, the use of artemisinin combination therapy (ACT) as first-line treatment for uncomplicated *Plasmodium falciparum*. In Brazil, artemether/lumefantrine was recommended by the National Malaria Control Programme in 2007. The identification and monitoring of genes and mutations giving rise to resistance to artemisinins and its derivatives are essential for evaluation of this strategy. A previous study with *P. falciparum* suggested that sarco/endoplasmic reticulum Ca^{2+} ATPase – type protein encoded by a gene denoted *pfatp6* might be the major chemotherapeutic target of these drugs. Consequently, *pfatp6* gene polymorphisms are being pointed out as markers of artemisinins resistance elsewhere. Jambou *et al.* reported a significant decrease of *in vitro* sensitivity to artemether in *P. falciparum* isolates from French Guiana, located along Brazil´s northern border. This reduced efficacy was significantly associated with a mutation encoding a S769N shift in *pfatp6* gene. Critically, French Guyana´s physical proximity to Brazil makes it possible for the potentially resistant parasites to be carried across the border and spread through local treatment-based selection. Other polymorphisms have been identified in *pfatp6* gene in Senegal, Thailand, Africa and São Tomé and Principe. There are scarce data on *pfatp6* gene in Brazilian isolates, especially from the Amazon. Recently, three mutations were described in samples from the State of Pará. This is an ecological study aimed to describe the molecular profile of *pfatp6* in *P. falciparum* isolates from different localities in the Amazonas State. *P. falciparum* field isolates were obtained: from the bank of samples from the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD) Laboratory of Malaria, collected from 2000 to 2005; and prospectively collected from 2009 to 2010 from eleven endemic municipalities of the Amazonas State. Parasite samples were collected from patients before unsupervised antimalarial treatment. The DNA polymorphism of the *pfatp6* gene in 80 *P. falciparum* isolates was analyzed by automatic sequencing. The mutations search in the gene was performed using the Mutation Surveyor v3.25 software. The *P. falciparum pfatp6* gene presented polymorphisms at codons 37, 630 and 898. The R37K mutation was found in 16% of the samples, A630S in 32% and I898I in 52%. However, no S769N mutation was detected in any samples analyzed. Despite the number of samples, data presented here provide baseline information about polymorphisms of *pfatp6* gene before and after exposure to ACT, in a

low transmission area, that will help to infer drug selection pressure in this area futurely. **Keywords:** malaria, *Plasmodium falciparum*, artemisinin, molecular marker, *pfatp6*, polymorphisms, Amazon. **E-mail:** larissa_brasil@hotmail.com

Mal24. **Experimental Validation of *Plasmodium* proteins reannotation proposed by signal peptides analysis in orthologous groups**

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Orthologous proteins of related species are generally conserved, sharing metabolic functions, subcellular localization and structural features. The signal peptide (SP) plays a key role in the targeting of proteins and hence in their biological role. Therefore, it is expected that orthologous proteins agreed for the presence of SP. By adding predicted signal peptide information with the orthology, was noted the presence of orthologous groups of proteins with divergent prediction of SP. The most likely explanation for this could be due to the presence of errors in the N-terminus annotation of proteins. Thus, our objective was to identify poorly annotated protein sequences by comparative analysis of SP prediction between orthologous and subsequent experimental validation. To test this hypothesis, all *Plasmodium* proteins were recovered in PlasmoDB, submitted to the SP predictor (SignalP) and aligned with their orthologous. We selected proteins groups (one of each available *Plasmodium* specie) that have divergent predictions of SP among its members (mixed group). These orthologous proteins were manually inspected for possible inconsistencies in its sequence. Proteins were reannotated, and an alternative gene model was proposed. After this, prediction of SP was performed again. Of the 541 mixed groups, 315 have already been inspected. Of these, 243 need to be changed, of which 197 have been fixed, which corresponds to 270 corrected proteins. Most reannotated proteins has changed its SP prediction (77%), generally to positive prediction (77%), resulting in a significant increase in proteins secretomes from various *Plasmodium* species. Some reannotated sequences were chosen for experimental validation by analysis of mRNA by RT-PCR. Seven proteins were selected, for which primers were designed and amplification reactions optimized. The gene PVX_081500 has been validated by PCR, and primers that amplify this gene from genomic DNA and cDNA works, demonstrating that our proposed gene model is correct. The reannotation method based on comparative analysis of SP prediction in orthologous proved to be very effective in the N-terminus reannotation of proteins. As candidates for vaccine antigens are normally secreted proteins, the correction of erroneously annotated proteins can increase the hall of potential vaccine targets.**Supported by:** FAPEMIG, CNPq, CPqRR, CAPES. **E-mail:** dama.danet@yahoo.com.br

Mal25. **Platform for malaria diagnosis applied to blood donors samples from endemic and non-endemic Brazilian areas processed in pool: determination of the frequency of positivity using real-time PCR**

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Introduction: Malaria transmitted by blood transfusion remains one of the most relevant infections in endemic and non-endemic areas due to the increasing number of individuals moving globally. Although the incidence of malaria by blood transfusion is unknown in Brazil, it may contribute to the spread of the disease whether clinical and epidemiological screening in blood banks is not carried out carefully. Asymptomatic infections with low parasitaemias are difficult to detect by haemoscopy and represent a challenge for control strategies. Therefore, it is essential to apply more sensitive tools for a safer screening of blood donors. Considering that results of assays using pools are comparable to the individual analysis in previous study, the diagnostic platform using pooled samples could be applied in

special situations as hemotherapy services, reducing the processing time and cost. **Material and Methods:** For assembling the pools, 9,280 blood samples from 147 Brazilian blood banks were arranged in 10 samples/pool. DNA was extracted with Qiagen® QIAamp DNA Blood Mini Kit. Real-time PCR for genus-specific amplification targeting the gene encoding the small subunit 18S rRNA of Plasmodium was modified by reducing reagent volume and increasing number of cycles to obtain a protocol with lower cost and higher sensitivity. Reaction was prepared with 2.5 µL of gDNA, 12.5 µL of TaqMan® Universal PCR Master Mix 2x, 500nM of each primer M60 and M61, and 300nM of M62 probe marked with FAM™ and TAMRA™, assayed in duplicate on the ABI PRISM 7300. **Results:** From the 928 pools tested by Real Time PCR, 30 were positive for Plasmodium, with Cts lower than the cut-off established of 37.28. **Main Conclusions:** Our results showed that 3.2% of pools assayed by real-time PCR amplified Plasmodium gDNA, indicating an important positivity rate that contributes for transfusional malaria. Our previous study showed that the sensitivity of individually processed samples was similar to the pooled ones, demonstrating that the platform is indicated for processing a large number of blood tests. Samples from positive pools will be assayed individually using nested PCR to determine species. **Acknowledgments:** This study was supported by SUCEN/Instituto de Medicina Tropical de São Paulo (technical cooperation agreement); CAPES; CNPq; LIM 49 HCFMUSP. **E-mail:** giselledecastro@usp.br

TRYPANOSOMIASIS

CHAGAS DISEASE

Morbidity and epidemiology of Chagas disease

Chag1. **Mode of Death and Degree of Myocardial Impairment in Chronic Fase of Chagas' disease**

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Introduction: The aim of this study is to assess modes of death in the chronic phase of Chagas' disease (CD), correlating them to myocardial involvement levels, closer to death. **Patients and Methods**: A descriptive study of a consecutive series of 136 cases of death for which there was a possibility to determine the mode of death in the period from March 1990 to December 2009. Cases of deaths were collected from the follow up cohort of CD patients from Evandro Chagas Clinical Research Institute / Oswaldo Cruz Foundation. All patients underwent a protocol of clinical, electrocardiographic (ECG), radiological and echocardiographic at admission and followed-up. The review of the evaluation made closer to death was done through the chart review. Assessing the severity of myocardial impairment, we used the classification recommended by the Brazilian Chagas disease consensus (2005) and the left ventricle ejection fraction (LVEF) estimated in the echocardiographic. **Results:** 136 deaths were reviewed, 111 were related to chronic Chagas' disease (CCD) and 25 not related (NCCD). CCD: 63 sudden death, 39 refractory congestive heart failure (CHF) and 9 embolic encephalic vascular accidents (EVA). The mode of death changed with the cardiopathy stage ($p<0.0001$) and the level of ventricular dysfunction ($p<0.0001$). Sudden death was the most frequent mode of death at all stages except stage with normal ECG (only NCCD deaths) and stage D (only CHF deaths). One third of deaths caused by EVA and one third of sudden deaths occurred in patients without CHF. The average LVEF differed in the various modes of death: 23±7% of the deaths by refractory CHF, 37±16% for sudden deaths and 39±21% for deaths due to embolic EVA ($p<0.0001$). **Main Conclusions**: The CCC is the main cause of deaths in Chagas' disease patients. The modes of death related to CCC are sudden death, CHF and EVA, in order of frequency. The extension of myocardial involvement changed according to the mode of death and was

more severe in patients who died of CHF, compared to cases of sudden death and EVA. Sudden death was the most frequent mode of death in all ventricular dysfunction levels and in most stages. **E-mail:** sergiosallesx@gmail.com

Chag2. **Chagas disease in the Brazilian Amazon: present situation at Rio Negro microregion and control perspectives**

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Introduction: Chagas disease in the Brazilian Amazon can be considered an *enzootic infection* of wild animals or an *anthropozoonosis*, when humans penetrate a wild ecosystem or when wild triatomines infected with *T. cruzi* invade human dwellings. It can also be considered a *foodborne illness*, by oral transmission of juices and other infected foods. In the microregion of Rio Negro it can be considered an *occupational disease* of piaçava's gatherers. **Materials and Methods:** Three preliminary serological surveys, to evaluate the *T. cruzi* infection, by indirect immunofluorescence was carried out in a sample of 2,254 persons, representing nearly 25% of the resident population in the town of Barcelos, in the microregion of Rio Negro, Amazon State. A specific serological study with IIF, ELISA and Western-blot was performed in 244 persons heavily exposed to triatomines bitten. A parasitological study (xenodiagnosis, PCR and hemoculture) was performed in 46 patients with positive serology and in 240 wild animals captured in the piaçava's gatherers settlements, and also parasitological exams of 949 wild triatomines. An epidemiological, clinical, electro and echocardiogram study was carried out in about 200 patients with positive serology for *T. cruzi* infection. **Results:** The three preliminary serological surveys showed a mean prevalence for *T. cruzi* infection of 13%, but the confirmatory techniques by IFF, conventional and recombinant, ELISA and Western blot confirm only 2.8 – 5%. The specific serological study with IIF, ELISA and Western blot of 244 persons heavily exposed to triatomines bitten showed 27 (11%) with positive serology for *T. cruzi* infection. From 46 patients serological positives from all surveys 9(19, 5%) had xenodiagnosis and PCR positives for *T. cruzi* and only one (2.17%) had positive hemoculture. From the 240 wild animals captured 54 (22, 5%) were positive for *T. cruzi* and from 949 wild triatomines (*Rhodnius brethesi*), only 19 (2%) were infected. The people seropositives for *T. cruzi* were 10 times more frequent among the piaçava's gatherers, and among them typical and fatal cases have been described. **Conclusions:** In the microregion of Rio Negro, Chagas disease can be considered an occupational endemic disease of piaçava's gatherers. Control measures to avoiding endemic Chagas disease in the Amazon Region should be the following: improving health education in communities, training public health officials and communities for vector and Chagas disease surveillance and training local physicians to recognize and treat acute and chronic cases of Chagas disease as soon as possible. **E-mail:** coura@ioc.fiocruz.br

Chag3. **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 peri-urban 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%) peri-urban 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:** gvbarbosa@fmt.am.gov.br

Chag4. **Occurrences of Chagas disease in serological exams at a clinic analyses laboratory in Pontal do Triângulo, Minas Gerais State, Brazil**

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Introduction: The Chagas disease is considered nowadays a neglected disease, causing 50 thousand deaths per year approximately and about 18 million people stricken by the parasite. In Brazil, around two million infected people are in the chronic stage of the disease. There are no studies of epidemiological character in the area of Pontal do Triângulo Mineiro that demonstrate the real prevalence of people affected by the disease. **Objective:** To evaluate the CHAGAS-positive prevalence to Chagas disease in people that was submitted to serological exams at a clinic analyses laboratory in Ituiutaba, Minas Gerais. **Material and Methods:** 2431 exams from assisted persons were analyzed at a clinic analyses laboratory. The variables analyzed from patients with Chagas disease were: age, prevalence and gender. The serological tests used for the diagnosis were: tests using antigens *T. cruzi:* indirect hemaglutination (HA), indirect immunofluorescence (IFI) and enzyme-linked immunosorbent assay (ELISA). **Results:** 2431 patients submitted to the exam 169 (6.9%) of them have presented suggestive serology for Chagas disease, 134 (5.5%) of the cases were considered positive. The total patients diagnosed for Chagas disease: 70 (2.9%) of cases are females and, 64 (2.6%) males, with 18 (0.7%) and 17 (0.7%) are uncertain cases, respectively. The 60-80 year-old age group had the highest confirmed prevalence for Chagas disease, contemplating 95 cases, followed by the 40-60 year-old group with 58 cases, 14 cases in patients with more than 80 years old have been found, 2 cases in the 20-40 year-old group and any case at all in patients with ages below than 20 years old. **Conclusion:** There was a meaningful prevalence of seropositive persons for Chagas disease. There were no significant differences between men and women as for Chagas-positive cases for Chagas disease in patients submitted to exams. The highest prevalence of disease was registered in patients with more than 40 years old. **E-mail:** patty_lopes19@yahoo.com.br

Chag5. **Pilot program for surveillance of Chagas disease in pregnant women and newborns, Colombia**

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Introduction: Colombia is an endemic country for Chagas disease; however, it does not have a surveillance program in pregnant women and their children. Migration from rural to urban areas, improvement of vector and transfusion control, and recent reports on some congenital cases indicate the relevance of congenital transmission research. **Materials and Methods:** From January 2010 to December 2011, a cross sectional study was conducted in five endemic departments of Colombia. 4,417 pregnant women were enrolled in primary care institutions. They were tested by ELISA and IFA IgG anti-*T. cruzi,* and answered a validated questionnaire. A monitoring program of the positive women and their children, from birth to one year of age, was developed. It included home visits, clinical evaluation and laboratory tests such as serology, PCR and hemoculture. The prevalence of infection in pregnant women, the incidence of congenital cases in newborns and the risk factors were estimated. A performance evaluation of the program was carried out by measuring timely access to health care, health insurance and tracking cases. **Results:** Chagas disease was found in 119/4417 women, with an overall prevalence of 2.70% (95%CI: 2.0-2.9) in both rural and urban areas. The prevalence in each department was: Arauca 2.15% (95%CI: 1.19-3.54), Boyacá 3.20% (95%CI: 1.83-5.18), Casanare 3.97% (95%CI: 2.84-5.28), Meta 0.23% (95%CI: 0.03-0.76), and Santander 3.36% (95%CI: 2.53-4.36). Factors associated with infection in pregnancy were: older age (p=0.00), housing conditions (p=0.00), previous vector contact (p=0.00), level of education (p=0.00), and history of Chagas in relatives (p=0.00). Of 47 children who had completed 12 months of age, 34 (82%) were followed up. None was identified as positive by hemoculture or serology. Health insurance, distance from house to hospital, cultural, and monetary factors were identified as the main barriers in the access to health care. **Main conclusions:** Chagas disease in pregnancy is an important health issue in both rural and urban areas in Colombia. Health insurance problems were identified as barriers to diagnose, treat, and monitor Chagas disease in women and newborns. National regulations are needed to achieve the coverage of pregnant women, newborns and relatives. **E-mail:** zcucunuba@ins.gov.co, zcucunuba@gmail.com

Chag6. **Word-of-mouth and social networks are key to Bolivian migrants seeking Chagas screening in Barcelona, Spain.**

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Introduction: The arrival of large numbers of Latin American migrants to Spain has set new challenges for the national health system. These challenges are associated with migrants' lack of familiarity with local health services and the increased prevalence of diseases previously unfamiliar to health professionals. The Tropical Medicine Unit (Hospital Clinic Barcelona) has taken a holistic approach, incorporating clinical and non-clinical elements to the management of Chagas disease. This study explores how patients came to know about the availability of Chagas screening in Barcelona and the factors that led to them seeking the test. **Material and methods:** Retrospective analysis of responses to the standard questionnaire administered during consultation to patients that attended the Tropical Medicine Unit seeking a test for Chagas disease between November 2009 and February 2012. Questions included: how the patient found out about Hospital Clinic, knowledge of locations where the test for Chagas was offered, and whether they had previously been tested. **Results:** 623 patients completed the questionnaire (433 women, 190 men, 91% Bolivian and 9% from other Latin American countries). In 56% of cases, a friend encouraged the patient to seek Chagas diagnosis, whereas in 28% of cases, the patient was referred by his or her general practitioner. 4% attended the Tropical Medicine unit because the patient was aware of his or her Chagas infection and specifically sought information about where to be tested and receive treatment. Of the 623 patients, 30% had previously been tested for Chagas in their country of origin. If asked informally about why they had not been previously tested, patients mentioned a lack of financial resources, being unaware of Chagas disease or convinced that they could not be infected. **Main conclusion:** Communication within the local Latin American community is key to the dissemination of information to encourage Chagas disease screening. E-mail: **eposada@clinic.ub.es**

Pathogenesis and treatment of Chagas disease

Chag7. **Pathogenesis of Chagas disease: Rupture of the dystrophin gene by *Trypanosoma cruzi* kDNA minicircle**

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Introduction: Clinical manifestations of Chagas Disease can be seen in the heart (94.5%) and in digestive system (5.5%) of circa one third of the *T. cruzi*-infected humans. Genetically driven autoimmune rejection of target heart cells by the immune system effectors cells is involved in the pathogenesis of the disease. Transfer of mitochondrial DNA (kDNA) minicircle sequences to genome of *T. cruzi*-infected of rabbits, humans and chickens, is documented (Nitz et al, 2004; Hecht et al, 2010). Interestingly, the refractoriness of chickens to *T. cruzi* has fostered the study of kDNA transfer in a parasite-free, transkingdom model. The inoculation of virulent *T.cruzi* in fertile eggs generated parasite-free chicks, but the kDNA minicircle was retained in the genome. Adult kDNA-mutated chickens presented Chagas heart disease similar to that in humans. The birds showing cardiomyopathy had kDNA mutations in the dystrophin gene. Rupture of the dystrophin gene is associated with skeletal muscle weakness and the heart disease in the chicken model (Teixeira et al, 2011). The aim of this work is to describe further rupture of dystrophin gene by kDNA mutations in parental birds and their progeny. **Material and Methods:** A *tp*TAIL-PCR (Hecht et al, 2010) made with specific dystrophin primers and kDNA nested primers, annealing at 50º and 61 ºC, was used. Genomic DNA digested with *Eco*RI and *Xho*I was subjected to electrophoresis in 0.8% agarose gel and Southern blotting with radio labeled specific dystrophin and kDNA probes. **Results:** Chimera kDNA minicircle-host dystrophin sequences were obtained from a chicken family: F0 (2); F1 (3); F2 (5); F3 (4). The *tp*TAIL-PCR assessments revealed kDNA-dystrophin mutations in parental and progeny. Interestingly, each mutation showed different minicircle insertion at the same gene cluster base pair in *locus* NW_001471534.1 Mosaicism was observed at the chimeras as resulting from a shear mass (15 thousand of different copies) of kDNA minicircle integrations in the dystrophin gene, showing variegated profiles: 1) Different kDNA minicircle sequences in the dystrophin chimeras of parental and progeny; 2) Chimeras showing truncated kDNA minicircle-host dystrophin; 3) Hitchhiking different kDNA sequence-host retroelement DNA insertion into the dystrophin gene. The mosaicism profiles suggested a dynamic pattern of chimeric DNA mobilization, which differed from parental to progeny. Actually, the genomic Southern blot hybridizations revealed specific bands with migration differences, thus suggesting profile changes over time. **Main Conclusions:** Vertical transfer of *T. cruzi* kDNA minicircle sequences to dystrophin *locus* NW_001471534.1 is a non-Mendelian genetic inheritance; the kDNA variable region present in the parental is rarely documented in the progeny. Moreover, the kDNA mutation, showing rupture oh the dystrophin gene, can be associated with the pathogenesis of Chagas disease. **E-mail:** marianahecht@gmail.com

Chag8. **DNA damage and oxidative stress in patients with Chagas disease**

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Introduction: Chagas disease is caused by the parasite *Trypanosoma cruzi*. Nitric oxide (NO) produced during the inflammatory response is an important part of the host defense strategy to kill the parasite. However, it is not well known whether this intermediate can cause DNA damage in Chagas patients. Therefore, we aimed to investigate the DNA damage along with oxidative/anti-oxidative status of patients with chronic forms of Chagas disease. **Material and Methods:** We evaluated 20 patients in the chronic

phase of Chagas disease, in three different forms indeterminate, cardiac or digestive and 20 healthy controls, both groups from Botucatu Medical School University Hospital. Single-cell Electrophoresis (Comet assay) and Gress reaction were used to measure DNA damage and NO production, respectively. We also evaluated the total hydrophilic antioxidant capacity (THAC) and tocopherol levels in the plasma of Chagas patients and controls. **Results:** Chagas patients with the cardiac or digestive forms presented higher DNA damage and NO production when compared with patients with the indeterminate form and control individuals ($p<0.05$). However, the THAC and tocopherol levels were significantly lower in patients with the cardiac or digestive forms, when compared with patients with the indeterminate form and control individuals ($p<0.05$). **Conclusion:** In conclusion, our results indicate that patients with the cardiac or digestive forms of Chagas disease present more DNA damage then patients with the indeterminate form and control individuals, which is probably related with the high levels of NO. These findings support the notion that NO produced by the host as a defense strategy may not be only responsible for the parasite destruction, but in high levels may also induce oxidative damage in non-infected cells. **Keywords**: Chagas Disease, DNA damage, antioxidants, NO. **E-mail:** francilene_capel@hotmail.com

Chag9. **Predictive Value of Transforming Growth Factor--β1 in Chagas Disease**

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Introduction: Up to 30% of patients with Chagas disease progress to the cardiac phase of the disease, which has high mortality. The mechanisms underlying this progression are still poorly understood. Transforming growth factor-β1 (TGF-β1) may be implicated in the development of Chagas heart disease and our group has already reported high TGF-β1 values in the serum of patients with Chagas heart disease. **Materials and Methods:** We retrospectively analyzed the all-cause mortality of patients whose TGF-β1 serum values were determined in a previous publication of our group. Results: Sixty-eight patients (49±10 years old) were followed for a mean of 10.0±3.6 years. Eleven patients died during this period. There were a total of 22 patients at the indeterminate phase (32.3%), 20 (29.4%) patients at stage A (isolated changes in electrocardiogram), 13 (19.1%) at stage B1 (asymptomatic with mild changes at the echocardiogram), 8 (11.8%) at stage B2 (asymptomatic with moderate to severe left ventricular [LV] systolic dysfunction) and 5 (7.4%) at stage C (7.4% symptomatic heart failure). LV end-systolic diameter was an independent predictor of all-cause mortality (hazard ratio [HR] 2.8; 95% confidence interval [CI] 1.5 to 5.0; p=0.0007), while TGF-β1 was not. The optimal cutoff for LV end-systolic diameter to identify patients who died was 4.1 cm (area under the curve [AUC] 0.77, p=0.002, sensitivity 73%, and specificity 80%). However, in patients with normal to mild LV systolic dysfunction, TGF-β1 was higher among patient who died than in survivors (49.5 ± 15.5 ng/ml vs. 17.6 ± 3.1 ng/ml, p=0.003). TGF-β1 (HR 1.02; CI 1.0 to 1.04; p=0.01) and LV ejection fraction (HR 0.91; CI 0.83 to 0.99; p=0.02) were independent predictors of all-cause mortality. The optimal cutoff for TGF-β1 to identify patients who died among those with normal or mild LV systolic dysfunction was 12.9 ng/ml (AUC 0.82, p=0.003, sensitivity 100%, and specificity 57%) and for LV ejection fraction was 53% (AUC 0.74, p=0.009, sensitivity 50%, and specificity 90%). **Main Conclusions:** TGF-β1 was an independent predictor of all-cause mortality only in the group of patients with normal to mild LV systolic dysfunction. Therefore, TGF-β1 seems to be an important determinant of Chagas disease patients' outcome at earliest stages of the disease, while at the advanced stages, after moderate to severe LV dysfunction is established, LV systolic function and diameters become the main prognosis determinants. **E-mail:** roberto.saraiva@ipec.fiocruz.br

Chag10. **Immunogenetic analysis of Human Leukocyte Antigen (HLA) region for Chronic Chagas disease in Bolivia**

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Introduction: Chronic Chagas disease is characterized by the typical clinical forms namely cardiac and digestive but the pathogenesis is still unclear. To identify possible host genetic factors that may influence on the prognosis of Chagas disease, HLA region genes polymorphism was analyzed in the patients presenting characteristic clinical symptoms detected by Electrocardiogram (ECG) and colon barium enema X-ray exam (Colon X-ray). **Material and Methods:** Two hundred ninety one of the chronic Chagasic individuals from Santa Cruz, Bolivia, with positive serology for Trypanosoma cruzi were recruited. ECG and Colon X-ray could identify three different forms of Chronic Chagas, and finally combined with post-operational megacolon patients, 100 megacolons, 81 cardiac and 73 indeterminate were categorized for further analysis. HLA-A, HLA-B, MICA, MICB, DRB1 and TNF-alpha promoter region genes polymorphism were analyzed by the sequence based and SSO typing test and the V281L mutation for CYP21 gene by the RFLP technique. **Results:** The frequencies of HLA-DRB1*01 and HLA-B*14:02 were significantly lower in patients with megacolon as well as with ECG alterations compared with indeterminate. Because the DRB1*0102, B*1402 and MICA*011 alleles were in strong Linkage Disequilibrium (LD), we could not identify the primary association locus within this haplotype. The mutation of V281L was identified in the haplotype but there was no significant contribution to the resistant phenotype. **Main Conclusion:** The HLA-DRB1*01-B*14-MICA*011haplotype with V281L of the CYP21 in the class III region showed strong association to resistant to chronic Chagas disease. **E-mail:** colepuerto@hotmail.com

Chag11. **Evaluation of results of association of the *T.cruzi* chemotherapic Benznidazole with others drugs (Nifurtimox, Cetoconazole) in the treatment of the experimental Chagas disease**

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Introduction: Treatment of Chagas disease, determined by infection with *Trypanosoma cruzi* has been a challenge due to the different susceptibility of the parasite strains, characterized into different biological types or Biodemes. It has been shown that the Benznidazole (BZ) is the drug of choice for the treatment in humans but the results are irregular in patients of different endemic areas. Experimental data have shown a high resistance of the strains of Biodeme type III (*T. cruzi* I), and irregular results in the treatment of infections with Type II strains (*T. cruzi* II). The same results were obtained in the treatment with Nifurtimox (NF), in respect to the resistance of different strains. Other drugs in clinical use for different diseases have been assayed experimentally as the case of the Cetoconazole (Ceto). Probably the association of different drugs could improve the results of treatment in humans. Combinations of drugs may shorten the period of treatment and diminish the collateral toxic effects. **Objective:** To evaluate the effect of treatment with binary combinations of BZ with NF and of BZ with Ceto on the evolution of infection with the Y strain of *T. cruzi* (Biodeme Type I – Z2b). **Material and Methods:** Swiss mice inoculated with the Y strain ($5x10^4$ trypomastigotes blood forms) were divided into two experimental groups: G1 (Association of BZ+NF) and G2 (Association of BZ+ CETO). G1: I – Infected, untreated; II – Infected, treated with BZ (100mg/kg/day; III – infected and treated with NF (50mg/kg/day) - Infected and treated with Benz + NF. G2: I - infected, untreated; II – Infected, treated with BZ (100mg/kg/day; III – infected and treated with CETO (120mg/kg/day); IV – infected, treated BZ+ CETO. Cure tests: Parasitaemia after use of Cyclophosphamide, xenodiagnosis and hemoculture. **Results:** G1 – 100% mortality in the untreated controls until the 12^{nd} day of infection; Groups treated with BENZ and BENZ + NF – 94.7% of survival and with NF: 100% of survival. Cure rates were of 94% for the mice treated with

BZ and for the treated with the combination of BENZ + NF. G2 - Survival for the groups treated with BENZ and BENZ + CETO was of 100%. Cure testes: 100% of negativation for the treated with BENZ, 94, 73% and with CETO and 100% for those treated with BENZ + CETO. **Conclusion:** The present study did not indicate a significant difference between the treatment with Benznidazole as compared with the association with Nifurtimox or Cetoconazole. **E-mail:** marcioalmeida14@hotmail.com

Chag12. **Evaluation of the response to treatment with Benznidazole of mice with triple infection with clones 0f 21SF strain (S. FELIPE-BA) of *Trypanosoma Cruzi* with different degrees of susceptibility to chemotherapy in comparison with mice with single infection**

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Introduction: People living in endemic areas of Chagas disease are submitted to multiple infections during their lives and could be infected with strains or clones with different virulence and susceptibility to chemotherapy. This is an important factor in the development and morbidity of the disease. Strains of *Trypanosoma cruzi* represent complex multiclonal populations, which can be homogeneous or heterogeneous with predominance of a principal clone. The strains are biologically classified in different Biodemes (Types I, II and III) which disclose different degrees of resistance to chemotherapy. Type I strains are very susceptible to treatment. Type II strains disclose medium susceptibility (21SF strain); strains of Type III are very resistant (Colombian strain). The clones isolated from different strains can also present different degrees of resistance. In the present study the results of treatment of mice triple infected with clones of the 21SF strain is evaluated, in comparison with the infected with the parental strain. **Materials and Methods:** 50 Swiss mice were infected with the 21SF strain (single infection).The inoculum was of 5 x 10^4 blood trypomastigotes. 100 mice were infected successively with 3 clones of the 21SF strain (C6, C7 and C8) inoculum: 1x10^4 trypomastigotes (triple infection). Single infection with each Clone was also done. The mice of both groups were divided into 2 sub-groups: treated with Benznidazole –BZ: (100mg/kg/day – 60 doses) and untreated controls. After 60 days of the end of treatment, surviving mice were killed by exsanguinations after anesthesia; the blood was collected for indirect immunofluorescence serological test; cure tests were performed (parasitaemia, xenodiagnosis and hemoculture). **Results:** Cure rates varied in each group (26.6% - 66% - single infection and 73.3%- triple infection). Serology (IIFT) titles varied from 1:20 a 1:280 for the infected with parental strain treated with BZ and from 1:640 to 1:1280 for untreated controls. Serology titles in the single infection with each clone varied from 1:10 to 1:1280 in treated mice and from 1:160 to 1:1280 in the untreated controls. **Conclusions:** In all the groups, treatment with BZ determined comparable results (parasitaemia, serology titers, mortality rates and cure rates). These data are compatible with previous studies that demonstrate a medium susceptibility of Type II strains and the predominance of a principal clone in the endemic area of S. Felipe-BA. **E-mail:** mc_reboucas@hotmail.com

T. cruzi – diversity

Chag13. **Biological and molecular characterization of clonal populations of *Trypanosoma cruzi* strains: 21SF – Biodeme Type II and Colombian strain – Biodeme Type III, isolated from infected mice, treated with Benznidazole and not cured**

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Introduction: Different strains and clones of *Trypanosoma cruzi* present different degrees of susceptibility to treatment with chemotherapic drugs. Several studies have been developed to evaluate the response to different drugs concerning the strains prototypes of Biodemes Types I, II and III according to the biological characterization. Results have shown that the strains prototypes of the Biodeme Type I (Y and Peruvian strains) disclosed a high susceptibility to treatment with Benznidazole and Nifurtimox; strains of the Biodeme Type II (prototype: the 21SF strain) showed a medium susceptibility; the strains of the Biodeme Type III (Colombian strain) were highly resistant. Considering that *T. cruzi* strains represent complex multiclonal populations, differing in their genetic and biological characteristics, clones of two *T. cruzi* strains were analyzed with the objective of to investigate if the treatment with chemotherapics anti-*T. cruzi* could conduct to the selection of resistant clones differing or not in their biological and molecular characteristics. **Objective:** In the present study we investigate the biological and molecular characters of clones of the 21SF strain (Biodeme Type II) and of the Colombian strain (Biodeme Type III) isolated from mice treated with Benznidazole, but not cured, in comparison with clones isolated from untreated mice, with the objective of to investigate possible differences in the biological and molecular characteristics of these resistant clones. **Material and methods:** 18 clones were isolated from mice infected with the Colombian strain and 08 clones isolated from those infected with the 21SF strain, treated and uncured. The clones were characterized according to biological behavior (parasitaemia, mortality, virulence, histotropism) and molecular characters, evaluated through the restriction fragment length polymorphism (RFLP) of the k DNA for each isolated clone, using restriction enzymes RSA I, HINF I and ECO RI. With the intent to distinguish between individual clones of the 21 SF and Colombian strains we made a microsatellite characterization, a very sensitive technique. Here were chosen five *loci* which are very polymorphic: TcTAT20, TcAAAT6, SCLE11, SCLE10 and MCLF10. We observed that the microsatellite profiles between the strains and their clones were very similar, showing that these populations are probably monoclonal and the treatment with Benznidazole did not alter their molecular structure. **E-mail:** sgandrade@bahia.fiocruz.br

Chag14. **Strains of DTUs TcI and TcV with Parasitaemia subpatent Involved in Transmission of Experimental Congenital Chagas Disease**

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Introduction: The congenital transmission of Chagas disease can occur in acute and chronic phases of maternal infection. It has been suggested that the parasite reaches the fetus by crossing the placental barrier. Many studies have linked high maternal parasitaemia and the DTU TcV with the congenital Chagas disease. However, in this study, we observed, experimentally, high rates of congenital transmission induced by strains of TcI and TcV characterized by producing parasitaemias subpatents. **Material and Methods**: We evaluate the congenital transmission in mice infected with the strains 3048 (TcV), isolated from a child infected by congenital transmission, and AQ1-7 (TcI), isolated from *Triatoma sordida*. Twenty female BALB/c mice, ten for each strain, were infected with 1 x 10^5 trypomastigotes by the intraperitoneal route. The females were mated after 35 days of infection. The technique of microhematocrit and hemoculture in LIT (Liver Infusion Tryptose) medium was used to evaluate the parasitaemia during pregnancy. Congenital infection was diagnosed by hemoculture and polymerase chain reaction (PCR) with 121 and 122 primers. **Results**: During mating occurred the death of a female (1/10) infected by AQ1-7 and none infected by 3048. The fecundity rate of infected female was 90% (9/10) for strain 3048 and 88.89% (8/9) for AQ1-7 ($p>0.05$). Parasitaemia during pregnancy presented subpatent and was detectable by microhematocrit for 3048 and only by hemoculture for AQ1-7. The mean litter size in offspring born was 9.22 ± 2.22 ($n = 83$) for 3048 and 5.38 ± 2.13 ($n = 43$) for AQ1-7 ($p= 0.003$). The offspring mortality rate was 12.05% (10/83) for 3048 and no mortality was observed for AQ1-7 ($p=0.017$). The Hemoculture resulted negative for all offsprings. However, the PCR was positive in

46.58% (34/73) of the offspring for 3048 and 58.14% (25/43) of the offspring for AQ1-7 (p>0.05). **Conclusions**: Females infected with AQ1-7 displayed a significantly reduction in the litter size suggesting the occurrence of fetal resorptions. The female mice infected by TcI and TcV strains presented high rates of congenital Chagas disease transmission even in the presence of a low maternal parasitaemia. We did not observe any relationship between *Trypanosoma cruzi* maternal parasitaemia and congenital transmission. The hemoculture showed itself ineffective in the diagnosis, indicating that PCR is the best tool to detect *T. cruzi* in congenital transmission. We showed that the DTU TcI also has a great potential to transmit the congenital Chagas disease and should be better studied in the context of vertical transmission. **Supported:** Capes, CNPq Universal 2008; FUNEPU and FAPEMIG **E-mail:** salkmim@hotmail.com

Chag15. **Widespread genetic exchange and the origins of domestic *Trypanosoma cruzi* I in Colombia**

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Introduction: Clonal propagation is considered to be the predominant mode of reproduction among many parasitic protozoa. However, this assumption may overlook unorthodox, infrequent, or cryptic sexuality. *Trypanosoma cruzi*, which causes Chagas disease, is known to undergo non-Mendelian genetic exchange in the laboratory. In the field, evidence of extant genetic exchange is limited. **Materials and methods:** In this study we undertook intensive sampling of *T. cruzi* Discrete Typing Unit (DTU) I in endemic Eastern Colombia. Using Fluorescence Activated Cell Sorting we generated 269 biological clones from 67 strains. Each clone was genotyped across 24 microsatellite loci. Subsequently 100 representative clones were typed using 10 mitochondrial sequence targets (3.76 Kbp total). **Results and Main conclusions:** Clonal diversity among humans, reservoir hosts and vectors suggested complex patterns of super-infection and/or co-infection in oral and vector-borne Chagas disease cases. Clonal diversity between mother and foetus in a congenital case demonstrates that domestic TcI genotypes are infective *in utero*. Importantly, gross incongruence between nuclear and mitochondrial markers is strong evidence for widespread genetic exchange throughout the dataset. Furthermore, a confirmed mosaic maxicircle sequence suggests inter-molecular recombination between individuals as a further mechanism of genetic re-assortment. Finally robust dating based on mitochondrial DNA indicates that the emergence of the domestic TcI clade (formerly TcIa / VEN_{Dom}) occurred 23,000±12,000 years ago and was followed by population expansion, broadly corresponding with the earliest human migration into the Americas. **E-mail:** david-r@uniandes.edu.co

Chag16. **Immunopathological response in mice with triple infection with *Trypanosoma cruzi* strains of different Biodemes submitted to treatment with immunossupressor drugs**

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Introduction: An important factor that contributes to the severity of Chagas disease in people living in endemic areas is the occurrence of multiple infections with *Trypanosoma cruzi*. Experimental studies have shown the increased morbidity due to successive reinfections with different strains, with the possibility of to evaluate the influence of multiple infections upon the tissue lesions. Multiple infections could also influence an activation of the disease in patients with HIV infection (AIDs) or immunosuppressed in cases of organs transplantation. In such cases, a reactivation of virulent strains could occur with aggravation of the disease. **Objective:** to evaluate the immunopathological response in mice triple infected with different *T. cruzi* strains and treated with immunosuppressor drugs. **Material and**

Methods: Inbred Balb/c mice were successively infected with *T. cruzi* strains of different Biodemes: 1) Colombian strain (Biodeme Type III – *T. cruzi* I); 2) re-infections with 21SF strain (Biodeme Type II – *T. cruzi II);* 3) third infection with Y strain (Biodeme Type I, Z2b). For each *T. cruzi* strain a group of mice with a single infection was included as a control group. Twenty days after the last infection, groups of triple infected surviving mice were submitted to different schedules of treatment with immunosuppressor drugs: *Schedule 1* – Betamethasone (2mg/kg/day) plus Cyclophosphamide (250mg/kg/day), during four weeks; *Schedule 2* – Azathioprine (2mg/kg/day) plus Betamethasone (1mg/kg/day) and Cyclosporine (during 4 weeks). Results were evaluated by the evolution of parasitaemia and mortality, histopathology and serological specific responses. Evaluation of immunoglobulin isotypes (ELISA method) and skin test for delayed hypersensitivity (DTH). **Results**: A reactivation of *T. cruzi* infection in mice with triple infection and treated with Betamethasone and Cyclophosphamide was detected, with increased parasitaemia and mortality rates, macrophagotropism, arterites and peri-arterites intensification of perivascular mononuclear cells infiltration and of extracellular matrix deposits. Combined treatment with Azathioprine, Betamethasone, Cyclosporine in the triple infected mice did influence neither parasitaemia levels nor mortality rates. However the histopathological study demonstrated aggravation of the necrotic inflammatory lesion with the presence of arterites and peri-arterites in the myocardium and skeletal muscles. DTH cutaneous test, disclosed significant differences in the 48 hours point between untreated triple infected mice and the treated with either one of the treatment schedules. The immunosuppression with Betamethasone plus Cyclophosphamide determined reactivation of the acute phase of the infection with *T. cruzi,* comparable to that observed in immunosuppressed patients. Treatment with Azathioprine, Betamethasone and Cyclosporine determined aggravation of the immunopathological lesions, characteristics of DTH alterations, without re-agudization of *T. cruzi* infection. **E-mail:** sgandrade@bahia.fiocruz.br

Chag17. **Differences in the infecting potential and pathogenicity of *Trypanosoma cruzi* I strains in various mice lineages**

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Introduction: *T. cruzi* (Tc) is divided into six DTU (*Discrete Typing Units*) (TcI - TcVI) which demonstrate a high parasite's heterogeneity. The influences of these genotypes in the evolution of experimental and human infection with diverse clinical forms are little studied. TcI lineage can be associated with several infections. In Colombia mixed infections with TcI and TcII lineages were already demonstrated in patients with cardiac and digestive forms. In this research, we evaluate the experimental behavior of three TcI strains isolated from triatomines from different regions in Brazil and analyzed the molecular and biological behavior in different mice lineages during acute phase of experimental Chagas disease. **Material and Methods:** The following mice lineages were used: Swiss, Balb/c and C57BL/6 mice, respectively susceptible and resistant to *T. cruzi* infection. 15 animals of each lineage were infected with 10.000 trypomastigotes/mL from each strain obtained from MK2 cells culture. The following Tc I strains were used: ALVANI (*Panstrongylus megistus* – Uberaba, MG), AQ1-7 (*Triatoma sordida* – Água Quente, Ba), MUTUM (*Panstrongylus megistus* – Uberaba, MG). Parasitaemia was carried out during 30 days both by microhematocrit (MH) and fresh examination (FE). At the 15th p.i., blood culture (BC) in *Liver Infusion Tryptose* (LIT) medium was also performed. 35 days p.i. fragments of various organs were taken. Tissue samples were stained using hematoxilin and eosin (HE) technique. Subsequently, to detect tissue and blood parasitism the PCR technique was also performed using 121 and 122 primers. **Results:** No blood parasites were detected using the MH or FE during the first 30 days in all infected groups and animals. BC was positive in 13.3% specimens from Balb/c mice infected with ALVANI and AQ1-7 strains and a positive rate of 55.5% ($p < 0.005$) was detected in infected animals with MUTUM strain. C57BL/6 and Swiss mice showed the lowest rate with 6.6% of positive specimens when infected with AQ1-7 and ALVANI strains and in an opposite manner these mice lineages showed the highest rates when infected with MUTUM strain 55.5% Swiss lineage ($p < 0.005$) and 88.8% in C57BL/6 ($p < 0.005$). The PCR analyses from blood samples were positive in 16.6%, 33.3% and 83.3% ($p < 0,005$) respectively from ALVANI, AQ1-7 and MUTUM strains. Nevertheless, tissue samples (PCR) were positive in 92.5% of

examined organs from C57BL/6, 100% from Balb/c and 88.8% from Swiss lineages when infected with ALVANI strain. A positivity rate of 92.5% was observed from C57BL/6 and Balb/c mice, and 74.0% of Swiss mice when infected with AQ1-7 strain. MUTUM strain was positive in 38.8% ($p < 0,001$) from Balb/c, 61.1% and 66.6%, respectively from Balb/c, Swiss and C57BL/6 mice. The HE were positive in 10.4% for examined organs from animals infected with ALVANI strain, 45.8% with AQ1-7 strain and none parasites were detected in animals infected with MUTUM strain independently for mice lineages used. **Main conclusions:** These results demonstrate a characteristic disposition of *T. cruzi* in tissues from acute phase, and also an interaction between parasite tissues and systems. An adaptive capacity to different hosts was observed. AQ1-7 and ALVANI strains showed a tissue tropism whereas MUTUM strain showed a blood tropism confirming the strain-dependent profile and the low pathogenicity in the MUTUM strain independently for mice lineages. **E-mail**: mardenbiomed@hotmail.com

Chag18. **Molecular characterization of *Trypanosoma cruzi* and diagnosis in Colombian children treated with Nifurtimox (Lampit®)**

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Introduction: Chagas disease caused by the parasite *Trypanosoma cruzi* is a neglected pathology of major importance in the American continent. There are about 1.2 million people infected in Colombia, and 5,250 new cases of vector transmission annually. Since 2007, the only available treatment in the country is Nifurtimox (Lampit®), and its efficiency has not been evaluated yet. **Material and Methods:** Sixty-two scholar children on an average age of 11 years were found infected with *T. cruzi* by serological methods and treated with Nifurtimox according to WHO recommendations. Samples of guanidine-EDTA blood pre and post treatment were collected, and analyzed by conventional PCR and indirect immunofluorescence. Molecular characterization of *T. cruzi* was performed using SL-IR and 24S regions. **Results:** Monitoring of treatment by indirect immunofluorescence showed reduced percentages of positiveness through time: pre-treatment 98%, day 60 posts treatment 98%, and at 6, 12 and 18 months post treatment 89%, 71% and 25%, respectively. Meanwhile, results of conventional PCR showed percentages of positive tests as follow: 94% pre-treatment, day 60 post treatment 70%, and at 6 and 12 months post treatment: 78 and 81%, respectively; 18 months post treatment is still under evaluation. Discrete typing units (DTUs) were characterized in 46 of the 62 patients (74%). TcI was the predominant DTU, detected in 28 patients (45%), DTUs TcII-TcVI where identified in 12 patients (19%) and mixed infection TcI and TcII-TcVI in 6 patients (9.6%). Amplification of the 24Sα-rDNA region, showed 2 samples as TcII/TcVI (11%) and one sample as TcIV (5, 5%). Finally, TcI samples were genotyped as: TcIa (62%), TcId (20.5%) and TcIb (15%). **Main Conclusions:** This is the first report in the country evaluating Nifurtimox as a treatment for Chagas disease. Implementation of real time PCR will be included in the study as a more accurate method. We were able to find a variety of DTUs, showing the predominant presence of TcI in the region, and reporting for the first time the detection of TcIb in patients' samples. **E-mail :** f.bianchi58@uniandes.edu.co

AFRICAN TRYPANOSOMIASIS

AfrTryp1. **Deep annotation of Trypanosoma brucei endogenous siRNAs yields insights into their sources, variation and functions in two life stages of the parasite**

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Trypanosoma brucei, the pathogen of trypanosomiasis of human and domestic animals, is an early evolved protozoan with a complex life cycle. The majority of its genes are post-transcriptionally regulated. The regulatory mechanisms and the molecules involved in remain largely unknown. We have deep-sequenced the small RNAs of two stages in the life cycle of this parasite, the blood slender form and the insect procyclic form, and have analyzed the sources and functions of endo-siRNAs. Surprisingly, we found that T. brucei siRNAs could be generated from multiple sources, including TEs (transcriptional elements), bidirectional transcriptions (BTRs) and natural antisense transcripts (NATs). Widespread variation among these endo-siRNAs was observed in different developmental stages. The evidence indicated that the endo-siRNAs could regulate the expression of genes through an RNAi pathway. Our results demonstrated a novel function of siRNAs on *T. brucei* gene expression regulation, which implied a more complex picture than previously thought. The results also shed light on questions regarding the origins and evolution of siRNA-based mechanisms of gene expression regulation. (This work was supported by grants from the National Basic Research program (“973” program, No: 2011CB811300) to L.-H.Q. and from the National Natural Science Foundation of China (No: 31071995) to Z.-R.L. The authors would like to take this opportunity to thank the persons from our labs who provided comments and suggestions on this project and manuscript.) **Key words:** sleeping sickness, Nagana disease, high through-put sequencing, RNA interfering, gene regulation. **E-mail:** lsslzr@mail.sysu.edu.cn;/ lssqlh@mail.sysu.edu.cn / fjayala@uci.edu

AfrTryp2. **Immunotherapeutic efficacy of DNA vaccines in the experimental model of African trypanosomiasis**

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Introduction: The administration of plasmid DNA encoding a target gene is capable of generating cytotoxic T lymphocyte, T helper cells and antibody responses in a variety of animal models. However, a Th1 immune response profile is preferentially produced by this genetic immunization methodology, probably due to the effect of the plasmid DNA adjuvant. In this context, African trypanosomiasis (AT) may be a good example of a polarized Th1 immune response activation. The causative agent of AT is the extracellular protozoan *Trypanosoma brucei,* which is transmitted by the bite of the infected tsetse fly (*Glossina* sp.). In the natural course of AT ailment, the interactions between *T. brucei* and the host preferentially induce an early Th1 polarized immune response profile, characterized by the release of inflammatory mediators and the secretion of IFN-γ, TNF, and IL-2 in response to some of the parasite antigens. Thus, the Th1 immune response may be important in the immunopathological consequences of AT since relative resistance is associated with a strong Th1 immunity and IFN-γ production. In order to investigate this hypothesis, we will evaluate the immunotherapeutic efficacy of DNA vaccines in the experimental mouse model of AT. **Material and Methods:** The pVAX1 plasmid (Invitrogen, USA) was

used to build the plasmid DNA vaccines. As vaccine prototypes we will a multivalent preparation of three plasmids: ISGpVAX1, nTSApVAX1 and PLCpVAX1, encoding respectively the Invariant Surface Glycoprotein (ISG), the n-terminal sequence of *trans*-sialidase (nTSA), and Phospholipase-C (PLC) from *T. b. brucei* parasites. Mice were infected intraperitoneally with 500 parasites prior to the immunization protocol. Two strains of mice, CD-1 and BALB/c, were immunized subcutaneously with either 0, 1 mg of the multivalent vaccine preparation. As control groups, mice were injected with 0, 1 mg of plasmid pVAX1LacZ (Invitrogen – USA) or 0, 1 mL of PBS by the same route. Mortality was recorded from this day forth and parasitaemia was evaluated at days 7, 13, 20 and 60 post-infection. Sera from all mice were obtained at the same time. ELISA titration for immunoglobulin subclasses were performed using a total purified protein extract from *T. b. brucei* as antigen source. **Results and main conclusions:** This work allowed the studying of the humoral response after immunotherapeutic with DNA vaccine and the examination of the progress of infection caused by *T. b. brucei* in mice. The results obtained so far seem to corroborate this finding, with an increase of total IgG and IgG2a over time. In conclusion, this study shows that this process allowed us to control partially the mortality rate of the mice infected with *T. b. brucei*. These results open up the possibility of the use of new attractive targets and approaches for vaccine development against AT. **Acknowledgments:** This work is supported by grants from *Fundação para a Ciência e Tecnologia* – Portugal (PTDC/CVT/102486/2008) and *Fundação Calouste Gulbenkian* – Portugal (Proc105228). **E-mail:** mssilva@ihmt.unl.pt

AfrTryp3. **Evaluation of the Trypanocidal activity of diamines and ferrocenyl diamines against *Trypanosoma brucei* strains**

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Introduction: Trypanosoma brucei is the etiologic agent of sleeping sickness, transmitted by flies of Glossina genus, known as tsé-tsé flies. Pentamidine is the mainstay of treatment for stage I infection with T. brucei. Novel drugs with less adverse effects than pentamidine are urgently needed. In this work, we evaluated the trypanocidal activity of a series of N1,N2-dibenzylethane-1,2-diamine hydrochlorides (diamines) and N1benzyl,N2-methyferrocenylethane-1,2-diamine hydrochlorides (ferrocenyl diamines, chart 1) against T. brucei parasite strains. Chart 1: Structures of the diamines and ferrocenyl diamines investigated. **Material and Methods:** Procyclic forms of T. brucei (427 and 29-13 strains) were treated with the diamines and ferrocenyl diamines for determination cytotoxicity index (CI50) using the MTT colorimetric assay and was also carried out to evaluate the toxicity of the substances in HepG2 cells. HepG2 cells are a hepatoma cell line used as a model to simulate human hepatic functions. **Results:** The diamines showed lower cytotoxicity when compared to pentamidine (control). Furthermore, RAC03 showed higher activity than pentamidine against both strains (Table 1) which is interesting for further studies, because pentamidine has limited action in stage II of the disease where the other different diamines derivatives are active. The ferrocenyl diamines showed CI50 values slightly higher than pentamidine. Table 1. Results of diamines and ferrocenyl diamines activities against T. brucei strains (CI50). Cell viability by the MTT colorimetric assay in HepG2 cells showed that the diamines and ferrocenyl diamines have low toxicity towards the cells (graph 1). Graph 1. Cell viability by the MTT colorimetric assay towards HepG2 cells (a) and CI50 (µg/mL) (b). Our results indicate that these diamines are interesting compounds for further studies using these parasites as model. **E-mail:** avellangel@gmail.com

AfrTryp4. **Dose regimen assessment for oral Fexinidazole**

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Introduction: Fexinidazole, a 5-nitroimidazole, is being developed as a new oral treatment for Human African Trypanosomiasis. The aim of the present study was to determine the efficient dosing schedule for a phase II/III study based on a population pharmacokinetic (PK) model. **Material and Methods:** The population PK analysis was performed using NONMEM VI and SAS 9.2 based on plasma samples from data collected through three phase 1 studies, validated using the data of a fourth study (Multiple Ascending Dose over 10 days under fed condition using two different loading dose regimen). **Results:** The PK of fexinidazole was best described by a two compartment model with a zero order absorption process. Integration of M1 and M2 metabolites in the model was successful and allowed simultaneous fitting of the three compounds. Population PK parameters of the parent drug estimated in the complete model were in agreement with those reported by non-compartmental analysis. The model simulates the time course of concentration of fexinidazole, M1 and M2 in a typical subject, following multiple once daily oral administration of fexinidazole under fasting and fed conditions. The following dosing regimens under fed conditions were determined from the simulations and implemented in the fourth phase I study:

-1800 mg fexinidazole or placebo from Day 1 to Day 4, and 1200 mg fexinidazole or placebo from Day 5 to 10

-2400 mg fexinidazole or placebo from Day 1 to Day 4, and 1200 mg fexinidazole or placebo from Day 5 to 10. This treatment regimen was found to be poorly tolerated and was stopped.

Observed data of the first dosing regimen were in agreement with simulated data obtained with the first treatment schedule. Safety and tolerability were acceptable. Active metabolite M2 plasma concentration was reached rapidly and maintained for 3 to 4 days in all cases and more than 80% of the subjects had pre-dose plasma levels above 10 mg/L. **Main Conclusions:** Based on these results, the dosing regimen of phase II study was defined and is about to be tested. **E-mail:** atarral@dndi.org

AfrTryp5. **Effect of food on the pharmacokinetic of single dose of Fexinidazole in healthy male volunteers of sub-Saharan African origin**

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Introduction: Fexinidazole is a 2-substituted 5-nitroimidazole that exhibits activity against *Trypanosoma brucei rhodesiense* and *T.b. gambiense* parasites, the causative agents of human African trypanosomiasis (HAT). Two bioavailability studies were conducted, the first comparing high fat rich meal to fasting, the second to assess the effect of two different field-adapted types of meal, Plumpy'Nut® (meal 1) or rice and beans (meal 2) versus fasting according to a three-way crossover design. **Materials and Methods:** Twelve subjects were enrolled in each study. Each subject received a single dose of 1200 mg of fexinidazole on three occasions separated by a wash-out period of at least 14 days. The drug was administered fasting or 30 min after the meal. The determination of the free fraction of Fexinidazole and metabolites was included in the second study. Subjects were assessed for clinical and laboratory safety and electrocardiogram (ECG) recordings. Fexinidazole and its metabolites, fexinidazole sulfoxide (M1) and fexinidazole sulfone (M2), were quantified in plasma and urine by LC-MS/MS**. Results:** 12 subjects were dosed in each study. The tolerability was excellent. No relevant changes from baseline were observed in laboratory parameters, vital signs or in ECG parameters. Concomitant food intake, such as high-fat breakfast (first study), induced a marked increase (4-fold, based on $AUC_{0-\infty}$) of the relative bioavailability of fexinidazole. In parallel, M1 and M2 levels increased proportionally. In the second study, whatever the meal, overall exposure was increased on an average of 200%, 190% 160% (based on $AUC_{0-\infty}$) for Fexinidazole, M1 and M2, respectively. The mean half-life ($T_{1/2}$) remained in the same range with or without food, around 10h for Fexinidazole and M1 and around 20h for M2. Inter-individual variability, as expressed by the coefficient of variation (CV) of the C_{max} and AUCs of M2, was reduced under fed conditions, as compared to fasting condition (39% *vs.* 46-53%). Free fractions were 2% for Fexinidazole, 59% for M1 and 43% for M2. Metabolic ratios (metabolite vs. fexinidazole) of C_{max} and

AUCs were comparable between fasted and fed conditions. Plasma levels higher than the Minimum Inhibitory Concentration (MIC) of M2 (2200 ng/mL) could only be reached under fed conditions (between 6 and 48 hours after dosing). **Main Conclusions:** Fexinidazole given with food was well tolerated. The high M2 free fraction predicts good availability to the brain. Food effect could be used to reduce the treatment dose of Fexinidazole. **E-mail:** atarral@dndi.org

AfrTryp6. **Multiple dose safety and Pharmacokinetics study of Fexinidazole dosed under fasting conditions in health male volunteers of sub-Saharan African origin**

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Introduction: Fexinidazole is a 2-substituted 5-nitroimidazole that exhibits activity against Trypanosoma brucei rhodesiense and T.b. gambiense parasites, the causative agents of human African trypanosomiasis (HAT). This first-in-man study tested multiple doses of the drug under fasting conditions in healthy male volunteers of sub-Saharan African origin, in order to assess its safety and tolerability compared to placebo, and to determine the pharmacokinetic (PK) parameters of fexinidazole and its metabolites. **Materials and Methods:** 24 subjects were randomized in 3 cohorts of 8 subjects (6 active + 2 placebos) to receive ascending doses of 1200, 2400 and 3600mg fexinidazole under fasting conditions. Subjects were assessed for clinical and laboratory safety, electrocardiogram (ECG) and 24hour Holter recordings. Fexinidazole, fexinidazole sulfoxide (M1) and fexinidazole sulfone (M2) were quantified in plasma and urine by LC-MS/MS. **Results:** In total, due to 3 replacements, 27 subjects were dosed. Fexinidazole was well tolerated up to 3600 mg for 14 days. One related serious adverse event (elevation of transaminase levels) was reported in the highest dose cohort after 14 days and spontaneously subsided. Treatment-emergent adverse events consisted mainly of headaches and gastrointestinal disorders. No relevant changes from baseline were observed in vital signs or in ECG parameters. On categorical ECG analysis, all QTc values were below 450 ms. Isolated changes from baseline above 30 ms, but none above 60 ms, were reported for all fexinidazole doses. Within the 1200-3600 mg dose range, the Cmax and AUCs of fexinidazole and its metabolites (M1/M2) increased less than expected assuming dose proportionality, under single or repeated doses. There was a small accumulation of Fexinidazole, M1 and M2 on day 7 as compared to day 1, which remained comparable through day 14. After single or repeated administration, circulating levels of M1 and M2 metabolites were higher than those of the parent compound. The AUC ratios of M1/fexinidazole and M2/fexinidazole were comparable on Day 7 and on day 14. Steady-state was reached on Day 4 for fexinidazole and M1 and on Day 9 for M2. **Main Conclusions:** Multiple repeated administration of Fexinidazole, up to 3600 mg, was well tolerated. However, liver function enzymes were detected as target parameters after 14 days of administration, calling for the consideration of a shorter duration of exposure. **E-mail:** atarral@dndi.org

OTHER TRYPANOSOMIASIS

OTryp1. **Assessment of the Species Involved in Livestock Trypanosomiasis in the Magdalena Medio of Colombia Using a Molecular Approach**

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Animal Trypanosomiasis is an economically important disease, widely distributed in several countries of South America, Africa, and Asia. In South America, it is caused by hemoflagellated protozoan of the genus *Trypanosoma,* transmitted mechanically by hematophagus vectors. In Colombia, this disease is spread throughout the country, especially in tropical and subtropical regions such as the Magdalena

Medio. Currently the gold standard method to confirm animal trypanosomiasis is the optical microscopy. This technique, although specific, lacks sensitivity and does not provide reliable information regarding the species of parasite. To contribute to the solution of this problem, in this work 200 blood samples from large ruminants (cattle and buffaloes) growing in ten farms of the Colombian Magdalena Medio were analyzed by traditional and molecular methods. To accomplish this objective, PCR coupled with restriction analysis based into Ssu-rDNA genes proposed by Geysen *et al.,* (2003) was evaluated, optimized and applied for *Tripanosoma sp., T. vivax*, *T. theileri*, *T. evansi*. In this study, the blood samples evaluated by the microscopy (MHCT) exhibited: 3.9% for *Trypanosoma sp.,* 28.4% for *Anaplasma sp.* and 3.9% for Babesia. Conversely, the analysis species-specific with modified PCR-RFLP-Ssu rDNA, showed that, the sensibility of molecular assays was more than four times that MHCT, with 17% of infection frequency in the samples tested. Normal range of PCV, white blood cell count and eosinophils, were found to be protective factors for *Trypanosoma*. Protective association with race was also found. Significant differences in clinical and hematological manifestations were observed between two populations tested, indicating that may be exist trypanotolerance mechanisms associated with some buffalo races. Anemia, leukopenia and thrombocytopenia were found associated with trypanosomiasis. Positive association was found hemoparasites with environmental and nutritional factors, such as floods, forest ratio, presence of vectors and nutritional supplements. In our region, was determined the presence of three species of Trypanosome, being *T. vivax,* the most prevalent in both, bovines and buffaloes. Our results confirmed the low sensitivity of parasitological method compared with the molecular assays and demonstrated the feasibility of using this technique as a diagnostic tool in studies of field samples. Additionally, this work would constitute the first study using molecular technology to identify all the species causing trypanosomiasis in cattle and buffaloes in our country. **E-mail:** torcoromin@hotmail.com

OTryp2. **Molecular Epidemiology of *Trypanosoma rangeli* in Ecuador and assays on the parasite's cell invasion**

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Introduction: *Trypanosoma rangeli* is a protozoan able to infect mammals, humans, and Triatominae insects. *T. rangeli* shares vectors and reservoirs with *T. cruzi*, the Chagas disease parasite. This can result in misdiagnosis of Chagas in areas where the two parasites overlap spatially and temporarily. The objectives of the study were to determine the presence of *T. rangeli* in Ecuador and to investigate by molecular tools the relationship parasite-vectors-reservoirs. The second part concentrated on revealing the mechanism that *T. rangeli* uses to invade cells, and to compare such mechanism with that of *T. cruzi*. **Materials and Methods:** Triatomines were collected in two provinces (10 villages in Manabi and 10 villages in Loja). Vectors were identified to species level and samples taken (hemolymph, intestinal contents, salivary glands, feces). Several sets of primers (for kDNA minicircles, 24Sα rRNA, nuclear elements) were used to run PCRs to identify and characterize trypanosomes in the samples and to identify the blood meal source (cytB primers). Fluorescent antibodies were used to target markers of early events in the invasion process. *T. rangeli* Choachi strain and BALB/c fibroblasts were used. Timed experiments were performed to analyze the interaction parasites-cells and the infection by *T. rangeli*. Inhibitors of cell processes such as Cytochalasin D and Wortmannin were used to observe the effect of cellular functions in invasion. **Results:** Specimens of *Rhodnius ecuadoriensis* (113), *Panstrongylus howardi* (39) and *Triatoma carrioni* (14) were found and included in the study. Individuals of each species were naturally infected with *T. rangeli* (8.4% *Re*; 4% *Ph*; 3.7% *Tc*). Mixed infections *T. rangeli*-*T. cruzi* were observed (5.6%, 4.9%, and 4%, respectively). Blood meal analysis revealed that vectors had fed on several species (common rat, mice, dog, cat, goat, guinea pig, human, chicken). *T. rangeli*-infected cells did not show signs of intracellular division up to 288 hours post-infection and parasitaemia in BALB/c mice was transient and declined rapidly overtime. Cell invasion was reduced drastically when the cells were pre-treated with Cytochalasin D (potent inhibitor of actin polymerization) and Wortmannin (inhibitor of PI3 Kinases). **Main Conclusions:** *T. rangeli* seems to be widely distributed in Ecuador and probably infecting a variety of vectors and mammals. The species of vectors reported here as naturally infected

with *T. rangeli* had not been reported before, but further studies on vector competence are necessary. Also, identifying blood meal sources can contribute to understanding the epidemiology of transmission cycles. *T. rangeli* invades cells primarily in a lysosome-dependent way similar to that of *T. cruzi*, but *T. rangeli* seems to lack the alternative lysosome-independent pathway described for *T. cruzi*. *T. rangeli* invasion of mammalian cells seems to depend to a great extent on actin polymerization that takes place in the host cell upon contact with parasites. PI-3 kinase activity seems to play an important role in promoting an efficient cell invasion by *T. rangeli*. **E-mail:** slascano@cdc.gov

OTryp3. **Timing of morphological events during the Trypanosoma rangeli cell cycle in vitro**

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Introduction: Trypanosoma rangeli is a protozoan parasite closely related to *T. cruzi* but considered non-pathogenic to the mammalian host. Studies regarding the *T. rangeli* cycle in mammals are controversial, remaining unknown essential aspects of the parasite biology, such as multiplication within the host. The present study addresses the time course of morphological events during the *T. rangeli* cell cycle in vitro. **Materials and Methods:** Choachí strain epimastigotes were cultured in LIT+15%SBF and counted in 12-hour intervals up to 72h. To assess morphological changes, parasites were incubated with 5-bromo-2-deoxyuridine (BrdU) and evaluated by immunofluorescence assays using antibodies anti-BrdU and anti-flagellum calcium-binding protein (FCaBP), following DAPI staining. The duration of each event within the cell cycle could be determined from the frequency of cells showing that event because i) the position of a particular stage in the cycle is known, ii) the culture is asynchronous and iii) the generation time is constant. Duration of *T. rangeli* G2, mitosis (M) and cytokinesis (C) were calculated according to Williams (1971). To determine the length of S phase, the sum of G2 phase, M and C events, the duration of BrdU labeling period and the proportion of cells exhibiting labeled nuclei and kinetoplast were applied to the formula described by Stanners and Till (1960). **Results:** Independent experiments resulted in an average generation time of 26.2h for *T. rangeli* Choachí strain, being close to the 24h estimated for *T. cruzi* Y strain. This generation time represents a specific growth rate of 0.02 per hour. According to morphological stages previously described for *T. cruzi* and *T. brucei*, the *T. rangeli* cycle is arranged as follows: cells containing one nucleus (1N), one kinetoplast (1K) and one flagellum (1F) are in G1 or S; cells containing 1N1K2F are in G2; 1N2K2F cells are in mitosis (M); and 2N2K2F cells are about to undergo cytokinesis (C). Thus, in *T. rangeli* Choachí strain G1/S lasts 18.5h, accounting for 71% of the cycle, G2 lasts 4.4h (17%) and M and C last, respectively, 1.6h and 1.7h. So far, the extent of the S phase estimated for *T. rangeli* has an average of 5.3h (± 1.5h). **Main conclusions:** The present study describes for the first time the *T. rangeli* cell cycle duration, which revealed to be very similar to the *T. cruzi* cycle and almost three times longer than the *T. brucei* cycle. Further studies using markers revealed by the *T. rangeli* genome are in progress to assess the parasite's biology. Support: CNPq/CAPES/FINEP and UFSC. **E-mail:** edmundo.grisard@ufsc.br

OTryp4. **Characterization of Trypanosoma evansi antigen by SDS-PAGE and Western blot**

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Trypanosomosis caused by Trypanosoma evansi, is a haemoparasitic disease of domestic animals and widely distributed throughout the world in area with hot weather and mechanically transmitted by sucking insect. The present study was attempted to provide some information through Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and western blot of the T.evansi isolated from mice which inoculated with blood from naturally infected camels. SDS-PAGE of T.evansi antigen after staining with coommassie blue stain showed the presence of 26 bands ranging between 222.56 – 9.0 KDa. Western blot analysis of antiserum from 4 naturally infected camels (by mice inoculation and card

agglutination test for trypanosomosis) and 2 mice experimentally infected with T.evansi, showed immunoprecipitation only with 2 proteins components with camels' positive serum and had molecular weight of 98.976 and 16.678 KDa. While with mice positive sera revealed only one immunoprecipitation with protein component had molecular weight 16.678 KDa. **Key words:** Trypanosoma evansi; Camel; SDS-PAGE; Western blot. **E-mail:** dochakim2000@yahoo.com

OTryp5. ***Trypanosoma caninum*, a new species described in domestic dogs, is found in different Brazilian states**

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Introduction: *Trypanosoma caninum* is a parasite of the *Trypanosoma* genus recently described in the natural infection of dogs in the municipality of Rio de Janeiro, Brazil. Suspecting the existence of a natural cycle and the circulation of this new species, the objective of this study was the taxonomic identification of 33 samples of *Trypanosoma* spp. isolated from dogs in different Brazilian regions. **Material and methods**: The samples studied were obtained during surveys with domestic dogs conducted in the states of Rio de Janeiro, comprising the municipalities of Rio de Janeiro, Niterói and Maricá; São Paulo (Bauru); Minas Gerais (Belo Horizonte); Mato Grosso (Cuiabá) and Goiás (Brasília). All samples were obtained from intact skin fragments, cultured and characterized by nested-PCR targeting the partial sequence of 18S rRNA gene. The PCR products were sequenced and the phylogenetic tree was constructed based on the neighbor joining analysis. **Results:** All isolates showed similar morphological aspects in culture and the results of PCR assays showed the same amplification pattern for all 33 samples. Sequencing analysis showed that the isolates were genetically identical or closely similar and confirmed *T. caninum* identify. **Main conclusions:** We demonstrate that the analysis of partial SSU ribosomal DNA sequence was enough to taxonomically identify the 33 samples isolated from different Brazilian regions and grouped all stocks reported to date in a single clade. In Brazil, visceral leishmaniasis is a serious public health problem and the domestic dog is one of the targets for control actions. The evidence of autochthonous cases of infection by *T. caninum* in areas where VL is endemic constitutes an alert for epidemiological surveillance. The results presented in this study clearly show the circulation and the existence of a natural cycle of *T. caninum* and broadens the geographical distribution of *T. caninum* reported in Brazil. **E-mail:** fatima.madeira@ipec.fiocruz.br

LEISHMANIASIS

Mixed and infection by Leishmania

Leish1. **Mixed Infections by *Leishmania* spp. and *Trypanosoma cruzi* in the Amazon**

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Introduction: Chagas disease and leishmaniasis are endemic in the Amazon. The insect-vectors of the flagellate protozoan *T. cruzi* and *Leishmania* spp are sympatric in those regions where cases of mixed infections have been reported in humans. Differential diagnosis among these close related

trypanosomatids is based on the detection of specific antibodies against *T. cruzi* epimastigotes and *Leishmania* promastigotes. Cross reactivity of antibodies against *Leishmania* spp and *T. cruzi* antigens is a major caveat on the differential diagnosis of these endemic protozoan infections. Usually, parasitological methods have low sensitivity because parasitaemia are low during chronic infections. Presently, molecular methods based on PCR assay emerge as a powerful tool for diagnosis screenings, showing high sensitivity and specificity. In this study, we evaluate the PCR performance in differential diagnosis of individual living in endemic area for Chagas disease and leishmaniasis. **Material and Methods:** Blood samples from 109 individual from Pará State, Brazil, were analyzed by IF and ELISA, and by PCR with specific primer sets annealing to *T. cruzi* nuclear DNA (nDNA, Tcz 1/2), and to *L. braziliensis* mitochondrial DNA (kDNA primers Lb1/Lb2). **Results:** The *T. cruzi* infections were diagnosed in 35, 7% of the individuals (39/109) by IF and ELISA. These assays revealed 46.7% (51/109) individuals were positive for the *Leishmania braziliensis* antigens. The PCR results for *T. cruzi* nDNA showed 76, 1% (83/109), whereas the *L. braziliensis* PCR showed the kDNA marker in 90, 1% (46/51), in those cases of positive serology against *L. braziliensis* and *T. cruzi* antigens. **Main Conclusions:** Discrepancy between the serological results and the PCR finds confirm the higher sensibility and specificity of molecular methods than those obtained with immunological tests. In this regard, the immunological tests underestimated the true prevalence of the *T. cruzi* infections in endemic areas. Of great interest, the immunological tests usually do not make the differential diagnosis between these infections. This is a major limitation towards the serum epidemiologic surveys that have based on the identification of antibodies to determine the prevalence of these trypanosomatid infections, which are sympatric in the Brazilian territory. The occurrence of mixed-infections by *T. cruzi* and *Leishmania* spp. has been reported, which were suspected by serological assays end-point dilutions and by immunobloting. In the study reported here we used PCR assays to show that asymptomatic mixed infections by leishmaniasis and *T. cruzi* are frequently present in humans living in the Amazon. **E-mail:** nnitz@unb.br

Leish2. **Mixed infection in rodents captured in Belo Horizonte, Minas Gerais State, Brazil, endemic area for visceral and cutaneous leishmaniasis.**

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Introduction: The municipality of Belo Horizonte, capital of Minas Gerais State, is an area densely populated and urbanized, where the transmission of leishmaniasis occurs in all districts of the city. The simultaneous occurrence of different forms of leishmaniasis in the same area can favor the emergence of mixed infections by *Leishmania* spp in the hosts. In 2006, it was reported the first case of co-infection with *L. (V.) braziliensis* / *L. (L.) infantum* in a dog from the city of Rio de Janeiro, Brazil. The authors suggested the collection of different tissues, especially in animals from areas where species of the genus *Leishmania* overlap. **Material and Methods:** The present study was conducted in Belo Horizonte during the period between June 2006 and November 2007. Sixty-two rodents were captured including 24 *Mus musculus*, 19 *Rattus rattus*, 9 *R. norvegicus*, 5 *Necromys lasiurus* and 5 *Cerradomys subflavus*. After euthanasia (in accordance with the ethical principles of animal experimentation), samples from skin (ear and tail), liver, spleen and bone marrow were collected. *Leishmania* nested PCR was used to detect the parasite DNA, followed by sequencing for species identification. **Results:** Among the 62 rodents captured 42 (67.7%) were infected at least in one tissue. Of these, 4 (9.5%) were infected by *L. infantum*, 24 (57.1%) by *L. braziliensis* and 3 (7.2%) had mixed infection by *L. braziliensis* / *L. Infantum.* In one specimen of *M. musculus* was observed infection in the liver (*L. Infantum*) and spleen (*L. braziliensis*), another *M. musculus* presented tail skin and bone marrow infected by *L. infantum* and liver and spleen infected by *L. braziliensis*, one specimen of *R. rattus* showed infection by *L. infantum* in samples from liver and bone marrow infected by *L. braziliensis*. It is important to emphasize that these animals were collected in the peri-domicile area. In 11 (26.2%) specimens it was not possible to identify the *Leishmania* involved in the infection. **Main Conclusions:** These results are worrying as these two rodent species with mixed infections are commonly found in urban areas, in close relationship with houses, especially in poor housing and health conditions. The detection of different species of *Leishmania* among rodents confirms

the simultaneous occurrence of active transmission cycles in the study area. **Financial Support:** CAPES, FAPEMIG, CNPq / FIOCRUZ, the European Community.**E-mail:** ecferreira9@yahoo.com.br

Leish3. **Leishmaniasis visceral human and canine in the city of Parnaíba, Piauí, period 2007-2011**

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Introduction: Visceral leishmaniasis (VL) is a chronic infectious disease, considered reemerging, often lethal if untreated, is caused in the Americas by *Leishmania (Leishmania) infantum chagasi*, the main vector of the *Lutzomyia longipalpis* and dogs as an important source of infection. The LV is currently in the process of increasing urbanization and growth in various regions of Brazil with a high prevalence in the northeast, and the state of Piauí one of the main areas of endemic disease. This study aimed to investigate and analyze the cases of canine and human VL in the city of Parnaíba-PI in the period 2007-2011. **Methodology:** A survey of human cases of VL reported in Parnaíba from January 2007 to December 2011, through the records SINAN provided by the Municipal Epidemiological Surveillance of Health. Were also investigated cases of canine leishmaniasis in different city districts, and the data collected by the Center for Zoonosis Control (CCZ) and Regional Health (FUNASA) of Parnaíba, in the period 2007-2011. **Results:** There were 76 reported cases of VL in humans and 453 confirmed cases of the disease in dogs in the investigated period. The largest number of cases of human VL was recorded in 2007 and 2011 with 20 cases, where there was a record 16 cases of the disease, following the canine LV in 2007 that showed the highest frequency with 187 cases, decreasing in subsequent years and returning to grow in 2011. Additionally, it was found that neighborhoods with the highest number of cases of canine leishmaniasis were those with the highest frequency of sandflies in other research. **Conclusion:** The present study showed high frequency of canine visceral leishmaniasis in the municipality of Parnaíba with considerable number of cases of human disease, being appointed a growth of cases in 2011. The data indicate the need for epidemiological surveillance in the region with continuity and intensification of control measures of LV in the area investigated. **E-mail:** izebaraujo@yahoo.com.br

Leish4. **Molecular detection of *Leishmania* sp. in wild small mammals in the Federal District of Brazil**

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Introduction: Autochthonous human cases of cutaneous leishmaniasis have been reported in the Federal District of Brazil (FD) since the 1990s. In the case of visceral leishmaniasis, autochthonous cases (human and canine) were detected since 2005, mainly in the administrative region of Sobradinho. However, the role of wild small mammals as reservoirs of *Leishmania* is still poorly characterized in FD. Here, we describe the occurrence of *Leishmania* sp. among wild small mammals in the Contagem Biological Reserve (CBR), DF, adjacent to Sobradinho. **Material and Methods:** Rodents and marsupials were captured in baited traps (Young and Sherman) distributed in grids located in cerrado (open shrubland) and gallery forest. Traps operated over four consecutive nights between 4 and 8 November 2011; total sampling effort was 664 trap-nights. All the animals were identified and marked. Ears tip scraps were taken from the captured animals. The detection of *Leishmania* sp. infection was performed through the amplification of 120 bp from the conserved region of *Leishmania* minicircle (kDNA) by PCR. **Results:** In the CBR, we captured 23 individuals of six species: *Rhipidomys macrurus* (6), *Necromys lasiurus* (6), *Nectomys rattus* (3), *Oecomys bicolor* (3), *Cerradomys scotti* (1), and *Gracilinanus agilis* (4). PCR was positive in the skin of 15 (65.2%) individuals captured. **Main Conclusions:** The studied area is located bordering the Sobradinho urban areas, where canine and human cases of visceral

leishmaniasis were detected since 2005. The high proportion of infected rodents and marsupials in the area and the habitat urbanization/fragmentation processes occurring in the region alert to the possibility of interaction between wild and domestic cycles with deleterious results for human and animal population. **E mail:** rgurgel@unb.br

Leish5. **Mapping of leishmaniasis disease research: Analysis of publications output during 1945-2010**

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Introduction: Publications are often used as a measure of success of research work. Leishmaniasis is still one of the world's most neglected diseases; 350 million people are considered at risk of contracting leishmaniasis, and some 2 million new cases occur yearly. The aim of this study was to investigate leishmaniasis research output using PubMed over a period of 66 years (1945 to 2010). **Material and Methods:** The PubMed database was selected as the most suitable for references to leishmaniasis publications. PubMed was accessed online on 10th February 2012 (http://www.ncbi.nlm.nih.gov/pubmed). For retrieving documents, a search was composed with the Medical Subject Headings (MeSH) terms or descriptors "Leishmaniasis" or "Leishmania". The period of study was from 1945 to 2010. **Results:** 20,780 references were retrieved for the whole study period, with 3,380 (16, 3%) publications from 1945 to 1980, 3,567 (17.2%) from 1981 to 1989, 5,566 (26.8%) from 1991 to 2000, and 8,267 from 2001 to 2010 (39.8%). The main language was English (82.8%) followed by French (4.2%), and Portuguese (2.8%). These articles were published in 1,846 scientific journals. Eight journals contained 21.4% of the leishmaniasis journal literature. About half the literature was concentrated in 50 journals, while the remaining half was scattered throughout 1,796 journals. The main journal was *Transactions of the Royal Society of Tropical Medicine and Hygiene* (1,010; 4.9%), followed by *American Journal of Tropical Medicine and Hygiene* (779; 3.3%), *Molecular and Biochemical Parasitology* (599; 2.9%) and *Memórias do Instituto Oswaldo Cruz* (438; 2.1%). The main MeSH was 'animals' (72.2%), 'humans' (52.6%), 'leishmaniasis visceral' (42.7%), 'mice' (19.4%) and leishmania donovani (14.5%). The most common document type was 'journal articles', accounting for about 86.5% of the total (n=17,982). The institutional address of the first author of the publication was present in 8,785 of the 13,989 articles (67.8%). USA was the predominant country (16.8%). The second country was Brazil (14.9%). The third country was India (9.1%). The other main countries were UK (7.1%), France (5.8%), and Spain (5.3%). **Conclusions:** We have found an increase in the number of publications in the field of leishmaniasis. USA and Brazil led scientific production on leishmaniasis research. Efforts should be made to help countries with the highest prevalence of leishmaniasis to come to develop a network of scientific research (collaborative platform) with North American or Western European countries to increasing the research. **E-mail:** diemen@coma.es

Diagnostic and treatment of Leishmaniasis

Leish6. **Canine visceral Leishmaniasis diagnosis: comparison of molecular methods and clinical samples**

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Leishmania (Leishmania) chagasi is responsible for visceral leishmaniasis (VL) in Brazil and the dog is the main domestic reservoir. Disease control is based on the euthanasia of infected animals, therefore is necessary to use a sensitive and specific diagnostic tests that can prevent the disease transmission or unnecessary culling of dogs. The control program of VL uses serological surveys performed by indirect immunofluorescence reaction (RIFI) and ELISA (Enzyme Linked Immunosorbent Assay). Such techniques, however, have showed problems related to specificity and sensitivity, so that even today remains the challenge to obtain an appropriate method. The Polymerase Chain Reaction (PCR) has been recognized as a valuable tool for the identification of *Leishmania*. However, one problem that limits the use of molecular methods is the lack of standardization and few studies comparing the existing methods are available. In this study was compared the sensitivity of four molecular methods used for *Leishmania* detection: kDNA PCR-hybridization, Internal Transcribed Spacer 1 Nested PCR (ITS-1 nPCR), *Leishmania* Nested PCR (Ln-PCR), Semi-nested kDNA PCR (kDNA-sn PCR). Two of the methods (kDNA PCR-hybridization and kDNA snPCR) used primers addressed to kinetoplast minicircles and the other two methods used primers to the coding (LnPCR) and intergenic noncoding regions (ITS-1 nPCR) of the ribosomal rRNA genes. The comparison was performed on different clinical samples: conjunctival swab, blood, skin and bone marrow. The study group was composed of thirty symptomatic dogs, positives in serological and parasitological tests. When comparing PCR methods for each clinical sample, there was a lower performance of the techniques addressed to the mini-circles of kDNA in blood samples (KDNA-sn-PCR and kDNA PCR-hybridization). No statistical difference was found among the methods for skin and bone marrow samples. The KDNA-PCR-hybridization showed the best sensitivity for conjunctival swab. When comparing samples based on the positivity obtained by the sum of all methods, blood showed the worst result (76 /120), statistically lower than the others samples. The bone marrow showed the highest positivity (106 /120), followed by conjunctival swab (100/120) and skin (89 /120). No statistical difference was verified between conjunctival swab and bone marrow. Given that bone marrow samples are unsuitable for routine epidemiological surveys, the conjunctival swab was recommended because it allows high sensitivity, mainly when associated with kDNA PCR hybridization method, and is a noninvasive sampling method. **E-mails:** melo@icb.ufmg.br alineleandra@hotmail.com / vidasnino@yahoo.com.br/ streptos@hotmail.com/antero@cdtn.br

Leish7. **Multiplex real time PCR for simultaneous detection and discrimination of species of *Leishmania***

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Introduction: Leishmaniasis is a serious public health problem in several countries. Part of this problem is never to know which species are circulating in certain regions. This differentiation is needed especially in geographic regions where there are overlapping areas of transmission of cutaneous and visceral forms of the disease and due the disease presents a wide spectrum of symptoms. It can also be used in epidemiological studies, documentation and monitoring the circulating species to help in planning measures to control domestic reservoirs and vectors. In this study we describe a three real-time fluorogenic (TaqMan) multiplex PCR for the detection of several species of *Leishmania* and discrimination between subgenus *Viannia* and the *donovani* complex. **Methods**: The samples were divided into two groups. Group I was composed of 220 samples of blood and tissue from dogs and humans with positive diagnosis for several species of *Leishmania*. Group II was composed of 93 samples of blood, tissues and stools from humans without leishmaniasis but with others parasites like *Plasmodium* spp., *Acanthamoeba spp., Balamuthia mandrillaris, Naegleria fowleri*, *Entamoeba histolytica, E. dispar, and Trypanosoma cruzi.* Molecular markers were designed for the identification of genus *Leishmania spp*. based on 18S ribosome subunit labeled with the fluorophore CY-5. Molecular markers to discriminate subgenus *L. Viannia* and *donovani* complex were designed from sequences of *Leishmania* actin and were labeled with FAM and HEX respectively. The reactions were performed in an ABI 7500 Real Time PCR System (Applied Biosystems). **Results:** Group I**,** 59 (89.4%) of 66 positives isolates from species of the subgenus *Viannia* were positives for CY-5 and 60 (90, 9%) were positives for FAM. From 110 isolates of *Leishmania donovani* complex, 100 (90, 9%) were positives for CY-5 and 104 (94.5%) were positives for HEX. All the samples from the subgenus *Viannia* were negative for HEX (*donovani* complex marker)

and all samples from *donovani* complex were negative for FAM (subgenus *Viannia* Marker). Samples of *L. (L.) amazonensis, L. (L.) mexicana* and *L. (L.) major* were positives only for CY-5. The other species belonging to the subgenus *Leishmania*, such as *L. (L.) tropica* and *L. (L.) aethiopica*, also tested positive for both markers CY-5 and HEX. The group II, 93 negative samples for *Leishmania spp*. showed no Ct in any analysis. **Conclusions:** A multiplex PCR was effective to discriminate between species of the subgenus *Viannia* and the *donovani* complex. The molecular marker Cy5 was able to detect all species of *Leishmania spp.* analyzed and was useful for detecting species with lower circulation. **Email:** colombofabio@uol.com.br

Leish8. **Plasmid pUC18 as control of extraction method of the DNA in multiplex PCR to American Cutaneous Leishmaniasis diagnosis**

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Introduction: The American Cutaneous Leishmaniasis (ACL) is a parasitic disease, clinically heterogeneous, caused by protozoa of genus *Leishmania.* Early diagnosis and implementation of treatment are important to prevent severe damage to the patient and spread of the disease in the community. The limitations of current diagnostic methods and the absence of a "gold standard", provides a necessity to develop an accurate diagnosis. In this context, the molecular methods have been developed, specially based in polymerase chain reaction (PCR) technology. However, to a safe diagnosis, with a minimal margin of error, inclusion of samples quality control becomes necessary. The detection of a DNA added to the biological sample (external control) will demonstrate possible losses during the extraction process. This work aims to develop and standardize a multiplex PCR able to detect small amounts of *L. braziliensis* DNA with an internal control (G3PD gene of mammal), including a new external control system (plasmid pUC18), in the same reaction. **Materials and Methods:** Two sets of primers (P1 and P2) were designed to detect the pUC18. A preliminary test was made to evaluate the sets in conditions of duplex PCR (for detection of *L. braziliensis* and G3PD gene) already developed by the team for diagnosis of American cutaneous leishmaniasis (mACL), according to Gonçalves (2011). From this test, one system was chosen to be included in the multiplex PCR. For determination of detection limit, a dilution curve (from 0, 5 fg/µL to 50ng/ µL, factor 1:10) of pUC18 plasmid was built, using the primers concentration of 20 pmol/µL. After determination of the limit detection, a dilution curve of primers was made (from 1 pmol/ µL to 20 pmol/ µL) to determine the lowest concentration being included in duplex reaction. **Results:** The P1 set showed the best performance, and was chosen for optimization. The limit detection obtained was 10 pg of pUC 18 in 25µL of the reaction, the lowest concentration of primers able to detect 10 pg of pUC18 was 10 pmol / µL. **Conclusion:** The first results obtained with P1 system, showed compatibility with the conditions already standardized by Gonçalves (2011) in a duplex PCR in which the pUC18 will be included as extraction control. The first step to development of triplex PCR was given, for the construction of a diagnosis tool with a minimum margin of error. The validation of tests that allow the evaluation of the sample quality will ensure the results of the diagnosis method, contributing to early treatment of patients and disease control actions. **Financial Support:** CNPq, FACEPE and FIOCRUZ .**Email:** mp@cpqam.fiocruz.br

Leish9. **Lupane-Liposomal system as alternative chemotherapy against cutaneous leishmaniasis: macrophage as target cell**

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Introduction: Leishmania amazonensis causes human diseases that range from self-healing to diffusion cutaneous lesions. Macrophages are important cells involved in the immune response against pathogens due to their phagocytic capacity. The chemotherapy of leishmaniasis requires long-term treatment and

has been based on the use of pentavalent antimonials. Drug delivery systems are promising pharmaceutical formulations used to improve the therapeutic outcome of drugs. In this context, liposomes have been used as antileishmanial drug carries and have adjuvant activity in vaccines against several protozoan or bacterial organisms, representing an important option to the development of new therapeutics for the disease. In this study, we developed a liposomal formulation of a lupane 3ß, 6ß, 16ß-trihydroxylup-20(29)-ene]. This triterpene, isolated from fruits of *Combretum leprosum*, has pharmacological properties as antinociceptive, anti- inflammatory, antiulcerogenic and anti-leishmanial activities. The aim of the present study was to evaluate in vitro/ex vivo the efficacy of liposomal- lupane in Leishmania amazonensis-infected macrophages**. Material and Methods:** Liposomes were prepared with DPPC, DPPS and cholesterol at 5:1:4 weight ratio. The lupane (2 mg/ml) was added to the lipid mixture, solubilized in chloroform and dried under nitrogen flow. The lipid vesicles were formed homogeneously by the extrusion method. The activity of lipossonal-lupane was conducted in vitro with mouse peritoneal infected macrophages. Furthermore, BALB/c mice were infected in the right hind footpad with 105 stationary growth phases of *L. amazonensis* promastigotes. After six weeks, the animals were treated with liposomal- lupine for 15 days by intraperitoneal injection. The evolution of disease was monitored weekly by measuring footpad thickness with a caliper. Three days after the treatment, mouse peritoneal macrophages were collected, plated and the production of the cytokines IL-6, IL-10 and IL-12 was evaluated in supernatants of the cultures after 24h. **Results:** *In vitro* phagocytic index of infected macrophages showed that lupane-liposomes significantly decrease the number of amastigotes inside the cells compared to control liposomes. The lesion size of mice treated with liposomal-lupne showed a reduction of 33% compared with the untreated infected group. It was observed a significant reduction in the production of IL-10 by liposomal- lupane treated macrophages when compared with untreated negative control or uncharged liposomes. Also, there was an increase in the production of IL-6 and IL-12 elicited by liposomal- lupane treated macrophages. Main Conclusions: The results indicate that the liposomal system containing lupane achieved in this study is a promising tool to confer anti-leishmanial activity to infected macrophages. **E-mail:** izaltina@gmail.com

Leish10. **Use of a combination therapy between miltefosine and trifluralin to treat murine cutaneous leishmaniasis by *Leishmania amazonensis***

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Leishmaniasis belongs to the group of neglected tropical disease caused by protozoan parasites of the *Leishmania* genus, affecting about 12 million people around the world. At least four clinical manifestations can be described: Visceral Leishmaniasis (VL), Mucocutaneous Leishmaniasis (MCL), Cutaneous Leishmaniasis (CL) e Diffuse Cutaneous Leishmaniasis (DCL). In Brazil, *Leishmania amazonensis* is responsible for cutaneous and diffuse cutaneous forms of the disease, which is highly mutilating and incurable using currently available drugs. The chemotherapy employed today is based on pentavalent antimonials, amphotericin B and pentamidine. More recently, miltefosine has been used as oral treatment for visceral and cutaneous leishmaniasis in several countries. However, miltefosine remains still controversy to the treatment of leishmaniasis caused by some species of the New World. Trifluralin is a dinitroaniline used as plant herbicide that binds specifically to plant's tubulin; however it also presents activity against several protozoan parasites. Thus, the aim of this work was evaluate the combination therapy between miltefosine and trifluralin against murine models of cutaneous leishmaniasis caused by *L. amazonensis*. BALB/c mice were inoculated with infective promastigotes of *L. amazonensis* through subcutaneous injections at the base of the tail. After the development of the lesions, mice were divided into eleven groups: control and vehicle groups, Glucantime® group (50 mg/kg/day), trifluralin groups (30; 50 mg/kg/day), miltefosine-treated groups (20; 30; 40; 50 mg/kg/day), and two combination doses of miltefosine/trifluralin [(20/30 mg/kg/day); (20/50 mg/kg/day). Miltefosine and trifluralin were administrated daily by oral gavage, while Glucantime by intraperitoneal route, during 21 days. To confirm the efficacy of the treatment, different approaches were employed: evaluation of lesion sizes, skin smears, histopathology and transmission electron microscopy. The results confirmed the dose-dependent

response induced by miltefosine alone, which was not observed after treatment with trifluralin alone. On the other hand, combination treatment between miltefosine and trifluralin revealed a significant reduction of lesion sizes similar to those observed after treatment with high doses of miltefosine, indicating that this combination could be effective to reduce the doses of miltefosine's dose. Histophatology of the lesions revealed an important modulation of the immune response in the treated-groups with the miltefosine alone and in the combination doses. At the site of infections, we observed a reduction of foam macrophages and resident inflammatory cells, with a concomitant recruitment of cells involved in the humoral immune response. Thus, this study contributes for the search of new treatments for the leishmaniasis, suggesting that a possible combination of miltefosine with other drugs could be more interesting than the monotherapy. **E-mail:** address: joseanegodinho@biof.ufrj.br

Leish11. **Leishmaniasis East Africa Platform: an integrated endemic country approach to implementing new treatments and building capacity for Leishmaniasis**

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Introduction: Current treatments for Visceral Leishmaniasis (VL) are toxic, expensive and difficult to administer. In addition, increasing parasite resistance calls for the development of new treatments that also take into account differences between patient populations. **Materials and Methods:** The Leishmaniasis East Africa Platform (LEAP) was launched in Khartoum, Sudan in 2003, bringing together over 50 members from 20 institutions (local and international research institutions, local Ministries of Health, NGOs) in Ethiopia, Sudan, Kenya and Uganda and in developed countries. LEAP aims to evaluate new treatments and diagnostics and to facilitate their registration and implementation in East Africa, by developing clinical trial capacity and serving as a base for continuous training and standardization of procedures and practices. **Results:** LEAP conducted a multi-country trial involving over 1,000 patients that led to the development of sodium stibogluconate with paromomycin (SSG&PM) as a new, improved combination treatment for VL in East Africa, where it is now recommended by the World Health Organization (WHO) as 1st-line treatment. Following data generated by LEAP, the WHO Expert Committee recommended treatment with 30mg AmBisome as second line in East Africa, versus 10mg recommended for South Asia, reflecting the differences in the response of populations to treatments. Other combinations of AmBisome and SSG or AmBisome and Miltefosine are currently being tested as part of a phase II clinical trial, and depending on the outcome, one of these combinations will be evaluated in a phase III clinical trial. In addition to providing a new VL treatment, LEAP has enabled sustainable capacity strengthening in Ethiopia, Kenya, Sudan and Uganda. Examples include Good Clinical Practice / Good Clinical Laboratory Practice training of clinical research staff and laboratory technicians, the establishment of a Data & Safety Monitoring Board, as well as the creation of a data management centre. **Main Conclusions:** The South-South and international collaborations within the LEAP platform have led to a new, improved combination treatment for VL in East Africa and provided important data to policy-makers. Local teams have been trained to internationally recognized standards, a model that is being applied to other similar platforms, notably for the treatment of sleeping sickness and Chagas disease. **E-mail:** mkwasunna@yahoo.com

Characterization of Leishmania

Leish12. **Insights on Taxonomy, Phylogeny and Population Genetics of Leishmania (*Viannia*) Parasites Based on Multilocus Sequence Analysis**

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Multilocus enzyme electrophoresis is still considered the gold standard for *Leishmania* identification, but DNA based methods have been proven useful to study *Leishmania* genetic diversity. PCR-RFLP of *hsp70* genes were used successfully for the identification of *Leishmania* parasites, although too conserved for intraspecific diversity studies. Highly polymorphic markers, such as microsatellites, perform poorly at taxonomic levels higher than species, while most other genotyping methods rely on multicopy genes that are more difficult to analyze. A standardized, sensitive, and reproducible typing method, such as multi-locus sequence typing (MLST), may form the basis for a robust classification of *Leishmania* species. Here we propose four genomic targets to be used in multilocus sequence typing of the sub-genus *Leishmania (Viannia)*, also providing new insights on its taxonomy and phylogeny as well as a tool for population genetics. Ninety six strains were chosen to be representative of the zymodeme and geographical diversity of the sub-genus *Leishmania (Viannia)*: *L. (V) braziliensis*, *L. (V) guyanensis*, *L. (V) naiffi*, *L. (V) lainsoni*, *L. (V) shawi*, *L. (V) utingensis* and *L. (V) lindenbergi*. Fragments of four genes already employed in MLEE studies, G6PDH, 6PGD, MPI and ICD were amplified by PCR and directly sequenced. The alignments were edited and used as an input in appropriates software for the determination of: SNP specificity; haplotype diversity (HD), diploid sequence types (DST), concatenated NeighborNet construction and e-BURST analysis. Putative species specific SNPs were detected in all species except *L. braziliensis*. Overall HD was highest for ICD (0.85). However, upon analysis by species, MPI was the least polymorphic marker for *L. guyanensis* ($P<0.001$) and G6P was the least polymorphic for *L. lainsoni* ($P<0.001$). Among the *L. shawi* studied isolates, ICD was the only polymorphic marker. Seventy five DSTs were assigned. Four DSTs were identified in several isolates of *Leishmania braziliensis*. The NeighborNet obtained were in agreement with MLEE for species groups. *Leishmania utingensis* and *L. lindenbergi* branched off the closest to *L. guyanensis* and *L. naiffi* clusters, respectively. Geographical clustering was not observed. The BURST algorithm generated six clonal complexes (CC) corresponding to *Leishmania* species, with eighteen singleton DSTs. The four loci had different degrees of diversity, and are thus suitable to be used in combination for intra and inter-specific inferences. SNP markers can be used for species identification, but with care. We detected a high number of unique and some prevalent DSTs, widely distributed and presenting temporal stability. MLST approach allows not only the detection of different genotypes - as other markers do – but also the level of separation between strains through the number of polymorphic sites. The level of phylogenetic precision obtained is needed for many molecular epidemiologic studies, since several *Leishmania* properties are probably strain-specific. **E-mail**: boitemc@ioc.fiocruz.br

Leish13. **Intense pro-inflammatory response in treated VL/HIV-1 co-infected patients can be due to microbial translocation and *Leishmania* infection**.

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Introduction: We have shown that LPS were implicated in T-cell activation and pro-inflammatory cytokines in visceral leishmaniasis (VL) patients. In addition, the heightened cellular activation observed in anti-leishmanial treated VL/HIV co-infected patients was not due to viral load, but probably to *Leishmania* infection. Thus, we investigated whether parasite persistence or LPS from microbial translocation were responsible for the maintenance of cellular activation in remission VL/HIV co-infected patients. **Methods**: 10 healthy volunteers, 17 AVL/HIV-1 and 16 HIV-1/AIDS patients were recruited. $CD4^+$T counts and $CD8^+$T cells expressing CD38 were analyzed by flow cytometry. Blood parasite load was quantified by real-time PCR. LPS and sCD14 levels were measured by enzymatic assays. Plasmatic pro-inflammatory cytokines (IL-1β/IL-6/IL-8/IL-17/IFN-γ/TNF-α) were assessed by multiplex analysis. The macrophage migration inhibition factor (MIF) and intestinal fatty acid binding protein (IFABP) were quantified by ELISA. Multivariate linear regression (MLR) was used to determine influence of intervenient factors over T-cell activation. **Results**: A significant reduction of number of promastigotes/mL was

detected after anti-*Leishmania* therapy. Despite this, elevated levels of $CD8^+/CD38^+$ were seen independently of leishmaniasis clinical phase when compared to HIV/AIDS cases (p<0.05). We observed that co-infected and also HIV-1 patients presented higher LPS and IFABP levels than healthy donors (p<0.001). Pro-inflammatory cytokines levels were significantly augmented in active and remission co-infected cases. LPS levels were positively correlated with IFABP (r=0.45; p<0.05). MLR analysis showed that LPS levels were positively correlated to CD38 on $CD8^+$T cells (p<0.001), independently of $CD4^+$T counts, HIV viremia, sCD14, MIF and IFAB levels. The variable *Leishmania* infection was also associated with activation levels (p<0.001). Finally, LPS and *Leishmania* infection were positively correlated with IL-6 (p<0.05) and IL-8 (p<0.01) levels. **Conclusions**: VL/HIV patients had an exacerbated pro-inflammatory response. High levels of T-cell activation were maintained even in remission phase despite reduction of parasite load. Also, LPS probably due to microbial translocation can be involved in the cytokine storm, which in turn may increase T-cell activation. These factors can impair the effector function enabling further parasite reactivations, worsening the prognosis of VL/HIV patients. **Support:** IOC, CNPq, PN-DST/AIDS. JRSO is FAPERJ 10 sponsorship.**E-Mail:** alda@ioc.fiocruz.br

Leish14. **Regulatory T cells (T reg) and its relationship with the splenic changes and clinical manifestations in dogs naturally infected with *Leishmania (Leishmania) chagasi.***

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Introduction: Visceral leishmaniasis (VL) is a zoonosis caused by *Leishmania (Leishmania) chagasi*. It is transmitted by the bite of the sandfly *Lutzomyia longipalpis*. The dog is the main reservoir of the disease in urban areas. The spleen is one of the hematopoietic and immunocompetent organs involved in the VL, as a blood filter. The immunosuppressive role of regulatory T cells may adversely affect the fight against VL, where the reports about the frequency and function of these cells are not conclusive. This study evaluated the dogs' spleen in the participation of T reg cells in modulating of the response against the Leishmania infection and its presence correlated with the intensity of clinical manifestations and the organ's changes. **Material and Methods:** We used 39 animals, males and females of unknown age. The diagnosis of leishmaniasis was realized through the serology's exams (ELISA), parasitological (the marrow's puncture, and the *imprint* of spleen). The animals were divided in four groups: Infected by *Leishmania (Leishmania) chagasi* and asymptomatic, oligosymptomatic, polysymptomatic and control group (uninfected). We collected blood and fragments of spleen. We prepared the organs' *imprints* in slides for cytological analysis, and fragments were fixed in 10% formalin for the histological and morphometric analysis, and RPMI 1640 for performing of the flow cytometry. **Results:** The most frequent clinical findings were skin lesions (76.9%) and lymphadenopathy (65.38%). The Histopathology analysis of the spleen revealed changes as: neutrophilic inflammatory infiltrate in the capsule, granuloma, hyperplasia of follicles and depletion of T cells. The hyperplasia of follicles was the most common group of asymptomatic animals compared to oligosymptomatic and polysymptomatic (p = 0.000117). Hypercellularity was higher in asymptomatics compared to other groups (P = 0.0000145). In Polysymptomatic, there was a greater depletion of cells of the periarteriolar sheath with a degree of intensity ranging from normal to moderately severe (p = 0.000191). Hyperplasia of T cells was higher in asymptomatic (P = 0.00000103). Granuloma of medium intensity was observed in higher quantities in the group of asymptomatic animals (p = 0.000580). In this study, T CD4+ cells were marked using anti-canine CD4+ antibody (clone YKIX302.9) and Foxp3, anti-mouse antibody (clone FJK-16s) and analyzed by flow cytometry. Quantification of $TCD4^+$ cells and $TCD4^+Foxp3^+$ showed no significant difference between groups of dogs examined, however, there was a tendency numerically higher of $TCD4^+$cells in control animals compared to infected animals and T cells $TCD4^+Foxp3$ in polysymptomatic animals. **Main Conclusions:** The clinical manifestations of canine visceral leishmaniasis progresses with the intensity of the changes in the spleen, as well as the amount of T reg cells tend to be higher in animals with the largest number of clinical manifestations. In oligosymptomatic animals there are a low number of T reg cells. **E-mail:** simonemousinho@yahoo.com.br

Leish15. **Role of IGF-I expression in bone marrow in the pathogenesis of pancytopenia in dogs naturally infected by *Leishmania (Leishmania) chagasi***

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Introduction: During active visceral leishmaniasis (VL) pancytopenia is a frequent pathological alteration that can contribute to the lethality. It has been attributed to hypersplenism, but the mechanisms are not completely known. Some growth factors (Colony-Stimulating Factors) and cytokines such as TNF-α are implied in the alterations in hematopoiesis in human VL, and the insulin-like growth factor (IGF)-I acts as promoter of growth of hematopoietic cells *in vitro*. Therefore, in this study, we evaluated the pathogenesis of pancytopenia and the expression of mRNA IGF-I in the bone marrow in dogs naturally infected by *Leishmania (L.) chagasi*. **Material and Methods:** We evaluated five naturally infected dogs from endemic area in the Northeast, Brazil and five control dogs from non-endemic area in the Southeast, Brazil. Dogs infected with *Ehrlichia canis* were discarded by specific PCR. We collected whole blood to hematological exam and sternal bone marrow to analyze IGF-I mRNA expression by Real Time PCR and myelogram. **Results:** The hematological alterations in VL dogs were normocytic normochromic anemia, leukopenia and thrombocytopenia. In the myelogram we observed dysgranulopoiesis (100%), dyseritropoiesis (100%) and dysmegakaryocitosis (40%) and more frequently an erythroid hypoplasia in infected dogs. In 60% of infected dogs the myeloid: erythroid ratio was lower than normal that suggested either the erythroid hypoplasia or myeloid hyperplasia. Furthermore, we saw an increase of precursor erythroid cells and a decrease in mature erythroid cells in infected dogs compared with control dogs. The precursor erythroid: mature erythroid ratio was greater in the infected dogs (0.118 ± 0.037) than control dogs (0.032 ± 0.009). In the differential count we saw a significant decrease in plasma cell in infected dogs compared with controls dogs. mRNA IGF-I expression in bone marrow was lower in infected dogs compared with control dogs. **Conclusions:** In VL dogs anemia was the major hematological alteration, seemingly due to the maturation arrest, and these hematological changes may be related at least in part to the reduced IGF-I expression in the bone marrow. **Supported by:** FAPESP, CAPES, CNPq, LIM-38 (HC-FMUSP) **E-mail:** flavianeealves@usp.br

Leish16. **Characterization of a Presenilin-like in *Leishmania chagasi***

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Introduction: Leishmaniasis, caused by parasites from *Leishmania* genus affects about 12 million individuals worldwide. In some regions approximately 80% of cases exhibit resistance to the drugs used. Currently there are not species-specific diagnostic methods or more efficient drugs available. By previous studies from our laboratory, a membrane aspartic protease called Leishpsina-I was isolated which shares similar characteristics to presenilin from human gamma secretase complex. This work aims to continue the characterization studies of Leishpsina-I from *Leishmania chagasi* parasite. Our specific objectives are to identify B linear epitopes and perform biochemical characterization of this protein and evaluate the inhibitory capacity of peptidomimetics anti-presenilin, trying to validate the enzyme as a chemotherapeutic or diagnostic target. **Material and Methods:** Through parallel synthesis on membrane technique, we identified all major antigenic determinants of the protein. Some were selected, synthesized in solution and used to immunize rabbits for obtaining monospecific antibodies. Antibody's specificity was determined by western blotting and celluspot. **Results:** Only one band compatible with the molecular weight of the aspartyl protease has been identified by western blotting and specific spots were revealed by celluspot. Cellular localization of Leishpsina-I is being performed by confocal and electron microscopy. Even results obtained by immunofluorescence evidenced punctiform staining in a large part of the parasite's body.

Main conclusions: This is the first study confirming the presence of Leishpsina-I in *L. chagasi*. This information may contribute to the knowledge of a new mechanism of membrane proteins processing from the parasite and also of its infectivity and maturation processes, in addition to providing subsidies for the discovery of novel therapeutic targets or general diagnostic. **Supported by:** CNPq, FAPERJ, CAPES **E-mail:** srpinto@yahoo.com

Leish17. **Characterization of an atypical aspartyl protease presenilin-simile from *Leishmania amazonensis***

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Introduction: Previous work in our laboratory led to the characterization of several serine proteinases from *Leishmania* sp promastigote cells. In this work we describe the molecular, biochemical, enzymatic and structural characterization of an atypical aspartyl protease (AP) from *Leishmania amazonensis*, a member of the presenilin (PS)-simile proteins, enzyme that cleaves type I transmembrane (TM) proteins. **Material and Methods:** The nucleotide sequence of PS-simile of *L. amazonensis* was provided by the genome sequence consortium (IOC/FIOCRUZ and U. Glasgow). The nucleotide sequence of PS-simile was translated to the primary sequence of protein and this sequence was aligned to *L. major* and *L. infantum/chagasi* PS sequence from database, using BLAST. TM topology was investigated by bioinformatics approaches and hydropathy plots. This protein was purified from promastigote cells through chromatographic steps and the enzymatic parameters were evaluated. **Results:** The PS protein of *L. amazonensis* presented high degree of sequence identity with PS from *L. major* (93.75%) and *L. chagasi* (94.32%). Its TM topology studies demonstrated that *L. amazonensis* PS contains eight hydrophobic regions that can function as TM domains. Using the selective peptide substrate for aspartyl-proteases [Phe-Ala-Ala-Ala-Phe-(4NO_2)-Phe-Val-Leu-O4MP], it was possible to demonstrate the presence of peptidase activity in the PS of *L. amazonensis* promastigotes. The substrate was hydrolyzed by the PS at pH 3.5 with an apparent *K*m of 3, 38 µM. Among proteinase inhibitors AP specific tested, the 4-β-phorbol 12 myristate 13 acetate (4β-PMA) showed the highest Km, with an IC_{50} of 5.71 µM, and the Statin showed the lowest Km, with the IC_{50} of 40.27 µM. **Conclusions:** In this work we have demonstrated that *L. amazonensis* has a PS protein with aspartyl peptidase activity and eight TM domains, indicating it is an integral membrane protein. We have also showed that the PS sequence is conserved among different species of *Leishmania*. Characterization of PS protein in *L. amazonensis* is not only of particular interest for drug development but also because it may facilitate a better understanding of the host-parasite interaction, since it is involved in the cell surface proteins maturation/turnover. **E-mail:** degrossoli@ioc.fiocruz.br

TOXOPLASMOSIS

Toxo1. **Congenital toxoplasmosis in Brazil: modeling the cost of maternal screening**

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Introduction: *Toxoplasma gondii* is a protozoal parasite infecting a high proportion of the world's population, although infection is generally asymptomatic in immunocompetent people. Congenital infection, however, can result in fetal death or mild to profound visual, cognitive, and hearing impairment. Symptoms can be present at birth or develop in early childhood or adolescence. Some high-prevalence

European countries have universal maternal screening/ treatment programs. France almost completely eliminated serious injury from congenital infection. A decision-analytic model applying the French protocol to the low-prevalence US population found cost saving of $1 billion and prevention of avoidable injury in thousands of children every year. **Material and methods:** Using TreeAge Pro Suite software, we constructed a decision-analytic model to estimate costs of untreated toxoplasmosis and costs of screening, treatment, and follow-up for 3 high-prevalence Brazilian states. The model includes probabilities of maternal and fetal infection, fetal loss due to congenital toxoplasmosis (CT), post-natal infection, distribution of visual, hearing, and central nervous system injury, treatment efficacy, and non-probabilistic variables, such as costs of screening tests and treatment. **Results:** Brazil has very high prevalence of toxoplasmosis, from 30% to 80% in different states, with different ecologies and different quality of water and sanitary infrastructure. High adult prevalence is associated with high incidence during pregnancy due to acquisition in adolescence and young adulthood. High incidence of CT is compounded by a more virulent strain than found in Europe. The Brazilian strain affects 1 in 500 births and also can produce blindness when acquired post-natally in immunocompetent persons. Clinical experience in Brazil indicates that the local strain, if untreated, produces more profound injuries than the European strain, but that prenatal treatment is equally effective in preventing or mitigating injury. High levels of exposure, including from the water supply, make pre-natal and post-natal incidence a serious public health problem. In this high-incidence population, maternal screening is found to be cost-saving. Universal screening also has spillover benefits in community education, reducing post-natal infection and visual injury. **Main conclusions:** Very high prevalence and serious visual and neurologic injuries make prenatal intervention a high priority for public health in Brazil. Maternal screening for CT could prevent extensive injury and avoidable loss of capacity at considerable savings in societal costs in Brazil. The Brazilian case is important because local strains are highly prevalent and thought to be especially virulent. **E-mail:** stillwaggon@gettysburg.edu

Toxo2. **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

Toxo3. **ROP16 genetic analysis in Colombian patients with and without ocular toxoplasmosis**

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Introduction: *Toxoplasma gondii* is an obligate intracellular protozoan parasite with very tight and intimate host-parasite interactions. This parasite presents an apical complex which include rhoptries, a secretion organelle that contains a diverse number of injected protein kinases called ROPs that play an essential role in parasite virulence. It has been previously demonstrated that the protein codified by the ROP16 gene displays a paramount role in the cell invasion process. This study was focused on the evaluation of the Single Nucleotide Polymorphisms (SNPs) in the ROP16 gene, as a plausible genetic marker in Colombian patients with and without ocular toxoplasmosis and different clonal type strains. **Material and Methods:** For this study, the clinical samples were divided in two main groups; patients with ocular symptoms associated with a *Toxoplasma* infection (clinical samples) and a second group of patients with asymptomatic toxoplasmosis (soldiers in jungle or urban regions). A total of 197 samples were evaluated, 64 clinical and 123 from soldiers that were previously diagnosed as IgG anti-*T.gondii* positive and the parasitic infection was confirmed through a Real-Time PCR of the RE gene. Additionally, 10 meat isolates and 18 reference strains from the three clonal types were evaluated. A nested PCR was standardized with specific primers for both rounds. The DNASP v.5 software was used to determine SNPs characteristics. Additionally, the Network 4.6 and MEGA 5 software were applied to confirm the genetic relationships. **Results:** A total of 64 sequences were obtained for the ROP16 gene that included 18 sequences reported in Genebank from different clonal types, 12 from ocular toxoplasmosis patients, 2 from urban soldiers, 12 from soldiers operating in jungle and 7 from meat isolates. A total of 26 polymorphic sites were identified, including 27 mutations and 11 parsimony informative sites. SNPs analysis through Median-joining algorithm showed an haplotype network which discriminated the samples in two main haplotypes, the first one compose by sequences from patients with ocular toxoplasmosis, urban soldiers and type I and III strains. A second haplotype grouped sequences from meat, jungle soldiers and atypical strains. Similar results were obtained by a Neighbor-joining tree where two main taxa were clearly identified. **Main Conclusions:** The SNPs analysis showed a remarkable difference that discriminate ROP16 sequences from patients with ocular toxoplasmosis and atypical strains. These results indicate a particular molecular epidemiology model for the ROP16 gene in different samples from Colombia. **E-mail:** mc.alvarez68@uniandes.edu.co

Toxo4. **Synergism among endosomal Toll-Like Receptors imparts host resistance to *Toxoplasma gondii* infection**

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One third of the human population in the world is chronically infected with *Toxoplasma gondii.* While the majority of infected individuals are asymptomatic, toxoplasmosis is a major cause of congenital disease, abortion, and a life threatening opportunistic disease in immunocompromised individuals. Early activation of the innate immune system and cytokine production (i.e. IL-12 and IFN-y) by myeloid cells is required for establishment of protective immunity to *T. gondii* infection. In mice, a mutation in the *UNC93B1* gene abolishes signaling via the intracellular innate immune receptors, namely Toll-like receptors (TLR) 3, 7 and 9, thus, named triple-deficiency (3d) mice. Our group has demonstrated that 3d mice are highly susceptible to infection with *T. gondii*, suggesting the possibility that the combined deficiency of intracellular TLRs are critical for host resistance to *T. gondii* infection. When infected with *T. gondii,* TLR3/7/9 knockout mice have a normal immune response and are only partially susceptible to infection.

TLR11 knockout mice have a defect in IL-12 production but a minor defect in IFN-y, which make these mice resistant to infection with *T. gondii.* Evaluating sub cellular localization of TLR11 and TLR12, we identified those as an Endoplasmic Reticulum proteins, and like TLR3, 7 and 9, the activity is mediated by UNC93B1. By FRET we identified that TLR11 and TLR12 form heterodimer upon stimulation, and after infection the quadruple TLR3/7/9/11 deficient mice is highly susceptible. When treated with recombinant IL-12, 3d and quadruple deficient mice were rescued from death. We demonstrated in our work the TLRs involved in mice resistance to *T. gondii* infection and we also identified a new TLR heterodimer, formed by TLR11 and TLR12. **E-mail:** warrisonbio@yahoo.com.br

Toxo5. **Toxoplasma gondii genotypes and parasite loads in 84 congenital infections**

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Introduction: In congenital toxoplasmosis, the disease severity depends on many factors, the main one being the gestational age at the time of transmission, but also the parasite genotype, the parasite load assessed in amniotic fluid samples or fetal blood, and genetic characteristics of patients. *Toxoplasma gondii* has been described as a low genetic diversity parasite with a clonal population structure. Three *T. gondii* genotypes (I, II, III) were described according to the severity of disease, and congenital infections were associated with the less pathogenic genotype II in Europe and the USA. In South America, some reports found a higher percentage of atypical and more pathogenic parasites, especially among HIV patients with toxoplasmic encephalitis, and in ocular toxoplasmosis. A few congenital infections were reported in South America with predominance of genotype III in Colombia, and atypical genotypes in some Brazilian cases. With respect to the parasite load, it is considered an independent risk factor associated with fetal prognosis. **Aim:** To determine whether the parasite genotype is associated with the parasite burden in amniotic fluid samples of pregnant women who transmitted infection to their offspring. **Patients, Material and Methods:** Eighty-four DNA samples from amniotic fluid samples of confirmed congenital toxoplasmosis cases and negative controls were subjected to a quantitative PCR with SYBR Green and B1 gene primers. Parasite genotyping was carried out by a multi-loci nested-PCR-RFLP of four markers: 3'-SAG2, 5'-SAG2, SAG3, GRA6 genes. **Results:** Among the 84 samples, 5 were type I (5.8%); 35 were type II (41.9%); 41 were type III (48.8%) and 3 samples were RFLP-inconclusive (3.5%). The parasite load presented a high dispersion in all groups, and only 7 cases had loads above 10^4 parasites, even though all patients received treatment. These 7 cases belonged to genotypes II and III, and none to the genotype I (the most pathogenic). The distribution of genotypes with respect to the parasite loads was: type I - 5 cases - (median 1,048 parasites/mL; minimum 330; maximum 2,734); type II - 35 cases - (median 1,752; minimum 302; maximum 1,523,648); type III - 41 cases - (median 2,066; minimum 222; maximum 82,352); RFLP-inconclusive - 3 cases - (median 1,768; minimum 380; maximum 7,368). The Wilcoxon test showed no difference between the median values of type II and III parasite loads ($p< 0.0001$). **Conclusion:** The results presented herewith add some important information on *T. gondii* genotypes in Brazilian congenital infections. In our casuistic, most amniotic fluid samples contained type II or type III parasites. To our knowledge, this is the first report of type II parasites in Brazilian congenital infections. Whether or not they are clonal type II or type II-derived parasites has still to be determined by DNA sequencing. In addition, it was shown that parasite loads are highly dispersed irrespective of the parasite genotype. **E-mail:** thelma.okay@usp.br

Toxo6. **Toxoplasmosis: knowledge and risk factors of infection for pregnant women assisted in the University Hospital of Rio Grande – RS**

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Introduction: Toxoplasmosis is an anthropozoonotic disease caused by the protozoan *Toxoplasma gondii*. Humans are infected by eating oocysts released in the feces of young cats which may be present in food or water, also by eating raw or undercooked meat containing tissue cysts of the parasite. Another important form of transmission is transplacentally from mother to fetus. In this case, tachzoites cross the transplacental barrier, reach the fetus, causing mental complications and it can affect other organs such inner ears, most commonly the eyes and may also cause fetal death. This study aimed to evaluate risk factors for *Toxoplasma gondii* infection in pregnant women treated at University Hospital (HU) of the Federal University of Rio Grande (FURG), in Rio Grande, Rio Grande do Sul. **Material and Methods:** The study was cross-sectional with convenience sample of 80 patients treated at HU/FURG in the period from April to July 2011. This study was approved by the Ethics Committee in Research in Health Area of the University. Was applied a questionnaire about eating habits and familiarity with cats, as well as evaluating the knowledge of pregnant women about toxoplasmosis. **Results:** Risk factors associated with infection by *T. gondii* in pregnant women treated in the HU/FURG (n=80)

Risk factors for infection	Pregnant women (n)	%
Own cat(s)	44	55.0
Use of the sandbox for cat feces	09	11.3
Residence where cat defecate outside the home	35	43.8
Pregnant women that clean the sandbox	06	7.5
Ingestion of raw or undercooked meat	25	31.3
Ingestion of sausages	75	93.8
Information about the risks of infection	35	43,8

This study showed that pregnant women are exposed to risk factors for the high ingestion of sausages and raw or undercooked meat. Furthermore showed that pregnant women are responsible for cleaning the feces of cats characterizing exposure factor for infection. In most homes, the cats have access to the street to defecate, representing an important factor in relation a possible environmental contamination. **Main Conclusions:** Toxoplasmosis during pregnancy may be responsible for miscarriages and cause serious neurological problems to the fetus, however, the study showed that pregnant women remain vulnerable to risk factors. Although, the prevention of toxoplasmosis is part of prenatal, 56.2% of pregnant women do not received any information about this parasitic disease during the pregnancy. The prevention of congenital infection depends on an efficacy serological screening of pregnant women for antibodies to *T. gondii* and provides guidance to these women about the risk factors and prophylatic measures during the pregnancy. **E-mail:** paulavet10@hotmail.com

GIARDIASIS

Giard1. **Prevalence and Genotyping of Giardia lamblia in Dogs in City of Rio de Janeiro, RJ**

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Background: Giardiasis is a disease caused by intestinal protozoan Giardia, can infect a broad host range. Among the different species of Giardia, Giardia lamblia is known to infect mammals, including humans and domestic animals. This disease is widespread, but their prevalence rates are underestimated, due to the occurrence of asymptomatic cases and misdiagnosis. The genotypes of G. lamblia are rated A to G, in accordance with host. Genotype A and B are found in humans host and other mammals, while the other genotypes are species specific, like C and D that are specific for dogs. Therefore, giardiasis is a major risk to public health due the antropozoonotic potential of this G. lamblia. Thus, the objective this work is determine the prevalence of G. lamblia in fecal samples of the canine population of Rio de Janeiro by different diagnostic methods and determine the genotypes of G. lamblia circulating in this population. **Methods:** We collected 60 fecal samples of dogs from different districts of

Rio de Janeiro, RJ. These samples were subjected to three diagnostic techniques: parasitological diagnosis, according to the method RITCHIE; immunodiagnosis, performed with the SNAP*Giardia, according to the manufacturer's instructions; molecular diagnostics according to PCR. In molecular diagnosis, DNAs were extracted from the samples using the QIAamp kit according to manufacturer's instructions. The obtained DNA fragment was a gene encoding the 753pb β-Giardina amplified using the primer G7 and G759. Since the amplification of the nested-PCR was performed with the primers βGiaF and βGiaR, producing a fragment of 511pb. The products found positive in β-nested-PCR were purified and sequenced using the BigDye Kit according to manufacturer's instructions. The chromatograms were analyzed and compared with the nucleotide sequences available in GenBank. **Results:** Of the 60 samples studied, 10% (6/60) samples were positive for G. lamblia, according to the diagnosis parasitological and immunodiagnosis and 20% (12/60) samples, the molecular technique. The result of the three techniques converged in four samples. Two samples were positive only in the immunodiagnosis and two others were the result of convergent parasitological and molecular diagnosis. The 12 PCR positive samples were sequenced and all were grouped in genotype A. **Conclusion:** The positivity of G. lamblia in the canine population of Rio de Janeiro is 20%, according to the most sensitive analytical technique that has been PCR. And the population has the genotype A and no genotype-specific host dog, C or D, suggesting the existence of a cycle antropozoonotic. **E-mail:** maria_fantinatti@hotmail.com

Giard2. **Immunoenzymatic detection of *Giardia duodenalis* in ancient material**

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Introduction: A wide diversity of parasites has been recovered from ancient material so far, but these findings have been limited mostly to helminthes. Although intestinal protozoa present a worldwide distribution, their recovery from archaeological contexts is still exceptional. This scarcity might be associated with difficulties in their detection through traditional microscopy techniques and the sensibility of their parasitic structures, which present a rapid degradation after desiccation. Immunologic techniques have successfully detected protozoa in ancient material before. The aim of this research was to apply an immunologic assay (ELISA) to identify the presence of *Giardia duodenalis* (*G. duodenalis*) in archaeological samples from human and animal origin (coprolites or sediments). **Material and Methods:** We selected 80 samples from 20 archaeological sites in three different countries (Brazil, Chile and Peru). In order to standardize the identification of protozoa in ancient material, the tests were also performed in an experimental coprolite. This was prepared by desiccating a stool sample, positive for *G. duodenalis*, until there was no more weight loss. Each sample was rehydrated, sedimented according to the Lutz technique, and stored without any preservatives. Immunoenzymatic detection was performed using an IVD Research Kit for *G. duodenalis*. Results were measured by spectrophotometric absorbance and direct visualization. Results: 40% (32/80) of the samples were positive for *G. duodenalis* by ELISA, from which 68.8% (22/32) were human, 3.1% (1/32) were canid, 3.1% were felid, 15.6% (5/32) were rodent and 9.4% (3/32) was sediment corresponding to the same human burial. **Conclusions:** *G. duodenalis* was detected in archaeological samples, both from human and animal origins, by immunoenzymatic techniques. These results suggest that giardiasis might have already been a zoonosis in earlier times and that further analysis by molecular biology would offer an interesting approach to the study of zoonosis in the past. **E-mail:** adauto@ensp.fiocruz.br

CRYPTOSPORIDIUM

Crypt1. **Comparison between immunoassay and modified Ziehl-Neelsen for detection of *Cryptosporidium* in fecal samples**

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Introduction: The protozoan *Cryptosporidium* is an important etiological agent of chronic diarrhea in immunocompromised individuals, and may spread to others organs, leading to a more severe disease or even to death. In children under 5 years old, the parasite can also interfere with physical and cognitive development, justifying the importance of its correct diagnosis and treatment. However, specificity and sensitivity of routine parasitological methods for *Cryptosporidium* identification are not well defined. The aim of this study was to compare the modified Ziehl-Neelsen (mZN) with the coproantigen enzyme immunoassay (ELISA) for *Cryptosporidium* diagnosis. **Materials and Methods:** A total of 207 stool specimens were analyzed by mZN and ELISA (*Cryptosporidium* II TECHLAB). The sensitivity and specificity of both methods were calculated considering as true positives *Cryptosporidium*-samples identified by one or both methods. *Cryptosporidium* samples detected in this study, along with previously identified positive samples, were subjected to the 18S rRNA gene amplification by nested Polymerase Chain Reaction (PCR) to confirm the diagnosis and to compare with the results of ELISA and mZN. **Results:** Out of the 207 samples analyzed, 8 were positive for *Cryptosporidium*. Four isolates were identified by both ELISA and mZN, one by mZN and three only by ELISA. The sensitivity and specificity found for mZN was 62.5% and 98.5%, and for ELISA 87.5% and 99.5%, respectively. To compare the concordance of previous methods with the molecular detection of *Cryptosporidium*, 17 samples were submitted to the 18S rRNA gene amplification, revealing 13 (76.5%) samples with the expected 819-825 bp DNA amplicons. Of the four non-amplified samples, one was positive in both mZN and ELISA, and three were positive only by ELISA. Despite the high sensitivity of PCR, it is not possible to affirm that these three latter were ELISA false-positive cases, since the PCR of fecal samples may be influenced by many factors, such as a mutation in the target gene or the presence of PCR inhibitors in stool. Moreover, the PCR confirmed three isolates of *Cryptosporidium* only detected by ELISA, but not identified in microscopy. **Main Conclusions:** The detection of coproantigens by ELISA demonstrated high sensitivity and specificity when compared to the mZN for *Cryptosporidium* laboratory diagnosis. However, the PCR confirmation of a positive sample identified only by mZN, emphasizes the need to perform different methods to diagnose *Cryptosporidium*, especially in high risk groups, such as immunocompromised patients and children under five years. Support: FAPESB. **E-mail:** rknrs7@gmail.com

Crypt2. **Coccidian Parasite Prevalence in Children with Diarrhea in the Province of Eskisehir/Turkey**

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Introduction: Coccidian parasites are an intestinal protozoon that has emerged as an important cause of endemic or epidemic diarrhoeal disease in children and adults worldwide. They are easily overlooked due to lack of inspection methods, therefore the actual number of unknown. Detailed studies are usually done in patients with immunocompromised patients. In our study, prevalence of intestinal coccidian parasites was investigated with different analysis methods in children from the emergency department with complaints of diarrhea. Material and methods: In this study, total 578 fecal samples were collected at 2 years period in emergency department in University and Gowerment hospital with complaints of children with diarrhea. All stool samples were examined macroscopic and wet saline mounts and then, formalin-ethyl acetate sedimentation procedure. Initial identification of Coccidia was carried out on stool samples by direct microscopic examination and modified Ziehl Nelseen acid-fast staining method of stool smears was used to identify Cryptosporidium parvum, Cyclospora cayetanensis, Microsporidia sp.and Isospora belli. Results: Our studies found 5.1 % different intestinal parasites investigation of direct microscopic examination of diarrhoeal stool samples. Coccidian oocysts were detected using modified acid-fast staining. Coccidian parasites were observed in 6.2% (3.13% Cryptosporidium spp, 2.2% Microsporidia spp., 0.03% Cyclospora and Isospora spp.). Coccidian parasites were the most recurrent parasite found in this study with diarrheic children. Conclusion: Acute diarrhea is a major problem with high morbidity and mortality rates in developing countries, especially in children. Coccidia infection should be considered in children with prolonged or severe watery diarrhea in healthy children. Complex laboratory investigations are required to define the etiology because of the broad spectrum of etiological agents and the non-specific clinical signs. In the last years, **E-mail:** nihaldogan42@gmail.com

DISEASES BY HELMINTHS

SCHISTOSOMIASIS

Schisto1. **Specific antibody isotype response to *Schistosoma mansoni* in resistant individuals living in low endemicity area**

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Introduction: Isotypic response against *S.mansoni* antigens is extensively studied to characterize resistance to re-infection after re-exposure. However, continuous exposure to *S.mansoni* is not always equal to infection and "putative" or "natural" resistance can be demonstrated in individuals not infected with *S.mansoni* living in endemic areas. Putative resistance is characterized by low levels of specific IgGE and IgG4. Since antibody isotypic responses are different in infected population resident in areas of high or low endemicity, we hypothesized that non-infected individuals living in low endemicity areas present a distinct immunoreactivity from residents of a high endemicity area. **Objective:** To determine specific isotypic response in individuals resistant to *S.mansoni* infection. **Material and Methods:** Study population comprised 108 individuals from rural area (Sumidouro, Rio de Janeiro) being 53, 7 % males (mean age of 35 years) followed from 2003 to 2011. After informed consent was obtained from each participant, enrolled individuals in study approved by HUCFF/UFRJ Ethics Committee (n°058/09) provided fecal and blood samples. *S.mansoni* infection was determined by Kato-Katz (two slides/stool sample, K-K). Specific IgG1, IgG4 and IgE anti-adult worm (SWAP) were measured by ELISA. Real-Time PCR (qPCR) was used for DNA detection in fecal samples. Probes and primers targeted the cytochrome c oxidase subunit 1 (cox) gene in the mitochondrial genome. **Results:** Individuals with history of exposure and no previous use of specific chemotherapy who were K-K, qPCR and/or serology negative during the period of the study were selected as resistant group. Individuals were considered infected if K-K+ was positive in addition to PCR + and/or serology. Concentrations of IgE anti-SWAP were lower in the resistant group when compared to infected individuals (median arbitrary units (mau) = 0. 39 x 0.98 [IQR=0.25], 0.86 x 1.27 [IQR=0.50], 1.39 x 2.04 [IQR=0.75], $p < 0.01$). But, if infected group was exclusively defined as K-K positive no difference was observed when compared to resistant group (median arbitrary units (mau) = 0.87 x 1.19 [IQR=0.50] and mau = 1.39 x 1.90 [IQR=0.75], $p > 0.05$). In addition, IgG4 levels showed no statistical difference between resistant and infected groups despite which criteria were applied. Data suggest that low levels of IgE but not IgG4 in non-infected individual resident in low endemicity areas is presumptive of natural resistance against *S.mansoni* infection. **Conclusion**: Putative or natural resistance can be differently characterized in low endemicity areas by a distinct isotypic response which contrasts with high endemicity areas results reported in other studies. **E-mail:** cavalcanti.marta66@gmail.com

Schisto2. **Looking for the haemolysin of *Schistosoma mansoni*: Are Saposin-like proteins the answer and can we exploit them?**

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Introduction: Schistosomiasis affects 200 million people in developing nations and is second only to malaria in terms of morbidity for an infectious disease. It is essential that new prophylactic and therapeutic interventions are found to complement praziquantel for sustained control of schistosomes. Schistosomes are voracious blood feeders with females estimated to consume 400,000 red blood cells

per hour. Thus, their alimentary system can be viewed as an excellent organ in which to search for potential targets of drugs and vaccines. The proteolytic digestive cascade in the schistosomes' gastrodermis has been partially elucidated. However, the molecules involved in red blood cell lysis have not been identified. Saposin-like proteins (SAPLIPs) are a diverse family of proteins whose common functions include lipid perturbation. Despite their similar structures, SAPLIPs have adapted to carry out a number of different functions at biological membranes. Some SAPLIPs of protozoan parasites have been found to be haemolytic. SAPLIPs are expressed in schistosomes and it is possible that one or more of them is a haemolysin. SAPLIPS also have diagnostic potential. Importantly, previous research of other helminths shows that SAPLIPs can provide high levels of protection in vaccine trials, that is better than or comparable to other vaccine candidates. **Materials and Methods:** A comprehensive search algorithm has identified 15 SAPLIPs from the *S. mansoni* proteome. Quantitative real-time PCR (qPCR) was used on specific life cycle stages to determine gene expression from eggs to mature adults. RNA interference (RNAi) was employed against schistosomula and adult life cycle stages to investigate the effects of gene knock-down on digestion. **Results:** Life cycle expression data eliminates five of the 15 SAPLIPs as potential haemolysins, while a further five have been identified as the most promising candidates. These five have been characterised further through RNAi and recombinant protein technologies. Solving the 3D-structure is currently underway. Here we present data on expression patterns, sites of expression and functional characterization of these schistosome SAPLIPs. **Main Conclusions:** Due to the great diversity in functions among SAPLIPs that outwardly appear similar, it is not possible to infer function by homology searches of sequences. We are characterising five SAPLIPs of *S. mansoni*. Our approach is a combined structure-function analysis to identify SAPLIPs that might be targetable as innovative vaccine candidates. **E-mail:** charlene.willis@qimr.edu.au

Schisto3. **Activation of sinusoidal endothelial cells correlates with fibrosis stage evaluated by ultrasound and it is mediated by hedgehog pathway in human schistosomiasis mansoni**

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Introduction: One of the most remarkable characteristics of schistosomiasis fibrosis is the vascular remodeling. Hedgehog (Hh) pathway regulates fibrogenic liver repair and there is growing evidence that it also regulates angiogenesis and vascular remodeling. Our aim was to evaluate if the hedgehog pathway regulates the vascular remodeling that occurs in schistosomiasis. **Material and Methods:** A total of 28 wedge liver biopsies (Universidade Federal de Minas Gerais) from patients with different grades of schistosomiasis fibrosis staged by ultrasound (WHO protocol; pattern A=3 patients, D=5, Dc=2, Ec=17, F=1) were used in this study. Fragments of three donor livers that were used for split liver transplantation at Duke Hospital were also included as controls. This project was approved by the Ethics Committee of UFMG and Duke University Ethical Board (204-06). Activation of the Hh pathway (Patched and Gli-2) and activation of endothelial cells (CD31) were evaluated by immunohistochemistry and double immunohistochemistry. The number of positive cells was counted in ten 400x fields per patient (Gli-2 and Gli-2/CD31) or quantified using morphometry (Patched). Primary human sinusoidal endothelial cells (SEC) were isolated by elutriation from residual healthy liver tissues of two donor livers that were utilized for split liver transplantation at Duke University Hospital. Cells were incubated with 5µM Cyclopamine (Hh pathway inhibitor) or its inactive analog Tomatidine and total RNA was collected 0h and 48h and analyzed by Real time PCR. **Results:** We found that patients with schistosomiasis mansoni had more Hh activation markers (receptor and target gene Patched and transcription factor Gli-2) than healthy individuals and the number of hedgehog responsive cells correlates with fibrosis ($p<0.00$; $r=0.64$ and $r=0.83$, respectively). Bile ducts, stromal cells and endothelial cells were Hh responsive. We observed that activated SECs (CD31+) accumulated in patients with schistosomiasis and the majority of CD31+ cells were also responsive to Hh (Gli-2+) and this correlates with fibrosis stage by ultrasound ($p<0.001$; $r=0.97$). Even patients with schistosomiasis but without detectable fibrosis by ultrasound had an increase number of

CD31/Gli2 positive cells ($p<0.05$). Primary human SEC fresh isolated do not express activation marker CD31 and are not hedgehog responsive but express the hedgehog inhibitor Hhip. When those cells are activated in culture they repress Hhip, express CD31 and become hedgehog responsive and the inhibition of the Hh pathway repress their activation phenotype ($p<0.005$), suggesting that Hh pathway regulates activation of SEC. **Main conclusions:** Activation of sinusoidal endothelial cells is mediated by Hh pathway and correlates with fibrosis stage by ultrasound in human schistosomiasis mansoni. **E-mail:** dealmeida.thiago@gmail.com

Schisto4. **Osteopontin production by bile ductular cells and the ductular reaction correlates with schistosomiasis fibrosis stage evaluated by ultrasound**

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Introduction: Osteopontin is a profibrogenic extracellular matrix protein that plays a role in fibrosing viral hepatitis, alcoholic and non-alcoholic steatohepatitis. Recently this molecule was implicated in murine schistosomiasis japonicum fibrogenesis. Our aims were to investigate if expression of liver osteopontin correlates with human schistosomiasis fibrosis stage by ultrasound. **Material and Methods:** A total of 28 wedge liver biopsies (Universidade Federal de Minas Gerais) from patients with different stages of schistosomiasis fibrosis as assessed by ultrasound (WHO protocol; pattern A=3 patients, D=5, Dc=2, Ec=17, F=1) were identified. Fragments of three donor livers that were used for split liver transplantation at Duke Hospital were included as controls. This project was approved by the Ethics Committee of UFMG and Duke University Ethical Board (204-06). Expression of liver osteopontin, keratin 7 (KRT7) and keratin 19 (KRT19) (both bile ducts markers) were evaluated by immunohistochemistry. The number of immunoreactive cells was counted in ten 200x fields per slides. Pearson's correlation analysis and Student's t test were performed using SPSS version 11.5. **Results:** Ductular reaction was present in patients with schistosomiasis, and the number of Krt7 and Krt19 positive cells increased with fibrosis stage ($r=0.784$ and $r=0.850$ respectively; $p< 0.00$). The greatest expression of osteopontin was seen in ductular cells, and liver osteopontin mirrored fibrosis stage ($r=0.856$; $p<0.00$). **Main conclusions:** Our study confirms that ductular proliferation occurs during fibrosing schistosomiasis, and expression of liver osteopontin correlates with fibrosis stage. Since osteopontin is a profibrogenic molecule, additional studies will be needed to examine if osteopontin could be a novel anti-fibrotic target. **E-mail:** dealmeida.thiago@gmail.com

Schisto5. **Prospects for Integrated Control of Asian Schistosomiasis**

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Introduction: Schistosomiasis japonica is a serious parasitic disease and remains a public health issue despite intensive control. The relaxation or termination of mass drug-treatment has seen a dramatic recent rebound in both its prevalence and associated morbidity in China and the Philippines. The recent completion of the Three Gorges Dam, which crosses the Yangtze River in southern China, will result in exponential expansion of the habitats for the intermediate snail host *Oncomelania hupensis*, thereby increasing the risk of human and bovine infection, and resulting in potentially severe consequences for control. Asian schistosomiasis is a zoonosis and can be transmitted by a range of different mammalian hosts, including water buffaloes. Despite complicating control efforts, this characteristic provides a practical method for attacking the causative schistosome, *Schistosoma japonicum*, through development and deployment of a transmission blocking veterinary vaccine. A major block to success has been the low

ceiling efficacy achieved with many anti-schistosome vaccine molecules, although our recent successful experimental testing of two DNA vaccines (SjCTPI and SjC23) in field trials in China has renewed the prospects for success. As well, the publication of the complete genomic sequence for *S. japonicum* by the author and his Chinese colleagues, and new advances in post-genomics technologies, provide an unparalleled opportunity to identify new molecules exploitable as vaccine targets for schistosomiasis. One such molecule is the insulin receptor which, in vaccine trials in mice, has shown high levels of protective efficacy in terms of reduced fecundity of adult female worms. This and other vaccine candidates will need to be produced in quantity and rigorously tested first in the laboratory and then in the field. **Material and Methods**: I will describe details of a large field trial we are currently undertaking in China – in the highly endemic Dongting Lake area downstream of the Three Gorges Dam - aimed at field-testing integrated strategies for schistosomiasis control. The trial involves a multi-factorial randomised design and the strategies we are employing are: 1. Combined human mass chemotherapy and bovine vaccination; 2.Combined bovine vaccination with molluscicide of snails. **Results and Main Conclusions:** Our recently completed bovine intervention trials and mathematical modelling of the transmission of *S. japonicum* underpin the concept that such a vaccine, targeting buffaloes, could have major implications for future integrated schistosomiasis control in China. When developed, the transmission blocking vaccine used in water buffaloes could, in combination with other control strategies including human treatment, result in the elimination of *S. japonicum* from China and other parts of Asia, particularly the Philippines, where our recent studies have indicated bovines (carabao) likely play a similar major role in transmission as in China.**E-mail:** donm@qimr.edu.au

Schisto6. **The role of Carabao in transmission of *S. japonicum* in Northern Samar, the Philippines**

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Introduction: *Schistosoma japonicum* is the causative agent of schistosomiasis in China, the Philippines and Indonesia. In the Philippines, 10 out of 16 regions (administrative divisions) have reported cases of clinical schistosomiasis, with 6.7 million people living in these endemic areas [1-3]. Furthermore, within these endemic areas, 1.8 million are considered to be directly exposed to potential infection through daily lifestyle water contact activities that include farming, fishing, domestic activities (bathing and washing) and recreation. As a zoonotic disease it can infect over 40 mammalian species which can act as reservoirs of infection. In China, water buffalo have been shown to be major reservoirs of human infection however, in the Philippines, carabao have not been considered important reservoir hosts for *S. japonicum* due to the low prevalence and intensity of infections found in previous studies. The aim of this work was to address this confusion and identify a reservoir host, if any, in the Philippines. **Materials and Method:** To examine the role of animals, specifically bovines and dogs, in *S. japonicum* transmission in the Philippines we examined a number of animals and humans from a selection of endemic barangays in Northern and Western Samar provinces, the Philippines. For both dogs and humans Kato-Katz and real-time PCR (qPCR) were used and for bovines (both carabao and cattle) the newly developed FEA-SD technique and qPCR were used. **Results:** High prevalence of infection was found in bovines (95.5%) and humans (90.38%) from Western Samar. These results show a higher prevalence in bovines and humans than has previously been recorded. FEA-SD and qPCR were also more sensitive than Kato-Katz. From the bovine intensity data we were able to calculate the animal contamination index and found that each bovine is excreting nearly 30, 000 *S. japonicum* eggs into the environment each day when using a conservative estimate of total daily fecal excretion rates for bovines. **Main Conclusions:** From this study we have been able to show that bovines are likely to play a larger role in transmission of *S. japonicum* in the Philippines than has previously been thought. As a result the introduction of an animal component, such as a vaccine or Praziquantel treatment of animals, to national control programs, currently only consisting of infrequent mass treatment, will result in a decrease in human prevalence. The FEA-SD technique has been developed for use in ruminants and is ideal for correctly identifying infections and infection intensity in these large animals. **E-mail:** catherine.gordon@qimr.edu.au

Schisto7. **Investigating the local immune response against migrating *Schistosoma japonicum* larvae in the natural host, the water buffalo**

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Introduction: Schistosomiasis remains a major disease of tropical developing countries, despite the availability of an effective drug, praziquantel. Several limitations in current control strategies of the parasite mean that novel measures are required, such as a vaccine. In the Asian context water buffaloes are a major source of human transmission, and an effective livestock vaccine could simultaneously reduce human infection and improve animal health. However, there is a lack of information on the buffalo's immune response during infection, especially against the migrating larvae (schistosomula), which are considered an ideal target for vaccination. Previously we established a method to examine the local immune response induced by schistosomula in a rat model. In this study we used this method to examine the larval migration in the natural host, the water buffalo, in order to firstly identify novel larval-specific antigens recognised by the buffalo, and secondly to establish the type of immune response the schistosomula elicit. **Materials and Methods:** The lymph nodes (LN) draining larval infection sites (skin and lung) were removed at optimal time points from buffaloes following an experimental infection. Tissue samples were also taken for histology and RNA extraction, while samples from un-infected buffaloes were taken for comparison. Cells from the LN were cultured in order to collect antibody secreted into the culture media, which is specific to the larval infection drained by the LN. The type of response the larvae elicit in the buffalo was investigated by histological examination of target tissues and cytokine analysis of cultured LN cells and PBMCs. **Results:** The antibody response in skin lymph nodes from infected buffalo produced significant amounts of antibody, compared to control animals, and will be used to discover novel larval-specific vaccine targets. A strong inflammatory response was observed in skin tissues shortly after infection indicative of a type-2 immune response. Cytokine analysis is underway to characterize this response further. **Main Conclusions:** This study is the first to investigate the immune response of the natural host, the buffalo, against the migrating larvae and is anticipated to provide new targets for generating a transmission-blocking vaccine. **Email:** hamish.mcwilliam@monash.edu

HELMINTHIASIS

Helm1. **Echinococcus *granulosus* aldolase and enolase: two potential moonlighting proteins**

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Glycolytic enzymes, such as fructose-bisphosphate aldolase (FBA) and enolase, have been described as complex multifunctional proteins that may perform non-glycolytic moonlighting functions, but little is known about such function, especially in parasites. We have carried out *in silico* genomic searches in order to identify FBA and enolase coding sequences in *Echinococcus granulosus*, the causative agent of cystic hydatid disease. Three FBA genes and 3 enolase genes were found, and their sequences and exon-intron structures were characterized and compared to those of their orthologs in *Echinococcus multilocularis*, the causative agent of alveolar hydatid disease. To investigate the possible involvement of *E. granulosus* FBA (EgFBA) and enolase (EgEno) in non-glycolytic functions, the expression patterns of these enzymes were analyzed in the pathogenic larval form (hydatid cyst) of this parasite. Antibodies raised against recombinant EgFBA1 and EgEno1 were used to assess the expression profile of FBA and

enolase in the hydatid cyst. EgFBA1 and EgEno1 expression was detected in protoscolex and germinal layer cells, as expected, but it was also found in the hydatid fluid, which contains parasite's excretory-secretory (ES) products. Besides, both proteins were found in protoscolex tegument and *in vitro* ES products, further suggesting possible non-glycolytic functions in the host-parasite interface. EgFBA1 modeled 3D structure predicted an F-actin binding site, and the ability of EgFBA1 to bind actin was confirmed experimentally, which was taken as an additional evidence of FBA multifunctionality in *E. granulosus*. Overall, our results represent the first experimental evidences of alternative functions performed by glycolytic enzymes in *E. granulosus* and provide relevant information for the understanding of the host-parasite interplay. **Supported by:** CNPq, CAPES .**E-mail:** karilorenzatto@gmail.com

Helm2. **Genetic polymorphisms of Diphyllobothrium species found in fishes from Lake Llanquihue, southern Chile**

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Diphyllobothriasis is a fish-borne cestodiasis caused by infection of adult tapeworms belonging to the genus Diphyllobothrium and a limited species can cause diphyllobothriasis in humans. The disease occurs sporadically in South America, including Chile, Argentina, Peru, Ecuador, most recently in Brazil. In Chile, it has been known that three species, *D. latum*, *D. dendriticum* and *D. pacificum*, exist and D. latum is more frequent than D. pacificum in human diphyllobothriasis1-3). Some human infections caused by D. latum seem to be closely linked to the consumption of undercooked salmons which are aquacultured in the southern Chile2, 4). In 2009, the presence of Diphyllobothrium plerocercoids in fishes inhabiting in Lake Llanquihue, southern Chile was studied and found D. latum and D. dendriticum plerocercoids in coho salmon (Oncorhynchus kisutch). It was aimed to clarify the origins of D. latum and D. dendriticum in Chile and phylogenetic relationships to related species based on the molecular analysis of mitochondrial cytochrome c oxidase subunit 1 (cox1) and cytochrome b genes (cob). To collect Diphyllobothrium plerocercoids, the survey was twice conducted in Lake Llanquihue in 2009 and 2012, but no plerocercoid was obtained in 2012. Thus, D. dendriticum (n=58) and D. latum plerocercoids (n=2) collected in 2009 were used for the molecular analysis. In D. dendriticum, genetic polymorphisms were confirmed, and 10 and 9 haplotypes were identified in cox1 and cob, respectively, suggesting that D. dendriticum was not affected by a strong selection pressure. In contrast to D. dendriticum, haplotypes of both cox1 and cob in D. latum were identical to those from European isolates, suggesting that *D. latum* was introduced into Chile from Europe. In addition, phylogenetic analysis revealed that D. dendriticum from Chile is genetically distinguishable from D. dendriticum distributed in Europe and Russia. To clarify the origin of D. dendriticum, phylogenetic new studies are needed comparing D. dendriticum isolates from South America and North America. **References:** 1)Torres P. et al. (1993) Bol Chil Parasitol. 48: 39-43; 2) Torres P. et al (2004) Comp Parasitol 71: 111-117; 3) Sagua H. et al (2001) Bol Chil Parasitol 56:22-25; 4) Mercado R. et al. (2010) Parasitol Res 106: 995-1000;5) Yamasaki et al. (2010) XIIth ICOPA 2010, Merbourne, Australia. **E-mail :** hyamasak@nih.go.jp

Helm3. **Pathological features of livers and mesenteric polycystic hydatidosis from Brazilians patients**

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Abstract: Polycystic echinococcosis (PE) also referred as Neotropical Hydatidosis is an emergent parasitic zoonosis of public health concern due to human infection by the larval stage of *Echinococcus vogeli* in the tropical forests of Central and South America. The life cycle includes canines such as bush dog (*Speothos venaticus*) and domestic dogs (*Canis familiaris*), that harbor the tapeworm after ingestion of tissue from an infected competent intermediate host (*Agouti paca*), which develops the larval form (metacestodes). Human became accidentally infected after ingesting eggs shed in the environment by

definitive hosts. Although PE importance tends to be underestimated due to underreporting and to the lack of compulsory notification, literature review refers to 181 known cases. Most of these cases have especially the liver or lung as sites of infection. Clinical presentation, radiological imaging and surgery procedures have been studied among medical aspects of polycystic echinococcosis, but their pathology has not been examined in detail. This study was aimed to perform histopathological analysis of hepatic and mesenteric tissues with suspected PE from patients derived from States of Acre and Amazonas. Liver and mesentery fragments were fixed in buffered formaldehyde and subjected to routine histological processing. The sections were stained with haematoxylin and eosin, Lennert's Giemsa and Picrosirius. Liver cysts showed three characteristics layers (adventitious, laminated and germinal). In contrast, mesenteric cysts did not show an organized training of layers, given that the adventitious layer was absent, whereas the laminated layer is the membrane that stands out in the cyst. In this organ, mononuclear cells were main constituent of the leukocyte infiltration, whose intensity was related to the proliferative capacity of germinal layer. Moreover, the immunological response was linked with the hepatic cysts development (acute, sub-acute and chronic). Mesenteric and hepatic cysts showed protoscolices and calcareous corpuscles at different stages of development, showing internally large and small hooks and two pairs of dorsal and ventral suckers. We can conclude that the developments of mesenteric and hepatic cysts follow different pattern, as well as the immunological response in each organ. **E-mail:** almeida@ioc.fiocruz.br

Helm4. **Health Education Works! A Video-Based Intervention Reduces Soil-Transmitted Helminth Infections in Chinese Schoolchildren**

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Introduction: Over a third of the world's population is infected with soil-transmitted helminths (STH), mainly in developing Asian, African and Latin American countries. STH are intestinal parasitic nematode worms and comprise the most common of the 13 major neglected tropical diseases (NTDs) causing disabling chronic infections globally. We examined the effect of a video-based health education package in rural schools in Hunan province, China, where soil-transmitted helminth (STH) infections are prevalent and are strongly associated with poor hygiene and inadequate sanitation. The intervention aimed to increase knowledge of STH, induce improved hygiene practice and reduce infection. **Materials and Methods:** We undertook a single-blind unmatched cluster randomized intervention trial in 38 schools (N=1718, aged 9-10 years). Schools were randomly assigned to either a health education intervention package that included an educational cartoon video (19 schools) or to a control package (19 schools) where a traditional health education poster only was installed. Infection rates, knowledge and hand washing behaviour were assessed at the beginning and at the end of the school year. Albendazole treatment was given to all the participants at baseline and all positive cases at follow-up. **Results:** The intervention had a significant impact on all three outcome measures. There was a 50% decrease in incidence of STH ($P<0.0001$) and a 90% increase in knowledge scores between the intervention and control groups ($P<0.0001$). The proportion of students washing hands after using the toilet increased by a factor of two in the intervention group ($P<0.02$). To our knowledge, a 50% reduction in incidence is unprecedented for school-based health educational interventions targeting STH. The research objectives, study design, and main results will be discussed; and we will show selected scenes of the animated narrative cartoon video that has been professionally developed within this project. **Conclusion:** New control strategies to tackle STH are urgently needed, since current control efforts in form of mass drug administration (MDA) have shown to be unsustainable due to rapid re-infection. The video-based educational package presented here provides a promising new tool for STH control. The video can readily be adapted to different cultural settings, and incorporated into existing MDA programs. As part of a multi-component integrated control strategy combining health education, improved sanitation and chemotherapy, it can potentially prevent millions of STH infections in other areas with high STH endemicity across SE Asia, Latin America and sub-Saharan Africa. **E-mail:** franziska.bieri@qimr.edu.au

Helm5. **Antigenic extracts of *Strongyloides venezuelensis* by ELISA test in immunodiagnosis of human strongyloidiasis: preliminary results**

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Introduction: Strongyloidiasis is a parasitic infection caused by the nematode *Strongyloides stercoralis* with worldwide distribution, especially in tropical and subtropical regions. Definitive diagnosis is possible by detecting larvae in faeces, but parasitological methods have low sensitivity. Thus serological tests show variable sensitivity and specificity depending on antigen preparation. *S. venezuelensis* antigen can be recognized with accuracy and specificity, by the way of anti-*S.stercoralis* antibodies present in serum samples of strongyloidiasis patients. The aim of this study was to evaluate different antigenic fractions of *S. venezuelensis* by ELISA test in immunodiagnosis of human strongyloidiasis. **Material and methods**: Infective larvae utilized as antigens were obtained from animals experimentally infected with *S. venezuelensis*. Saline four antigen preparations were used: Fraction soluble (SP) and of membrane (MP) in phosphate buffered saline (0.01 M PBS pH 7, 2), soluble fractions (ST) and of membrane (MT) in Tris-HCl (25mM). Serum samples from 10 patients infected with *S. stercoralis* (group I), 10 patients with other parasitic infectious (4 *Schistosoma mansoni*, 2 *Ascaris lumbricoides*, 3 hookworm and 1 *Hymenolepis nana* - group II) and 10 patients (group III) with negative stool tests by performed by the methods of Lutz, Rugai and agar plate were tested. For ELISA were used 10 μg/mL of antigen, serum diluted 1:200 in PBS 0.05% Tween 3% milk (PBSTM) and secondary antibody (anti-human IgG conjugate peroxidase, Sigma) diluted 1:30000 in PBSTM. Values of reactivity index greater than one were considered positive. **Results:** The positivity rate for IgG against S. *stercoralis* in group I by MP extract was 90%; SP, ST and MT was 80%. There was no positivity rate in group II and III, resulting in a specificity of 100%. **Conclusions:** MP extract can be used as a tool for the diagnosis of human strongyloidiasis, after being evaluated by a large number of samples. **Financial support:** FAPESP (2010/51110-2). **E-mail:** marcelo.corral@usp.br

Helm6. **Proposal for an expert system for aid to the diagnosis of visceral toxocariasis: methodology and preliminary results**

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Introduction: The visceral toxocariasis is a worldwide zoonosis. It is the human infection, particularly by larvae of *Toxocara canis*, a nematode common in dogs. The definitive diagnosis is accomplished by viewing the larvae of *Toxocara spp* in the host tissues, however, even in liver biopsy, this finding is rare, and thus it requiring other laboratory tests for aid in the diagnosis. Fuzzy logic has been applied in various areas of knowledge, especially useful in medical applications, since the information used in decision-making are uncertain. The aim of this study was to develop an expert system based on fuzzy set theory to aid the diagnosis of visceral toxocariasis (VT). **Materials and methods:** was analyzed a database containing characteristics of 100 individuals aged between 1 and 12 years from the study of Cassenote (2010). The presence of disease was defined by infectious and parasitic physician using a medical history, laboratory tests and clinical evaluation. The prevalence of visceral toxocariasis this group was 12%. To assist in the diagnosis of VT was developed a language model based on fuzzy rules, which has as input variables: the epidemiological score (ES) that considers geophagy, onychophagy, habit of washing hands, habit of putting objects in the mouth, the laboratory score (LS) which is the optical density from the serology and eosinophil count, and clinical score (CS), which considers the presence of hepatomegaly, splenomegaly, adenopathy, and pulmonary symptoms (cough, wheeze, and asthma). The fuzzy sets were defined based on the distribution of the disease in a scatter plot taking into account the frequency of disease according to the distribution SE, LS, CS; the rule base comprises 27 rules, was used the inference method Mandani, that consider fuzzy sets both in their antecedents as and in their consequents; the output variable is a "risk" to present the outcome (varied between 0 and

100), characterized by four groups: absent, mild, moderate and severe. **Results:** in this review we consider the output as a binary event where absent and mild refers to the individual without disease and moderate and severe, with disease. The area under the ROC curve resulted in 0,93 (95% CI: 0.87-0.99) with statistical significance ($p \leq 0.001$).The optimal cutoff point based on the Youden index results in sensitivity and specificity of 72% and 96%. With this cutoff the Kappa concordance coefficient results in 0, 95 when compared with reference diagnostic (set by the infectious and parasitic physician). **Main conclusions**: the preliminary results showed that it is feasible and useful to the physician the use of expert system based on fuzzy set theory in the diagnosis of visceral toxocariasis. Further tests will be performed with another database to support the performance of the model and compare it with the opinion of various experts. **E-mail:** cassebote@usp.br

Helm7. **Expression of the TES30 Protein of *Toxocara canis* by *Pichia pastoris* in bioreactor**

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Introduction: *Toxocara canis* is the main agent of toxocariasis in humans, becoming a public health problem. The prevalence of this zoonosis and its impact on health are underestimated around the world. The product of secretion and excretion of *T. canis* larvae (TES) is the most important antigen used in the diagnosis of this disease; however, its obtaining is laborious and unproductive. The objective of this study was to produce, TES-30 antigen of *T. canis* in *Pichia pastoris*, in a bioreactor. **Materials and Methods:** For this purpose, a synthetic gene was cloned into the expression vector pPICZαB (Invitrogen). Competent cells of *P. pastoris* KM71H (Mut^S) were transformed by electroporation with the recombinant vector (pPICZαB/TES30), according to the protocol described by Invitrogen. Recombinant clones were cultivated in YPD agar containing 500 µg/mL of zeocin and subsequently selected by Dot blotting with mAb anti-his-tag conjugated with peroxidase (Sigma). After selected the clone, the pre-inoculum was cultivated in YPD in orbital shaker, overnight, at 28°C with 125 rpm. The inoculum was performed in three flasks containing 250 ml of BMGY medium and incubated for 24h at 28°C (125 rpm), making 10% of the volume of initial fermentation. Thereafter, the total volume of inoculum was added to the basal medium of salts (¼ of salts and ½ of glycerol). The temperature of 28ºC was maintained constant during the experiment, and stirring of 500 rpm also was constant until arrived DO spike. After that, the stirring (200-1000 rpm) was controlled by the dissolved oxygen maintained constant at 30% after the beginning of induction. The total consumption of glycerol was observed by two peaks of high rates of dissolved oxygen and supplemented with 50% (v/v) of glycerol was continued for 1h. When glycerol was depleted, 0, 5% of methanol was added every 24h to induce expression during 4 days. Recombinant TES30 expression was detected by Dot blotting using samples of the supernatant and pellet by lyses of the culture with mAb anti-his-tag conjugated with peroxidase and serum of sheep experimentally infected with *T. canis*. The samples were also tested by indirect ELISA. **Results:** The presence of recombinant TES30 was observed, by Dot blotting, just in the pellet, and the results of ELISA suggested that the protein was recognized by *T. canis* positive sera. **Main Conclusion:** These results demonstrated the successful of utilization the yeast *Pichia pastoris* expression system of recombinant protein TES30 of *Toxocara canis* in a bioreactor. **Email:** carolgmagalhaes@gmail.com

Helm8. **Evaluation of the effect of probiotic *Saccharomyces boulardii* on the intensity of infection by larvae of *Toxocara canis* in mice**

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Introduction: The syndrome of visceral larvae migrants (VLM) or visceral toxocariasis is a parasitic tissue chronic disease distributed worldwide, the nematode *Toxocara canis* is the main etiological agent of this disease. The activity of probiotic microorganisms have been used in the prevention and treatment of diseases and for modulating the immune response. In studies in which probiotic activity was observed

(*Enterococcus faecalis* and *Saccharomyces boulardii*) in acute visceral toxocariasis and chronic infectious visceral toxocariasis used doses were 100 eggs of *T. canis*. However, it is important to investigate the effect of probiotics in infected mice with lower inoculum in order to simulate what likely occurs in humans. The aim was to evaluate the effect of the probiotic *Saccharomyces boulardii* on the intensity of infection of larvae of *Toxocara canis* in mice inoculated with low numbers of eggs. **Materials and Methods:** Four groups of nine Swiss mice were formed. Groups I and II were inoculated 25 eggs of *T. canis* and Groups III and IV 50 eggs were inoculated by intragastric probe. Group I and Group III received a diet supplemented with the probiotic *S. boulardii* (107 CFU / g of feed). Groups II and IV were the control of Groups I and III, respectively. After, the research of larvae was performed by tissue digestion technique. **Results:** This was the first study to evaluate the effect of the probiotic *S. boulardii* on the intensity of infection of larvae of *T. canis* in mice inoculated with low infective doses. The administration of the probiotic in mice inoculated with 25 and 50 eggs of *T. canis* promoted, respectively, reducing the intensity of infection of 22.6% and 55.3% compared to control groups. **Conclusion:** The probiotic *S. boulardii* induced a reduction in the intensity of infection in mice with acute toxocariasis, inoculates with low infective doses.**Email:** anacbeheregaray@hotmail.com

Helm9. **First molecular identification of the *Angiostrongylus costaricensis* in paraffin-embedded worms taken from a human case**

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A boy was brought to the emergency department with twelve days of abdominal pain, fever, vomiting and diarrhea, which evolved to acute abdomen. A laparotomy was indicated, which resulted in segmental enterectomy and four enteroanastomosis. The microscopy of the small bowel showed mural infiltration of neutrophils and eosinophils, arterial thrombosis, eosinophilic vascular granulomas and reactive lymphadenopathy. Two worms of *Angiostrongylus costaricensis* were identified inside the mesenteric artery, in which a PCR testing specific to parasite was positive. The final diagnosis was abdominal angiostrongyliasis (AA) associated with multiple bowel infarcts. The definitive diagnosis of AA depends on the identification of parasitic structures in histological sections of surgical specimens. For the microscopy is necessary to include the specimens in paraffin block, in an attempt to find parasitic forms, which results in various histological slides. When the parasitic structures are not found, the diagnosis is presumptive. The PCR technique can be helpful in the identification of fragments of the parasite in histological sections of surgical specimens. The parasite DNA was extracted from sections with 10 mm of thickness. About 25 mg of tissue was paraffin embedded in xylene, centrifuged and washed twice with 100% ethanol, and dried at room temperature. For extraction we used the DNeasy Tissue kit. The primers were designed based on published sequences from mRNA of *A. cantonensis* (Genbank, U17585). The amplification reaction was performed in a final volume of 25 mL of 0.4 mM of the primer R1 Angio Rev (5'-CTCGGCTTAATCTTTGCGAC-3') and Angio F1 For (5' AACGAGCGGCAGTAGAAAAA-3'), using AmpliTaq Gold PCR Master Mix, on the following conditions: 94 ° C for 4 min, 35 cycles of 94 ° C for 1 min, 58°C for 2 min and 72°C for 10 minutes in the thermocycler FTC405. For the verification of amplicon with the expected product of 232 bp we used the horizontal electrophoresis in agarose gel with ethidium bromide. Visualization was performed in transluminator. Negative controls were used for specificity, including *Trichuris* and *Enterobius* and positive controls in all reactions. Amplification of DNA from *A. costaricensis* was also seen in paraffin material containing parasitic granulomas, in the absence of worms, larvae and eggs. In conclusion, PCR can be a valuable tool in the diagnosis of AA in which parasitic structures are not found in surgical specimens. Additional studies are needed to determine its diagnostic performance. **E-mail:** rubens.rodrigues@terra.com.br

Helm10. **First report of *Angiostrongylus cantonensis* in Porto Alegre, Rio Grande do Sul, south of Brazil**

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Angiostrongylus cantonensis is parasitic nematode discovered in pulmonary arteries and hearts of domestic rats in Guangzhou (Canton), China, by Chen in 1935. The life cycle requires intermediate host mollusks and definitive host rodents. Accidental human infection occurs by ingestion of raw or undercooked snails or slugs, paratenic hosts such as prawns, or contaminated vegetables containing the infective larvae (L3).The ingested L3 penetrate into intestinal wall and migrate to the brain and spinal cord where they may develop into fifth-stage larvae (L5) causing eosinophilic meningitis. Parasitological diagnosis is a challenge since larvae forms in the CSF is seldom founded, thus requiring the use of molecular methods for diagnosis. According with (Wang et al, 2008) more than 2820 cases have been reported in approximately 30 countries mostly in Asia and the Pacific Islands. The occurrence of *A. cantonensis* was reported for the first time in Brazil in the state of Espirito Santo. So far Pernambuco, Rio de Janeiro, Santa Catarina and São Paulo indicated the parasite has been circulating in those areas in view of the fact that intermediary and definitive host have been found infected. Also human cases were reported in these areas. Recently, we have been engaged in a study of *Strongyloides* spp. For this research was necessary capture rats naturally infected. One of the rats was capture in Vila Fátima, a poor neighborhood of Porto Alegre. The rat was euthanized and taken to necropsy to collect *Strongyloides* spp. worms. During necropsy the lugs reveled a suspected appearance of *Angiostrongylus* infection, thus, lungs were removed and analyzed under a stereomicroscope. In the pulmonary arteries, 11 females and 2 males of *Angiostrongylus cantonensis* were found. These worms were clarified and mounted as permanent slides in creosote solution and examined under a light microscope. For measurement proposes drawings of the nematodes were made with *camera lucida*. The average length of the females was 275mm and the males 210mm, which is in accordance with literature to characterize the specie. In addition, we used real time PCR for DNA confirmatory assay.10, 000 first sate larvae (L1) isolated from rat feces were used to infect two mollusks of *Bionphalaria glabratta* specie in order to produce L3. After 30 days snails were digested and L3 (104/rat) were used to infect two rats, *R. norgevicus* Wistar for maintenance of *Porto Alegre* isolated in our laboratory. This strain could be useful for further studies on phylogenetic dispersion of *A. cantonensis.* **E-mail:** bicognato@hotmail.com

Helm11. **Recombinant protein of *Angiostrongylus cantonensis* expressed in insect cells system**

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Angiostrongylus cantonensis is a nematode parasite which may cause eosinophlic meningoencephalitis (EM) in humans. EM is an acute disease caused by the presence of larvae in the meninges. The diagnosis is based on parasitological or molecular findings of either parasites or DNA in CSF or specific antibodies in the CSF or serum. The current immunological test detects antibodies to a 31 kDa protein on Western blot using crude extracts of nematode. Using mass spectrometry to identify the 31 kDa protein we discovered three potential antigens: the 14-3-3 phosphoserine-binding protein, a protein containing a nascent polypeptide-associated complex domain, and the putative epsilon subunit of coatomer protein complex isoform 2. Also, we demonstrated the 31 kDa band antigenicity is dependent on protein glycosylation. To produce large scale quantities of the glycosylated recombinant proteins we expressed all three proteins using an insect cell system. Genomic sequences were obtained by parallel tag sequencing using a Roche 454- instrument and the corresponding cDNAs were amplified by PCR using Platinum Taq DNA polymerase. The fragments were cloned into the 6xHis-tag pFastBac vector (Bac-To-Bac® Invitrogen) as a donor plasmid. Recombinant viruses were constructed by homologous recombination using site specific transposition into DH10Bac *E.coli* cells. DNA from the constructed

Bacmids were purified and used to transfect Sf-9 insect cells for recombinant virus production. Cells were cultivated in serum free medium. Viability and diameter were monitored every day by Vi-Cell XR cytometer. After cells reach about 18 µm and 50% of viability they were harvested by centrifuging (1,500 rpm) and suspended into 50 mM Tris-HCl, pH 7.4, Tween 20 1% for cell lyses and centrifuged at 20,000 x g to pellet insoluble proteins. Supernatants were passed through a cobalt column for recombinant protein purification and eluted with an imidazole gradient. SDS-PAGE and Western blot analyses using an anti-6xHis monoclonal antibody revealed all three proteins were expressed with their expected molecular weights. 14-3-3 was secreted during expression which facilitated its purification. Antigenicity of the recombinant proteins was tested using a pool of 20 positive infected patients. Only 14-3-3 was recognized by the infection sera. However the signal was very weak indicating that carbohydrate incorporated during insect cell expression may not represent the correct sugar moieties necessary for antigenicity. Ultimately another expression system may be necessary to produce those glycoproteins. Further analysis of the glycosylation patterns of *Angiostrongylus* may be warranted. **E-mail:** almorassutti@gmail.com

Helm12. **Structural characterization of adenylate kinase newly identified from *Clonorchis sinensis***

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Introduction: Adenylate kinase (AK) is a ubiquitous phosphotransferase enzyme that plays an important role in cellular energy homeostasis. AK catalyzes reversible phosphoryl transfer reactions between ATP and AMP to generate ADP. In this study, new AK of *Clonorchis sinensis*, an oriental liver fluke was characterized by 3D structural modeling. **Materials and Methods:** AK gene of *C. sinensis* was identified from EST database by BLAST search. Nucleotide and deduced amino acid sequences of eukaryotic AKs were aligned using Clustal W. PSIPRED, Geno3D and SWISS-MODEL were used for prediction of secondary structure and 3D modeling. Specific inhibitor (GP5 or AP4) -bound human AK4 and Escherichia coli AK were used as templates. The recombinant *C. sinensis* AK protein was produced bacterially and purified by affinity chromatography. Antigenicity of the recombinant *C. sinensis* AK was examined by immunoblot with various helminth-infected human sera. **Results:** A cDNA clone Cs63 was identified to be AK gene of *C. sinensis* by BLAST search and named CsAK. A putative protein encoded by CsAK was constituted with 198 amino acids of which predicted theoretical pI/Mw was 6.45/22294.72. Multiple amino acid sequence alignment of CsAK with the other helminthic AKs revealed 56% to 74% of sequence identities. Catalytic domains of CsAK were conserved well. CsAK contained nine helices and four strands in its secondary structure. 3D structural modeling of CsAK revealed localization of canonical ATP binding sites inside the pocket formed by ligand binding. Immunoblot of the recombinant CsAK Cs63 performed with various helminth-infected sera showed no specificity to clonorchiasis. **Main Conclusions:** In the present study, a newly identified AK of *C. sinensis* was examined. Results obtained from the study provide structural basis of *C. sinensis* AK for the development of new anthelminthic drugs. **E-mail:** tsyong212@yuhs.ac

Helm13. **Bile-chemotactic behaviors of Clonorchis sinensis**

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Clonorchis sinensis habits in bile ducts of man and mammalian hosts. In vitro the C. sinensis newly encysted juveniles (CsNEJs) migrated toward 0.001-0.01% bile. Of bile components, cholic acid attracted chemotactically the CsNEJs and it moved fast. After cholic acid equilibrated the second and third addition of cholic acid to the assay system re-activated chemotactic migration of the CsNEJs. Neurons transmitting bile sense was analyzed using neuroreceptor inhibitors of glutamate, serotonin, dopamine and acetylcholine neuron groups. Bile chemotaxis of the CsNEJs was inhibited strong by nano molar of

dopaminergic antagonists D1, D2, D3 and dopamine uptake inhibitor BTCP, but much less even by higher concentration of serotoninergic, glutaminergic and cholinergic neuroinhibitors. To trace in vivo migration, the CsNEJs were radio-labeled with 18F-fluorodeoxyglucose (18FDG) by incubating in 1x Locke's medium containing 18FDG at 37°C. After 12 minutes cholecystokinin was injected to stimulate bile released from gall bladder, about 3,000 radio-labeled CsNEJs were inoculated at middle duodenum of a rabbit using catheter under anesthesia. Photon signals emitted from the 18FDG-labeled CsNEJs were collected and in vivo images were reconstructed using positron emission tomography-computed tomography. Signals emitted from the radio-labeled CsNEJs were detected from 9 minutes and increased rapidly until 18 minutes after the CsNEJs injection. The CsNEJs migrated up fast with bile-chemotaxis from duodenum and into the bile duct through the ampulla of Vater. Collectively, the CsNEJs sensed cholic acid of bile in the duodenum and migrated up quickly and chemotactically into the bile duct. It is proposed that dopaminergic neurons play a major role of bile-chemotactic behavior of the CsNEJs. **E-mail:** hongsj@cau.ac.kr

Helm14. **Neurocysticercosis in a pig rearing community of Assam**

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Introduction: Pig rearing within household is a common in tea garden community of Assam. Neurocysticercosis is a major cause of epilepsy in India and prevalence of epilepsy in tea garden communities of Assam is not known. We conducted this study in order to find out the frequency of epilepsy and presence of NCC amongst those cases and also to provide a baseline information regarding prevalence of taeniasis and cysticercosis in tea garden communities of upper Assam. **Material and Methods:** We conduct door to door a cross sectional study in Basmotia tea estate of Dibrugarh district in Assam. 1028 cases were screened to identify patients with history of epilepsy. Out of them 46 patients out of 52 with suggestive history were investigated with contrast enhanced computerized tomography (CT) scan of brain to find out any brain lesion. Serological test including ELISA and EITB were also performed on samples collected from 987 individuals. 497 stool samples were examined under microscope in search of *Taenia solium* eggs. **Results:** Out of 1028 individuals screened, 52 had history of active epilepsy. CECT brain form 46 active epilepsy cases revealed that 43.4% had cystic lesion in brain of which 60% were multiple cystic lesions and 40% had solitary cystic lesions. Prevalence of cysticercosis and taeniasis was 295.8 and 66.4 per 1000 respectively. **Main conclusion:** Prevalence of active epilepsy is very high in tea garden communities of Upper Assam. NCC is the major cause of active epilepsy. **E-mail:** kanwar_narain@hotmail.com

Helm15. ***Echinostoma*** **spp. Molecular Diagnosis in experimental coprolites: methodology standardization for its use in archaeological material**

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Introduction: Echinostomiasis is a zoonosis caused by intestinal parasites, transmitted mainly by contaminated food, and attributed to at least 16 trematodes species. The human infection is endemic in Southeast Asia and Far East, and has been recently found in other regions. The study of these parasites in ancient materials can provide valuable information about the origin and spread of these parasites. The paleoparasitology studies the origin and evolution of parasites through archaeological and paleontological materials. Parasite eggs, identified as *Echinostoma* sp, were found in a coprolite of a mummy from Minas Gerais State, Brazil. However, microscopic diagnosis was not able to determinate the parasite species. **Objectives:** The aim of this research was to standardize a methodology for molecular diagnosis in

modern stool samples and experimental coprolites, all positive for *Echinostoma* spp., and further applying it to archaeological material. **Material and Methods:** The recent samples (adult worms, isolated eggs and feces) were obtained from experimental infection with the species *Echinostoma paraensei*. The series of 1, 3 and 5 isolated eggs were placed into a 0.2 ml PCR tube with 5 µl of ddH2O. Eggs and fecal samples were submitted to alternate boiling and freezing (100 °C and liquid nitrogen) for 3 cycles to break the egg shell. DNA extraction from isolated eggs was only physical. The adult worm was macerated using liquid nitrogen. Following this physical treatment, DNA extraction from feces and adult worm were conducted submitting 200 µl of sample to treatment with the commercial kit QIAamp DNA Stool Mini kit (Qiagen), with modifications (Proteinase K digestion at 55–60 °C for 2h and final elution in 50µl). PCR for a 126 bp fragment of the 18S-ITS region (*primers in house*) of *Echinostoma* spp., followed by nucleotide sequencing were performed. The same methodology applied to feces was used with experimental coprolite. **Results:** The methodology was able to amplify specific DNA fragment for the genus *Echinostoma* sp. in all samples: adult worm, feces, and of a single egg isolate. **Conclusions:** As the methodology applied to the stool yielded successful PCR amplifications also in experimental coprolite, it suggests that it can now be applied to archaeological material. **E-mail:** dleles@id.uff.br

Helm16. **Determining the End Point of Mass Drug Administration for Lymphatic Filariasis Elimination in Ghana**

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Introduction: The Global Programme for the Elimination of Lymphatic Filariasis estimates 6 effective annual rounds of mass drug administration with ivermectin and albendazole to achieve interruption of transmission of lymphatic filariasis. Ghana's programme for lymphatic filariasis elimination has completed 10 rounds of implementation activities with regular monitoring of impact and process indicators. **Materials and Methods:** Parasitological methods and coverage survey have been undertaken to evaluate the impact of mass drug administration in Ghana. Transmission assessment surveys (TAS) have also been conducted in 1 evaluation unit representing 4 districts to determine the end point of mass drug administration (MDA). **Results:** Nationally reported coverage has ranged from 63.9% in 2007 to 77.3% in 2009. Reported coverage has ranged from as low as 37.6% in Accra Metropolitan district in 2008 to over 80% for many other districts. Annual parasitological assessments have demonstrated marked reduction in microfilaria prevalence in all districts assessed. One of the 5 start-up districts qualified to stop MDA based on recently developed protocol for conducting transmission assessment surveys. Microfilaria prevalence is less than the required 1% in many sentinel sites but only one districts recorded prevalence of less than 1% in all its monitoring sites. Sensitization of the communities in the districts that will stop the MDA, stopping the MDA, undertaking a 2 year post-MDA passive surveillance and then a repeat TAS prior to possible certification remain the next steps. Districts have completed 6 rounds of MDAs with parasite prevalence of less than 1%. Transmission assessment surveys are required in other districts to help determine the end point of MDAs. Other districts have completed 10 rounds of MDA and yet have sites with microfilaria prevalence greater than 1% and do not qualify for the surveys. An exit plan based on the number of completed rounds of MDA by the districts is in place. Coverage reliability assessments data is also required to back up the reported coverage. Determining the end point of mass drug administration is constraint by human capacity, logistics and funding. Clear methods for undertaking these TAS though recently developed require further testing. **Conclusions:** The Lymphatic Filariasis Elimination Programme in Ghana has been effective. A decision to stop MDA in 4 of its 74 endemic districts is in place. Further TAS will provide evidence to further scale down the programme and provide the Global Programme the opportunity to further test the protocol for conducting TAS in countries that have reached the end point of lymphatic filariasis elimination. **Email:** nanakwadwo.biritwum@ghsmail.org

Helm17. **Assessing the epidemiological status of Lymphatic Filariasis on the islands of Zanzibar, Tanzania through a Transmission Assessment Survey (TAS)**

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Introduction: Zanzibar was one of the first countries to start MDA targeting Lymphatic Filariasis with drug treatments for the whole population in 2001[1]. Five successful rounds where administrated by 2006 with coverage exceeding 65% in all 5 of the rounds[2] were found to have significantly reduced the prevalence of *Wuchereria bancrofti* infections[3]. Coverage Surveys conducted after the completion of the first MDA showed that coverage for the islands was 79% on Unguja and 71% on Pemba which corresponded to 92.1% and 85.6% of the eligible population swallowing the drugs[4]. A transmission assessment survey (TAS) is conducted after a country or set of implementation units (IUs) have completed a minimum of 5 success MDA rounds. They are designed to assist programme managers in determine whether the prevalence of lymphatic filariasis has reached levels below the critical threshold required for tranmission[5]. Programmes will be able to assess whether MDA has succeeded in lowering the prevalence of infection to a level where recrudescence is unlikely to occur. Children aged 6 to 7 years old are surveyed because they should have been protected from lymphatic filariasis infection if transmission was successfully interrupted by the MDA[5]. Antigenemia in young children is used as a marker for relatively recent transmission as in older children or adults the presence of antigens may be related to past infections[5]. This is measured using immunochromatographic test (ICT) cards in areas with *W. bancrofti* infections where a positive result indicates the presence of an adult worm, thus a measure of on-going transmission[5]. The TAS is being done to determine whether the control programme has been successful in eliminating the transmission of the parasite *W. bancrofti* since the cessation of the MDA programme. The objectives of the TAS being conducted on Zanzibar are twofold; firstly to determine the residual prevalence of *W. bancrofti* and determine if the prevalence has been maintained at levels where transmission is not likely to occur. The results of the TAS will tell if Zanzibar is ready for verification of the absence of transmission of Lymphatic filariasis**. Material and Methods:** A total of 2 evaluation unit (EU) were used for this survey, one for each islands (Unguja and Pemba). **Survey Sampler Builder:** The Survey Sampler Builder (SSB) was used to select the appropriate survey strategy as it automates these calculations[5]. Based on total primary enrolment figures it is estimates that 73,000 children aged 6-7 attended primary school in 2011 with an enrolment rate of 96% in 2010 [6]. This information along with school numbers, absentee rates and main vector species was entered into the SSB. Based on this information a school based survey will be used for the TAS with 30 schools in each of the EUs (Table 1) with a critical cut-off of 18 on the island of Pemba and 20 on the island of Unguja. **Sample Collection:** As *W. bancrofti* is the endemic on the islands ICT cards were used to measure the antigenemia prevalence in Zanzibar. The ICTs require a blood samples, totalling 100uL, in order to detect the Ag of the adult worms, this was collected from each student using a finger prick on the inside of their index finger or thumb. **Survey Teams:** Survey teams from each of the EU are conducting the survey in 30 schools in each of the EU. Each team consists of 3 members who are responsible for recording the student's information (Name, Age and Gender), assigning then there identification number, collection the blood sample and recording the ICT results. **Student Selection:** Students are selected using a sampling interval. Upon arrival at each of the schools boys and girls are separated and assembled in lines and a sampling interval was calculated based on the number of students available and the number required for sampling. The first student to be selected is based on the selection of an arbitrary starting number between '1' and the sampling interval; from that location every 'SI' student was selected for sampling. **Data Management and Analysis:** All results obtained from the ICT were recorded and later entered using the double entry system of data entry in the programmes database. These will then be exported and analysis will be conducted on them to determine if the TAS has pass or failed based on the number of positive ICT results that are recorded per EU. **Results:** In the moment of the abstract submission 21 schools has been already surveyed in Pemba with 46 positives found so far. Preliminary results in Unguja do not allow making conclusions yet, with 27 schools surveyed and 14 positives found. **Main Conclusions:** The preliminary results in Pemba shows that transmission is still on-going and more efforts are required to eliminate LF as a public health problem. Preliminary results in Unguja do not allow making conclusions yet, though the survey on the island of Pemba has already exceeded the critical cut-off point

of 18 positives; thereby requiring further MDA to take place on the island. This will be conducted in conjunction with the Ministry of Health and the NTD control programme on the island. **E-mail:** maria.rebollo@liv.ac.uk

Helm18. **RNAi Mediated Silencing of Paramyosin Expression in Trichinella spiralis is Specific, Persistent and Results in a Phenotype**

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Introduction: Trichinella spiralis is an important human and animal parasitic nematode which can lead to serious zoonosis called trichinellosis. The entire genomic sequencing of Trichinella has been finished, but a lot of gene function remains unclear. The development of tools for the genetic manipulation, based on the recent decoding of the genome sequences of trichinella parasites, is an important priority for gaining a fuller understanding of the cellular and molecular biology of trichinella parasites and offering the identification and validation of potential new drugs for controlling the disease. RNAi technology, a successful and useful approach for the elucidation of gene function has been established in parasitic nematodes, but there are no known reports of the application of RNAi to T. spiralis. In this study we selected paramyosin (Pmy), target gene, a highly conserved structural protein and a major protective antigen of T. spiralis, to examine whether the efficacy and specificity of the current RNAi methodologies is suitable for the larvae and adult worms of T. spiralis and the roles of paramyosin in modulating worms grown and survival of T. spiralis. In the present study, siRNAs, delivered by soaking or electroporation, succeeded to trigger consistent Ts-Pmy transcript and protein translation levels knock-down in larvae (57.5% and 49.7%, respectively) and adult worms (66.3） % and (69.6)%, respectively) by real-time PCR and Western-blot analysis; dsRNA were also effective (60.2% and 79.3%, respectively in larvae). SiRNA treated worms displayed significant reductions in both molting (40%) and viability detected by microscope recording. Furthermore, the RNAi of Pmy could results in a 37.5% reduction in adult burden and 23.4% reduction in larvae burden. These results indicated that loss of Paramyosin expression may cause delays in larval development. These data demonstrated a functional RNAi pathway in T. spiralis and led to significant phenotypic changes for the first time. These and earlier findings collectively suggested that Paramyosin could be developed as a target for novel anti-*T. Spiralis* interventions **Keywords:** RNA interference; Trichinella spiralis; Paramyosin; siRNA; molting; viability. **E-mail:** zhuxping@ccmu.edu.cn

MYCOSIS

Myco1. **Paracoccidioidomycosis epidemiological profile in Rondônia, 1997 to 2008**

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Introduction: Paracoccidioidomycosis (PCM) is the most important systemic mycosis in Brazil. The country represents the largest endemic area in the world, with 80% of the cases. It is caused by a dimorphic fungus (*Paracoccidioides brasiliensis*) encountered in rural areas. The most common lesions frequently occur in the lungs bucopharinx mucosa and linphonodes. It is not a disease of mandatory notification, what doesn't allow the real knowledge of the magnitude of problem. This research aimed to evaluate the PCM Rondônia's epidemiological profile between the years 1997 and 2008. **Methods:** We analyzed retrospectively 1856 PCM reported cases to State Epidemiological Surveillance System. Patient information was accomplished in standardized protocol. The data were organized and analyzed using Epi Info 3.51, Tabwin and Excel 2007. **Results:** The incidence was more important in central and southern

state (80% of PCM cases) and 83% of patients related rural work. More than 97% were adults and 90% were male, average age 48 years old (range 4-80). The clinical chronic presentation was present in 92%, with lungs 84%, mucosa 10%, skin 4% and linphonodes 2%. The diagnosis was confirmed in 40% of PCM cases by direct examination 37% clinical and epidemiological, 12% serology, 6% histopathologic and 3% culture. More than 90% received itraconazol for treatment. The mortality rate was 6.6 cases per 100.000 inhabitants. **Conclusion:** The males of productive age in central and southern rural areas in Rondônia were the most affected. The local PCM incidence increased during the last 12 years, and PCM is considered as an emerging disease. The compulsory notification allowed this epidemiological analysis. The availability of the drug has been an important factor for therapeutic management and clinical monitoring. There is need of training more health professionals in clinical diagnosis, laboratory, treatment and epidemiological surveillance in order to control program sustainability, promotion, prevention and assistance to the PCM patients. **E-mail:** rdurlacher@yahoo.com.br

Myco2. **Prevalence of relapse in paracoccidioidomycosis-patients and its identification by an agar gel precipitin test**

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Introduction: Treatment of paracoccidioidomycosis-PCM leads to clinical and radiological cure, negativation of agar gel precipitin tests and recovery of the cell mediated immunity. However, persistence of *Paracoccidioides brasiliensis* latent foci can be responsible for a disease relapse. Double agar gel immunodiffusion test-DID has been the choice for serological diagnosis and patient's follow-up, due to its specificity. This study aims to evaluate the frequency of relapse in PCM-patients and DID test positivation. **Patients and Methods:** Three hundred thirty-five patients with PCM confirmed by identifying the typical *P. brasiliensis* yeast forms and/or by DID test, 77 of whom with the acute/subacute (AF) and 258 with the chronic form (CF), were evaluated. Relapse was defined as the recurrence of signs and symptoms compatible with PCM, associated with the identification of the typical *P. brasiliensis* yeast forms in any clinical specimen after appropriate treatment. Treatment was considered appropriate when symptomatology disappeared, erythrocyte sedimentation rate (ESR) returned to normal values and antibody serum levels evaluated by DID were persistently negative for one year with antifungal compound therapy. Frequencies were compared by Fisher's exact test and significance was set up at p 0.05. **Results:** Twenty patients (6.0%) relapsed 48 to 300 months (MD=96) after the beginning of the treatment, and 4 to 264 months (Md=60) after its discontinuation. Relapse frequencies did not differ as to clinical form (AF=7.8%; CF=5.4%; $p > 0.05$). Among the twenty relapsed patients, only nine (45%) showed positive DID test. **Conclusions:** Relapses showed low prevalence and late occurrence. As it must be soon diagnosed, other serological tests should be studied, and evaluation of the enzyme-linked immunosorbent assay (ELISA) is in progress. **E-mail:** mip.ricardo@gmail.com

Myco3. **Evaluation of kidney blood chemistry before and during follow-up of paracoccidioidomycosis-patients treated with cotrimoxazole or itraconazole.**

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Introduction: Itraconazole-ITC and cotrimoxazole-CMX are antifungal compounds used to treat paracoccidioidomycosis-patients (PCM-). This study aims to evaluate the blood kidney chemistry before and during therapy. **Methodology:** We studied 200 PCM-p confirmed by identification of the typical Paracoccidioides brasiliensis yeast form, or by detecting specific antibodies by agar gel precipitin (DID) test, 55 of them with the acute/subacute severe form (ASF), and 145 with the chronic form (CF), 39 of them severe (SCF), 97 moderate (MCF), 9 mild (MiCF). Forty patients were treated with ITC and 160 with

CMX. Serum levels of sodium, potassium, blood urea nitrogen- BUN and creatinine were evaluated. Patients were evaluated at different moments: M0 before treatment; M1: 4-6, M2: 7-10, M3:11-14, M4: 15-18, M5: 19-22 weeks after the beginning of the treatment; M6: at clinical cure and normal erythrocyte sedimentation rate. Statistical analysis was carried out by Kruskal-Wallis, Mann-Whitney and Friedmann tests; significance was set at $p \leq 0.05$. **Results:** 1. At M0: a) sodium levels showed tendency to be lower in ASF than in MiCF [p=0.07]; b) BUN levels were higher in CSF and lower in ASF, but MCF and MiCF did not differ of the other groups [p = 0.022]; c) potassium and creatinine levels were not altered in any group. 2. Effect of therapy: a) ASF: lower potassium levels in CMX-treated patients [p<0.001] at M2 and M3 [p<0.001]; b) ASF: higher potassium levels at M5 and M3 in ITC-treated patients [p<0.004]; c) CF: lower potassium levels in CMX-treated patients at M2 and M3 [p<0.001]; d) CF: potassium levels lower at M2 and M3 than M0 in ITC-treated patients. 3. Comparison among clinical forms, moments and antifungal compounds: a) ASF: higher potassium levels in CMX-treated than ITC-treated patients at M1 [p=0.035] and M4 [p=0.049]; b) CF: higher potassium levels in CMX-treated than ITC-treated at M4 [p=0.012] and M5 [p=0.023]; c) CF: higher creatinine levels in CMX-treated than ITC-treated patients at M2[p=0.002] M3, M4 , M5 [p <0.001] and M6[p=0.02]; d) ASF: higher BUN levels in ITC-treated than CMX-treated patients at M3 [p = 0.03]. **Conclusions:** At admission, PCM-p presented lower values of sodium in the acute/subacute severe form. There was no alteration during ITC-treatment, while mild increase of sodium, potassium and creatinine were observed in CMX-treated patients, within normal limits. These data demonstrate the safety of both PCM treatments, mainly ITC. **Email:** mip.ricardo@gmail.com

Myco4. **Evaluation of liver blood chemistry before and during follow-up of paracoccidioidomycosis-patients treated with cotrimoxazole or itraconazole**

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Introduction: Itraconazole-ITC and cotrimoxazole-CMX are antifungal compounds used to treat paracoccidioidomycosis-patients (PCM-p). Liver blood chemistry was evaluated before and during therapy. **Patients and Methods:** We studied 200 PCM-p confirmed by identification of the typical *Paracoccidioides brasiliensis* yeast forms, or by detecting specific antibodies by agar gel precipitin (DID) test, 55 of them with the acute/subacute severe form (G1), and 145 with the chronic form (CF), 39 of them were severe(G2), 97 moderate (G3), and 9 mild(G4). Forty patients were treated with ITC and 160 with CMX. Serum levels of total (TB) and direct bilirrunbin (DB), alkaline phosphatase (AP), aminotransferases (ALT and AST) and γ - glutamyl transferase (γ GT) were evaluated. Patients were studied at different moments: M_0 before treatment; $M_{1:}$ 4-6, M_2: 7-10, M_3: 11-14, M_4: 15-18, M_5: 19-22 weeks after the beginning of the treatment; M_6: at clinical cure and normal erythrocyte sedimentation rate. Statistical analysis was carried out by Kruskal-Wallis, Mann-Whitney and Friedmann tests; significance was set at $p \leq 0.05$. **Results:** 1. *At M_0*: a) TB, DB, ALT and γ-GT levels were not altered in any PCM clinical form; b) AP: G1 was mildly increased and higher than G4, while G2 and G3 showed intermediate values [p<0.01]. 2. *Effect of therapy*. Alterations were not observed in ITC-treated patients. CMX-treated patients showed: a) CF: increased AST levels from M_3 to M_6 [p<0.001] and decreased γ- GT levels from M_3 to M_5 [p <0.001]. 3. *Comparison among clinical forms, moments and antifungal compounds*: a) G1: higher DB levels at M_3 [p = 0.002] and M_5 [p = 0.005] in ITC-treated than CMX- treated patients; b) G1: higher AP levels in CMX-treated than in ITC-treated patients at M_1 [p <0.001], M_2[p=0.001], M_3[p=0.003] and M_5[p=0.02]; c) CF: higher TB levels in ITC- treated than in CMX-treated patients at M_2 [p = 0.0015] and M_3 [p = 0.002]; d) CF: higher AST levels in CMX-treated than in ITC-treated patients at M_1 [p=0.04] and M_3 [p=0.02]; e) CF: tendency to higher ALT levels at M_1[p=0.008] and AP levels at M_3[p=0,008] in CMX-treated than in ITC-treated patients. **Conclusions:** At admission the patients presented normal values of the liver blood chemistry, independently of the clinical form. There was no alteration during ITC-treatment, while mild increase of AST and AP were observed in CMX-therapy, within normal limits. These data demonstrate the safety of both PCM treatments, mainly ITC. **E-mail:** mip.ricardo@gmail.com

Myco5. **Treatment of refractory feline sporotrichosis with potassium iodide capsule**

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Introduction: Sporotrichosis is an infectious disease caused by the dimorphic fungus *Sporothrix schenckii*, which affects humans and a wide variety of animals, especially cats. In the state of Rio de Janeiro, Brazil, a zoonotic form of this disease has been emerging over the last 13 years in a region with socioeconomic and environmental difficulties. The domestic cat is involved in zoonotic transmission through scratches, bites and contact with lesion secretions. Saturated solution of potassium iodide, the first successful drug for the treatment of sporotrichosis was replaced by azolics due to adverse effects. Ketoconazole and itraconazole are the most common azoles used, being itraconazole considered the drug of choice. Although treatment with itraconazole has proved to be effective in cats, the clinical response is unsatisfactory in some cases, especially in those with respiratory signs and nasal mucosa lesions. In refractory cases to azolics, potassium iodide capsule might be an alternative. **Materials and Methods:** Fourteen cats with sporotrichosis confirmed by isolation of *S. schenckii* in culture and persistence of skin or nasal lesions refractory to treatment with itraconazole (n=10) or ketoconazole (n=4) for a minimum period of 8 weeks were included in the study. Treatment was exclusively potassium iodide 2.5 mg/kg to 20 mg/kg q24h. The scaling period of the drug was initially established with 2.5 mg/kg q24h. Then, doses were progressively increased at each 5-day period until a clinical response was achieved or signs of toxicity appeared as follows: 5 mg/kg, 10 mg/kg, 15 mg/kg and 20 mg/kg q24h. The cats were followed monthly by clinical examination and laboratory tests (hematology and biochemistry). All procedures undertaken during the study were signed by the owners in terms of informed consent and approved by the Ethics Committee on the Use of Animals (CEUA-Fiocruz). **Results:** Twelve (85.7%) of the 14 cats achieved clinical cure, being 9 from itraconazole group (n=10) and 3 from ketokonazole group (n=4). Treatment failure was observed in 2 cases, one from each group. Clinical adverse effects were observed in 35.7% (n=5) of the cases. Hyporexia and vomiting were the most frequents signs related to iodide, followed by lethargy, weight loss, anorexia and diarrhea. **Conclusions:** Potassium iodide capsule is a promising alternative for treatment of feline sporotrichosis refractory to azolic antifungals agents especially in socioeconomic disadvantaged areas. **E-mail:** ericaguerino@gmail.com

Myco6. **A semi-nested multiplex PCR for detection and identification of seven *Candida* species in blood samples from critically ill pediatric patients**

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Nosocomial candidemia is associated with high crude mortality rates among immunocompromised and critically ill pediatric patients. The detection and identification of *Candida* species in clinical samples are important to allow prompt initiation of correct antifungal therapy. PCR assays may provide more rapid and reliable candidemia detection with more sensitivity and specificity than blood cultures. This study aimed at developing a semi-nested multiplex PCR for detection and identification of the seven *Candida* species more frequently involved in invasive nosocomial infections (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. guilliermondii* and *C. lusitaniae*), comparing results with those obtained by blood cultures. Seventeen patients admitted to the pediatric intensive care unit of a tertiary hospital in the city of São Paulo, Brazil presenting predisposing conditions to develop candidemia, and 15 healthy children were included in the study. Nested amplifications were targeted to *Candida* ITS sequence. The first round employed universal primers to amplify the partial 18S, ITS1, 5.8S, ITS2 and partial 28S ribosomal DNA. The second round was performed in two separated amplification systems: one employed primers corresponding to *C. albicans, C. glabrata* and *C. tropicalis*; and other system using primers for *C.*

parapsilosis, *C. krusei*, *C. guilliermondii* and *C. lusitaniae*. The PCR products were fractionated by electrophoresis, resulting in single DNA fragments of the expected size for each *Candida* species, as visualized in 2.5% stained agarose gels. The assays detection limit corresponded to 1 *Candida* genome equivalent/ml of blood samples for all seven species. No amplifications were observed with genomic DNA from bacteria and other pathogenic yeasts frequently involved in sepsis pediatric infections. Blood cultures were positive in five patients (29.4%), whereas the semi-nested-PCR identified *Candida* species in six (35.3%), including all cultures positive patients. PCR was 100% concordant with blood cultures species identification, but only the molecular technique identified dual candidemia in one patient. None of the blood samples from 15 healthy children were positive by this technique. These are preliminary results of an ongoing study indicating that this multiplex semi-nested-PCR can be a useful tool for rapid detection and identification of *Candida* species in clinical samples. FAPESP 2010/02626-6 and 2011/09715-4 **E-mail:** gildamdn@usp.br

Myco7. **Prevalence and antifungal susceptibility of *Candida parapsilosis* complex isolated from the oral cavity of HIV-infected individuals**

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At present few data are available on prevalence and susceptibilities of *Candida parapsilosis* complex, which contains 3 species *C. parapsilosis, C. metapsilosis* and *C. orthopsilosis*, in HIV-infected individuals. Fifteen isolates of 318 *Candida* spp. were identified as *C. parapsilosis* complex by means of polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Prevalence of the *C. parapsilosis* complex was 4.7%, with 2.2% being *C. parapsilosis* and 2.5% *C. metapsilosis,* while no *C. orthopsilosis* was isolated. This is the first study that identifies isolates of *C. metapsilosis* obtained from the oral cavity of HIV – infected individuals. *In vitro* studies demonstrated that all isolates were susceptible to amphotericin B (AMB), fluconazole (FLC), ketoconazole (KTC), itraconazole (ITC), voriconazole (VRC) and caspofungin (CASPO). Studies with *C. parapsilosis* and *C. metapsilosis* showed minimum inhibitory concentration MIC_{50} (mg/L) and MIC_{90} (mg/L), were similar for all drugs tested. There were no marked differences in the MICs for *C. parapsilosis* and *C. metapsilosis* isolates for all antifungal compounds tested, except for FLC, which was significantly higher for *C. metapsilosis* than *C. parapsilosis*. Based upon the frequency of candidiasis and that certain isolates of *C. parapsilosis* complex respond differently to FLC therapy, our data may be of therapeutic relevance with respect to susceptibility to specific antifungal agents and the potential for acquirement of drug resistance. **E-mail:** danimoris@yahoo.com.br

Myco8. **Species distribution and antifungal susceptibility profiles of invasive and colonizing *Trichosporon* isolates from hospitalized patients**

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In the last three decades the incidence of fungal infections has increased significantly, with high associated mortality and morbidity rates. Among the factors contributing to this phenomenon are the increase population of patients with severe immunosuppression and those solid organ and hematopoietic transplant recipients. In this scenario, some fungi, especially those of the genus *Trichosporon*, are considered emergent pathogens. Infections caused by this genus are difficult to diagnosis, resistant to

many current antifungals, and are associated with high mortality rates. The elevated rates of drug resistance, together with the vulnerability of the immunosuppressed patients and the lack of knowledge of the putative virulence factors, warrant investigations comparing invasive and colonizing isolates. Our study aimed to evaluate the species distribution and susceptibility profiles between invasive and colonizing *Trichosporon* isolates from hospitalized patients. Seventeen isolates obtained from blood samples and other sterile sites of the organism (invasive isolates), and 42 isolated from urine and/or catheter (colonizing isolates) were evaluated. The phenotypic identification was performed by microscopic morphological features and by the VITEK 2® system. All isolates were tested for antifungal susceptibility by the EUCAST broth microdilution reference method employing the following compounds: amphotericin B, 5-flucytosine, fluconazole, itraconazole, cetoconazole and voriconazole. The 17 invasive isolates were identified as follow: 15 *T. asahii*, one *T. inkin* and one *T. mucoides*. All 42 colonizing isolates were identified as *T. asahii*. Of the invasive isolates, around 50% exhibited intermediate susceptibility to the azole drugs, except voriconazole for which 82.4% were susceptible. With regard to the colonizing isolates, the susceptibility profiles varied according to the azoles evaluated, but voriconazole was active against 95.2% from total. Of note, 82.4% of the invasive and 65.9% of the colonizing isolates exhibited resistance to amphotericin B. These are preliminary results of a study that aims to addressing the prevalence and the antifungal susceptibility profiles of the nosocomial *Trichosporon* species in some medical centers of the city of São Paulo, correlating with co-morbidities involved in this emerging fungal infection. FAPESP 2010/20187-0 E**-mail:** gildamdn@usp.br

DISEASES BY BACTERIA

BACTERIA

Bact1. **Cloning, expression and purification of *mecA* from methicillin-resistant *Staphylococcus aureus* as vaccine candidate**

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is the major pathogen involved in nosocomial infections, leading to high rates of morbidity and mortality in hospitals worldwide. The methicillin resistance occurs due to the presence of an additional penicillin-binding protein, PBP2a, which has low affinity for *β-lactam* antibiotics. PBP2a is encoded by the *mecA* gene, a foreign gene integrated into the chromosome of methicillin susceptible *S.aureus* (MSSA). In the past few years, vancomycin has been the only antibiotic option for treatment of infections caused by multiresistant MRSA; however, reports of vancomycin-resistant strains have generated great concerns regarding the treatment to overcome these infections. The aim of this study was to clone, express and purification of *mecA* as vaccine candidate. **Material and methods:** The 637bp fragment of *mecA* gene was amplified by PCR which were extracted from *S.aureus* COL strain (methicillin-resistant *S.aureus*). This fragment was cloned into prokaryotic expression vector pET24a. The pET24a-*mecA* plasmid was transformed into competent *E.coli* BL21 (DE3). Recombinant protein was overexpressed with isopropythio-β-D-galctoside (IPTG) and affinity purification was done by Ni-NTA agarose. SDS-PAGE and western blotting were performed for protein determination and verification. **Results:** The *mecA* clone was confirmed by colony-PCR and enzymatic digestion as well as sequencing. SDS-PAGE analysis indicated that the constructed

prokaryotic expression system pET24a -*mecA*-Origami efficiently produced target recombinant protein with molecular weight of 13.5 kDa. The recombinant *mecA* was over expressed as inclusion bodies by the use of 1.0 mmol/L IPTG. **Conclusion:** This prokaryotic expression system provides a simple method for producing recombinant *mecA* in high concentration and good conformational structure quality and may also be useful for the production of other bacterial outer membrane proteins for vaccine studies. **Keywords:** *S.aureus* COL strain, mecA, pET24a.**E-mail:** d.siadat@gmail.com

Bact2. **Antimicrobial resistance in *Salmonella* spp isolated from poultry carcasses**

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Introduction: The improvement of poultry industry in Brazil results from a general improvement in industrial processes. To prevent poultry carcass contaminations, it is crucial to control pathogen dissemination along the food production chain. Because of this, a number of actions have been taken to reduce the prevalence of pathogen dissemination with public health significance in food-producing animals. Due to their high incidence rates, *Salmonella* spp., are considered as the main poultry pathogen in both developed and emergent countries and, an important zoonotic pathogen of economic significance in both humans and animals. **Methods:** The aims of the present study were to detect main *Salmonella* serotypes isolated from poultry carcasses and evaluate the emergence of antimicrobial resistant strains to last generation human and veterinary drugs. From 2009 to 2011, 243 *Salmonella* spp., isolated from frozen (n=84) and refrigerated (n=159) poultry carcasses commercialized in Brazil were received for serotyping. All strains had been tested for resistance to several antimicrobials by disk diffusion method, following CLSI guidelines. **Results**: Thirty-three *Salmonella* serogroups were identified, being *S.Mbandaka* (10.3%); *S.Minnesota* (7.4%); *S.Enteritidis* (7.4%); *S.Typhimurium* (7.0%), and *S.Infantis* (6.2%) the most frequent serovars. The decrease of *S.Enteritidis* isolation rate might be due to implementation of control programs in poultry industries, such as vaccines, and probiotics. Among the 243 *Salmonella* isolates tested, 70% were resistant to one or up to seven antimicrobial drugs. Antimicrobial susceptibility identified a total of 64 resistance profiles, being 4.1% to fluoroquinolones and, 9.6% to 3rd generation cephalosporin. Discontinued drugs (MAPA resolution, 1998; 2003) not allowed being used showed resistance to Tetracycline 31.5% and 28.7% to Nitrofurantoin and the occurrence of resistance to chloramphenicol in *Salmonella* ssp. was either low, or very low from 2009 to 2011. **Conclusions**: In food-producing animals, particularly of certain species, sub-clinical infections can be common. Those species can spread pathogens resistant to antimicrobials to people via foodborne routes but also by routes such as water and by direct animal contact. Our antimicrobial resistance results highlighted a Public Health concern and the infections with *Salmonella* which are resistant to antimicrobials may result in treatment failures or necessitate the use of second-line antimicrobials for therapy. The selective pressure exerted by the use of antimicrobial drugs and the degree of spread of clones of *Salmonella* can also is influenced by factors such as foreign travel in humans as well as animal movements. Monitoring the *Salmonella* transmission along the food chain and the potential mechanisms to acquire or transfer a multiple antibiotic resistance elements can create strategies to identify and control these *Salmonella* strains. **E-mail:** renata@ioc.fiocruz.br

Bact3. **Identification of toxin co-regulated pilus A (*tcpA*) gene expression in *Vibrio cholerae* O26 strains isolated from human cases in Brazil**

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Introduction: Cholera is an acute intestinal infectious disease caused by *Vibrio cholerae*. Epidemic strains of *V. cholerae* have two important genetic elements, the CTX prophage encoding cholera toxin, and *Vibrio* pathogenicity island (VPI). The toxin coregulator pilus (TCP) main subunit is encoded by the *tcpA* gene, present in VPI, and allow the colonization of the intestinal epithelium by *V. cholerae*, beyond is the receptor for the entry of the CTX prophage in new cells. Molecular analysis of strains of *V. cholerae*

non-O1/non-O139 isolated between 1992 and 2000, revealed the presence of genes of CTX prophage in 14 strains of *V. cholerae* O26 and one non-typable, however, only two of these strains were detected gene *tcpA* El Tor. Since the product of this gene is the cellular receptor of the CTX prophage the absence in detection of *tcpA* gene pointed to the presence of a variant allele in the strains in question. Corroborating this hypothesis, using primers external to the *tcpA* gene was observed fragment with the expected size in all strains. **Material and Methods:** This study aimed to verify the presence of protein in *tcpA* strains that have the gene variant. For this purpose, the *tcpA* gene fragment, from a strain of *V. cholerae* O1 was amplified by PCR and cloned into vector pET-21a. *Escherichia coli* competent cells were transformed with the plasmid constructs *tcpA*/pET-21a and their DNA extracted using "miniprep". Some clones were sequenced to assess the quality standard of the sequences, and used to transform competent cells of *E. coli* for recombinant protein expression TcpA. The protein was used to immunize rabbits to obtain polyclonal serum anti-TcpA. Bacterial extracts were produced from strains of *V. cholerae* O1 and non-O1/non-O139, and strains not belonging to the genus *Vibrio*. Later, *Western blot* tests were performed to verify the recognition of the TcpA protein by polyclonal serum in bacterial extracts. **Results:** The strains of *V. cholerae* showed protein expression TcpA, while in other strains; its expression was not detected. **Main Conclusions:** The results of this study point out the pathogenic and possible epidemic potential of strains that do not belong to serogroups O1 and O139, by expressing one of the most important virulence factors in *V. cholerae*, TCP, thus reinforcing the importance of studies to *V. cholerae* non-O1/non-O139.. **E-mail:** mariananunes@cpqam.fiocruz.br

Bact4. **Survey Coverage, Risk Factors Associated in an Outbreak of *Pertussis* and Effectiveness of the *Pertussis* Component Vaccine in a Brazilian city, 2011**

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Introduction: *Pertussis* is an important cause of morbidity and mortality in children. During May 2011, following 20 *pertussis* reported cases in Jaramataia municipality/Alagoas, Brazil, an investigation was conducted and the objectives were: conduct survey coverage to DPT, identify risk factors and analyze the effectiveness of *pertussis* component vaccine. **Material and Methods**: We performed a cross-sectional study to assess vaccination coverage in children less than 15 years of age. We defined as basic immunization schedule, children under one year with three dosis of DPT and the complete scheme, from one to eight years with five dosis of vaccine and nine to 14 years with four dosis. In a case-control-design study (1:6, 7), confirmed case was a resident with positive laboratorial test for *pertussis* or attended the clinical or epidemiological criteria according Brazilian Surveillance Guidelines. Controls were randomly selected from a list of the residents and presented no respiratory symptoms or fever during November 2010 to May 2011. Pulsed-field gel electrophoresis (PFGE) was performed in a positive culture. Vaccine effectiveness was based on confirmed cases by laboratorial test (culture or RT-PCR). **Results:** Vaccination coverage in children under one year was 100%, followed by 56% (95%CI: 38-57) in children aged one to eight years and 43% (95%CI: 33-54) on nine to 15 years of age. In case-control, of 20 people meeting the case definition, 15 were men with a median age of seven (0.7 - 32) years and 75% presented complete schedule of vaccine for *pertussis* (five dosis). The disease was associated with having closed contact with coughing illness (OR: 11.2, 95%CI: 3.9-31.2) and attending a day care (OR: 11.7, 95%CI: 1.8-75.3), both with $p<0.01$. The strain of *Bordetella pertussis* was classified as serotype 1.3 and the vaccine effectiveness was 92.8%. **Conclusions:** This outbreak affected children aged one to four years; most of them had complete vaccination. The association with attending day care may be related to cluster of people. Vaccine effectiveness is consistent with literature and serotype 1.3 found in a patient was the same of the vaccine. This investigation emphasizes the importance to conduct studies to identify the circulating strain, the genetic profile of *Bordetella pertussis* and to evaluate the performance assessment of this disease in Brazil. **Email:** eduardo.saad@saude.gov.br

Bact5. **Epidemiology of Tick borne encephalitis and Lyme borreliosis in Slovenia-risk for travelers health**

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Introduction: Notification of Lyme borreliosis (LB) and tick born encephalitis (TBE) is mandatory in Slovenia. LB is endemic in whole Slovenia, while TBE cases occur mainly in northern, central and eastern regions of the country with expansion of new endemic foci in the last decade. An increasingly higher incidence rate of LB, but not for TBE, during the last 20 years is noticed. Reported incidence rates for TBE varied presumably due to climatic and some other factors. Slovenia is one of the 3 countries with the highest overall reported incidence rates of TBE in Europe. **Material and Methods:** Descriptive study of epidemiological data of notified LB and TBE cases was conducted and tourism data of Slovenian statistics bureau is presented to illustrate a travelers risk for tick borne diseases in Slovenia. **Results:** 2596 to 6304 cases of LB and 166 to 373 cases of TBE were reported yearly in period 2000-2010. Both diseases have typical seasonal pattern with majority of cases diagnosed from May to August (60% of LB and 76% of TBE cases). In analyzed period incidence of LB shows significant increase ($p<0.01$). In the last 5 years average incidence is 236/100.000 and 14/100.000 for LB and TBE, respectively. More than 3.000.000 tourists visit Slovenia yearly and spend on average 3 nights here. More than 70% of foreign tourist coming for holidays, leisure, entertainment and to visit friends and relatives and can be considered to come in contact with tick habitat. Approximately 2/3 of tourists travel to TBE most affected regions of Slovenia. **Main Conclusions:** In Slovenia increasing trend of notified LB cases and one of the highest incidences of TBE in Europe represent a high health risks also for travelers to this country in tick season. Information about tick bites prevention measures and vaccination against TBE is of high importance for travelers to LB and TBE endemic countries. **E-mail:** karl.turk@zzv-mb.si

Bact6. **Prevalence and distribution of trachoma in primary schools in State of Pará, Brazil**

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Introduction: Trachoma is the leading cause of preventable blindness in the world, but it is a neglected disease that demands great harm to the public coffers for lost productivity of people with visual impairments. In the last national survey of trachoma, carried out in the 1970s, the State of Pará appeared with the highest prevalence of the disease (26.2%). The campaigns against trachoma and economic growth of Brazil had a huge impact on the incidence and prevalence of the disease that was considered eradicated, culminating in termination of surveillance and control activities of the disease. **Materials and Methods:** A population-based cross-sectional study was conducted with random sampling of primary students from all public schools within municipalities were the human development index was less than national average, between March and September 2006. The sample size was calculated considering the population of students (N = 25.700), the national average prevalence of trachoma (5%), $\alpha = 0.01$ and confidence interval of 95%. The eye examination was conducted to detect the presence of clinical signs of trachoma and conjunctival sample collection to confirm the diagnosis by direct immunofluorescence (DIF). **Results**: The survey reached 95.94% (6.908/7.200) of the sample determined in school and 1.682 communicants. In total participated 98 schools in 73 municipalities and the prevalence ranged from zero to 29.37%.Was observed 479 (6.93%) students and 233 (13.85%) communicating with clinical signs of trachoma prevalence. The highest prevalence was observed among students younger than 10 years, males and rural residents. In communicating the prevalence was higher among younger than 10 years and living in rural areas with no differences between genders. The prevalence was respectively greater

than 5%, 10% and 20% in 35, 13 and four municipalities. Of 133 samples analyzed by the DIF, 27 (20.30%) were positive. **Main Conclusions**: Data from the survey showed that trachoma was not eradicated and revealed 35 priority municipalities in state of Pará and four characterized as pockets of endemic disease.**E-mail:** raymcarvalho@gmail.com.

Bact7. **Identification of bacterial diversity from digestive tract of *Triatoma brasiliensis using* 16S rRNA gene**

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Introduction: *Triatoma brasiliensis* is considered the most important vector of Chagas disease in Brazil, having its biggest dissemination rate in the states of Northeastern Brazil. Its life cycle occurs mainly in dry areas of this region. There are evidences that most of insect vectors have mutualism relationships with microorganisms from the environment, interfering in the vector-host relationship. Insect vectors can modify their life cycle by the action of these microorganisms depending on the characteristics of this interaction. Thus, this study proposes to identify the bacterial diversity that exists in the digestive tract of *Triatoma brasiliensis* using the sequence of 16S rRNA gene. **Materials and Methods:** The samples of *Triatoma brasiliensis* were obtained from a colony maintained in the Laboratório Central de Pernambuco (LACEN). The digestive tracts of three specimens were kept in sterile mili-Q water at -20°C until DNA extraction. After this, the cloning and sequencing of Bacteria 16S rRNA gene PCR products were done. The sequences were identified by comparison against 16S rRNA NCBI (National Center for Biotechnology Information) and RDP (Ribosomal Database Project) databases. **Results:** Representatives of four phyla were found distributed as follow: 49% phylum *Bacteroidetes*; 31% phylum *Proteobacteria*; 5% phylum *Cyanobacteria*; and 2% phylum *Actinobacteria*. The remaining 13% of the sequences did not fit into any of the phyla, being categorized as unclassified bacteria. In phylum *Bacteroidetes*, the family *Chitinophagaceae* was the most abundant (63% of identified sequences). The most represented genus was *Emticicia sp.* (order Cytophagaceae), comprising 26% of the sequences. This bacteria group is mainly related to digestive tract of hematophagous insects and mammals due to their level of specificity to extreme habitats. **Conclusion:** In this study, the phylum *Bacteroidetes* was the most abundant, whose frequency increase by blood ingestion. Although there is no information regard the association of these bacteria with the transmission of insect-borne parasites, those bacteria are connected with the process of hematophagy. This study was the first step towards the understanding of the bacterial population dynamics in the digestive tract of *T. brasiliensis*, which enables the use of this information to elaborate new strategies to control Chagas disease. **E-mail:** vqbalbino@gmail.com

Bact8. **HIV and Buruli Ulcer: which link?**

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Introduction: Buruli ulcer is caused by *Mycobacterium Ulcerans*. It is the third most common mycobacterial disease after tuberculosis (TB) and leprosy. Mycobacterium Ulcerans is responsible for extensive undermined cutaneous ulcers inducing esthetical and functional deformities. The impact of HIV on Buruli Ulcer is not clarified yet, unlike other mycobacterial disease: TB is the most common opportunistic disease in HIV infection, whereas HIV has no effect on leprosy. A few studies have investigated HIV as a risk factor for Buruli and they show contradictory results. Some case-reports show extensive and disseminated Buruli lesions in HIV+ patients, but similar cases are also reported in HIV-patients. The aim of our research is to investigate the impact of HIV on *Mycobacterium Ulcerans* clinical manifestation. **Method:** Retrospective analyses are done using data from the MSF-CH project in Akonolinga, Cameroon. This project has been running since 2002, with the goal of treating Buruli ulcer. The first analysis measures HIV prevalence in Buruli patients who have been treated since 2008 (introduction of HIV systematic testing in Buruli patients treated at Akonolinga). The second analysis

compares various Buruli indicators to show the differences between HIV+ and HIV- adult patients. The same indicators are compared in the HIV population between two groups of CD4. Kruksal-Wallis and T-test statistical tests were used. In a third part, a Kaplan-Meier survival analysis has been performed to compare the follow-up duration until complete Buruli ulcer healing, by using two groups of CD4. Finally a cox model (uni-/multivariate analysis) is used to investigate the factors influencing the follow-up duration. **Results:** Since 2008, systematic HIV testing has been introduced, resulting in more than 90% patients tested. HIV prevalence among patients with Buruli Ulcer is 5 to 6 times higher than the national prevalence. In the comparative analysis, HIV+ adults tend to have larger lesions and multiple Buruli lesions than HIV-. HIV+ adults also need more than one excision and longer time to heal. The tendencies are similar when we make the comparative analysis according to CD4 groups. These results are consistent with the survival analysis of the follow-up duration, which shows a significant longer time needed to heal the Buruli lesion in immunosuppressed patients. In a multivariate analysis with cox model, baseline CD4 count is the only significant predictor of the follow-up duration until complete healing of the Buruli lesions. **Conclusions:** Our results indicate a negative effect of HIV on Buruli clinical manifestation. First because of the extremely high HIV prevalence in a group of patients treated for Buruli. Second because of the high number of HIV+ patients who show serious indicators for Buruli, which is consistent with what has been observed in the analysis of the most immunosuppressed group of patients. Finally in the third analysis: baseline CD4 count appears to be the exclusive significant predictive factor of the follow-up duration until healing of the Buruli ulcer. These results strengthen the hypothesis that HIV immunosuppression has an effect on Buruli ulcer. **E-mail:** vchristinet@swissonline.ch

LEPROSY

Lepr1. **Risk factors for disease in a cohort of leprosy contacts in Rio de Janeiro, a parametric survival model analysis**

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Introduction: Leprosy is still a public health problem in the tropical zones of the planet. The leprosy elimination program, sponsored by the World Health Organization (WHO), which is aimed at the detection and treatment of newly detected cases, has reached a relative success in the reduction of global prevalence of the disease. WHO's objective is to reduce the prevalence rate below 1 case by 10.000 inhabitants. Though in Brazil there was a marked reduction in prevalence rates and in detection of new cases in recent years, it was not yet possible to come to the elimination cut-off. In that context, contact surveillance seems to be still a valuable tool for leprosy control. The objective of this study was to characterize risk factors for disease among followed contacts. **Methods:** A dynamic cohort study for leprosy contacts was initiated in 1987 at Fiocruz, Rio de Janeiro. Three thousand contacts were analyzed in this study. Demographic, socio-economic, environmental, and clinical variables have been collected and analyzed. Parametric accelerated survival models have been used to model incidence and study risk factors for contacts to become diseased in that cohort. This approach is relatively efficient, and handles right censoring as well as more complicated censoring schemes, as is the case for co-prevalent cases among leprosy contacts. R software was used for that modeling. **Results:** The incidence rate of leprosy among contacts was estimated as 0, 01694 persons-year in five years of follow-up (the first five years of follow-up). The starting time for follow-up was the date of diagnosis of the index case. Factors associated with becoming diseased were: 1) non-vaccination with BCG (p=0,002); 2) a negative Mitsuda reaction (p=0,007); and 3) a multibacilary clinical form of leprosy (p=0,007). Particularly, contacts whose index-cases had a high Baciloscopic Index at the end of treatment (> 1) were at risk. **Discussion and Conclusion:** The main conclusions of this study point to additional strategies for leprosy control in areas with a high incidence and prevalence of the disease, including contact vaccination with BCG, follow-up of newly treated patients, with characterization of their Baciloscopic Index, and active surveillance of contacts of leprosy cases in primary health care settings. **E-mail:** haroldodematos@gmail.com

Lepr2. **Serum and salivary anti-PGL1 antibodies and detection of *M. leprae* DNA in leprosy household contacts**

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Introduction: Leprosy household contacts are at high risk of developing the disease. Clinical examination of the contacts does not detect a subclinical infection. In the present work we investigated the presence of *M. leprae* DNA in nasal mucosa and anti-PGL1 serum IgG/IgM and salivary IgA/IgM antibodies in leprosy household contacts living in two endemic regions, Ceara, Brazil. **Methods:** A total of 135 leprosy contacts were included in the study. Among them, 50 (37%) were paucibacillary (PB) leprosy contacts and 85 (63%) were multibacillary (MB) leprosy contacts. They had a median age of 29 years old (age range, 7 to 81 years old). Saliva and serum samples were analyzed by the enzyme immunosorbent assay for the measurement of anti-PGL1 antibodies (Nagao-Dias, et al 2007) and molecular analysis from nasal swabs were conducted according to Torres, et al 2003. A questionnaire was also applied for evaluation of the family history. The project was approved by the Ethics Committee of Universidade Federal do Ceara and each participant or his guardian was asked to sign a written informed consent. **Results:** Good correlation between serum IgM and IgG isotypes was observed both in MB and in PB leprosy household contacts (r = 0.39, p <0.0001). However, their levels were much different (p <0.0001). Among the contacts positive for serum IgM, 74 (87%) were found to be negative for serum IgG. In respect to the salivary antibodies, PB leprosy household contacts showed correlation between IgA and IgM (r = 0.60, p <0.0001); the same was observed in MB leprosy contacts (r = 0.77, p <0.0001). It was observed that most of the leprosy household contacts who were positive to serum anti-PGL1, their salivary antibodies were negative. Bacterial load reduced at mucosal surfaces after the bacteria had translocated to systemic circulation what partially explained the negativity of salivary antibodies in some leprosy contacts. On the other hand, half of the leprosy household contact who was negative to serum anti-PGL1 antibodies, showed positivity for salivary antibodies. The contacts were infected by the mycobacteria but do not necessarily have major risk to develop the disease once they do not present serum antibodies. Finally, *M. leprae* DNA was found in nasal swab in 9 MB household leprosy contacts (10.6%) and in 3 PB leprosy contacts (6.0%). The frequency of positivity was 8.9%, considered to be similar to that found by other authors. **Main conclusions:** We concluded that semi quantitative analysis of serum and salivary anti-PGL1 in leprosy contacts associated with molecular markers is necessary for mounting strategies to survey subclinical leprosy infections in order to prevent development of the leprosy disease. **E-mail:** paulabbcc@yahoo.com.br

Lepr3. **Enrollment of Iron in the Immunopathogenesis of Lepromatous Leprosy**

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Introduction: Lepromatous leprosy is characterized by specific anergic to *Mycobacterium leprae* antigens and permissive bacterial proliferation. Macrophages (Mø) seem to have a crucial role driving the immune response to *M. leprae.* The immunosuppression in lepromatous leprosy implies modifications on iron metabolism proteins and its carriers. Previously, our group demonstrated that lepromatous Mø presents a higher expression of the scavenger receptor CD163 when compared to tuberculoid ones. CD163 recognizes Hemoglobin-Haptoglobin complex and we hypothesized that *M. leprae* increases CD163 in order to enhance iron storages in Mø, which contributes for mycobacterial persistence. Thus, the aim of the present work was to investigate the enrollment of iron in the immunopathogenesis of leprosy. **Materials and Methods:** Skin biopsies of leprosy patients classified by the Ridley-Jopling method were analyzed by optical microscopy following to stains with Prussian Blue, wade staining and immunohistochemistry analysis of ferritin light chain (FTL), CD163, heme oxygenase-1 (HO-1),

hemoglobin (Hb), haptoglobin (Hp) and transferrin receptor-1 (TfR1). Peripheral blood mononuclear cells (PBMC) or monocytes (Mo) from health donors (HD) were stimulated with $FeSO_4$ (100µM) and/or *M. leprae* (10:1) for 24 hours. The supernatants were measured to IL-4, IL-6, IL- 10, IL-12p70, IL-17, IL-23, IFN-γ, TNF-α and TGF-β. In addition, Mo was stained by flow cytometry to intracellular IDO. **Results:** We observed a highest expression of FTL, CD163, HO-1, Hb, Hp and TfR-1 in skin biopsies of lepromatous patients when compared to tuberculoid. The hemosiderin and FTL deposits colocalize with foamy Mø filled with bacilli. We observed that $FeSO_4$ could down modulate the increase of IDO expression induced by *M. leprae* in Mo from HD. In monocytes stimulated with $FeSO_4$ and *M. leprae* there was an increase in the secretion of both IL-12p70 and IL-10, whereas in PBMC there was a secretion of IL-6 when compared to cultures stimulated with *M. leprae*. **Conclusions:** Increased CD163 and TfR-1 expressions in lepromatous Mø are associated with augmented iron storages in cells from skin biopsies of lepromatous patients. In addition, free iron increased pro-inflammatory pathways in leukocytes stimulated with *M. leprae* which suggest that iron can induce a dual role in lepromatous leprosy pathogenesis by creating a favorable environment for mycobacterial persistence (mediated by IL-10) and by inducing pro-inflammatory cytokines (IL-12p70 and IL-6) that can contribute to the activation of antimicrobial pathways in the macrophages. **E-mail:** barbosa@ioc.fiocruz.br

Lepr4. **Role of autophagy in immune response to *Mycobacterium leprae***

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Introduction: Leprosy is a chronic infectious disease that can present different clinical forms and there is evidence that the establishment of different clinical forms is driven by host innate mechanisms. Macrophages from BT and LL patients seem to have a different behavior in relation to the bacteria. While in LL patients there are highly infected macrophages, in BT rare or few bacilli are found. Electron microscopy studies showed the presence of phagosomes with double membrane in macrophages exposed to *M. leprae*, suggesting a possible involvement of autophagy in the immunomodulatory response. In the present study we evaluated the role of autophagy in the immune response to *M. leprae*. **Methods:** We used the THP-1 monocytic cell line, monocytes from healthy subjects and LL and BT macrophages. The presence of autophagosomes was evaluated by electron microscopy. LC3 and Atg3 expression were evaluated by immunoperoxidase or Western blotting and the pattern of induced cytokines was evaluated by ELISA. **Results:** Ultrastructural analysis showed a higher number of autophagosomes in cells from skin lesions of BT patient compared to LL patient or normal tissue. A greater tissue expression of the autophagosome marker LC3 was observed in BT patients when compared with LL. Additionally, there was an increase on LC3-punctae expression in *ex vivo* macrophages from BT patient, in the presence or absence of IFNγ. IFNγ treatment in *M. leprae*-stimulated cells increased LC3-punctae expression compared with stimuli alone or non-stimulated monocytes and THP-1 macrophages. The pre-treatment with autophagic inhibitors wortmannin or 3-methyladenine was able to reduce IFNγ-induced LC3 expression. IFNγ treatment promotes *M. leprae*-LC3 colocalization in THP-1 macrophages, but did not in the presence of wortmannin. In addition, in the presence of both IFNγ and *M. leprae*, there was a higher expression of Atg3, the enzyme responsible for LC3 lipidation. IFNγ treatment in *M. leprae*-stimulated cells was able to increase IL-15 secretion in relation to non-stimulated cells, but not IL-10. Autophagic blockage by 3-methyladenine led to decreased IL-15 levels in response to stimulation with IFNγ and *M. leprae*, but did not affect the IL-10 production. In addition, IFNγ treatment led to reduction in macrophage-*M. leprae* association. **Conclusion:** Together, these data indicate that in *M. leprae*-stimulated macrophages, IFNγ induces the production of IL-15 which contributes to increase the microbicidal activity in host cells by autophagy induction. These findings may contribute to a better understanding of the mechanisms associated with leprosy immunopathogenesis. **E-mail:** rolmo@ioc.fiocruz.br

Lepr5. **Association between leprosy infection susceptibility and Ficolin 1 polymorphisms**

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Introduction: Mycobacterium leprae exploits complement activation and opsonophagocytosis to infect phagocytes. *M-ficolin* is encoded by the FCN1 gene and initiates the lectin pathway on monocyte surfaces. We investigated FCN1 promoter polymorphisms that could be responsible for the high interindividual variability of *M-ficolin* levels and for modulating leprosy susceptibility. **Material and Methods**: We genotyped rs2989727 (-1981G>A), rs28909068 (-791G>A), rs10120023 (-542G>A), rs17039495 (-399G>A), rs28909976 (-271IndelT), rs10117466 (-144C>A) and rs10858293 (+33T>G) in 400 controls and 315 leprosy patients from Southern Brazil, and in 296 Danish healthy individuals with known *M-ficolin* levels. **Results**: Ten haplotypes were identified with sequence-specific PCR and/or haplotype-specific sequencing. We found evidence for a negative association of FCN1*-542A-144C with leprosy in Euro-Brazilians (P=0.003, OR=0.243 [CI95%=0.083-0.71]) and a positive association of the -399A variant in Afro-Brazilians (P=0.022, OR=4.151 [CI95%=1.115-15.454], as well as a negative association of the FCN1*3A haplotype with lepromatous leprosy, compared with less severe forms of the disease (P=0.016, OR=0.324 [CI95%=0.123-0.858]). Danish individuals with this haplotype presented M-ficolin levels higher than the population average of circa 1000 ng/ml, and -542A-144C occurred in individuals with levels under the 25 percentile (P=0.031). **Conclusions**: Our data provide the first evidence that FCN1 polymorphisms are associated with the susceptibility to leprosy. *M-ficolin* may represent a novel key to understand the Immunopathogenesis of *M. leprae* infection. **Financial support:** CAPES/CNPq. **E-mail:** iarareason@hc.ufpr.br

Lepr6. **Live *M. leprae* induce expression of coronin1 through down regulation of cullin1 neddylation and proteasome pathway**

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Introduction: We have reported that live *M. leprae* covered by coronin1 (TACO protein) could inhibit phagosome-lysosome fusion which resulted in survival of it as same as in *M. tuberculosis*. However expression and degradation of coronin1 in the *M. leprae* infected macrophages remained and undefined, we investigated expression of coronin1, cullin1 and neddylation in macrophages with *M. leprae*. **Materials and Methods: 1)** Cell culture and *M. leprae* infection; **2)** RAW264.7 macrophage cell line (RAW) was cultured and infected1:20; **3)** Expression of coronin1 by Real Time PCR. Total RNA were isolated and synthesized. Primers of Coronin1 (NM_009898) were applied for Real time PCR; **3)** Isolation of protein and western blot analysis *M. leprae* infected RAW were lysed and quantified (used antibodies; Coronin1 Ab and Cullin1 Ab); **4)** Degradation of coronin1. We applied *M. leprae* and MG-132 (proteasome inhibitor) for 4 hours to RAW. Western blot applied for coronin1 degradation, and cullin1 expression and neddylation. **Results: 1)** Live *M. leprae* induced continuous expression of coronin1 gene. Live *M. leprae* at 1:20 rate of infection induced continuous expression of coronin1, but dead *M. leprae* could not show increment of expression during 4 hours of infection. **2)** Expression of coronin1 protein by *M. leprae.* Live *M. leprae* infected RAW revealed expression of coronin1 protein for 1 to 4 hours contrast with dead *M. leprae* which showed baseline level of it. **3)** Degradation of coronin1. Dead *M. leprae* inhibited expression of coronin1 to 26.2%, but proteosome inhibitor enhanced expression of coronin1 to 17.8%. Thick bands of NEDD8-Cul1 by proteasome inhibitor (MG 132) mean that ubiquitination is involving degradation coronin1. **4)** Changes of cullin1 neddylation by *M. leprae.* Dead *M. leprae* treatment showed increase of expression and neddylation of cullin1 for short period of RAW for 10, 20 and 40 min of time interval on the

histogram, but there are decrease of them for 1 to 4 hours. But live *M. leprae* treatment caused no change in expression of cullin1 expression, and decreased neddylation of cullin1 on the histogram. **Main Conclusions:** Live *M. lperae*, quite different from dead *M. leprae*, enhanced expression of coronin1 gene 1.21-1.51 folds and its protein increment of 16-28% for 4 hours. Live *M. leprae* induced decrease of neddylation of cullin1 which play an important role in involving ubiquitination and degradation of the coronin1. Thick bands of NEDD8-Cul1 by proteasome inhibitor mean that ubiquitination is involving degradation coronin1. These results suggest that Live *M. leprae* could inhibit SCF formation to result in halting cullin1 neddylation and degradation of coronin1. **E-mail:** guetae@catholic.ac.kr

TUBERCULOSIS

Tb1. **The isolation of suspected pulmonary tuberculosis patients based on a neural network model in a high prevalence general hospital**

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Introduction: To minimize the risk of nosocomial transmission, there is a need for respiratory isolation of patients with suspected pulmonary tuberculosis (TB), but the infrastructure for providing this isolation is scarce in developing nations. The different isolation strategies proposed at present in the literature have neither adequate specificity (SNS) nor sensitivity (SPC), thus having low performance on high prevalence settings. In our reality of a Teaching hospital in Rio de Janeiro, Brazil, with a high prevalence of TB (21%), the current isolation strategy that include clinical, images and laboratory results has a SNS of 77,4% and a SPC of 41,3%. Neural network (NN) technique has been used to build classification models for problems belonging to diverse domains, and its use in health care research has grown in different areas from tumor chemotherapy response to genetic polymorphism analysis. We aim at build a NN model that improves both SNS and SPC to have a better positive and negative predictive value (PPV,PNV) in our high prevalence situation. **Material and Methods:** A database with the patients under surveillance in the Hospital TB Control Program was built from 2001 to 2008, having 972 TB suspects, 210 of which had pulmonary TB confirmed by positive smear, Culture or Clinical Response to empirical Treatment, and that was used as the outcome for model building. A multilayer perceptron (MLP) NN was built based on 17 variables, which were selected using both statistics and the clinical significance as evaluated by a medical expert. Different training algorithms and number of neurons in the hidden layer were evaluated in order to achieve a better performance. As the data set suffers from class imbalance problem, different strategies were evaluated to mitigate this effect. The selection of training and testing groups was based on global K means clustering algorithm, whose number of clusters were defined using 5 fold cross-validations. **Results:** The best MLP NN model has 8 neurons on hidden layer, 1 neuron on output layer and used sigmoid as neuron activation function and RPROP training algorithm. This model achieved a sensitivity of 100% and a specificity of 91.4 when evaluated upon the test set, as a PPV of 75% and a PNV of 100%. **Conclusions:** The goal of having a better Clinical decision tool which is able to protect the hospital population and also help to rationalize the use of the isolation facilities may be achieved with the use of this tool. Future work will consist on make an external validation and impact assessment to evaluate if the performance of this clinical score is consistent for application. **E-mail:** galliez77@gmail.com

Tb2. **Rapid Diagnostic of childhood tuberculosis in blood and urine by Single tube Nested-PCR – Preliminary results**

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Introduction: On childhood's TB, the diagnosis is peculiar because it's difficult to confirm bacteriologically the disease. The child is frequently paucibacillary and doesn't have sputum in most of time. *Mycobacterium tuberculosis* complex is very infectious and the control of this is mainly through early diagnosis and an appropriate treatment. Gold standard have low sensitivity (Se), is nonspecific (*in acid fast bacilli* - AFB) and it takes at least 4 weeks for an accurate result. Molecular techniques are being proposed as an auxiliary tool in the detection of Koch's *bacillus* direct from clinical specimens. The PCR have a high Se and specificity (Sp), in addition to rapidity in obtaining the results. **Methods:** We collect peripheral blood (4mL) with EDTA and a pool of 3 samples of urine (10mL/day) from each patient. To extract the DNA from all samples, we used QIagen Midi kit. Then, we performed the single tube Nested-PCR (SNTPCR), which is composed for 15 (1st PCR) + 45 cycles (2nd PCR), using 2 sets of primers, an inner and another outer. The amplification products were visualized under UV light after the electrophoresis in 2% agarose gel. The used gold standard was clinical symptoms, epidemiology, laboratory findings and therapeutic response. Statistical analyses were done at SPSS 13.3, and the conclusions were taken for 5% of significance. **Results:** We used 53 children (54.2% of female sex), with the mean age 7.78 $\pm$ 4.65 (0-15 years old). Patients came from infirmary (59.3%) and outpatients of public hospitals of Recife-PE (Northeast of Brazil). The performance of STNPCR in blood was: the Se = 50.0% (CI = 31.1% – 68.9%), and the Sp = 92.0% (72.5% – 98.6%), the positive predict value (PPV) = 87.5% (60.4% – 97.8%) and negative predictive value (NPV) = 62.2% (44.8% – 77.1%). In urine, the performance of the PCR system was: Se = 34.6% (17.9% - 55.6%), Sp = 96.2% (78.4% - 99.8%), PPV = 90.0% (54.1% - 99.5%) and NPV = 59.5% (43.3% - 74.0%). When we calculate the performance of STNPCR putting together the blood and the urine, the values were: Se = 62.1% (42.4% – 78.7%), Sp = 88.5% (68.7% – 97.0%), PPV = 85.7% (62.6% – 96.2%) and NPV = 67.6% (49.4% – 82.0%). **Conclusions:** The performance of STNPCR for diagnosis of childhood TB proved to be efficient. It's faster than the culture, more specific than baciloscopy and can use samples minimally invasive. However, the positivity of PCR could indicate a recent infection or the disease in development, so, the clinic is still sovereign. With high PPV and Se, this system of PCR consists in a powerful tool to early confirm the *M. tuberculosis*, using blood or urine. When we use more than one clinical specimen of the patient, the accuracy and performance of STNPCR are improved. **Financial support:** FAPERJ, CPqAM/FIOCRUZ, PDTIS/FIOCRUZ, ICOHRTA/NIH. **E-mail:** jfcl@cpqam/fiocruz.br

Tb3. **Evaluation of Nested-PCR technique for detection of *Mycobacterium tuberculosis* in pulmonary and extrapulmonary samples from patients suspected of tuberculosis.**

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Introduction: The classical laboratory diagnosis of tuberculosis is performed by microscopy, which in despite of its speed and economy has low sensitivity (62%) and for culture, gold standard of the *Mycobacterium* diagnosis, in despite of being more sensitive, requires 4-8 weeks in way to obtain the result. Molecular technique has been used in diagnosis of infectious diseases by microorganisms specific nucleotide sequences detection. **Objective:** Evaluate the Nested-PCR (NPCR) technique for specific detection of the *M. tuberculosis (M. tuberculosis)* in pulmonary and extra-pulmonary samples. **Material and Methods:** The automated culture in BacT/ALERT system, and NPCR (*IS6110*) amplification of the insertion element IS6110 for investigation of *M.tuberculosis* in pulmonary (n = 20) and extrapulmonary (n = 78) samples were performed. For analyze of the parameters reproducibility, considering culture as the gold standard, were used McNamara's and Kappa tests (Biostatic v. 3.0), with a significance level of 5%. **Results:** TB was diagnosed in 11 patients, among of them 6 extrapulmonary cases and 5 pulmonary

cases. In all TB cases, the NPCR were positive, obtaining a 316 bp fragment in 2% agarose gel. Among 98 clinical specimens, the positivity results were 9.18% for culture and 13.26% for NPCR. Comparing the results obtained with NPCR for culture in pulmonary samples, there was obtained 100% sensitivity, specificity of 83%, 40% positive predictive value and 100% negative predictive value, and good reproducibility (K = 0.50, McNamara p = 0.25). For extrapulmonary samples these percentages were 83%, 96%, 62.5%, 98.5%, respectively, and good reproducibility (K = 0.6867, McNamara p = 0.625). **Conclusion:** This protocol of NPCR is a good complementary tool for the diagnosis of extrapulmonary TB. **E-mail:** adriana.cruz.furini@gmail.com

Tb4. **The Role of Biotransformation Enzymes in antituberculosis drug-induced Hepatotoxicity**

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Introduction: Antituberculosis drug-induced hepatotoxicity (ATDH) causes substantial morbidity and mortality. The biotransformation of drugs is an essential step in the process of elimination and reduction of toxicity. Genetic polymorphisms in drug-metabolizing enzymes can affect enzyme activity and this may cause differences in treatment response or drug toxicity, for example, due to an increased formation of reactive metabolites. The genotypes of the cytochrome P450 2E1homozygous wild type (*1A/*1A) and the glutathione *S-transferase* (GSTP1) gene polymorphisms with substitution (A>G) at position 313, which resulted in replacing isoleucine (Ile) with valine (Val), have been reported to increase the risk for ATDH. The aim of this study was to estimate the relationship of the CYP2E1 and GSTP1 gene polymorphisms to ATDH risks among a cohort of tuberculosis (TB) patients. **Methods:** This was a retrospective cohort study of 177 patients who had been treated for TB from 2006 to 2011 at Oswaldo Cruz Foundation (FIOCRUZ), Evandro Chagas Clinical Research Institute (IPEC), Rio de Janeiro, Brazil. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to measure GSTP1 (A>G) and CYP2E1 (C>G) gene polymorphisms. **Results:** Neither gene polymorphism was associated with the ATDH. The genotypes of CYP2E1 were analyzed in 177 patients and *1A/*1A genotype frequency were 92.7%, 6.2% (*1A/*5B) and 1.1% (*5B/*5B). Also, no association between GSTP1gene polymorphism and ATDH was found. The analysis of 104 patients showed, Val/Val genotype (7.9%); 26% (Ile/Val); 24% (Ile/Ile). Sixteen individuals had Val allele, of these fourteen developed ATDH, however no significance was observed. **Conclusion:** Neither *1A/*1A genotypes of CYP2E1 nor Val allele of GSTP1 gene may be considered as factors increasing the risk of ATDH in a group of patients undergoing anti-tuberculosis therapy. **E-mail:** **I**iane.castro@ipec.fiocruz.br

LEPTOSPIROSIS

Lept1. **Recombinant leptospiral flagellins towards developing new leptospirosis diagnostic tests**

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Introduction: More than 500,000 human cases of leptospirosis are diagnosed annually, with mortality rates >10%. This number is likely underestimated due to poor diagnostic tools. Early diagnosis of leptospirosis would aid patient management; however, the standard diagnostic test is the microscopic agglutination test (MAT). The MAT requires two serum samples collected at least one week apart. Recombinant protein-based ELISA are fast and relatively cheap alternatives. Leptospiral flagellins FlaA1

and FlaB1 are promising targets to leptospirosis diagnosis by ELISA. These proteins are immunogenic during infection and are present in all pathogenic *Leptospira* spp. The aim of this study is to produce and evaluate recombinant flagellins in diagnosis tests. **Material and Methods**: The *flaA1* and *flaB1* coding sequences were amplified from the *L. interrogans* serovar Copenhageni strain Fiocruz L1-130 genome by PCR. The amplified sequences were cloned into pAE, an *E. coli* expression vector that adds a 6xHis tag to the expressed protein. The plasmids containing FlaA1 and FlaB1 were characterized by PCR and by cleavage with restriction enzymes. After characterization, *E. coli* BL21 Star (DE3) competent cells were transformed with the plasmids and expression of the recombinant proteins was induced with IPTG. Protein expression was analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The recombinant proteins were purified under urea denaturating conditions by Ni+ affinity chromatography. After dialysis, purified proteins were separated by SDS-PAGE and visualized by western blotting. Proteins were quantified using a BCA protein assay kit. **Results**: The *flaA1* and *flaB1* coding sequences were successful amplified and cloned into pAE. The recombinant plasmids had the expected banding pattern following restriction enzyme cleavage. Recombinant FlaA1 and FlaB1 proteins were expressed in inclusion bodies with molecular weights of 34 kDa and 32 kDa, respectively, as determined by SDS-PAGE. Yields of 20 mg/L for rFlaA1 and 30 mg/L for rFlaB1 were obtained in *E. coli* BL21 Star (DE3) heterologous expression system. **Main Conclusions**: Recombinant leptospiral flagellins, rFlaA1 and rFlaB1, were successful cloned, expressed and purified. These proteins are undergoing evaluation for use in leptospirosis diagnostic tests. **E-mail:** grassmann.aa@gmail.com

Lept2. **Production of the recombinant protein OmpL37 of *Leptospira* interrogans for use as a subunit vaccine against Leptospirosis**

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Introduction: Leptospirosis is an emerging infectious disease caused by pathogenic spirochetes of *Leptospira genus*, of human and veterinary concern, with an incidence of over 500,000 human cases each year. This disease is associated with poor sanitation, overcrowding and recreational activities, resulting in frequent outbreaks in periods of heavy rainfall. Efforts to develop recombinant vaccines against leptospirosis have focused on outer membrane proteins (OMPs). OMPs are generally well-conserved and represent potential targets for immune-mediated defense mechanisms. OmpL37 is recognized by human and animal serum, binds human elastin, fibrinogen and fibronectin, and may be playing a role in transmission or pathogenesis of leptospirosis. **Material and Methods:** The DNA sequence encoding the OmpL37 (Lic12263) was amplified by PCR and cloned into the *E. coli* expression vector pAE. The resulting recombinant vector pAE/ompL37 was used to transform *E. coli* BL21 Star™ (DE3). Cultures were grown to OD600 0.5-0.8 and protein expression was induced with 1 mM IPTG. The His-tag OmpL37 was purified under denaturizing conditions using urea through immobilized metal ion affinity chromatography (IMAC). Dialysis was performed at 4 ºC with 100 mM Tris, 300 mM NaCl, pH 8.0, decreasing the concentration of urea in each step. The purified protein was then dialyzed against phosphate buffered saline, pH 7.2 at 4 ºC for 24 h and stored at −20 ºC. The ompL37 presence in pathogenic *Leptospira* serovars was assessed through PCR with the same primers as above. Pathogenic *Leptospira* evaluated include L. interrogans serovars Pomona, Canicola, Icterohaemorrhagiae, Autumnalis, Bataviae, Bratislava, Djasiman, Hebdomadis and Muenchen; L. borgpetersenii serovars Ballum, Castellonis, Mini, Poi, Sejroe and Javanica; L. kirshneri serovars Grippotyphosa and Cynopteri and *L. santarosai* serogroup Pomona. Template integrity was demonstrated by 16S rRNA gene amplification. **Results:** The OmpL37 recombinant protein was expressed as insoluble in *E. coli* host and presented the expected size of 37 kDa. The protocol using urea for protein solubilisation was efficient and the yield obtained was 10.4 mg L-1. The DNA fragment correspondent to ompL37 gene with 957 bp was detected in all pathogenic *Leptospira* strains investigated**. Main Conclusions:** OmpL37 is a potential antigen for subunit vaccine development against leptospirosis. Since it is conserved through pathogenic *Leptospira* species, it could have an advantage in inducing cross-protective immunity. Moreover, studies demonstrated that this protein is recognized by convalescent serum and binds host proteins. **E-mail:** thais.larreoliveira@gmail.com

Lept3. **The region shared by Leptospira immunoglobulin-like protein A and B (LigBrep) used as DNA vaccine afford partial protection against heterologous challenge**

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Introduction: Leptospirosis is considered to the most widespread zoonosis in the world. This infectious disease is transmitted through direct contact with an animal reservoir or an environmental contaminated with their urine. Current vaccines are based in bacterins that impart short-immunity with limited cross-protection. The leptospiral LigA and LigB proteins possess immunoglobulin-like domains with 90 amino acid repeats that have been identified in adhesion molecules involved in pathogenicity mechanisms. They are highly conserved in pathogenic *Leptospira* spp., thus are great interest for use as serodiagnostic antigens and recombinant vaccinogens. The N-terminus amino acid sequence of LigA and LigB proteins is identical, but the C-terminus varies. In this study we evaluated the immunoprotective potential of five truncated forms of LigA and LigB proteins of Leptospira interrogans serovar Canicola as DNA vaccine. **Material and Methods:** Gene regions corresponding to LigA and LigB proteins (named LigAni, LigBni, LigBrep, LigBct1 and LigBct2) were amplified by PCR and cloned into the pTARGET mammalian expression vector. The constructs were evaluated regarding functionality and expression of the recombinant proteins in vitro by indirect immunofluorescence (IFI), using mammalian cells (VERO). Female hamsters were immunized with 100 µg of DNA vaccines together with 15% of aluminium hydroxide, used as adjuvant. The hamsters were immunized twice with 21 days of interval. Forty-two days after the first dose all hamsters were subjected to heterologous challenge by intraperitoneal route with 101 L. interrogans serovar Copenhageni strain Spool (5x LD50). The efficacy of the vaccines was evaluated in terms of induction of specific IgG antibodies, survival and sterilizing immunity. **Results:** All target genes were successfully amplified and the constructs obtained were functional and expressed the proteins *in vitro*. Immunization with the LigBrep DNA vaccine protected 62.5% of the hamsters against lethal infection ($P < 0.05$). Additionally, this vaccine induced specific IgG antibody response and conferred sterilizing immunity to 80% of animals. In contrast, all hamsters that received the other DNA vaccines died during the experiment. **Main conclusion:** Our results indicate that the conserved region of LigA and LigB proteins (LigBrep) is an immunoprotective domain and can be used as a DNA vaccine for leptospirosis control. **E-mail:** thais.larreoliveira@gmail.com

Lept4. **Immune response elicited in mice by two adhesins of *Leptospira interrogans*.**

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Introduction: Leptospirosis is an emerging infectious disease caused by pathogenic *Leptospira*. The strategy for controlling the disease involved prophylactic measures that have been hampered by the lack of protective and conserved antigens. Since the sequencing of leptospiral genomes, putative membrane proteins conserved among *Leptospira* strains have been searched for the development of vaccines. The immune response characterization of antigen candidates is an important step in the investigation of subunits that may confer protection. **Material and Methods:** LIC11084 and LIC11228 are predicted hypothetical outer membrane proteins identified in genome of *Leptospira interrogans* sv. Copenhageni and chosen for our studies. These genes were amplified by PCR with specific primers and cloned into pAE expression vector. After expression in *E. coli* BL21 SI, recombinant proteins were purified through metal chelating chromatography. The interaction of these proteins with extracellular matrix or serum components was analyzed by ELISA. The characterization of immune response was evaluated in mice immunized with the proteins plus adjuvant ($Al_2 (OH)_3$) followed by two boosters, at two-week intervals.

Antibody titers, lymphocyte proliferation and cytokines measurements were performed. **Results:** rLIC11228 and rLIC11084 were expressed in insoluble and soluble form, respectively, and both were successfully purified. rLIC11228 interacts with laminin, collagen type I, plasma fibronectin, plasminogen and C4bp, while rLIC11084 binds to laminin, plasma fibronectin, cellular fibronectin and plasminogen. These proteins were able to stimulate mice immune system as titer of antibodies reached 1:200,000 and 1:25,000 for antigens rLIC11084 and rLIC11228, respectively. Moreover, statistically significant proliferation of lymphocytes was obtained with rLIC11084 immunized mice after *in vitro* stimulation of spleen cells. In addition, this antigen was able to elicit the production of IFN-γ cytokines. **Main Conclusions:** The rLIC1128 and rLIC11087 proteins are novel leptospiral adhesins and plasminogen binding receptors that may be involved in pathogenesis. The rLIC11084 protein is a potential vaccine candidate capable of inducing humoral and cellular immune responses. **Email:** souzanm@usp.br

Lept5. **Human *Leptospira* infection is associated to higher levels of plasminogen activators and metalloprotease activity.**

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Aspects of pathogenesis and virulence of *Leptospira* after entrance in the host are poorly understood. Recently, we have described the interaction of *L. interrogans* with the human fibrinolytic system by the ability of capturing plasminogen (PLG) on the surface. The proteolytic activity acquired by surface-bound PLG/plasmin renders the leptospires the capacity of degrading extracellular matrix components and of evading the immune system. Moreover, several outer membrane leptospiral proteins have been identified as PLG-binding receptors, suggesting the interaction with the fibrinolytic system might be important during leptospirosis. In the present study, we focused on the analysis of leptospiral activation of proteolytic systems during leptospirosis in humans. Sera from individuals with confirmed leptospirosis in the initial and convalescent phases of the disease were evaluated. The PLG activators uPA and tPA were quantified by commercial ELISA kits based on capture method, and the metalloproteases (MMPs) were analyzed by gelatin and collagen type I zymography and specific activity kits. The results indicate that leptospirosis seems to be associated to altered quantities of circulating uPA and tPA PLG activators in the sera when compared to healthy individuals. Furthermore, uPA activity is higher in leptospirosis condition. As demonstrated by zymography and activity tests, MMP-9 levels are enhanced in the sera from leptospirosis patients. The zymography profile of these sera seems to be specific to leptospira infection, as unrelated infectious diseases present different profiles with no MMP-9 stimulation. Altogether, the data suggest that leptospires interact with the fibrinolytic system in a way to stimulate the availability of PLG activators on the circulation. In addition, leptospires are able to elicit the secretion of MMPs by the host cells that in turn contribute to activate the proteolysis, disrupting the extracellular matrix and endothelial tissues, favoring the penetration and invasion of the bacteria. **E-mail:** monicalarucci@butantan.gov.br

Lept6. **Heterologous expression and characterization of two new proteins presents in *Leptospira***

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Introduction: Leptospirosis is an emerging zoonotic disease caused by spirochetes from the genus *Leptospira*. Found worldwide, leptospirosis is more common in tropical and sub-tropical areas where environmental and socioeconomic conditions favor its transmission. Humans most commonly become

infected through direct or indirect contact with the urine from carrier animals. Vaccines currently available are bacterins. These vaccines have short term immune response, several side effects and are protective only against the serovars included in the vaccine preparation. Recombinant leptospiral vaccines are currently being assessed, but to date only partial success has been obtained. In this study, two new recombinant leptospiral proteins were produced for future use as vaccine candidates. **Materials and Methods:** *In silico* analysis on *L. interrogans Icterohaemorrhagie* Copenhageni Fiocruz L1-130 genome was conducted to identify genes of potential subunit vaccine candidates. These sequences were amplified from the virulent *L. borgpetersenii* serogroup Ballum strain 4E by polymerase chain reaction (PCR). The sequences were cloned in pAE *E. coli* expression vector. The recombinant vectors were characterized by PCR and restriction digestion. *E. coli* BL21 Star™ (DE3) was electroporated with recombinant vectors and grown to exponential phase when expression was induced by IPTG. After solubilization with urea purification buffer, recombinant proteins were purified by Ni+ affinity chromatography and visualized in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot (WB) with anti-6xHis antibody. Finally, to assess how conserved these genes are throughout pathogenic *Leptospira* species the same PCRs above were carried out with genome DNA from 18 pathogenic serovars: *L. interrogans* serovars Pomona, Canicola, Icterohaemorrhagiae, Autumnalis, Bataviae, Bratislava, Djasiman, Hebdomadis and Muenchen; *L. borgpetersenii* serovars Ballum, Castellonis, Mini, Poi, Sejroe and Javanica; *L. kirshneri* serovars Grippotyphosa and Cynopteri and *L. santarosai* serogroup Pomona. **Results:** Two genes were selected for recombinant protein expression in *E. coli* heterologous expression system: *LIC11207* and *LIC20087,* a lipoprotein and an OMP, respectively. The cloning procedures resulted in pAE/*lic20087* and pAE/*lic11207* recombinant vectors as indicated by PCR and restriction characterization. Recombinant proteins were purified and the SDS-PAGE and WB revealed rLIC20087 and rLIC11207 with 33 kDa and 37 kDa, respectively. The genes were also amplified from all the genomes tested. **Main conclusions:** Recombinant proteins LIC20087 and LIC11207 were obtained by *E. coli* heterologous expression. The respective genes are present in different species and serovars, and a vaccine developed with them could induce cross-protection. The subunit vaccine potential of rLIC20087 and rLIC11207 is currently being assessed in hamster model. **E-mail:** f.e.valiati@gmail.com

Lept7. **Hamster IgG2-based immune response to leptospiral bacterin**

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Introduction: Leptospirosis is the most common zoonosis worldwide, occurs mainly in the tropics and mortality rates range from 10 to >50%. Vaccines are an effective strategy for the control of leptospirosis. However, protection is restricted to the serovar (or closely related serovars) used in the bacterin. Subunit vaccines have shown promising results, but little is known about the immune response required to induce protection. An understanding of the immune response caused by bacterins will improve the development of effective vaccines against leptospirosis. The aim of this study was determine which immunoglobulin G (IgG) isotype is related to protection in the hamster model of leptospirosis. **Materials and Methods**: Golden Syrian hamsters were distributed in four groups (n=7 by group) that were immunized with : A) Bacterin only; B) Bacterin followed by lethal challenge; C) PBS only; D) PBS followed by lethal challenge. Two doses of bacterin (10^8 heat-inactivated leptospires, *Leptospira interrogans* serovar Copenhageni) or PBS were administered on day 0 and day 14. Hamsters were challenged with 500 leptospires (5× the LD_{50}) 14 days post-immunization. Twenty-one days after challenge all surviving animals were euthanized. Blood samples were collected by phlebotomy of the retro-orbital venous plexus before vaccine and challenge administration and 8 days later. An ELISA was developed using recombinant LigBrep and LipL32 leptospiral proteins as previous described. Sera were diluted 1:100 in PBS-T and secondary peroxidase conjugated antibody to hamster IgG, IgG1, IgG2/3 and IgG3 were diluted 1:6000. Reactions were revealed with OPD and the absorbance was read at 492nm. **Results**: No deaths occurred in groups A, B and C, while all animals in group D died. Vaccinated animals showed a significant increase in IgG levels. Isotyping found no evidence of anti-IgG1 and IgG3 antibodies in the hamster sera. However, there

was a strong IgG2 response before and after challenge. In group D animals the IgG and IgG2 levels increased eight days after challenge. No significant antibody response was observed in animals that were immunized with PBS. **Main conclusions**: Bacterin, the only leptospirosis vaccine commercially available that offers 100% protection and sterilizing immunity, appeared to induce an IgG2 based antibody response. Currently, new experiments are on-going to elucidate whether the protective immune response is correlated with high IgG2 titres. If so, vaccine preparations based on recombinant proteins could be developed with adjuvants to stimulate an IgG2 response.**E-mail:** grassmann.aa@gmail.com

Lept8. **A novel point-of-care test for leptospirosis based on Dual Path Platform (DPP) technology to differentiate IgM and IgG antibody responses**

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Introduction: Improved diagnostics for leptospirosis are urgently needed, especially for resource-poor countries where leptospirosis is a major public health problem. The gold standard serodiagnostic test, the microagglutination test or MAT, is complex, time-consuming, labor-intensive, and unavailable in remote settings. **Materials and Methods:** A rapid (20 minutes) serodiagnostic test for leptospirosis was developed using recombinant leptospiral immunoglobulin-like (Lig) protein fragments and a modification of the Dual Path Platform (DPP) technology, namely dual DPP (D-DPP). The distinct feature of this new immunoassay format was that the test detects separately IgM and IgG antibodies such that each response can be measured semi-quantitatively by a reflectance reader device. The test was evaluated in a pilot study using acute (n=91) and convalescent-phase (n=96) serum samples from patients with confirmed leptospirosis and control individuals (n=50) from Brazil. **Results:** In this evaluation study, the D-DPP assay detected 80% of patients with acute-phase leptospirosis and 100% of patients with convalescent leptospirosis. The specificity was 100%. Among acute-phase cases, 60% developed both detectable IgM and IgG antibodies, 13% had only IgM, while 7% produced only IgG antibodies. During convalescence, 77%, 0%, and 23% of leptospirosis patients developed IgM and IgG, only IgM, and only IgG antibodies, respectively. **Conclusions:** We designed a recombinant Lig protein-based D-DPP assay which can rapidly identify patients with acute-phase or convalescent leptospirosis with high accuracy. The ability to detect IgM and IgG antibodies separately improves diagnostic specificity as well as detection of patients who present at different times in the course of illness. The test therefore has the potential to provide effective point-of-care diagnosis in field settings in Brazil and resource-poor countries worldwide where leptospirosis is an emerging health problem. **E-mail:**klyashchenko@chembio.com

VIRAL DISEASES

HIV/AIDS

HIV/AIDS1. **"Platelet activation and activation of cell death pathways in platelets isolated from HIV/AIDS patients"**

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Introduction: The acquired immunodeficiency syndrome (AIDS) is a pandemic threat. Around 1.800.000 deaths globally were registered only in 2010, accordingly with the Joint United Nations Programme on HIV/AIDS (UNAIDS). New data have emphasized long term complications of HIV infection such as cardiovascular disease and thrombotic events. Platelets have a paramount role on thrombus formation. Recent publications have demonstrated mechanisms of platelet-HIV interactions. Platelets are able to internalize HIV through surface molecules CLEC-2 and DC-SING. We thus hypothesized that platelets from HIV infected patients might have altered function profile compared with platelets obtained from healthy volunteers. The aim of this work is to characterize platelet response isolated from HIV patients. **Material and Methods:** Platelets were isolated from platelets-rich-plasma (PRP) from HIV/AIDS patients and healthy volunteers. *P-selectin* (CD62) was analyzed by flow cytometry (BD FACScan Excalibur) as a marker of platelet activation and degranulation. Mitochondrial dysfunction and cell death pathways were analyzed by mitochondrial membrane potential, caspase-9 activity and phosphatidylserine exposure. **Results:** We demonstrate a higher expression of P-selectin on the surface of platelets isolated from HIV/AIDS patients when compared with platelets isolated from healthy volunteers. In addition, we demonstrate that HIV platelets are less responsive to thrombin stimulus than control platelets. These data corroborate with the idea of "platelet exhaustion" previously described for HIV infection. As activation of cell death pathways has been described in agonist-stimulated platelets *in vitro*, we assessed whether it occurs in activated platelets during HIV infection. We observed that platelets from HIV patients show signs of cell death as mitochondrial alterations, caspase-9 activation and phosphatidylserine exposure. **Main Conclusions:** Our results suggest that platelet activation in HIV infection initiate cell death pathways in platelets, which might be an important mechanism of HIV-associated thrombocytopenia. Moreover, platelet dysfunction might correlate HIV infection towards AIDS. **Supported by:** CNPq, FAPERJ, FIOCRUZ. **E-mail:** emersom.mesquita@ipec.fiocruz.br

HIV/AIDS2. **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 Sao 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 Sao 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 deoxycholate (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 deoxycholate 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 coinfected individuals countrywide and efforts to that should be done by authorities. **E-mail:** igorthiago@usp.br / jlindoso@usp.br

HIV/AIDS3. **Oral treatment with HIV protease inhibitors lopinavir/ritonavir and atazanavir influenced the functions of BALB/c macrophages incubated *in vitro* with *Leishmania amazonensis*.**

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Introduction: HIV antiretroviral therapy has been associated to the worsening of the clinical manifestations of the tegumentary leishmaniasis and to the recurrence of visceral leishmaniasis after successful anti-*Leishmania* therapy. In this way, this work aimed to evaluate the influence of the treatment with HIV protease inhibitors lopinavir/ritonavir and atazanavir on the functions of BALB/c macrophages incubated *in vitro* with *L. amazonensis*. **Material and methods**: BALB/c mice were treated once a day, orally, for ten days with 200 mg/50mg/Kg lopinavir/ritonavir (LPV/RTV) or 90 mg/Kg atazanavir (ATV) or saline as control. Peritoneal macrophages were infected *in vitro* with stationary phase *L. amazonensis* (IFLA/BR/67/PH8) promastigotes, at a 2:1 ratio, for 24 hours. The average of *L. amazonensis*/macrophage and the permanence of macrophages adhered to coverslips were assessed by light microscopy. Nitric Oxide (NO) production, hydrogen peroxide and the cytokines IL-1β, IL-6 and IL-10 were determined by Griess reaction, Pick and ELISA methods, respectively. **Results**: The treatment with LPV/RTV and ATV influenced slightly the average number of *L. amazonensis*/macrophage since it increased the median from 1, 0 in the group treated with saline up to 1, 42 in the group treated with LPV/RTV (p=0,005, Mann-Whitney) and up to 1, 14 in the group treated with ATV (p=0,012, Mann-Whitney). However, they simultaneously elevated the resistance of macrophages against death since the mean ± SD of the number of macrophages per coverslip was higher (p<0, 05, t test) when mice were treated with LPV/RTV (13300 ± 7303) and ATV (15222 ± 5312) compared to those treated with saline (7352 ± 3739). Furthermore, the median of the NO production from macrophages treated with LPV/RTV (1, 89 µM) and ATV (1, 21 µM) was higher (p<0, 05, Mann-Whitney) than those treated with saline (0, 15 µM). Similarly, macrophages released more IL-1β (p=0,002, t test) when they were derived from mice treated with ATV (119, 6 ± 31, 0 pg/ml) than when mice were treated with saline (25, 60 ± 38, 88 pg/ml). Differently, the releasing of IL-6, IL-10 and hydrogen peroxide was not influenced by the treatment with LPV/RTV and ATV. **Conclusions**: Our results showed that the treatment with LPV/RTV and ATV slightly increased the average number of *L. amazonensis* by macrophage, but it also enhanced substantially the survival rate of infected macrophages. The *Leishmania amazonensis* down modulates the production and releasing of IL-1β and NO to survive and multiply inside macrophages, we believe that the elevated production of IL-1β and NO by macrophages from mice treated with LPV/RTV and ATV may have contributed to the increased survival of macrophages demonstrated in our assays. These data suggest that these medicines may collaborate to the defense against *Leishmania* parasite in patients co-infected with HIV/*Leishmania*. **E-mail**: ericaalvesvet1@gmail.com

HIV/AIDS4. **Efficacy and safety of nevirapine- versus efavirenz-based antiretroviral therapy in TB/HIV co-infected patients in Burkina Faso: a clinical and pharmacokinetic study.**

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Introduction: rifampicin (RIF) is an essential component of anti-TB regimen but concomitant use of this drug with non-nucleoside reverse transcriptase inhibitors is complicated by drug-drug interactions. Nevirapine (NVP) concentrations are significantly decreased by concomitant RIF use, with potential risk of sub-therapeutic plasma concentrations and virologic failure. However, NVP-containing regimes of ART are the most commonly used in Africa, and NVP has advantages over EFV, like safety in pregnancy, availability as fixed dose combinations, and lower costs. In the present study we aimed to evaluate the efficacy and safety of NVP- versus EFV-based ART in TB/HIV co-infected patients. **Patients and Methods**: from October 2008 to May 2010 an open-label, non-inferiority, randomized controlled trial was conducted involving adult TB-HIV co-infected patients in Burkina Faso. Anti-TB therapy consisted of rifampicin, isoniazid, pyrazinamid and ethambutol in fixed dose combination. After written informed consent, patients were randomized to a fixed combination of stavudine, lamivudine plus nevirapine 200 mg twice a day (NVP group) or efavirenz 600 mg once a day (EFV group). CD4+ cell count, viral load, and transaminases were measured at enrolment, at week 12, 24 and 48. Three NVP pharmacokinetic curves were obtained after 4 (T0) and 16 weeks (T1) of RIF+NVP and 30 days after termination of anti-TB therapy (T2). **Results:** 69 TB/HIV patients were enrolled: 33 in NVP group and 36 in EFV group. Patients had a median age of 37.8 years (range 23-50), and 49% (34) were male. Fifty-seven patients (83%) had pulmonary TB (70% smear-positive). CD4+ cells count; viral load and transaminases levels were similar in the two groups at enrolment. At week 48, 77% (22/33) in NVP group and 81% (29/36) in EFV group (51 in total) were evaluated (p=0.27). Nine patients (18%) died during follow-up, 78% in the first month of anti-TB therapy; 7 (32%) in NVP group and 2 (7%) in EFV group (OR= 6.30; 95% CI 1.16 – 34.26; p= 0.03). Eight adverse events grade III or IV occurred, 3 in NVP and 5 in EFV group (p= 1.0). There was no significant difference between groups respect to mean CD4+ cell increase at week 48 (219 ±150 in NVP group and 190 ±134 in EFV group). Viral suppression (HIV1-RNA<20 copies/mL) was reached by 71% of patients in NVP group and 64% in EFV group (p= 0.76). TB treatment success was obtained in 56 patients; 79% in NVP group and 83% in EFV (p= 0.9). The median NVP C_{trough} was significantly reduced at T0 (4.6 mcg/ml), and T1 (3.5 mcg/ml) compared to T2 (6.5 mcg/ml; p=0.006 and p=0.001, respectively). Two patients with virologic failure had NVP C_{trough} below the threshold level of 3 mcg/ml. **Main conclusions:** Immunologic and virologic responses in NVP group were not significantly different from those observed in EFV group at week 48. However, a higher number of deaths was observed in NVP group and the concomitant use of RFM reduced significantly NVP C_{trough} levels. **E-mail:** a.carvalho@libero.it

HIV/AIDS5. **Methodological flaws of STI-HIV prevention trials**

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Introduction: Substantial evidence indicates that sexually transmitted infections (STIs) promote HIV transmission and acquisition. Ten randomized controlled trials in sub-Saharan Africa examined effects of STI-treatment and prevention programs on HIV incidence. Only the trial in Mwanza, Tanzania, produced a statistically significant difference in HIV incidence between treatment and control arms. Consequently, support for STI treatment for HIV prevention has faded. **Material and methods:** We conducted an intensive review of methods and outcomes of the 10 trials and subsequent analyses of the trials. **Results:** The 9 trials after Mwanza had serious methodological problems that make it impossible for those trials to show whether STI-control programs reduce the spread of HIV; the trials' designs prevent them from answering the question they pose. Because of ethical imperatives to treat anyone presenting with disease, in trials since Mwanza, control participants received aggressive STI treatment, resulting in little difference in interventions between treatment and control arms, small differences in STI incidence, and insignificant differences in HIV incidence. Moreover, reports of post-Mwanza trials assert that interventions did not reduce HIV incidence, but their evidence cannot support those assertions since none actually measured change in HIV incidence because they did not have data on baseline incidence. The trials could only show that similar interventions led to similar levels of HIV incidence, but could not show whether interventions in either arm reduced, increased, or left HIV incidence unchanged. We derive evidence that suggests that most trials were very successful in reducing HIV incidence. **Main conclusions**: Evidence from the trials does not support recent published statements dismissing STI control for HIV prevention. The design of future trials would face similar ethical constraints to treat

controls, so more trials will still not answer the question, can STI control reduce HIV? Since there is abundant evidence that STI treatment reduces HIV transmission and since constructing trials that are both conclusive and ethical is not possible, policy makers should roll out STI control for HIV prevention now, not wait for the elusive 'crucial experimented**-mail:** stillwaggon@gettysburg.edu

HIV/AIDS6. **Designing and biological evaluation of bivalent HIV-1 and HCV candidate vaccine**

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Objectives: Infection with human immunodeficiency virus (HIV) and Hepatitis C virus (HCV) are the health problems worldwide. Therefore, they are considered for the huge number of studies looking for effective vaccines. In previous study, we introduced a single cycle replicable (SCR) HIV system that, completely maintained the antigenic structures of HIV-1, through its one cycle replicating properties represented a good implication as a potential vaccine candidate, Herein, we designed and constructed a novel HIV-1 virion, capable of expressing nonstructural 3 (NS3) protein of HCV as bivalent candidate vaccine that provides a more immunogenicity, while preventing any pathologic effects with further evaluated its biological properties. **Methods:** The pIPNL4-3/NS3 containing HIV genome of NL4-3 strain with a 2-kb deletion in reverse transcriptase (RT) and integrase (IN) genes and replacement of the deleted fragment with NS3 was constructed, confirmed by sequencing reactions and transfected into HEK 293T cell line. By further co-transfection of psPAX2 and pMD2.G plasmids, which encoded HIV Gag-pro-pol and vesicular stomatitis virus surface glycoprotein, into the same pIPNL4-3/NS3-harboring cells, pseudotyped virions were produced, evaluated by electron microscopy, quantified using P24 end-point ELISA assay and western blotting. Infectivity of recombinant virions and their efficiency towards the syncytium formation was evaluated on HIV-sensitive MT-2 cells. **Results:** Production of HIV virions was indicated by the level of P24 protein in culture supernatant of transfected cells and was further confirmed by electron microscopy. Also, expression of NS3 protein was confirmed using western blotting. Formation of syncytia in MT-2 cells also evidenced for the functionality of the surface glycoproteins in produced pseudotyped virions. Interestingly, infectivity analysis verified that the second generation virions were completely non-replicative. **Conclusion:** The results were shown that a new recombinant virion with capable to express NS3 protein completely maintained the antigenic structures of HIV-1, by its one cycle replicating properties , and represented a good implication as a potential bivalent vaccine candidate. Moreover, this guarantees further investigations toward the assessment of its immunogenicity, which are currently under process. It may also present another interesting approach towards the improvement of its application in bivalent HIV and HCV vaccine researches. **Keywords:** HIV-1, HCV, Bivalent vaccine. **E-mail:** mrasadeghi@pasteur.ac.ir

HIV/AIDS7. **High frequency of *MBL2* gene polymorphism in patients with human T-cell lymphotropic virus type 1/2 (HTLV-1/2)**

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The HTLV is endemic in Brazil and cause various clinical manifestations. However, most patients remain asymptomatic, which may be related to virus or individual genetic factors that influence the immune response. Mannose binding lecting (MBL) is an innate immunity molecule with important role in activating complement system. It has been reported that single nucleotide polymorphisms (SNPs) at *MBL2* gene related to low serum levels of MBL may contribute to an increase of the pro-viral load of HTLV and, therefore, could be involved with the clinical course of disease. Thus, the aim of this study was to investigate the influence of the *MBL2* gene SNPs in the promoter and structural regions in the clinical progression of patients infected with HTLV, compared to control group without infection. *MBL2*

genotyping was done by Real Time PCR technique, using Taqman probes for promoter regions (-550 and -221) and for the exon 1 region it was used the SYBER GREEN chemistry. We enrolled 232 control individuals and 117 patients with HTLV attended at HUOC/UPE, and the patients were separated in symptomatic (HAM/TSP) and asymptomatic groups. The results showed that wild genotype Y/Y was higher in asymptomatic patients with HTLV infection than in control group, with a frequency of 77,91% in asymptomatic patients and 56,5% in control individuals, compared to genotypes with variant allele Y/X + X/X, whose frequency was 22,09% and 43,6%, respectively (p=0,0008; OR=2,75; IC=1,48-5,17). The same relation was observed in the frequencies of patients vs. controls: Y/Y genotype had a frequency of 77,91% in all infected individuals and 56,5% in control group, while genotypes with mutant allele Y/X + XX had 70,3% and 52,9%, respectively (p=0,002; OR=2,26; IC=1,33-3,88). Nevertheless, no differences were observed in the -221 region genotypes between asymptomatic and symptomatic group. There was also no significant difference in the allelic and genotypic frequencies, as well as in haplotype, of -550 promoter and exon-1 polymorphisms in all groups investigated. Therefore, although it seems to be related to an increased susceptibility to HTLV, the genotype of high production of MBL apparently influences a clinical course of disease related to asymptomatic condition. However, a larger number of symptomatic patients are necessary to investigate if the YY genotype is exclusively associated to asymptotic course of HTLV disease. **Keywords:** HTLV, *MBL2*, polymorphisms, immunogenetics. **E-mail:** anessateixeira16@hotmail.com

DENGUE

Deng1. **Dengue Induces Platelet Activation, Mitochondrial Dysfunction and Apoptosis through Mechanisms that Involve DC-SIGN**

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Dengue is the most prevalent human arbovirus disease in the world. Subjects infected with dengue virus (DENV) usually have a self-limiting febrile illness but, in some cases, develop a life-threatening syndrome accompanied by bleeding and shock. Thrombocytopenia is also frequently observed in mild and severe disease. Although the mechanisms are incompletely understood, thrombocytopenia may occur as the DENV interacts with and directly activates platelets. Therefore, we examined responses of platelets that were freshly-isolated from patients with dengue. In parallel, we determined whether DENV induced direct activation of platelets that were obtained from healthy subjects. We found that platelets from dengue-infected patients display increased activation when compared to control subjects, a finding that is more pronounced in the presence of thrombocytopenia. DENV infection also induces mitochondrial dysfunction and activation of apoptosis pathways in platelets. Similar results are observed when platelets from healthy subjects are directly exposed to DENV. Our data also indicate that DENV activates platelets, in part, via DC-SIGN (*D*endritic *C*ell-*S*pecific *I*ntercellular adhesion molecule-3-*G*rabbing *N*on-integrin). Together these results demonstrate that DENV directly induces mitochondrial dysfunction and apoptosis in platelets, which may contribute to platelet activation and the genesis of thrombocytopenia in patients with dengue. **E-mail:** eugeniohottz@gmail.com

Deng2. **Experimental study on immune response following dengue virus infection in marmosets (*Callithrix penicillata*)**

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Introduction: Dengue is an arboviral disease caused by the four serotypes of *Dengue virus* (DENV, Flaviviridae, *Flavivirus*). The pathogenesis of DHF is poorly understood due to the occurrence of atypical immunologic responses involving the production of cytokines and chemokines, activation of T cells and imbalance of the coagulation system and homeostasis, and lack of an experimental model that mimic the human illness, and therefore the understanding of pathogenesis DENV infection remains a challenge. **Material and methods:** In this study we sought to investigate the susceptibility of marmosets (*Callithrix penicilata*) to sequential infections by DENV3 and DENV2 strains isolated from fatal human cases in Brazil. For such, twenty-two animals were primarily infected with DENV3 (3.23×10^3 PFU/ml) and, sixty days post-infection (dpi), eleven of them were secondarily infected with DENV2 (4.47×10^4 PFU/ml). Plasma and sera were daily collected for seven days and thereafter in specific time points. Viral RNA was assessed by real-time RT-PCR, while cytokines were detected by Cytometric Bead Array assays. **Results:** In the primary infection viremia lasted for 6 dpi; increased levels of TNF-α, IFN-γ and decreased levels of IL-5 were found. In secondary infection viremia lasted 7 dpi; decreased levels of IL-6, TNF-α and increased levels of IFN-γ and IL-5. The high levels of INF-γ and TNF-α indicates activation of the pro-inflammatory response with the subgroup Th1 differentiation (cellular response) and inhibition from proliferation of Th2 cells (humoral response) characterized by low levels of IL-5. Neutralizing antibodies may have led clearance of the inflammatory response during the secondary infection. **Conclusion:** Altogether, the results indicate the susceptibility of marmosets (*Callithrix penicillata*) to dengue field strains recovered from fatal cases and qualify this animal as an excellent model to evaluate the primate immune responses to dengue and dengue-vaccine strains. **Key words**: Dengue, primate model, viremia, cytokines. **Email:** mileneferreira@iec.pa.gov.br

Deng3. **Immunogenicity and Safety of CYD Dengue Vaccine in Healthy Children and Adolescents in Brazil**

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Introduction: Dengue is the most common arthropod-borne viral disease. Disease can range from mild febrile illness to life-threatening hemorrhagic conditions. There is currently no vaccine against dengue. A tetravalent dengue vaccine (TDV) comprising recombinant, live, attenuated viruses, one per serotype (CYD-1–4), is being evaluated for protective efficacy in phase III. Previous studies in both endemic and non-endemic populations found that this vaccine was safe and immunogenic, but no data are available for Brazil. **Material and Methods:** A randomized, controlled, double-blind phase II trial was conducted in Vitoria, Brazil, among healthy children aged 9 to 16 years (ClinicalTrials.gov NCT01187433). Participants received either three injections of TDV (Vaccine group, n=100) or placebo (Placebo group, n=50) at Months 0, 6 and 12. Solicited injection site and systemic reactions were recorded daily for 7 and 14 days after each injection. Plaque reduction neutralization test ($PRNT_{50}$) antibody titers against the TDV parental viruses were measured before and 28 days after each injection. Seropositivity was defined as antibody titers ≥10 (1/dil). **Results:** 150 subjects were enrolled, 54.7% were females and the median age was 12.6 years. No vaccine-related serious adverse events were reported. The percentages of subjects reporting solicited injection site reactions were 28.3%, 22.6%, and 16.9% in the Vaccine group and 30.6%, 14.9%, and 15.6% in the Placebo group after the 1st, 2nd and 3rd injections respectively. The percentages of subjects reporting solicited systemic reactions were 61.6%, 45.7%, and 40.4% in the Vaccine group and 53.1%, 44.7%, and 26.7% in the Placebo group after the 1st, 2nd and 3rd injections respectively. Most reactions were mild. Injection site pain and headache were most frequently reported. One month after the third vaccination seropositivity rates were 96.6%, 98.9%, 100%, and 100% for serotypes 1–4, respectively. The corresponding geometric mean titers were 267, 544, 741, and 432. All participants in the Vaccine group were seropositive for at least 2 serotypes after 2 vaccinations. The percentage of seropositivity after 3 vaccinations was 98.9% for at least 3 serotypes, and 96.6% for all 4 serotypes. **Main Conclusions:** A three-dose TDV regimen had a satisfactory safety and reactogenicity

profile in children and adolescents and elicited neutralizing antibody responses against all four serotypes in a population in Brazil. These findings support the continued development of this vaccine. **E-mail:** gustavo.dayan@sanofipasteur.com

Deng4. *In vitro* effects of DENV3 primary isolates that inhibit interferon in mammalian cells on mosquito antiviral responses

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Introduction: Anti-dengue responses in mosquitoes involve at least two immune signaling pathways (Toll and JAK-STAT) and the RNA interference pathway. Another immune pathway, Imd, may also have a role in anti-dengue defenses in insects. In order to continue its life cycle, the virus must survive immune responses of the invertebrate host. In the case of dengue virus (DENV), a recent study showed that it suppresses some of the mosquito immune defenses *in vitro*. Moreover, we recently showed that immune manipulation of mosquito defenses by the virus probably occurs *in vivo*. In vertebrates, the virus must also find a way to survive immune responses and one of the known mechanisms is the inhibition of the interferon (IFN) pathway. **Materials and methods**: Here, we selected four primary isolates of DENV serotype 3 (DENV-3) that inhibit or not IFN signaling in mammalian cells, to check for their effect on insect immune responses *in vitro*. For that, we are using three reporter genes under the control of promoters of molecules controlled by either Toll, JAK-STAT or Imd pathways upon stimulation. C6/36 (from *Aedes albopictus*) and S2 (*Drosophila melanogaster*) cells were then firstly transfected with the reporter plasmids and further infected or not with DENV3 isolates. **Results and Main Conclusions**: The magnitude of reporter expression was analyzed as a reflection of the respective pathway stimulation. Any significant difference of reporter expression between non-infected and infected cells is being analyzed as a reflection of virus manipulation of the immune pathways. This work will allow us to see if DENV-3 isolates that inhibit IFN signaling pathway in mammalian cells are also able to suppress any of the mosquito immune pathways, adding relevant information on virus adaptation in both hosts. **E-mail:** tecamagalhaes@hotmail.com.

Deng5. MBL serum levels and MBL binding activity in dengue infected patients

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Dengue is an important arbovirosis that causes an acute disease and it is endemic in Brazil. The complement system (CS) may be involved in dengue immunopathology associated with increased severity, influencing for example the occurrence of thrombocytopenia. Mannose-Binding Lectin (MBL) activates CS by recognition of pathogen-associated molecular pattern of Dengue virus (DENV). Thereby, the MBL activity could influence disease progression and has been associated with dengue severity. The exon-1 polymorphisms (SNPs) in MBL2 gene (allele O) may alter the conformation of MBL, reducing the quality/binding activity of this lectin. This would be beneficial because it would serve as a protective factor against progression to gravity, being a prognosis indicator of this disease. Thus, this study aimed to evaluate the relationship between the polymorphism in exon-1 region of the MBL2 gene, which influences the serum levels of functional MBL oligomers, and binding activity of MBL molecules present in the serum of patients infected with DENV. It was performed a MBL binding manana ELISA for detected the binding activity of MBL oligomers, besides a commercial ELISA kit (Bioporto Diagnostics) that detected high molecular weight oligomers of MBL, in the serum of 77 patients with positive serology for dengue, attended at HUOC/UPE in 2010, which were divided into 3 groups as follows: dengue fever (DF, n= 38), complicated dengue (DC, n=19) and dengue hemorrhagic fever (DFH, n=20). Genotyping of exon-1 region of MBL2 was performed using Real Time PCR technique by SYBR GREEN chemistry, and data

were analyzed using the software GraphPad Prism 5.0. The results showed significant differences in MBL binding activity between exon-1 genotypes, with high activity associated with the AA genotype ($p<0.001$). It was also seen the existence of correlation between the binding assay and the MBL serum levels ($p>0.001$, Spearman $r = 0.6655$). Thus, it seems that the polymorphism in structural region of MBL2 gene influences the molecule function and the binding activity in patients with dengue infection. However, no association was found between MBL levels or binding activity and dengue disease forms. **Keywords:** Dengue, MBL, binding activity, polymorphisms, complement. **E-mail:** vanessateixeira16@hotmail.com

Deng6. **Social representations about education and control of dengue campaigns**

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Health policies and actions to combat dengue are based on vector control, pushed into the background activities of health education. Understand the social representations of professionals and users of health on family education campaigns for dengue control is the goal of this research aimed at controlling the disease. Qualitative study was conducted with semi-structured interviews with professionals and users of the health strategy of the family of six selected cities, and the data tabulated by the technique of Collective Subject Discourse, resulting in four discourses: on the speech showed that the campaigns are of quality but no continuity found four distinct aspects: 1) on the means and forms of dissemination, with television and other media of mass appearing as a source of learning, and local media rarely used, 2) about the lack of continuity shows that the incorporation of the continuous character of an action is lacking in campaigns that occur at specific times, depending on the seasonality of the disease, 3) campaigns need to shift focus to highlight the occurrence of dengue and endemic growth of complications that increases the number of severe cases and deaths, and 4) campaigns have investments but the return does not appear, since the professionals perceive little attention with respect to distributed educational materials about dengue. About the discourse of shares affected by the requirement of productivity we see that even if they disagree with the methodology of work for goals, respondents accept the situation a decision to be vertical. In the third speech, it is observed that there is achieved by limiting public educational activities, taking advantage of the opportunities for discussion already in place, such as meetings of hypertensive, diabetics and pregnant women. On the last speech, it is observed that the information available but are not observed in daily life: there is high rate of knowledge of content, but insufficient knowledge about the disease severity by little emphasis on this aspect of the campaigns of dengue. We conclude that the social representations of dengue between both groups are very similar, showing similarities in many central ideas. The level of awareness of population about the disease is significant, but not reflected in preventive behavior, and communication between professionals and users based on the transfer of information on prevention and treatment. Thus, it considers it necessary improvements in the quality of health services at all levels of care, and implements specific actions aimed at changing behaviors and keep them long term. **E-mail:** cassia@uems.br

DISEASES BY OTHER VIRUS

Vir1. **Hantavirus in the State of Minas Gerais, Brazil: Analysis of the Clinical and Epidemiological Profiles in a Series of Cases between 1998 and 2007**

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Hantavirus cardiopulmonary syndrome (HCPS) is an emerging disease transmitted by the inhalation of wild rodent excreta and affecting mostly agriculture workers and other professionals in contact with these animals. HCPS transmission is relatively recent in the State of Minas Gerais, Brazil, but it has been persistent, which imposes constant challenges for control and disease surveillance. The objective of this study was to investigate the clinical and epidemiological aspects of HCPS cases that occurred in Minas Gerais in the period 1998-2007, started in the recording of the first case. This retrospective observational study was conducted by collecting data from notification forms and reports of epidemiological surveillance and Ezequiel Dias Foundation. For case definition were using the criteria established by the Ministry of Healthy. Of the 193 cases of HCPS, 77 died, representing a mortality rate of 39.9%, with kidney failure, dyspnea, hemorrhagic manifestations and hematocrit >45% as associated risk factors (p≤0,005). Males were most affected with 76.2% (n=147) of confirmed cases and median age of 34.9 years. There was an expansion of the occurrence area with 41 municipalities included, with emphasis on the Triângulo Mineiro/Alto Paranaíba region, concentrating 67.3% of cases. The cases were detected mainly between March and August, showing the seasonal pattern of the disease. Contact with rodents (40%) and activities associated which agriculture (53%) were the main epidemiological history mentioned, as well as the rural environment and work place as the probable local of infection (79.2%). Fever (96.3%), dyspnea (86.7%) and myalgia (79.9%) were the more frequent symptoms, and hemorrhagic manifestations were observed in 9.1% of the cases evaluated. Among laboratory and radiographic findings, there was a large proportion of cases with thrombocytopenia/plaquette counting below 150.000 mm^3 (89.9%) and thoracic X-ray showing bilateral interstitial infiltrate (83.4%). Regarding the evolution of the disease, from the onset of symptoms to death, the median duration were four days, and 82.9% of the patients died up to the seventh day after the onset of symptoms. Overall there was an increase in the number of HCPS cases and reduction of lethality during the analyzed period, which may suggest an improvement in the health system. However, there are still a large proportion of fatal cases, which requires vigilance and appropriate assistance. **E-mail:** mariana.gontijo@saude.mg.gov.br

Vir2. **Development of immunochromatographic test for the detection of HFRS and HPS Hantavirus antibody in the human and rodent serum**

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Introduction: Hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) are rodent borne zoonoses caused by hantavirus infection. ELISA and indirect immunofluorescent assay (IFA) have widely been used as serological diagnoses. However, more rapid, simple and specific diagnostic test has been required particularly in the field study. This study is aimed to develop immunochromatographic (ICG) test for diagnoses of hantavirus infection among human and rodent sera. **Materials and Methods**: Rabbit anti rat IgG labeled colloidal gold (Wine red chemicals) and Protein A labeled colloidal gold (EY Laboratories) were used. *N-ternimal* 103 amino acids of N proteins of Seoul type hantavirus (SEOV) and Puumala type hantavirus (PUUV) those are the causative agent for HFRS and Sin Nombre type hantavirus (SNV), that is a causative agent of HPS, were expressed in *E. coli* by using pET43.1 vector (Novagen) and purified by His-Trap column (GE). Serum specimens were examined at 1:75 dilution. **Results:** A total of 340 rat sera which consisted of 19 of experimentally infected laboratory rat sera, 38 of naturally infected sera and 283 of uninfected laboratory rat and urban rat sera were examined. ICG test detected antibody as same level as that of ELISA. The sensitivity and specificity of ICG compared to ELISA and/or IFA were 100% and 99.8%, respectively. A total of 122 human sera, which consisted of 28 of HFRS patients, 30 of HPS patients and 64 of NE patients were examined. The ICG test able to detect and serotyped antibody to SEOV, PUU V and SNV in human sera. The sensitivity of ICG test compared to ELISA was 100% for HFRS patient sera, 92% for PUUV patient sera and 100% for HPS patient sera, respectively. Whole bloods of human and rodent, instead of serum, were also applicable to ICG test. **Main conclusions:** The ICG test was considered as rapid, simple and safe diagnoses of SEOV infection in rats as well as HFRS and HPS virus infection among humans. **E-mail:** j_arika@med.hokudai.ac.jp

Vir3. **Genetic Diversity and Phylogeography of Asama virus in the Japanese Shrew Mole (*Urotrichus talpoides*)**

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Objective: Recent discovery of genetically distinct hantaviruses, including Asama virus (ASAV) in the Japanese shrew mole (*Urotrichus talpoides*) (Family *Talpidae*, Subfamily *Talpinae*) from Mie Prefecture, challenges the long-held view that rodents are the principal reservoir hosts and raises the possibility that soricomorphs may have played an important role in the evolutionary history of hantaviruses. The existence of two distinct chromosomal races of *U. talpoides*, geographically separated by the Fuji and Kurobe rivers in central Honshu, provided an opportunity to study the genetic diversity and phylogeography of ASAV in Japan. **Methods:** Lung tissues of 34 Japanese shrew moles, captured in Ehime, Niigata, Gunma, Okayama and Wakayama between 2007 and 2011, were analyzed for ASAV RNA by RT-PCR using oligonucleotide primers based on sequences of soricid- and talpid-borne hantaviruses. Host identification was confirmed by cytochrome *b* mitochondrial DNA (mtDNA) sequence analysis. **Results:** ASAV RNAs were detected in Japanese shrew moles from Niigata, Gunma and Okayama Prefectures. Pair-wise alignment and comparison of partial S- (432-bp), M- (847-bp) and L-segment (357-bp) sequences showed similarities of 72.7-83.2% and 75.9-96.6% against prototype ASAV strains at the nucleotide and amino acid levels, respectively. Phylogenetic analyses of the newly acquired sequences, generated by maximum likelihood and Bayesian methods using the GTR+I+Γ model of evolution, indicated topologies suggestive of geographic-specific clustering, similar to the phylogeography of soricid- and rodent-borne hantaviruses. Host mtDNA sequence analysis indicated two distinct lineages of Japanese shrew moles, congruent with the ASAV genetic diversity. **Conclusions:** The Japanese shrew mole is widely distributed throughout Japan (except Hokkaido) and is not found on mainland Asia. The close genetic and phylogenetic relationship between ASAV strains and hantaviruses harbored by soricine shrews in Eurasia suggests a possible host-switching event prior to the migration of ancestral shrew moles to Japan, hundreds of thousands of years before present. **E-mail:** arais@nih.go.jp

Arena Virus

Vir4. **Serological assays based on recombinant viral proteins for the diagnosis of viral hemorrhagic fevers caused by arenaviruses**

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Background: Viral hemorrhagic fevers (VHFs) caused by several arenaviruses are one of the most devastating emerging diseases in humans and lead serious public health concerns. It is of great importance to detect these pathogens rapidly and specifically to minimize the risk and scale of arenavirus outbreaks. However, these arenaviruses are classified as the BSL-4 pathogens, making it difficult to develop diagnostics for these virus infections in the institutes without BSL-4 facilities. To get around the difficulties, we have previously established recombinant viral nucleoproteins (rNPs)-based serological assays, such as IgG-enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence assay (IFA), and antigen (Ag)-capture ELISA for diagnosis of VHFs caused by Lassa and Junin viruses. We

applied this approach in developing serological assays for detection of the most recently recognized arenavirus, Lujo virus. **Methods:** Lujo virus rNP prepared by a baculovirus expression system and rNP-expressing HeLa cells were used as antigens for IgG-ELISA and IFA, respectively. Monoclonal antibodies (MAbs) to Lujo virus rNP were prepared and their antigenic epitopes were determined. These MAbs were used as capture antibodies in the Ag-capture ELISA. **Results:** In the IgG ELISA and IFA, rabbit anti-Lujo virus rNP antibodies were detected with a high sensitivity. Sera from Lassa fever patients or LCMV-infected mouse showed only weak cross-reactions to the Lujo virus rNP, indicating Lujo virus NP is antigenically less related to other Old World arenaviruses, as has been suggested by phylogenetical analysis. An epitope mapping demonstrated that the MAbs raised against Lujo rNP recognized specifically the NP of Lujo virus, but not of other arenaviruses. In the Ag-capture ELISA, Lujo virus rNP and authentic Lujo virus was detected as little as 0.8-3.0 ng and 230-470 TCID50 per well, respectively. **Conclusions:** These recombinant viral protein-based assays were proposed to be useful not only for etiological diagnosis of VHFs caused by Lujo virus but also in seroepidemiological studies. **E-mail:** fukushi@nih.go.jp

Arbovirus

Vir5. *In vivo* Identification and characterization of Arbovirus Variants with epidemic potential

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Introduction: Arboviruses (*arthropod-borne*) must initiate, establish, and maintain productive infections in both vertebrate and invertebrates hosts for a successful viral life cycle. These distinct infections ensure that these viruses face numerous selective pressures and bottlenecks, yet the molecular mechanisms involved in overcoming these processes are poorly understood. Chikungunya virus (CHIKV), an *Alphavirus* and member of the *Togaviridae* family, is a re-emerging and significant human pathogen. Genetic diversity and adaptive mutations have been shown essential for the evolution of CHIKV host tropism; thus, we hypothesized that genetic adaptations generated *in vivo* may play important roles in CHIKV transmission and pathogenesis as well. **Materials and Methods**: To address this hypothesis, we infected *Aedes* species of mosquitoes with wild type CHIKV and harvested infectious virus from individual insect organs and saliva at over two weeks of infection. We subsequently sequenced viral genomes present in each fraction to determine the genetic changes that occurred over the course of infection by standard and next-generation deep-sequencing. **Results:** We identified a major sub-population of viruses containing two previously undescribed mutations in the E1 glycoprotein present only in the saliva samples at significant frequencies. We introduced these mutations, both individually as well in tandem, into the chikungunya virus infectious clone, viruses were produced, and mosquitoes and mice were infected. We found both mutations to significantly increase infection, dissemination and transmission rates in mosquitoes compared to the wild type control. In addition, these mutations led to overall increased virulence in mice that correlated with higher viral loads in target organs. Finally, *in vitro* studies showed that the addition of these mutations lead to increased virion stability, as well as increased cell binding kinetics, shedding light on a possible mechanistic role for these mutations *in vivo*. **Main conclusions**: These studies not only highlight the possible epidemic potential of arbovirus adaptive mutations generated during the persistent infection of the mosquito vector, but also emphasize population-based deep sequencing approaches can identify and characterize these variants prior to their emergence in the field. **E-mail:** marco.vignuzzi@pasteur.fr

Flavivirus

Vir6. **A Rapid Non-nested Reverse Transcriptase-PCR Assay for Vertebrate Flavivirus Subgroups Using a Novel Universal Single Primer Pair Based on a Conserved Region of NS5 Gene Sequences**

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Background: Flaviviruses are important pathogens for humans in many regions of the world. The genus Flavivirus of the family Flaviviridae is a highly diverse group of over 70 viruses, including yellow fever (YF), dengue (DEN) types 1–4, Japanese encephalitis (JE), West Nile (WN) fever, and Russian spring and summer encephalitis (RSSE) viruses. Flavivirus infections are clinically often indistinct from and confused with other febrile illnesses. Thus, an essential component in the diagnosis of flavivirus diseases is the ability to have a rapid response so that the necessary treatment can be given quickly. **Objectives:** Transcription of RNA to complementary DNA, followed by the reverse transcription-polymerase chain reaction (RT-PCR), has been used widely for rapid and specific identification of RNA viruses, including flaviviruses. Several universal flavivirus primers have been reported, especially in the NS5 gene and 3'UTR region, but most of them are mixed primers and/or their sensitivity is unknown. The potential problem is that mixed primers may not be effective for detection because they necessarily contain too many varieties of molecular species due to many codon degeneracies. In the present study, we developed a simplified one step RT-PCR method using flavivirus universal primers for the presumptive identification of flaviviruses. **Methods:** We identified conserved sites and produced a non-nested degenerate primer pair covering a region within the encoded part of the RNA-dependent RNA polymerase (RdRp) of the NS5 protein region sequence. A pair of degenerate primer, FVXf and FVXr is designed from these regions and used to generate a 350 base pair cDNA product. We determined the reactivity and specificity of primers for a diverse panel of flavivirus isolates. **Results:** One-step RT-PCR with the universal primer pair test was successful using viral RNA from a wide range of flaviviruses, including mosquito-borne DEN, YF, JE and WN viruses, tick-borne RSSE and Powassan viruses, and also the vector-unknown Yokose virus. The cDNA from each virus isolate was direct sequenced and confirmed the identity with the template RNA. Furthermore, no cross-reaction with alphaviruses, including chikungunya virus, was found with the both primers. **Conclusions:** These findings clearly revealed the broad specificity against flaviviruses of these primers. Thus, the primers will serve as a useful tool for rapid diagnosis of flavivirus infections to distinct other febrile illnesses infected in endemic areas. **E-mail:** ck@nih.go.jp

Hepatitis

Vir7. **Construction and evaluation of optimized Hepatitis "A" virus recombinant vaccine prototype**

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Infection with Hepatitis A Virus (HAV) still affects millions of people around the world, causing a disease that can vary from asymptomatic to severe cases. Despite the existence of commercial vaccines produced from inactivated or attenuated viruses, they are not yet available for most of the world population, because of its high cost end. An attractive alternative is the research and development of DNA vaccines, which potentially offer some advantages over conventional vaccines as greater stability, greater security and possibility of being produced with a lower overall cost. This study aims to construct,

optimize and evaluate the expression and cellular immunogenicity of recombinant vaccine prototypes against Hepatitis A virus. The translatable segment of the genome of HAV is composed of P1 region, formed by structural proteins VP1, VP2, VP3 and VP4; P2 region, where are protein 2A (which has the important function of serving as the first signal for the assembly of the capsid proteins) and 2B and 2C (which participates in the process replication of viral RNA); P3 region, where are the 3C protease (responsible for processing all structural and non-structural proteins) and RNA polymerase 3D. The vaccine sequence was generated from the structural protein genes, and viral 3C protease, being called truncated polyprotein (HAV-PTRUNC). This sequence was also optimized, fused to the N-terminal region of the Lysosome Associated Membrane Protein (LAMP), aiming to promote the secretion of viral antigens and cloned into p43.2 eukaryotic expression vector, leading to the construction of p43.2_N-LAMP_HAV-PTRUNC plasmid. BHK-21 cells were transfected with the recombinant plasmid and the N-LAMP_HAV-PTRUNC expression was detected by immunofluorescence assay using monoclonal antibody raised against N_LAMP. After that, mice were immunized with the prototype and the magnitude of T-cell response was measured by ELISPOT assay using a library of 161 peptides spanning the length of the structural HAV proteins. The response to peptide pools was higher in p43.2_N-LAMP_HAV-PTRUNC (17 to 790 SFC/million splenocytes) than empty vector (4 to 40 SFC/million splenocytes) and PBS (9 to 28 SFC/million splenocytes). Currently we are performing an ELISA test intending to evaluate the capacity of naked DNA to induce humoral immune response and to elucidate if p43.2_N-LAMP_HAV-PTRUNC construct has the potential to be used as an immunogen. **E-mail**: renatosousa@cpqam.fiocruz.br

Vir8. **IL28b rs8099917 and rs12979860 polymorphisms in Brazilian patients infected with HCV GT 1 treated with PEG-IFNa + Ribavirin**

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Introduction: Several studies have demonstrated the importance of Interleukin-28b gene (IL28b) polymorphisms in predicting a successful of therapy for Hepatitis C (HCV). IL28b is a gene, which encodes an interferon type lambda (IFNλ), a new class of potent endogenous antiviral cytokine of innate immunity. Several genome-wide screen studies have demonstrated that SNPs rs8099917 and rs12979860 in IL28b gene strongly associates with sustained virologic response (SVR) in HCV treated patients. Our aim was to investigate the SNPs rs8099917 and rs12979860 in patients infected with HCV genotype-1 and associate these SNPs with the SVR to Peg INFa + Ribavirin therapy. **Methods:** A total of 102 HCV genotype 1 infected individuals with admixed ethnic background attended at the University Hospital Oswaldo Cruz-UPE and at the Liver Institute of Pernambuco-IFP (Recife, Brazil) treated with Peg INFa + Ribavirin was enrolled in this study. The patients were divided in SVR and Relapsers. The HCV-RNA was performed to define the SVR six months at the end of treatment; patients who achieved end of treatment response but became HCV-RNA positive after stopping therapy were considered relapsers. The SNPs were determined by specific TAQMAN probes using Real time PCR. **Results:** The frequency of CC genotype of rs12979860 was strongly associated to SVR compared to the relapsers, 56% vs 17% respectively (1.1×10^{-4} OR 6.25 IC 2.26-17.5). Regarding to rs8099917, the TT genotype did not differ statistically in groups SVR and relapsers, 75% vs 61% respectively (p=0.14). Regarding patients with HCV genotypes non-1, the frequencies of this SNP did not show statistical difference. The mean of GGT (Serum γ-Glutamyl Transferase) was higher in relapsers (p=0.01) compared with SVR patients (139U/L vs 70U/L) respectively. Other factors such as gender, alcohol intake (>40g/d), smoking, BMI, AST and ALT were not significantly different between the groups. **Main Conclusions:** The rs12979860 CC genotype was associated with SVR in patients infected with HCV genotypes 1. This study confirms the association of IL28b polymorphism with the SVR to the INFa + Ribavirin therapy in Brazilian patients infected with HCV. **E-mail:** luydson@yahoo.com.br

H1N1

Vir9. **Prognostic factors for death from influenza A (H1N1) new viral subtype, Case-control study, State of São Paulo, Brazil, 2009**

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Introduction: In April 2009 a new influenza A (H1N1) virus was identified in the United States and Mexico. The virus spread globally, resulting in the first influenza pandemic of the 21st century. Until August 2010 almost 214 countries had reported cases to the World Health Organization, with more than 18,449 deaths. In São Paulo, 6643 cases of Influenza A/H1N1 were confirmed, associated with Severe Acute Respiratory Syndrome and 586 deaths, with mortality rate of 1.4 per 100,000 inhabitants. **Methods:** A case-control study was conducted in the Greater Sao Paulo and Campinas. The cases were laboratory confirmed Influenza A/H1N1 2009, progressing to death. We studied cases reported during the period from 28 June through 29 August 2009. For each case, two controls were randomly selected by epidemiological week of hospital admission. The criterion for the definition of control was similar to the case, with the exception that they survived. Two questionnaires were used to collect social data, demographics, history of comorbidities, clinical and laboratory information in medical records and home interviews. Logistic regression was used to determine independent risk factors for death. **Results**: There were 193 cases and 386 selected controls. The analysis of the hospital (100% cases and controls) and home questionnaires (83,4% cases and 85,1% controls) showed that 47.9% of controls were under 20 years of age and among cases 57.5% were between 30 and 59 years. The cases over 18 years of age had lower education, when compared to controls, 27.8% and 22.3% respectively, OR 1.35 (0.81 to 2.25). The occupations that prevailed were domestic workers (29% of cases and 19% of controls), followed by managers (8% of cases and 11% of controls). The antiviral was introduced in 68% of cases and 76.4% of controls. Among the cases, in only 14.3% the treatment was initiated within 48 hours of symptom onset , while among the controls 45.4%, OR=0,20 (95% CI 0,12- 0,34). The following variables were significant in the final model: obesity OR=6.01 (95% CI 3.4 -10.8), heart disease OR=2.51 (95% CI 1, 1 -5.9), diabetes OR = 2.35 (95% CI 1.1 - 5.3) and antiviral treatment OR = 0.61 (95% CI 0.4 - 0.9). **Conclusions**: Pandemic influenza A/H1N1 represented major impact on morbidity and mortality in the state. The study showed as predictors of death, the presence of some conditions, especially obesity, diabetes mellitus and chronic heart disease. Use of antiviral treatment was a protective factor. **E-mail:** anafribeiro@uol.com.br

VECTORS

MOSQUITOES

Mosq1. **Variability of sodium channel region IIS6 in *Anopheles darlingi* populations from Brazilian Amazon.**

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The voltage-gated sodium channel located in the insects' nervous cells, is the main target of pyrethroid

insecticides, which are important chemical compounds used for controlling medically important mosquitoes, such as dengue and malaria vectors. Pyrethroid resistance has been a limiting factor in vector control programmes and, thus, its fast detection becomes very important, since no other suitable chemical compounds for replacing pyrethroids, are as yet available. In insects, the resistance to those insecticides is associated to a punctual mutation in the sodium channel IIS4-IIS6 region termed knockdown resistance (*kdr)*. Aiming to detect that resistance mechanism in *Anopheles darlingi*, the main goal of this work was to isolate and characterise that sodium channel gene region. Samplings of this species populations from Manaus, São Gabriel da Cachoeira, Coari and Amapá were submitted to amplified knockdown bioassay, adapted by Kawada et al. (2009) using deltamethrin. More and less resistant/tolerant individuals were selected and had their DNA extracted, amplified and sequenced. We obtained 278 sequences of good quality and homologous to those of sodium channel region IIS6 of anophelines displayed in the GenBank. Sequences were divided into nine distinct haplotypes. For this grouping only considered representative sequences of at least three clones, were considered. Twenty-three polymorphic sites were observed, being 10 substitutions synonymous in the exons and 13 in the intron. High divergence was observed within the same species and neighbouring localities. In samples of *Aedes aegypti* assessed in five regions of the country, despite a striking polymorphism in the region of intron (that served as basis for dividing sequences observed in two haplotypes: A and B in that species), only two synonymous substitutions were detected in a region homologous to that in the present study (Martins et al., 2009). Despite variability, no *kdr* mutation bearing haplotypes were detected in the analysed populations but, one notes its occurrence to be allowable since site 1014 codon shows to be similar to that of the remaining species where the same was detected. **Financial Support:** CNPq/ CT Amazônia, CNPq/FAPEAM Rede Malária, CT/Petro. **E-mail:** anna@inpa.gov.br

Mosq2. **Peritrophic matrix protein from the malaria vector in the Amazon, *Anopheles darlingi* root, 1926**

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Introduction: *Anopheles darlingi* becomes the main malaria vector in the Amazonian region from ingesting *Plasmodium*-contaminated blood*,* in this process this parasite must go through a major barrier that gets formed in the mosquitos stomach, the Peritrophic Matrix (PM), which presents itself as a thin acellular structure, constituted by a chitin, proteins, glycoproteins and proteoglycans mixture, surrounding the food bolus. Its functions are preventing or reducing the invasion of pathogens, and modulating blood ingestion following the meal. **Material and methods:** Adult three-four day old females were identified and then deprived of 10% sugared water for 12 h and afterwards, fed during one hour for PM induction. Instead of a blood-based feed, latex (16% v/v) in aqueous suspension was offered in the feeder. PMs were dissected 10 hours following the feeding, placed into tubes and stored at - 80ºC, until a total of approximately 300 PMs were accumulated. Out of nearly 5,000 *A.darlingi* specimens, we obtained almost 800 PMs and out of these we selected 300 that showed to be whole, which were analyzed by mass spectroscopy. **Results:** We analyzed the proteins and established experimental *Anopheles darlingi* PM induction procedures in adult females. In the standardization process, it was verified the time of 10hs post artificial feeding, with the latex solution, to be enough for the Peritrophic Matrix whole formation. This procedure assured success in the extraction of PMs in a whole form, so as to constitute an analysis sampling. Following dissection, PMs were submitted to protein extraction. Findings enable identification part in g eight proteins for the *Anopheles darling* PM*.* Similarity, analysis through NCBI data-bank, pointed out proteins AdP1, AdP3, AdP4, AdP6, AdP7 and AdP8 were being identified as proteins noted in *A. gambiae*; protein AdP2 in *Anopheles farauti*; and protein AdP5 in *Anopheles albimanus*, respectively. For analogy with the ontology of proteins of the genome of the anophelines already described, our findings suggest identified *A.darlingi* to present similar function. Proteins AdP3 and AdP5 take part in the

vector's immune response during *Plasmodium* invasion process. **Email:** simoes.rej@gmail.com, tadei@inpa.gov.br

Mosq3. **Morfometrical diagnosis for the malaria vectors *Anopheles cruzii*, *An. homunculus* and *An. bellator***

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Introduction: *Anopheles cruzii* is a primary vector of *Plasmodium* parasites in Atlantic Forest, Brazil. Adult females of *An. cruzii* and *Anopheles homunculus*, a secondary malaria vector, are morphologically similar and are taxonomically difficult to identify. These species may occur sympatrically and syntopically with *Anopheles bellator,* which is a potential vector of *Plasmodium sp.* morphologically similar to *An. cruzii*. and *An.homunculus*. Identification of these mosquitoes based on female external morphology is often jeopardised by the variability of morphological characters or damages in the specimen. Some researchers employ wing geometric morphometrics for distinguishing among species. The objective of this work was to assess the ability of wing geometrics morphometry to distinguish and characterize *An. cruzii*, *An homunculus* and *An. bellator*. **Materials and Methods:** Specimens were collected in the Atlantic Forest bioma (Cananeia, State of Sao Paulo, Brazil). Right wings of females of *An. cruzii* (n= 40), *An homunculus* (n= 50) and *An. bellator* (n= 27) were photographed. For each wing, 18 landmarks were marked for geometric characterization of shape. Discriminant analysis was performed to quantify shape variation. The following vein ratio was calculated: the distance between intersections "M + m-cu" and "M + r-m" was divided by the length of m-cu. **Results:** In the morphospace of canonical variables after discriminant analysis, individuals clustered into three distinct groups according to its species, with a slight overlap between *An. cruzii* and *An. homunculus* representatives. Consistently, Mahalanobis distances between these two species was lower (3.31) in comparison to pairs *An. cruzii* - *An bellator* (4.58) and *An homunculus* - *An bellator* (4.19). Pairwise cross-validated reclassification showed that geometric morphometrics is an effective method to identify the species, presenting reliability rates between 78-89% for the groups analyzed. The ratio was 0.97 for *An. cruzii*, 0.94 for *An homunculus* and, distinctively, 0.61 for *An bellator*. Discriminant analyses managed to distinguish between *An. cruzii* and *An homunculus*, and revealed that *An. homunculus* has more skinny wings. **Conclusions:** The species *An. cruzii*, *An homunculus* and *An. bellator* are distinct regarding wing shape. In addition, a vein ratio may be used to distinguish *An. bellator* from those congeneric species. **Financial Support:** CNPq 135207/2011-8; Fapesp 2005/53973-0. **E-mail:** linrocha@butantan.gov.br

Mosq4. **Anopheline (Diptera: Culicidae) in areas of environmental impact of the Serra da Mesa Hydroelectric Dam, State of Goiás, Brazil. Analysis of the reservoir influence**

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Have been studied five locations on influence of reservoir the Serra da Mesa Hydroelectric Dam (SMHD), covering cities directly affected: Site 1 - Minaçu, Site 2 - Campinorte, Site 3 - Uruaçu, Site 4 - Barro Alto e Site 5 - Niquelândia. The analysis of Shannon diversity index (H), Shannon evenness (Eh), abundance and frequency, of the Anopheline species captured in the studies accomplished by Guimarães *et al*., *Cad. Saúde Pública*, 2004, before, during and after of the filling of the reservoir of SMHD, were performed using the software SYSTAT 8.0. The specificity of the cover flora and agricultural crops, livestock facilities and timber and areas of mineral exploitation, gold prospectors especially, in each sampling point was determinant for the incidence of anophelines. The main captured species were: *Anopheles darlingi, An. albitarsis, An. triannulatus, An. evansae* and *An. oswaldoi*. Of those, *An. darlingi*, besides the most abundant, seemed to be related with the cases of malaria diagnosed in the area during the installation of the enterprise. In the general, the Site 5 was it of larger anophelines incidence, followed respectively by

the Sites 3, 4, 1 and 2. *Anopheles darlingi* was decisive in the anophelines total, respectively in the Sites 5, 3, 4, 1 and 2. *An. albitarsis* presented a low variation in that order, where at the Site 5 it was largest occurrence, followed by the Sites 3, 1 and 4, having smaller incidence in the Site 2. *An. triannulatus* had high active in the Site 5, following for the Sites 3, 1, 2 and 4. *An. oswaldoi* and *An. evansae* had very low frequencies. *An. darlingi* and *An. albitarsis* were the more presents in all of the sites of samplings. There was no significant difference in diversity index of anophelines before (H=0.9), during (H=0.8) and after (H=0.8) the of reservoir formation period. However, we verified alterations important in the specimens' abundance number. The largest indexes were reached in the samplings accomplished along the process of stuffing of the reservoir, with 80, 5% of the specimens. In the samplings carried out in the previous phase and soon after the conclusion of the reservoir and consequent beginning of operation dam, we obtained 2, 1% and 17, 4% respectively. The *An. darlingi* was the largest density specie (Fi=1, 00) and *An. evansae* the rarest specie (Fi = 0, 22). By biological specificities in larval habitat for immature development of the anophelines, the flooding and the decomposition of the vegetation the margins of the reservoir went decisive for the five species presence. In some cases, as for *An. darlingi* and *An. albitarsis*, the human communities, migrants of areas of the Amazonian and established the reservoir margins, were favorable. For *An. triannulatus, An. oswaldoi* and *An. evansae*, zoophilic species, the presence of livestock activities was positively. While the proximity with anthropic action places was unfavorable for this three species. On the other hand, we observed great adaptation capacity those circumstances for *An. darlingi* and *An. albitarsis*. **E-mail:** melandri@ioc.fiocruz.br

Mosq5. **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

Mosq6. **"I am a fire person": A qualitative investigation of the reasons why some Papua New Guineans who own mosquito nets choose not to use them**

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Introduction: Presents findings from a qualitative study designed to explore the reasons why some Papua New Guineans who own mosquito nets choose not to use them, whether on a regular or episodic basis. **Methods:** In-depth interviews (n = 44) were conducted with a sub-sample of participants in a countrywide household survey who reported owning or having access to a mosquito net, but not having slept under a mosquito net the night prior to survey. Analysis was informed by a general inductive methodology. All interview data were independently coded by two scientists using NVIVO 9 software. Inter-coder disagreement was resolved by consensus opinion or by the creation of new, mutually agreeable, codes/themes. **Results:** Multiple impediments to regular mosquito net use were identified by study participants, although all were broadly grouped into the inter-related categories of net, environmental or human factors. Apathy towards regular mosquito net use emerged as the most influential factor presenting as a general attitudinal context in which a majority of participant responses were grounded. A lack of knowledge regarding malaria transmission pathways or the utility of mosquito nets did not appear to underlie this apathy. Rather, the apathy appeared to be rooted in a lack of fear of malaria infection cultivated through lived experience. **Main Conclusions:** The impediments to mosquito net use reported by mosquito net owners appear many and varied and may, more often than not, be grounded in apathy. A wide range of interventions could potentially promote greater mosquito net use amongst this population. However, the basis of any intervention strategy, given the seemingly pervasive apathetic attitude towards regular mosquito net use, should be to render individual mosquito net use as easy and as convenient as possible and to promote complementary malaria control strategies where appropriate. **Email:** justin.pulford@pngimr.org.pg

Mosq7. **Dietary polyphenols modulates lifespan, metabolism and immunity of Aedes aegypti mosquitoes**

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Introduction: Insects are the predominant class in nature with over more than one million species, including mosquitoes, such as Aedes aegypti. They need to feed on blood of a vertebrate host to conclude oogenesis, giving them vectorial capacity to transmit disease, such as dengue. However, mosquitoes are able to feed on blood only 2-3 days after emergency and during these days they feed only on vegetal fluids, containing polyphenols that are metabolic compounds produced by plants under stress, which have several effects, well described in several models from yeast to mammals. **Objectives:** in this work we used some very well described polyphenols: Epigallocathequin gallate (EGCG), Genistein, Quercetin and Resveratrol (RV), in order to evaluate whether this kind of diet modulate changes in metabolism, immunity and lifespan of A. aegypti mosquitoes. **Materials and Methods:** Longevity was evaluated using Kaplan-Meyer test. Lipid content was measured by densitometry of Thin Layer Chromatography (TLC) and gene expression by qPCR Real –Time. Analysis of phosphorylation levels was made by western blotting. **Results:** the treatment of A. aegypti mosquitoes with 100 µM of RV increases at least 73% the mean survival in comparison with control. EGCG treated group showed an increase of mean survival of 93%, Genistein showed 37% and Quercetin showed 99%. About lipid content, it was verified that RV treatment changes lipid content (triglycerides) of those mosquitoes (26 % less). EGCG treated group showed 23 % less triglyceride and Quercetin showed 24% less than control. However, unexpectedly, Genistein treated ones did not show less lipid content. Its effect in lifespan may be related with an immune pathway, as antibiotic treated mosquitoes also showed an increase of mean survival. This result suggests that the lower numbers of bacterial colonies are beneficent for A. aegypti longevity. We selected RV to be our treatment model, and our data showed RV stimulates an increase of

autophagy in midguts of treated mosquitoes followed by a decrease of 50% in the number of copies of 16S (bacterial ribosomal gene). These autophagy-related effects and metabolism effects may be explained by increase of AMP-dependent kinase (AMPK) activity. Resveratrol-fed mosquitos showed an increase in the phosphorylation level of catalytic domain of AMPK. **Discussion:** dietary polyphenols modulates different aspects of mosquito biology and we hypothesize that these changes can be related with AMPK – dependent pathways. At our known this work is the first time that effects of dietary polyphenols in immunity, metabolism and lifespan of a disease vector are described. **Supported by:** FAPERJ E**-mail:** gventura@bioqmed.ufrj.br / maneto@bioqmed.ufrj.br

Mosq8. **An efficient method of DNA extraction of single egg of *Aedes aegypti***

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Introduction: In the last decades, the interest on phylogenetic studies based on molecular biology has increased. The molecular identification of species by analyzing small fragments of a genome represents the most promising approach to identify species. The molecular technics are limited to acceptable DNA quality and quantity for PCR amplification. In addition, DNA isolation of really small individualized organisms can be difficult. Therefore, a simple, fast and low-cost protocol to isolate DNA of culicid eggs has been developed in order to solve the DNA limitation problem. That way, there is no need to wait the eclosion of eggs till fourth instar of larvae, use big infrastructures and facilities as an insectary to obtain DNA molecules of these organisms. The quality of DNA template was evaluated by Cytochrome oxidase I (CO I), cause it is useful marker for phylogenetic and population genetic research. **Material and methods:** 636 eggs were used in triplicates, both viable and unviable eggs, in the following quantities: 1, 5, 10, 20, 30 and 40 eggs in 36 samples. Both, the individual and grouped eggs were placed in 1.5 ml plastic tubes, adding 100 µl of 5% Chelex$^{(R)}$ (BioRad). All the eggs were macerated until they were homogenized, and then they were kept at 54°C for 1 hour. After that, all samples were transferred to 200 µl tubes and they were kept at 94°C for 30 minutes in the thermocycler. The material was centrifuged at 13000 rpm for 6 minutes and the supernatant was separated in novel 1.5 ml plastic tubes. Then, DNA was quantified in nanovue (GE$^{(R)}$). PCR reactions for COXI gene were carried out as follow: 12.5 µl of Mix Go Taq Colorless (Promega$^{(R)}$); 7.5 µl of nuclease-free water; 1.5 of each primer (forward and reverse) and 2 µl of DNA template (45 ng x µl^{-1}). Then, the reaction was placed in the thermocycler with 35 cycles: 95^{o}C for 3 minutes for denaturation; 95°C for 30 seconds; 42.7°C for 1 minute; 72°C for 1 minute; and a final extension with 72°C for 7 minutes. Finally, the amplified products were sequenced in an ABI Prism^{tm} 3500 automated sequencer (Applied Biosystems). **Results:** All samples had the follow mean concentration: 47, 63, 77, 99, 121, and 147 ng x µl^{-1} for 1, 5, 10, 20, 30 and 40 eggs, respectively. After dilution, all DNA samples were successfully amplified generating a 540 bp fragment of COXI, which was confirmed by sequencing. **Conclusion:** The method provides a fast, simple and low-cost way of DNA extraction which could be an alternative method to acquire template DNA for phylogenetic studies when there was a limitation for the traditional taxonomy, as well as the insecticide resistance testing without the traditional Bioarray. **E-mail:** vqbalbino@hotmail.com

Mosq9. **Lime and chloride method for controlling *Aedes aegypti* in building sites**

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Introduction: In 2011 Manaus/AM went through the heaviest dengue epidemics in its history, with 45,509 recoded cases and 12 deaths Epidemiological assays demonstrate high *Aedes.aegypti* density in certain strata associated to house building activities. Thus, one noticed the need of inserting a new action modality into the Dengue Control Program. Multiple building procedures lead to the formation of mosquito breeding sites triggering a dengue transmission process within the building site itself, which also extends to the neighboring residing population. **Objective**: To introduce a new procedure – **Lime and Chloride**

Method: for the control of *Aedes aegypti* reproduction on building sites. **Methods:** This procedure consists of the lime mixture used for painting buildings and organic chloride used for swimming pool water maintenance. These powdered substances are put together in eight limes to two chloride portions concentration and applied directly on the places with accumulated water, in several building floors. Aiming to make feasible the process of preparing the mixtures in simple workable form in building site level, we suggest utilizing a (50 mL) disposable coffee cup as measuring container that originates 500 mL of mixture powders, in the preparation of eight little cups of lime and two of chloride. **Results**: Laboratory bioassays showed that the mixture of these two substances was effective to block egg hatching, kill larvae, and prevent pupae from emerging stop the mosquito's reproduction process. Then we passed to application in field, building sites, focusing on areas with accumulated water. In treatment of breeding sites, the product is used just as a powder and 180 m L disposable water cup, should be used as container for applying it over areas with water. A 180 mL cup is enough to cover one square meter, when water is just scattered on the pavement with no depth. This concentration must be doubled or even tripled depending on how deep the place is. In the tests for infiltrations into the paving stone, the sites should also be previously treated. Building sites routine needs to be monitored so that *Aedes aegypti* reproduction be stopped. So, we suggest builders to mobilize, among their employees, a brigade to make up a group in charge of mosquito monitoring. **Conclusion**: The use of the product resulting from lime and chloride mixture, therefore, is an alternative procedure in the actions for controlling the dengue mosquito, for the house building sites. This product holds environmental indication, since its components are bio degradable and don't affect the activities of the enterprise. Its alternative employment on building sites contributes to prevent the development of the mosquito's resistance to the routinely used insecticide. Monitoring the excess of the mixture applied is naturally incorporated into building routine activities. The action of the Dengue Brigade must be continuous and preventive, since where applications keep on occurring mosquito females lay no eggs and the ones that may be there will never hatch. **Funding:** FVS-AM/INPA-MCT E**-mail:** wptadei@gmail.com

Mosq10. **Kdr mutation in *Aedes aegypti*: resistance to pyrethroids and fitness cost.**

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Pyrethroids are widely applied against disease vectors due to their low impact to the human health and rapid neurotoxic effect to the insects, leading them to rapid and involuntary contractions followed by paralysis and death – the *knockdown* effect. The exacerbated use of these compounds, however, has been selecting resistant insect populations worldwide. This is the case of many *Ae. aegypti* populations from Brazil and other Latin American and Asian localities. One of the main mechanisms selected is a mutation in the voltage gated sodium channel gene (*AaNa$_V$*), causing the substitution Val/Ile in the aminoacid 1016 (Val1016Ile), known as the *kdr* mutation (from knockdown resistance) in this species. We were aware that the mutant allele was rapidly increasing in frequency throughout the country. In this work we aimed to evaluate the role of the kdr mutation itself not only in the insecticide resistance but also in eventual pleiotropic effects on life-trait parameters upon development and reproduction. First of all, we had to select a lineage homozygous for the mutant allele, based on the formation of individual couples from a natural population. Their genotypes were accessed by allele-specific PCR and the offspring of those which both male and female were homozygous for the mutation gave origin to the first selected lineage, LP32. In order to explore the effects of the mutation on the fitness in environment free of insecticides, we needed to compare life-trait parameters with Rockefeller (Rock), a known reference strain of susceptibility to insecticide and vigor in laboratory conditions. Then, successive crosses between the mutant linage and Rock were performed along eight sequential generations for introducing the mutant allele to a genetic background similar enough to Rock. The resulting lineage was named Aa-kdr. The activity of the main enzymes also related to resistance to insecticides was measured by biochemical colorimetrical assays. The life-trait parameters compared were: larval development time, pupae formation rates, adult longevity, circadian rhythm, locomotor activity, blood feeding, egg laying and viability of the eggs. Cage trial assays with the mutant allele under initial frequency of 50 or 75% were

kept in environment free of insecticides for 15 generations. We found that the Val101Ile mutation itself in fact confers resistance to pyrethroid in a recessive trait. Aa-kdr had lower activity levels of the main enzymes related to metabolic resistance compared to the original natural population, showing that its genetic background was closer to Rock. The larvae of Aa-kdr developed slower; the females laid a smaller number of eggs and showed an increased locomotor activity. Cage trial assays revealed that the frequency of the mutant allele diminished in all cages along 15 generations. These results strongly suggest that besides the *Ae. aegypti* kdr mutation Val1016Ile provides a high selective advantage in the presence of pyrethroids, its elevated fitness cost in other life-trait parameters makes the mutant allele disadvantageous in the absence of insecticide. **E-mail:** lpbrito@ioc.fiocruz.br

Mosq11. **The German Mobovirus Surveillance and Mosquito Monitoring Program, 2009 – 2011**

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Introduction: Human and animal diseases caused by mosquito-borne viruses (moboviruses) are of growing importance in many countries of Europe including Germany. Continuing eco-climatic changes and globalization create suitable conditions for the emergence of moboviruses in Germany. Up to now, four different moboviruses have been found in Germany. In 1968, Tahyna virus (TAHV) was isolated from mosquitoes that were trapped around Baunach, south-west Germany. TAHV is the causative agent of Valtice fever, an influenza-like illness occurring in summer and early autumn. Moreover, meningitis cases due to TAHV infection were also reported. Therefore, TAHV is the first human pathogenic mobovirus that was isolated from mosquitoes in Germany. Since these early discoveries in the late 60s of the last century, mobovirus surveillance in mosquitoes, humans and animals was not performed regularly and therefore, longitudinal data sets are missing, especially from Germany. Thus, we initiated a program that compiles and analyses mobovirus and vector data collected over a number of successive years. This provided a solid base to determine the underlying causes of the seasonal fluctuations in mobovirus activity and the relative abundance of the mosquito vector species. This information can be used as a basis for vector control programs and might provide an early warning of the presence of moboviruses in Germany. **Results:** Since 2009, mobovirus surveillance was performed mostly in south-west Germany. So far, more than 120.000 mosquitoes were captured and assayed for the presence of moboviruses. In 2009, Sindbis virus (SINV) was isolated from *Culex* and *Anopheles* mosquitoes that were exclusively trapped in the city of Weinheim, south-west Germany. SINV is the causative agent of a febrile illness in humans associated with maculopapular rash and joint pain. Consequently, a study was initiated to investigate the medical importance of SINV in that area. Only four out of 3389 investigated blood donor samples were tested positive for SINV-specific-IgG antibodies and all samples from 355 patients with clinically suspected acute SINV infections were tested negative for SINV-specific antibodies or SINV RNA, thus demonstrating the low medical importance of SINV in south-west Germany. In 2009, Batai virus (BATV) was isolated from *Anopheles maculipennis* mosquitoes trapped around the village of Waghäusel. BATV may cause a mild illness among sheep and cattle, but also, stillbirth and congenital abnormalities have been reported in association with BATV infections. Thus, 195 serum samples from cattles around the village of Waghäusel were investigated for BATV-specific-IgG antibodies and two samples were tested positive, demonstrating past BATV infections. In 2010, Usutu virus (USUV) was isolated from *Culex pipiens* mosquitoes trapped in the city of Weinheim. Since June 2011, considerable mortality in wild and captive bird species was observed in south-west Germany. Consequently, 168 dead birds were tested for the presence of USUV and USUV RNA was detected in 80 individuals from 6 species. Therefore, the mortality of birds was shown to be associated with the emergence of USUV. **Conclusion:** The early discovery of USUV in mosquitoes followed by the recent epidemic proof the importance and predictive value of our mosquito based monitoring program for zoonotic moboviruses. **E-mail:** jonassi@gmx.de

Mosq12. **Probing natural mosquito signaling pathway and vectorial capacity with Resveratrol**

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Mosquitoes are the major vectors of several human diseases. Usually, transmission relies on an effective and timely adjusted pathogen development within the mosquito in order to allow its transmission in the next meal. Thus understanding the molecular pathways that regulate mosquito lifespan may lead to novel strategies to shorten it. After emergence from the pupal stages, mosquito females undergo a hematophagic capacitation while feeding on vegetal fluids. However, the role of plant-derived molecules on the overall mosquito metabolism, immunity and lifespan has been neglected. Resveratrol is a naturally occurring polyphenol whose effects in several different cells and organisms are largely documented. It is currently a powerful tool to probe for overall effects of polyphenols. We have mapped the effects of Resveratrol and other common polyphenols on *Aedes aegypti* in order to gather information about the mechanisms that modulate its lifespan and vectorial capacity. The mean survival of polyphenol-fed mosquitoes was increased about 85% in females and 60% in males compared to controls. The tyrosine and serine phosphorylation profile was changed by Resveratrol. Triglyceride (TG) level is at least 25% decreased in polyphenol-fed insects. Such decrease is associated with modulation of AMP-dependent protein kinase activity (AMPK), as it is mimicked by pharmacological activation of AMPK pathway. Bacterial population isolated from *A. aegypti* midgut was 50% decreased by Resveratrol. In the same way, mosquitos fed with antibiotic showed the same increase on their average lifespan of Resveratrol-fed ones. However isolated midgut bacteria are little affected by Resveratrol exposition *in vitro*. Reactive species generation and antimicrobial peptides production, important immunity responses, were also not affected too. The autophagy was increased 10 times in Resveratrol-fed mosquitoes and, as expected these effects were also mimicked by pharmacological activation of AMPK and inhibited by AMPK knockdown. Finally Resveratrol-fed females have higher infection by Dengue virus than control. Thus meal-derived polyphenol may promote in nature an extension of average mosquito lifespan and modulate pathogen refractoriness. In conclusion, strategies targeting their effects on AMPK pathway should be designed in the future in order to block disease transmission by such organisms. **E-mail:** rdnunes@bioqmed.ufrj.br

SANDFLIES

Sandfly1. **Changes in intestinal environment from *Lutzomyia longipalpis* induced by *Leishmania infantum***

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Leishmania growth and development is markedly influenced by the pH in *in vitro* cultures and probably the same is truth for the parasite in the midgut of sandflies. Recently we measured the pH in non-infected *Lutzomyia longipalpis* after and during blood digestion. In non-infected sandflies, abdominal and thoracic midguts are kept acidic (pH 6.0). After blood ingestion, the pH was promptly alkalized to pH 8.1 in abdominal midgut. The aim of this study was to investigate if the presence of *Leishmania* or something produced by this parasite could interfere in the midgut physiology changing the intestinal environment in order to favor its development. The pH of the midgut of infected sandflies was measured during blood

digestion using H^+-sensitive microelectrodes and the indicator dye bromothymol blue was used to measure the intestinal pH six days after the infective blood meal. The time spent to complete the blood digestion was also investigated in infected and uninfected females. Complementary, we evaluated the interference of the presence of *Leishmania* or its secreted products in the activity of digestive trypsin from *L*. *longipalpis*. According to results, the pH in abdominal midguts remained more alkaline (pH 7.86 $\pm$ 0.65) in non-infected females than in infected ones (pH 6.84 $\pm$ 0.28) during blood digestion. After digestion, the pH was normally acidified in both infected and uninfected insects. In infected insects, trypsin production was lowered by the presence of *Leishmania* as well by its secreted products. We also observed a higher proportion of intestines with digesting blood in infected females than in uninfected ones. The set of data showed that the presence of *Leishmania* and its products inside midgut are able to acidify the pH. This precocious pH decreases can explain the delay in blood digestion, once proteases activity is diminished in more acidic pH's. The delay in blood digestion could favor *Leishmania* development because blood proteins and other nutritive molecules would be present in contact with the parasite. **Supported by:** FAPEMIG and CNPq. **E-mail:** vanietsc@yahoo.com.br

Sandfly2. **Ultrastructural aspects of Peritrophic matrix from *Lutzomya* (*Nyssomia*) *Umbralitis* (Diptera: Psychodidae: Phlebotominae)**

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Introduction: The species *Lutzomyia umbratilis* is the most important vector of *Leishmania guyanensis* in the Brazilian Amazon, responsible for human cases of cutaneous leishmaniasis, often with multiple injuries. During its development cycle in the sandfly the parasite must overcome several natural barriers imposed by the insect, produced in response to food, among these the peritrophic matrix (PM). This PM covers the food contents aiding in the digestion process, acting on the physical protection of the intestinal epithelial as a vector barrier. This study aimed to analyze the ultrastructure of the PM located at the midgut (MG) of *L. umbratilis* through analysis of Transmission Electron Microscopy (TEM) and scanning (SEM). **Materials and Methods:** The sandflies were collected in the Military Reserve (Instruction Base Marechal Rondon/CIGS, situated in 65 km of highway AM-010 /Manaus-Itacoatiara, AM). The insects (female) were blood-fed on anesthetized guinea pigs, and 24 h after feeding the PM were removed in a sterile solution of protease inhibitor cocktail, isolated and included in a fixative solution and maintained at 4^oC until the material being processed by procedure pattern to Electron Microscopy. The TEM analyzes were performed at the Evandro Chagas Institute - IEC (PA) and SEM at the National Institute of Amazonian Research - INPA (AM). **Results and Conclusions:** The results revealed the PM of *L. umbratilis* well formed in 24 h and completely degraded in 72 hours. The fresh dissections showed that MG of this species can be formed more than one PM presented a dense mass of gelatinous aspect. Observations by SEM showed the outer face of PM with a clear impression of the apex of cells of MG, possibly forming this one. The TEM, showed a close relationship between the PM and food with visible presence of chitin fibrils form a protein network around the food. **Financial Support:** FAPEAM/INPA.
Key-Words: Peritrophic Matrix, Midgut, *Lutzomyia umbratilis*. **E-mail:** luis@inpa.gov.br

Sandfly3. **Potential Leishmaniasis Vectors (Diptera: Psychodidae: Phlebotominae) of Ecotourism Area from Rio de Janeiro State, Brazil**

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Introduction: Ilha Grande (Angra dos Reis Municipality) is the greatest island of Rio de Janeiro State and an ecotourism hotspot, with constant visits of people from different parts of the world. The island records cases of American Cutaneous Leishmaniasis (ACL) since its first outbreak, in 1975, in Praia Vermelha. In 1978, the first sandfly survey demonstrated the importance of *Lutzomyia intermedia* and *L. migonei* on the disease's transmission. On the present study, over 30 years later, another survey was performed in the same locality, where ACL an American Visceral Leishmaniasis (AVL) human cases are notified. **Material and Methods:** Three monitoring stations (MS) were established: MS1, Vila do Abraão, which concentrates most touristic activities and has suffered environmental impacts; MS2, Enseada das Estrelas, region with sporadic ACL human cases and one recently detected (2005) AVL human case; MS3, Praia Vermelha, the 1975's ACL outbreak locality. From each MS, three residences were monthly surveyed, with HP light traps installed inside houses, in peridomicile and nearest forest. Additional capture sites were established to catch female sandflies for *Leishmania* natural infection survey, which was made by Multiplex PCR and Dot-blot Hybridization. **Results and Conclusions:** From July 2010 to July 2011, 931 sandflies were captured and 17 species detected. *L. migonei* and *L. intermedia* were the most abundant species, captured on the three MS. The vectors *L. longipalpis* (on MS2) and *L. flaviscutellata* (on MS1 and MS2) were also captured. The highest values of species richness and diversity were observed on MS2, while MS1 had the lowest values. Three females of *L. migonei* from MS3 were positive for *Leishmania (Viannia)* sp. Although *L. intermedia* is considered the main ACL vector of *Leishmania (V.) braziliensis* in Rio de Janeiro State, this result draws attention to the epidemiologic importance of *L. migonei*, which was observed in peridomicile strongly associated to chickens. Therefore, its role as ACL vector should not be underestimated. The capture of *L. longipalpis* in the locality of the AVL human case (MS2) was an important result, since when the case was notified the vector had not been found. This allowed the conclusion of the case´s notification on Ministry of Health's Notification System. The finding of *L. flaviscutellata* on peridomicile was also important, since this species is involved with the transmission of American Diffuse Cutaneous Leishmaniasis (ADCL). This species' abundance and distribution should be better studied, since the first human case of ADCL from Rio de Janeiro State was recently detected (2007) on Paraty, a neighbor municipality of Angra dos Reis with very similar environmental characteristics to those from Ilha Grande. The occurrence of leishmaniasis vector species in places where tourists are frequently trekking inside the forest highlights the need of proper health education practices as control measure of these diseases in Ilha Grande**-mail:** brunomc@ioc.fiocruz.br

Sandfly4. **Report of trypanosomatids in sandflies trapped in a cave of the Bambuí Speleological Province, Lassance municipality, Minas Gerais, Brazil**

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The sandflies are holometabolous insects that stand out in the neotropics, for presenting great epidemiological importance in the transmission of leishmaniasis. With regard to it biology, however, there is still a high gap in our knowledge of these insects, since only a few species were studied. Caves are ecotypes well differentiated of the external environment and among the insects that live or visit the inner area and adjacent environment, sandflies are found constantly. Based on this context and in the importance of sandflies as vectors, the objective of this work was to check the presence of flagellates protozoan on sandflies caught in a cave of Speleological Province of Bambuí. Catches were made with a Shannon trap, using light bait, in addition to an active search on the cave walls. This capture was performed every hour over all 24 hours of day, in August 2010, with the objective of verifying the activity period of most abundant species in this ecotype. However, females were separated and kept alive to verify natural infection by the technique of dissection of the digestive tract. We collected a total of 130 sandflies, 69 females and 61 males, represented by nine species, being more abundant: *Evandromyia spelunca* (36%), *Lutzomyia cavernicola* (27%) and *Ev. sallesi* (27%). Five specimens were found living flagellated forms: two *Ev.spelunca*, two *Ev.sallesi* and one *Sciopemyia sordellii*. A suspension containing PBS 1x with antibiotics (streptomycin and penicillin) and intestinal contents of the insects were inoculated in hamsters and in culture medium NNN-Schnneider. It was not observed the growth protozoa in culture,

and in all of them were visualized bacteria and/or fungi contamination that prevented the continuity of the same. No clinical signs were observed in hamsters which flagellates suspensions were inoculated. The presence of *Leishmania* DNA was investigated through ITS1-PCR, however, this technique also not allowed the identification of parasites found. The slides with the infected specimens were photographed and filmed and after the procedures for isolation, they were stained. The stained forms will be submitted for others methodologies in an attempt to identify the flagellates. Further studies of natural infection of sandflies caught in caves are important to formulate new hypotheses or unveil new cycles of disease in this ecotype, which has unique characteristics, ecological and biological aspects of importance. **E-mail:** gumayr@cpqrr.fiocruz.br

TRIATOMINES

Triat1. **Geographical distribution and entomological indicators of triatomines captured in the State of Minas Gerais, Brazil, 2001 a 2011**

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In the State of Minas Gerais, Brazil, after the elimination of *Triatoma infestans,* some species have merited the attention of entomological survey authorities. The aim of this study is to analyze the geographical distribution of triatomines and other entomological indicators, in Minas Gerais State, Brazil, in the period 2001 to 2011. For this purpose, we evaluated the information collected by the Chagas Disease Control Program (PCDCh) system: domiciliary unit infestation, domiciliary triatomine density, species captured, geographical distribution and vector infection by *Trypanosoma cruzi*. Over the period 345,791 specimens were captured, of these only 183 (0.05%) were other insects, 50.2% (n=173,286) were nymphs and about 6.4% (n=11.152) of these were captured indoor. The most prevalent specie was *Triatoma sordida,* that represented 90.1% (n=311,560) of the specimens captured, followed by *Panstrongylus megistus* (n=23,893; 6.9%) and others species (n=10,155; 3%). The general peridomiciliary infestation was 87.4%, with greatest intradomiciliar infestation for the *Triatoma sordida* (91.7%) and *Panstrongylus megistus* (54.7%). The specie *P. megistus* showed the highest dispersion and was registered in 412 municipalities in the analyzed period, while *T. sordida* was recorded in only 195 municipalities. The infection rate for *Trypanosoma cruzi* in the vectors was 1.4%, whereas for *P. megistus* was 6.1% and for *T. sordida* was 0.7%. From the results, we find that there is still a high rate of infestation of domiciliary units in the state of Minas Gerais, predominantly around the homes. In addition, there is a high percentage of records in households (12.6%), highlighting the potential risk of transmission of Chagas disease. Importantly, there is a low percentage of positive triatomines infected with *T. cruzi*, probably as a result of the habits of the prevalent specie *T. sordida,* mainly found in dwellings and associated with birds that are refractory to vertebrate parasite. However, species such as *P. megistus* has recorded high rates of natural infection, wide dispersion, and great potential of domiciliation and thus may potentially be involved in the transmission cycle of Chagas disease. Therefore, it is essential to direct current policies to the predominant species, 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. **E-mail:** marcela.ferraz@saude.mg.gov.br

Triat2. **Chagas disease vectors (Triatominae) in urban forest fragments of central Amazonia**

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Introduction: Chagas disease (CD) is endemic in the Americas, where ~7.5 million people are infected by *Trypanosoma cruzi*. CD transmission has classically been thought to require the colonization of human dwellings by triatomine bugs, the vectors of *T. cruzi*. Yet, the epidemiological importance of transmission without colonization of artificial structures has become evident in recent years. In fact, the invasion of houses and/or food storage/processing premises by wild adult triatomines probably underlies *T. cruzi* transmission to humans in Amazonia, where outbreaks of oral transmission are increasingly common. Vectors of the genus *Rhodnius*, which preferentially occupy large palm trees, seem to be involved in most transmission events, but whether palms may also pose a risk in urban landscapes remains unclear. **Materials and methods:** We investigated the patterns of *Rhodnius* spp. occurrence in palms along a disturbance gradient in the Brazilian central Amazon, including forest fragments within the urban-matrix environment of Manaus, a 1.8-million people metropolis. Infestation of *Attalea* (*n*=259) and *Oenocarpus* (*n*=44) palm trees was evaluated in preserved forests (*n*=113 palms), deforested rural areas (*n*=121) and urban forest fragments (*n*=69). We combined live-bait (Noireau) traps and manual bug searches in organic debris taken from palm crowns. **Results:** The overall palm infestation rate was ~20% (61/303; *Attalea*, 20.5%; *Oenocarpus*, 18.2%). Vector capture rates were lower in Noireau traps (4.3%, 37/861) than with manual collections, which were apparently more sensitive (13.6%, 33/243). Infestation rates were slightly higher in rural areas (23%) than in preserved forests (19%) or urban forest fragments (17%); these differences were statistically non-significant. However, more vectors were captured in urban forest fragments (45 bugs, 3.75/infested palm) than in better-preserved landscapes (forest: 35 bugs, 1.67/infested palm; rural: 57 bugs, 2.04/infested palm). **Main conclusions:** Palm tree infestation by *Rhodnius* spp. is similar in urban, rural, and forest landscapes in the central Brazilian Amazon. The higher apparent density of bug colonies in urban forest fragment palms might foster adult vector dispersal towards households, increasing the risk of both direct human-vector contact and foodstuff contamination by adventitious triatomines. Large peridomestic palm trees, whether in rural or urban settings, should therefore be regarded as major potential objects of entomological surveillance in relation with CD in Amazonia. Manually searching for vectors in decaying vegetable debris taken from palm crowns is a simple, feasible, and relatively effective means for ascertaining palm infestation under field conditions. **E-mail:** waltersantos@iec.pa.gov.br

Triat3. **Taxonomic and phylogenetic study in the municipality of Curaçá (BA) of a new rate of the Triatoma brasiliensis complex**

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Introduction: Triatomines are hematophagous insects, vectors of T. cruzi, the protozoan responsible for Chagas disease. Since Costa & Peterson 2012, Triatoma brasiliensis complex is known to be constituted by five species (T. b. brasiliensis, T. *b.macromelasoma*, T. *melanica*, T. *scherlocki* and T. *juazeirensis)*. Nevertheless, in a recent study carried out in the municipality of Curaçá, in the Juazeiro region (BA), a new pattern of this complex was found. In order to confirm the hypothesis of a new rate of the complex, 250 triatomines were collected in Curaçá. The new morphotype was found inhabiting both in wild and domiciliary ecotopes. The aim of this study is to determine the taxonomic status of the new morphotype. **Material and Methods:** The collect was carried out in the municipality of Curaçá, in the region of Juazeiro (BA). Triatomines were collected in three distincts ecotopes: wild, peridomiciliary and domiciliary. The insects were taken to the laboratory of Entomologic Biodiversity at IOC, where they were fed till they reached the adult stage. Fifteen males and fifteen females were used for the morphologic description, performed according to Lent & Wygodzinsky (1979). Another 150 insects are used for the molecular analyses. DNA is being extracted out of the legs by the fenol-chloroform method. In the next weeks, the mitochondrial genes Cyt b and COI will be amplified by PCR and purified with the Wizard® Genomic DNA Purification Kit de Promega. Then, a sequence reaction will be prepared with the kit ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction System, version 1.1. Sequencing will be done with a sequencer ABI Prism 3100 (Applied Biosystem). A phylogenetic reconstitution of Cyt b and COI sequences will be performed using the methodology described in Monteiro et al. 2004. **Results:** The

preliminary observations of the new morphotype showed new characteristics for this insect population from Curaçá, which presents a distinct color pattern from all other T. brasiliensis species complex. Measurements and analysis using sequences of Cyt b and COI genes are now being performed. **Main Conclusions:** Because all the members of the *T. brasiliensis* species complex present a distinct color pattern, the preliminary observation points out the existence of a new evolutionary unit. Nevertheless, further analysis must be carried out in order to confirm the taxonomic status of the new morphotype. A parallel study, which is being carried out, shows that the natural infection rate by *T. cruzi* of this population is at least of 20%. This highlights the importance to keep this population under constant monitoring since it is clearly maintaining the *T. cruzi* cycle in natural and artificial environments. **E-mail:** sophie.lorent@gmail.com

Triat4. **Geometric morphometry suggests the existence of plasticity in populations of *Triatoma sordida***

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Triatoma sordida (Stål, 1859) with wide geographic distribution in Brazil is found in the biome Caatinga, Cerrado, Atlantic Forest and Pantanal. Areas modified by human pressure contribute to the process of dispersion of the different populations of *T sordida*, modifying the mechanisms of association of birds in the natural environment. The size variation of morphological characters may often be influenced by environmental factors, while variations of conformation expressing changes in the genetic level. Therefore it is important to know and understand the dynamics of invasion/ infestation and reinfestation of domicile and peridomicile, even after control actions. By geometric morphometry female right wings of 10 populations of *T. sordida* (Tsor) originating from São Desidério (BA, n=7); Aparecida do Toboado (n=9), Paranaiba (n=15), Três Lagoas (n=8) (MS); Várzea Grande (MT, n=11); Bocaiúva (n=10), Itaobim (n=21) (MG); Aurora (n=6), Combinado (n=17), São Valério (n=7) (TO); and a population from Argentina (n = 6) were compared. *T. guasayana* Wygodzinsky & Abalos 1949 (Tgua) from Bolivia was used as external group (n = 21). The wings were removed, mounted between slide and coverslip in 70% ethanol, photographed under a stereoscopic for subsequent scanning of ten anatomical landmarks. Comparisons were carried out on the size (using isometric estimator as the centroid size) and conformation of the wings (using the method of Procrustes). Analyses were performed with JMP CLIC v45 and v4. Analysis of variance (ANOVA) revealed that the wings of Tsor from Bocaiuva (MG) are significantly higher ($p < 0.0001$) followed by Aparecida do Tobado (MS) and São Desidério (BA). However those from Várzea Grande (MT), are significantly lower ($p < 0.0001$) followed by the Tgua from Mataral (Bolivia) and Tsor from Três Lagoas (MS). Analyzing the variables of conformation, the map factor drawn from a canonical analysis showed a washout between the populations of Argentine and Brazilian Tsor, leaving the outer group separated from the other. Reclassification levels were high for most groups (approx. 83%). The canonical factors were 91%, 3% and 2% for the first three factors. Allometric effect was not found (Wilks' lambda = 0.14, F = 1.25, GL1 = 176, gl2 = 925.82, P: = 0.021). Although the map factor showed an undermining between populations Tsor, the Mahalanobis distances showed significant differences among most of them, indicating a strong population structure, confirming the use of geometric morphometrics to study populations. The present results are currently being assessed by molecular techniques. **Support by:** IOC/FIOCRUZ. **E-mail:** aniratac@ioc.fiocruz.br

Triat5. **Antennal phenotype of Amazonian species, *Rhodnius brethesi*, has a pattern of sensilla from other species of the genus Rhodnius**

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Of the 141 species of insect vectors of the protozoan *Trypanosoma cruzi*, the causative agent of Chagas disease (CD), the genus *Rhodnius* is described as having great epidemiological importance. Species of this genus are associated with palm trees and among them the *Rhodnius brethesi*, one of the vectors described in the Brazilian Amazon, in the states of Amazonas, Maranhão and Pará, with reports also in Colombia and Venezuela, has been associated with the *Leopoldinia piassaba* palm tree. Thus, this specie is involved in spots of sylvatic transmission of the human CD between fiber collectors of this palm tree. The triatomine can be distinguished by morphological characteristics and the antennal phenotype, therefore, deserving studies to understand the role of this species on the vector transmission. The antenna surface is covered with cuticular structures called *sensilla* (chemical and mechanical receptors). The antennal phenotype proves to be important in studies of population structure, as *sensilla* may be indicative of adaptation of triatomine in habitats of different complexity and stability. Antennas are divided into four segments (scape, pedicel, flagellum 1 and flagellum 2). *R. brethesi*, as in other species of the *Rhodnius* genus, presents only bristles (BR) on the pedicel. In the F1 and F2 segments, they present bristles (BR), thin walled trichoids (TH), thick walled trichoids (TK) and basiconics (BA). As our objective we verified the model of the antennal phenotype of *R. brethesi* triatomine reared on the colony of the Laboratory of Parasitic Diseased – Fiocruz/IOC, in order to know its populational profile. The results obtained were compared with data from other *Rhodnius* triatomine from the database of the Reference Laboratory for Sensory Patterns in CRILAR-Argentina. The study of the arrangement of these receptors will help us to clarify the relationship between insect-habitat and a possible populational variation. The antennas from each individual were removed, processed for the cuticle clearing and the visualization of the receptors. We obtained the following results: by measuring the lengths of the TPF and TPG *sensilla* present in F1 it was possible to verify that *R. brethesi* displays a greater length than other *Rhodnius* species; analysis on the number of *sensilla* demonstrated a significative phenotypic distance between *R. brethesi* when compared with *R. pallescens* and *R. pictipes*, and the existence of a populational difference between the analyzed specimens. We conclude that *R. brethesi* has a different pattern of sensory receptors compared with other *Rhodnius* species and the existence of population variation between individuals created in the laboratory. **E-mail:** junqueir.rlk@terra.com.br

Triat6. **Analysis of mitochondrial DNA applied to the taxonomy of triatomine (Hemiptera: Reduviidae: Triatominae) captured in the State of Tocantins, Brazil**

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The triatomine vectors of Chagas disease belong to Triatominae subfamily, which currently has 62 species with occurrence in Brazil. During 2004, monitoring studies of triatomine insect in the municipalities of Peixe, São Salvador and Paraná, southwest of Tocantins State, confirmed the occurrence of *T. sordida*, *T. pseudomaculata*, *T. costalimai* and *R. neglectus* in sylvatic environments[1]. However, some specimens, although similar to *T. costalimai* and *T. williami* could not be identified using the dichotomous key of Lent & Wygodzinsky (1979). In order to verify if these specimens should be considered as a new species geometric morphometric analysis of wings was performed. This study showed significant differences both in the size and in conformation between *Triatoma* sp and *T. costalimai,* as well as the separation from *T. williami* and *T. sordida*[3,4]. Morphological studies of the female external genitalia in triatomines, disregarded since the 1960, and retaken by Rosa et al (2010), was also used to confirm the taxonomic status of *Triatoma* sp. By scanning electron microscopy, significant taxonomic differences in the female genitalia of Triatoma sp, *T. costalimai* and *T. williami* were observed concerning to the posterior edge of urotergites as well as the form and pattern of bristles, and spines of the median area of gonapophise[6]. Based on these results, this study aimed to analyze the genetic distance and phylogeny of these specimens to confirm if it is a new taxon-specific or a population variation. Genomic DNA was extracted, followed by PCR amplification and sequencing of the 16S gene fragment of about 530pb. The genetic distance was performed according to Jukes-Cantor and phylogenetic by neighbor-joining method using the program MEGA 5.0. The reliability of the clade was estimated from 1000 bootstrap replicates. Among the species *Triatoma* sp and *T. costalimai, T. williami*

and *T. sordida* distances of 0.024, 0.051 and 0.054 was obtained, respectively. The analysis of genetic distance and phylogeny of a gene fragment of mitochondrial DNA 16S corroborate the morphology and geometric morphometric results, in other words, the separation of different clades, *Triatoma* sp and *Triatoma costalimai* well as the relationship between the species *T. williami* and *T. sordida*. [1] Gonçalves et al. 25ª Reunião de Pesquisa Aplicada em Doença de Chagas (2009) p.47; [2] H. Lent, and P. Wygodzinsky. *Bull. Am. Mus. Nat. Hist. Mus.* 163 (1979) 127-520; [3] Teves-Neves et al. Livro de Resumos I Simpósio de Entomologia do Rio de Janeiro (2011) p.109; [4] Teves-Neves et al. Anais da 25ª Reunião de Pesquisa Aplicada em Doença de Chagas (2011) p.27; [5] Rosa et al. *Mem Inst Oswaldo Cruz* 105 (2010) 286-292; [6] Teves-Neves et al. Anais do XXIII Congresso da Sociedade Brasileira de Microscopia e Microanálise (2011). **Support:** ENERPEIXE/SA, SESAU-TO / FIOCRUZ, Platform PDTIS - FIOCRUZ and Department of Genetics, Federal University of Rio de Janeiro RJ. **Email:** tcmonte@ioc.fiocruz.br

Triat7. **A strategy for population control of insect vectors of Chagas disease: targeting rhodtestolin, a cardio-inhibitor from *Rhodnius prolixus***

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Introduction: In a comparison of several species of insect vectors of Chagas disease, we have found that the male reproductive organs are morphologically similar whereas the female organs show subtle but significant variations in structure. This difference suggests that population control strategies focusing on males will potentially affect a wide range of vector species in this family of bugs. The present study further supports this focus by demonstrating that a cardio-inhibitor, first discovered in the testes of *Rhodnius prolixus*, is also present in males of *R. brethesi*, *Triatoma dimidiata*, *T. klugi* and *Nesotriatoma bruneri*. **Materials and Methods:** Sexually mature insects were obtained from the laboratory of National and International Reference on Triatominae Taxonomy at the Institute of Oswaldo Cruz-Fiocruz. Insects were secured with plasticine placed over their legs, and the dorsal abdominal cuticle, dorsal epidermis and the digestive system removed to expose the reproductive system. Six to 12 tests were placed in a glass homogenizer containing 0.5 ml of insect saline, and homogenized. The homogenate was centrifuged at 2000 *g* for 5 min. The supernatant was tested by using an automatic pipette to apply a 100 µl stream of test saline over an exposed female heart of the same species. Concentrations tested ranged from 4 to 12 gland-equivalents per ml, with the insect saline serving as control. An immediate inhibition of the heart beat upon application of the test saline followed by a rest period lasting at least 1 min indicated the presence of a cardio-inhibitor. **Results:** The major circulatory organ of Reduviid is the dorsal vessel that extends anteriorly from the posterior end of the abdomen along the midline to the head. The heart is the region of the dorsal vessel in the two most posterior full-sized abdominal segments. The heart is associated with alary muscles which contract to expand the heart to fill it with haemolymph. Contraction of the heart propels haemolymph anteriorly. An exposed heart beats spontaneously at rates ranging from 4 to 25 beats per minute, and the application of a stream of control saline tends to increase the resting rate momentarily. Upon application of a test saline from testes homogenates of R*. brethesi*, *T. dimidiata*, *T. klugi* or *N. bruneri*, to the female heart of the species, the heart immediately becomes flaccid and stops beating. **Main conclusions:** A cardio-inhibitor associated with the testes of insects was first discovered in *R. prolixus* and was given the name rhodtestolin. Rhodtestolin activity has now been observed in another four species of Reduviid bugs, indicating that it likely has a basic function needed for reproductive success. This discovery suggests that control strategies focusing on rhodtestolin may be applicable to more than one species of Chagas disease vector, an important consideration since Chagas disease can be transmitted by several species of Reduviid. **E-mail:** gchiang@redeemer.ca

Triat8. **Development of molecular and immunochemical techniques for the detection of *Triatoma virus* (TrV) in human and animal samples**

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Introduction: TrV (*Dicistroviridae: Cripavirus*) is a natural enemy of *Triatoma infestans* (*Hemiptera: Reduviidae*), one of the main vectors of the Chagas disease. To date, TrV is the only entomopathogenic virus found in triatomines (Muscio OA *et al*. J. Invert. Pathol, 49:218–220, 1987). It can also infect natural populations of *T. sordida* and several experimental populations of triatomines as well (Muscio OA *et al*. J. Med. Entomol., 34: 253-256, 1997; Marti GA *et al*. J. Invert. Pathol, 102: 233–237, 2009; Rozas-Dennis GS, *et al.* Mem. Inst. Oswaldo Cruz, 97: 427-429, 2002). In this work we study the infectivity of TrV in mice, and we search anti-TrV antibodies in sera of patients with Chagas disease. **Materials and methods:** The infective and non-infective TrV particles were purified from faces coming from infected insects (Agirre J, *et al*. Virology, 409: 91-101, 2011). Female *Mus musculus* BALB/c mice were used in the experimental infection with TrV. The mice were inoculated with 3.0 µg of empty TrV particles (uninfected *T. Infestans*) and different concentration of TrV. In the PCR reactions were used two primers pairs: TrV sense: 5´TCAAAACTAACTATCATTCTGG 3´ (nt 7427 to 7448 in TrV ORF2 sequence) and TrV anti-sense: 5´TTCAGCCTTATTCCCCCCC 3´ (nt 8240–8258 in TrV ORF2 sequence), with an expected product of 832 bp. Total protein extract from empty TrV particles were used to adsorb on micro-plates for using in indirect enzyme-linked immunosorbent assays (ELISA), using sera from inoculated mice and sera of patients with Chagas disease from Brazil, Cuba and Portugal. **Results:** Mice inoculated with TrV did not show any behavioral alteration or clinical signals of viral infection (e.g. leg paralysis, food intake rate, loss of weight, motility decrease and death), when compared with mice inoculated with empty TrV particles or saline solution. No PCR products were detected from blood samples of any groups of mice inoculated with TrV or mice inoculated with empty particles of TrV. Anti-TrV antibodies results show that there is no significant difference in the IgG2a/IgG1 ratio between inoculated mice with TrV to the non-inoculated mice or mice inoculated with empty particles of TrV. When analyzed by ELISA, we observed that there are no significant levels of anti-TrV antibodies in sera of patients with Chagas disease from Brazil, Cuba and Portugal. **Conclusion:** The results of the ELISA anti-TrV together with the results of TrV vRNA search by RT-PCR in blood samples from mice inoculated with TrV indicate that this virus is not infective in mice at least under the conditions of this study. Moreover, the results of antibodies in sera from patients with Chagas disease indicate that there is no significant level of anti-TrV antibodies which can be explained by the absence of TrV these countries as well as non-infectivity of TrV in humans. **Acknowledgements:** This work was partially supported by Acción Especial AE-2009-1-21, Spain and CYTED 209RT0364 action (RedTrV: www.redtrv.org). **E-mail:** mssilva@ihmt.unl.pt

OTHERS

Other1. **Snakebites-induced acute kidney injury in Northeast Brazil**

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Introduction: Acute kidney injury (AKI) is a common complication of snakebites and is a public health problem in tropical countries. There are four venomous snakes genus involved in this complication: *Bothrops*, *Crotalus*, *Lachesis* e *Micrurus*. The aim of this study is to investigate the occurrence of AKI after snakebites in a reference hospital in Brazil. **Material and Methods:** This is a retrospective study including all patients victims of venomous snakebites admitted to the José Frota Institute, a reference emergency hospital in Fortaleza city, Northeast Brazil, from January 2003 to December 2010. Patients with AKI (group I) were compared to those without AKI (group II). Statistical analysis was done by the SPSS program and p values<0.05 were considered significant. **Results:** A total of 233 patients were

included. The majority of patients were male, precedent of rural areas (85.4%) and the most frequently affected body area was the lower limbs (62.2%). The prevalence of AKI was 10.3%. The main involved snake was *Bothrops sp* (62% of cases). The mean age of group I was 42±20 years, while in the group II it was 33±21 years (p=0.04). The time between the accident and medical care was higher in group I (23±24 hours) than in group II (14±17 hours), p=0.02. The time between the accident and the administration of the antiophidic sera was also higher in group I (24±24 hours) than in group II (13±15 hours), p=0.001. Serum sodium in group I was 134±6.9mEq/l, while in group II it was 139±4.8mEq/l (p=0.0001). The length of hospital stay was higher in group I (14±12.5 days) than in group II (3.3±2.2 days), p=0.0001. Factors associated to the development of AKI in the multivariate analysis were time between the accident and the administration of antiophidic sera, the dose of antiophidic sera and length of hospital stay. AKI was predominantly oliguric (54.2%), with a mean creatinine of 3.3±3mg/dl, need of dialysis in 29.1% of cases and complete renal function recovery in 50% of cases. There was no death in this cohort. **Main Conclusions:** AKI is an important complication of snakebites, being characterized by its severe course, in which half of the patients do not present complete recovery of renal function. The delayed administration of antiophidic sera is an independent risk factor for the development of AKI, which should guide preventive measures for providing these sera in the areas where accidents occur. **Financial Support:** CNPq (Brazilian Research Council). **E-mail:** ef.daher@uol.com.br

Other2. **Evaluation of crotamin, crotapotin, convulxin and gyroxin isolated from *Caudisona durissa terrificus* snake venom as dengue virus inhibitors**

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Dengue virus (DENV) represents a major human arbovirus that occurs throughout the tropics and affects 50-100 million people each year. DENV belongs to the genus *Flavivirus*, family *Flaviviridae*, and includes four antigenically distinct serotypes (DENV 1-4). In the absence of licensed vaccines or antiviral agents, there is an urgent need to develop an effective antiviral strategy against DENV. Snake venoms are a rich source of bioactive compounds with antiviral potential effects. The aim of this study was to analyze the anti-dengue virus activity of four isolated toxins (crotamin, crotapotin, convulxin and gyroxin) from *Caudisona durissa terrificus* snake venom. The antiviral activity against DENV-2 (NGC) was evaluated by three methodological strategies: virucidal, pre-treatment and post-infection assays. The effect of the samples was evaluated by replication inhibition in VERO cells, which was measured by plaque-reduction assay. The cytotoxic concentration for 50% of the cell monolayer (CC_{50}) and the concentration that inhibited 50% of the viral infection (EC_{50}) were used to calculate the selectivity index ($SI = CC_{50}/EC_{50}$). Toxins showed no cytotoxicity up to the higher concentration tested (500ng/µL); thus, this concentration was considered as the CC_{50} to calculate the SI. Crotapotin and gyroxin showed a potent antiviral effect in the virucidal assay (SI of 609.75 and 2083.33, respectively) and a lower effect in the pre-treatment assay (SI of 12.80 and 10.57, respectively). The high antiviral activity on virucidal assay suggests a direct action on the virus particle, while the effect in the pre-treatment assay also suggests a possible action on VERO cells. These results suggest that both toxins have a potential anti-dengue virus activity and could be used in the prospection of new antiviral drugs. **Email:** raquelrrusso@gmail.com

Other3. **Clinical utility of antivenom in the treatment of scorpion stings and envenomations: systemic review**

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Objective: Scorpion stings in Morocco are a significant public health issue and children under the age of 15 are the most severely affected. Consequences can be severe, possibly producing multi-system organ-

failure and death. The Moroccan poison center (CAPM) uses a systematic four level envenomation classification system. Scorpion antivenom in North Africa has been controversial in the past and is not currently in use in Morocco. In 2005 a synthesis of Foëx has demonstrated that intravenous administration of antivenom reduces serum venom concentrations, but the question remained open for antivenom clinical relevance. A Systematic review was carried out to assess the clinical utility of antivenom in scorpion envenomation and health outcomes. **Method:** search strategy: Medline 1974-2011 using the OVID interface. Comparators: treatment with or without scorpion antivenom. A clinical bottom line is stated. The author, date, and country of publication, patient group studied, study type, relevant outcomes, results, and study weaknesses of these best papers are tabulated. **Results:** While there are many case series and retrospective reviews in the literature (101 papers) suggesting that scorpion antivenom is safe and effective, there is only ten clinical trial of this treatment. Six of them showed no improvement in symptoms or in preventing symptom progression. There was no difference in hospital admission rate or duration of stay, and no difference in mortality. One study found any clinical improvement and this was mainly for local symptoms. Another study demonstrate that intravenous administration of scorpion-specific F(ab')(2) antivenom resolved the clinical syndrome within 4 hours and reduced the need for concomitant sedation with Midazolam. Two studies proved that recovery from scorpion sting is hastened by simultaneous administration of scorpion antivenom plus Prazosin compared with Prazosin alone. **Conclusions:** There is very little evidence that giving antivenom will improve clinical outcome in scorpion stings. Multi-center trials are needed to determine the effectiveness of scorpion antivenin. Scorpion envenomation syndrome without antivenom persists for greater than 4 hours in North Africa and in North America. Recent clinical trials in North America indicate that severe *Centroides* envenomation resolves within 4 hours when promptly treated with effective antivenom. Taken together, these findings suggest that the 4 hour endpoint in a similar population could be used to test efficacy of antivenom specific to North African species. **Keywords:** Scorpion, Antivenin, systemic review, Morocco. **E-mail:** asmaekhattabi@yahoo.fr

Other4. **Efficacy of four environmental insecticides against premature stages of the jigger flea *Tunga penetrans* (Siphonaptera)**

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Introduction: Infestation with the jigger flea *Tunga penetrans* (L., 1758) is a Neglected Tropical Disease causing substantial health burden in endemic areas. As part of integrated control measures, eggs found in the environment may be treated with insecticides. However, so far there are no systematic data available on the efficacy of environmental insecticides against premature stages. **Material and Methods:** The study was carried out in the city of Uberlândia (Minas Gerais State) in Brazil's savannah region. To obtain fertile *T. penetrans* eggs, Wistar rats were exposed on compounds in the outskirts of Uberlândia where tungiasis occurs in the human and animal population. Eggs were collected from embedded fleas. We tested four environmental contact insecticides *in vitro* against *T. penetrans* eggs: the synthetic pyrethroids deltamethrin and bifenthrin, etofenprox, and the organophosphate dichlorvos. In each insecticide and control group, 40 eggs were tested. Eggs were incubated and controlled for hatching after 3, 5 and 7 days. **Results:** No larvae hatched after treatment with the organophosphate dichlorvos (100% efficacy; 95% confidence interval [CI]: 92.1%-100%). The efficacies of the other products tested were: etofenprox 57.1% (95% CI: 29.3%-75.1%); bifenthrin 51.4% (95% CI: 30.0%-67.2%); and deltamethrin 17.1% (95% CI: 0%-35.7%). Dichlorvos showed significantly higher efficacy than all other insecticides ($p<0.0001$). On day three, 21/29 (72%) larvae in the deltamethrin group showed spontaneous movement. No larvae in the other intervention groups showed vital signs. In the control group, 35/40 (87.5%) of larvae hatched, and all were fully active. **Main Conclusions:** Only the organophosphate dichlorvos had a

good *in vitro* efficacy against eggs. The use of dichlorvos can be directed to typical spots where early stages of *T. penetrans* are expected, considering its toxicity. Disease control should also consist of measures concerning housing and environmental conditions, and animal and human reservoirs. **E-mail:** heukelbach@web.de

Other5. **Microorganisms from different sources in students of medicine**

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Introduction: Medical students are in constant contact with patients, personal health care and medical and diagnostic devices. This study aims to identify microorganisms and antibiotic resistance pattern isolated from nasal and oropharyngeal cavities, hands and stethoscopes of medical students during their training in a University Hospital. **Material and Methods:** This is a cross-sectional study in medical students. Samples were taken for each student from nasal cavity, oropharynx, hands and stethoscopes; they were cultured on blood agar-AS and gram stained. Gram-negative bacilli then were culture in eosin methylene blue agar, and biochemical test, the alpha hemolytic gram-positive cocci were subjected to catalase and optochin, gamma hemolytic gram-positive cocci to catalase and bile esculin agar, beta hemolytic gram-positive cocci to catalase, mannitol salt agar, novobiocin, coagulase and bacitracin. The gram-positive bacilli were culture in sugar media and motility test. Susceptibility to antibiotics was assessed using the Kirby-Bauer technique. For gram-positive cocci were tested oxacillin, ampicillin-sulbactam and cephalexin, for gram-negative bacilli: ampicillin-sulbactam, metronidazole, trimethoprim-sulfametoxasole TMP-SXT and gentamicin, for gram positive bacilli: penicillin, ciprofloxacin, metronidazole and clindamycin. Incubation was performed at 37°C under 5% CO_2 during 24 hours. **Results:** Of 100 women (64.5%) and 55 men (35.5%) were collected 848 isolates: 22.7% from nasal cavity, 19.7% from oral cavity, 28.8% from hands and 20.2% from stethoscopes, finding gram-positive cocci in 73.4%, gram negative bacilli 6.6%, gram positive bacilli 7.4%, nontypeable 1.4% and no growth 11.2%. *Staphylococcus aureus* 41.7%, Viridans Group Streptococci 15.4%, *Staphylococcus* coagulase-negative 10.2%, *Streptococcus* spp 7.8%, *Escherichia coli* 2%, *Listeria* spp 6.7%, *Enterococcus* spp 0.4%, *Yersinia* spp 0.5%, *Klebsiella* spp 0.9%, Group A Streptococci 0.2%, *Enterobacter* spp 0.2%, *Proteus* spp 0.5%, *Serratia* / *citrobacter* 0.1%, *Burkoldheria* spp 0,1%, *Aeromonas* spp 0.1%, *Hafnia* spp 0.1%, *Acinetobacter* spp 0.1%, *Erisipelothrix* spp 0.4%, *Propionibacterium* spp 0.4%, *Eubacterium* spp 1.9%, and other with less than 0,3%; negative isolates 8.6%.The resistance to oxacillin, ampicillin-sulbactam and cephalexin is greater than 22% in *Staphylococcus* spp and *Streptococcus* spp. In hands were found *E. coli*, *Klebsiella* spp, *Enterobacter* spp, *Acinetobacter* spp and *Burkholderia* spp, in stethoscopes, *Klebsiella* spp, *Proteus* spp, *E. coli*, *Aeromonas* spp, *Citrobacter* spp and *Serratia spp*, here was the only place where we identified the presence of *Enterococcus* spp. Most of gram negative bacilli showed sensitivity to TMP / SXT, ampicillin-sulbactam, gentamicin and metronidazole-resistant. No strains of *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and *Stenotrophomonas* spp were found. The four sources had a high frequency of *Listeria* spp with more than 75% of resistance to penicillin, metronidazole and clindamycin. **Conclusions:** It highlights an important variety of microorganisms, with a high prevalence of gram-positive cocci (*Staphylococcus aureus* and coagulase-negative *Staphylococci*). These findings reiterate the need to conduct good personal hygiene and care of the things of work in medical practice to minimize the circulation in community and health care facilities of identified pathogens. **E-mail:** ivan.mendez@unimilitar.edu.co

Other6. **The environmental epidemiology of climate-sensitive diseases in the northern Micronesian region of the Pacific**

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Introduction: The health impacts of climate change are an issue of growing concern in the South Pacific region. During 2010 and 2011, the World Health Organization supported the Ministries of Health in the Federated States of Micronesia (FSM), Republic of the Marshall Islands (RMI) and Republic of Palau (Palau) in performing climate change and health vulnerability and adaptation assessments. This process involved analysis of the available long-term data on climate and climate-sensitive diseases (CSD's) in each of the three countries. **Material and methods:** The material used in the analysis included daily inpatient and outpatient data from the four State hospitals in FSM and two main hospitals in RMI between 2000 and 2010; monthly cases of dengue fever and leptospirosis in Palau between 2002 and 2010; and monthly rainfall and temperature data from weather stations in all of the above locations. Time-series analyses (including generalized Poisson regression and distributed lag non-linear models) and correlations of health and climate data were used to demonstrate associations between monthly climate variables and cases of CSD's. **Results:** In FSM, a significant association was observed between monthly maximum temperature and monthly outpatient cases of diarrhea and respiratory disease in Pohnpei, both at a lag of one month. In RMI, significant associations were observed between monthly rainfall and temperature and monthly outpatient cases of gastroenteritis and respiratory disease and presentations related to diabetes. In Palau, significant associations were observed between monthly rainfall and cases of dengue fever and leptospirosis, with the effects on leptospirosis cases strongest at a lag of one month. **Main conclusions:** Analysis of the available climate and health data in these three small island developing countries in northern Micronesia demonstrated some significant relationships between climate variables and disease. This information proved useful in providing an evidence basis for proposed adaptation activities in the health and other sectors in each of the three countries aimed at avoiding the most serious impacts of climate change on the health of these communities. **E-mail:** lachlan.mciver@gmail.com

Other7. **Travel medicine: travelers' adherence to medical recommendations and prescriptions**

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Introduction: Due to the growing number of trips into and from Brazil, travel medicine has become more useful, aiming the reduction of travelers' morbidity and mortality and preventing the transmission of diseases amongst countries. Pre-traveling consultation intends to investigate the destination, the sort of trips travelers have planned and, by doing so, the risks associated with them. It also provides preliminary guidelines for prevention of insect-borne, water-related / foodborne diseases and accidents. Another important issue discussed on the consultation is malaria chemoprophylaxis prescription and self-treatment of travelers' diarrhea. The main goal of this research was to evaluate the compliance with recommendations offered during consultation by travelers who were assisted at Unifesp travel clinic**.** **Methods:** Data were obtained from chart review from January 2007 - November 2010, and interviews through telephone contact were carried out with travelers who had already returned from their destination, between June and November 2010. **Results:** We checked 708 medical records. Of these, 52.3% were female subjects. The predominant age group was 18-45 years of age. The African continent accounted for 25.4% of the reported destinations, followed by Asia, accounting for 21.4%. Self-treatment medication for traveler's diarrhea was prescribed to 303 individuals (42.8%). Seventy six travelers out of 708 records were selected for interview, and 41 were actually interviewed. Among the 41 respondents, 14 reported at least one health problem while traveling. Of these, 3 sought for medical care in the country of destination. Five out of 14 travelers reported two health problems during the trip, a total of 20 injuries (one injury every 2 travelers). Among the 41 respondents, 35 had their compliance with traveler's guidelines analyzed. The proportion of travelers who complied with protection against insect bites was 71.4%, and with consumption of bottled water was 85.7%. The compliance with not consuming ice was 80%, and with not eating raw meal was 77.1%. The observance for not eating from street vendors was 91.4%. Five out of nine travelers (55.5%) who were prescribed malaria chemoprophylaxis reported correct use of medication. Among the travelers who were interviewed, 26 had a prescription for traveler's diarrhea. Six of the respondents had a diarrhea episode over the trip, and 2 of them used the medication as instructed. **Conclusion:** Travelers assisted at Unifesp travel clinic are predominantly young. They travel for business

or leisure and have no underlying diseases. The African continent was the predominant destination by most of them. Pre-traveling consultation has proved itself efficient to ensure good compliance with traveler's guidelines for protection against insects and food selection. However, the difficulty for the correct use of malaria chemoprophylaxis observed in other studies was also identified among Brazilian travelers, being dosage and side-effects the major obstacles for compliance. The study finding that travelers' diarrhea was the most common infectious illness is consistent with the literature. Health guidelines before traveling are crucial for the travelers' safety and to tame diseases transmission between countries. **E-mail:** puccinipedro@gmail.com

Other8. **Traveler's vaccinations – current knowledge of Polish patients**

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Background: The number of traveling Polish people is increasing and they more often choose overseas destinations. International travelers require vaccinations and general knowledge concerning chemoprophylaxis and safe behavior. The aim of the paper was to estimate knowledge of traveling people concerning obligatory and recommended vaccinations. **Material and methods:** Anonymous self-fulfilled survey was distributed among 1000 adult healthy patients aged 19-64 years (67% were men) coming for a vaccination or waiting for a visit to a travel medicine specialist. Final destination for 50% of responders was Asia, for 26% - Africa, for 24% - Europe. **Results:** 94% of responders declared they are aware of epidemiological risks at the place of destination, 88% found their knowledge concerning vaccinations before travel as good and enough. 54% of patients consulted with a physician only sometimes before travel, 32% of responders declared they look for the medical consultation always before travel. The main source of knowledge concerning vaccinations was internet (78%), the medical professional (general practitioner) was considered as the source of knowledge concerning travel vaccinations only by 12% of responders. The infectious diseases which may be dangerous for travelers were found malaria (66%), typhoid fever (50%) and hepatitis A (42%). Recently (during last 6 months) conducted vaccinations among responders were: vaccination against tetanus (82%), hepatitis B (80%) and hepatitis A (78%). 84% of patients used to conduct all vaccinations recommended by a physician. 74% of responders did not know which vaccination was obligatory for travelers according to International Health Regulations. **Conclusions:** General knowledge concerning vaccination before travel is not good and enough among patients planning international travel. Not all of patients consult a physician before travel. The main source of knowledge concerning vaccinations for travelers is internet. It is recommended to increase the role of general practitioners in pre vaccine consultations for traveling patients. **E-mail:** anitsch@amwaw.edu.pl

Other9. **Predictors of mortality among patients with tropical diseases admitted to a specialized intensive care unit in Northeast Brazil**

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Introduction and Aims: Tropical diseases are important morbidity factors and mortality causes, accounting for more than 13 million deaths a year, mainly in developing countries. The aim of this study is to investigate risk factors for death in critically ill patients with tropical diseases. **Methods:** This is a retrospective study conducted at a tertiary infectious diseases hospital in Fortaleza city, Northeast Brazil, from 2003 to 2009, including 247 patients with confirmed diagnosis of tropical diseases admitted to the intensive care unit. Acute kidney injury (AKI) was defined according to the RIFLE criteria ("Risk, Injury, Failure, Loss, End-stage renal disease"). Severity was assessed through APACHE II criteria. A comparison between survivors and non-survivors was done. Statistical analysis was done with SPSS program, version 16.0, considering as significant $p<0.05$. **Results:** Patient`s average age was 46.5±16.5 years, and 76% were male. The main causes of hospital admission were HIV (27, 5%), Tuberculosis (8, 22%), Leptospirosis (7, 7%), Dengue (5, 6%) and Visceral *Leishmaniasis* (3, 2%). Death was observed in

159 cases (64.4%). The comparison between survivors and non-survivors showed no difference regarding age (46±17 vs. 47±16 years, p=0.66) and gender (male 78.4% vs. 70.4%, p=0.08), serum urea (108±62 vs. 104±73mg/dL, p=0.67) and creatinine (3.2±1.8 vs. 3.1±1.7mg/dL, p=0.73). Non-survivors presented lower levels of systolic (112±26 vs. 123±28mmHg, p=0.002) and diastolic blood pressure (68±17 vs. 73±42mmHg, p=0.04), arterial pH (7.25±0.13 vs. 7.36±0.10, p<0.0001) and HCO3 (15±5.9 vs. 18±6.2mEq/L, p=0.001). Need of dialysis was higher among non-survivors (66.2% vs. 33.8%, p=0.04), as well as the frequency of respiratory insufficiency (65.3% vs. 34.7%, p=0.02). APACHE II score was higher among non-survivors (58±20 vs. 34±19, p<0.0001). There was no significant difference regarding the RIFLE criteria comparing survivors and non-survivors: Risk (44.4% vs. 55.6%, p=0.31), Injury (32.7% vs. 67.3%, p=1.66), Failure (40.5% vs. 59.5%, p-1.20). **Conclusions:** Tropical diseases are important cause of intensive care unit admissions, which can have a severe course. The main diseases observed in our study were HIV (27, 5%), Tuberculosis (8, 22%), Leptospirosis (7, 7%), Dengue (5, 6%) and Visceral Leishmaniasis (3, 2%). Factors associated with death were hypotension, metabolic acidosis, acute kidney injury and respiratory insufficiency. APACHE II was significantly associated with mortality. **Financial Support:** CNPq (Brazilian Research Council). **E-mail:** ef.daher@uol.com.br

Other10. **Health concern and chronic poisoning of heavy metals for drinking water consumers in rural regions in the west area of Iran**

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Introduction: Water pollution due to toxic heavy metals has been a major cause of concern. For these reason the aim of this study was to evaluate contamination of trace element in drinking water and the health status of inhabitants with respect to multi-chronic arsenical poisoning. **Material and Methods:** Concentrations of 8 heavy metals (As, Se, Hg, Cd, Ag, Mn, Cr and Pb) were measured in drinking water sources from 530 villages in Kurdistan Province in the west of Iran by graphite furnace or flame atomic absorption spectroscopy method. **Results:** The results showed that the level of As, Cd and Se in 28 village drinking water sources exceeded WHO or National Standard Limits. The levels of concentration of arsenic in drinking water ranged from 42 to 1500 µg/L. Then in a cross-sectional survey, 587 people from 211 households were chosen for clinical examinations of multi-chronic arsenical poisoning including pigment disorders, keratosis of palms and soles, Mee's line in fingers and nails and the gangrene as a systemic manifestation. Of 587 participants, 180 (30.7%) participants were affected by representing the type of chronic arsenical poisoning. The prevalence of Mee's line, keratosis, and pigment disorders were 86.1%, 77.2% and 67.8% respectively. Therefore, the prevalence of Mee's line between inhabitants was higher than the other disorders. The results show a strong linear relationship between arsenic exposure and occurrence of multi-chronic arsenical poisoning (R^2=0.76). The association between age for more than 40 years and gender for more than 60 years with chronic arsenical poisoning is significant (p<0.05). Also, there is a relationship between subjects who were affected with disorders and duration of living in the village. Except for gangrene disorder, the odds ratio of prevalence of other disorders with arsenic exposure level in drinking water show a highly significant relationship between arsenic content and the risk of chronic disorders (p<0.01). **Conclusion:** These results confirm the need to further study trace elements in drinking waters, food products and other samples in this area and the relationship to other chronic diseases arising out of arsenicosis. **Keywords:** Chronic poisoning; drinking water; heavy metals. **E-mail:** alasvand50@yahoo.com

Other11. **SEM method in Paleoparasitology: known techniques for unknown future**

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Introduction: Paleoparasitology emerged as a new science almost 35 years ago. Since the first record of parasites in coprolites[1] optical microscopy is the main tool for diagnosis. Over the years, new

techniques were introduced and the findings of parasites in archaeological material increase, shedding light to new possibilities. The increase in paleoparasitological record led to the application of new techniques such as the use of commercial kits for immunological diagnosis of *Giardia duodenalis*[2]. Later, by applying techniques of molecular analysis to mummified tissues and coprolites, new records of Chagas disease[3] and distribution of *Ascaris lumbricoides* and *Trichuris trichiura* in pre-Columbian America were obtained[4]. Electron microscopy has been also a powerful tool to characterize and help in the diagnosis of archeological samples. Although after 50 years since the first publication, few publications appeared showing good results using electron microscopy, either by transmission (TEM) or scanning (SEM). Data obtained from studies that use novel methods of diagnosis can increase the knowledge about these diseases, lifestyle, and behavior of ancient populations. **Material and Methods:** Optical microscopy- coprolites samples were rehydrated in a 0.5% Na_3PO_4 solution for 72h[5] followed by spontaneous sedimentation[6]. The sediment was mounted in glass slides and observed under bright field microscopy. SEM- small fragments of coprolites were crushed using a glass stick. The pieces were separated using a stereomicroscope. The smallest grains were set apart with a paintbrush and mounted on scanning electron microscope stubs with double-sided carbon tape, sputter-coated with gold and examined under a scanning electron microscopy. **Results:** Using SEM in coprolites we visualized eggs to define size and shell layout. Coprolites observed were identified as of *Tamandua tetradactyla*, dated 8870±60 years BP. After crushed, eggs of *Acanthocephala sp*. were identified. Average size was 114.1x66.60µm for dehydrated external shell and 115.3x67.58µm after rehydration. Another coprolite used for SEM came from a mummy of a priest, housed in the Piraino Cathedral with a high infection by *T. trichiura*[7]. Eggs measured 59.6-61.78x31.09-33.54µm. Now we are applying this technique to helminth eggs identified as *Echinostoma* sp. This Digenea was found associated with a partly mummified body from Minas Gerais dated 670 years[8]. Species identification is difficult without adult worms but SEM techniques may help to identify this and other parasites and bring new details of the structure of these eggs. **Supported by**: CNPq and FAPERJ **E-mail:** jsantiago@ensp.fiocruz.br