Vectors and the spread of urban visceral leishmaniasis in South America

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The spread of visceral leishmaniasis (VL) in Latin America in recent decades is a complex event that could be analyzed from different scales of time and space. The macroscale analysis shows that human VL incidence increased associated with records of *Lutzomyia longipalpis*, the main vector, in urban environments and places without previous records. This event was recorded in Brazil since the 70`s spreading from the historical NE foci and reaching Mato Grosso do Sul-MS around the year 2000, then in Paraguay from the border with MS to Asuncion and Department Central and to W, and then in Argentina since the record of the vector in the border with Paraguay in 2004 to new ones that were reported successively in four provinces, the southern states of Brazil, and two localities of Uruguay in 2010. Other phenomena related to the spread could be not associated with time-space changes of *Lu. longipalpis* but with the increase in the awareness about VL, and the intensified parasite and human-pet circulation: dispersion of parasite strains/typical-atypical VL, canine VL, vector capacities of *Lu longipalpis* complex/other species. The mesoscale analysis al locality level suggested that the distribution of the main vector in time and space behaves as a metapopulation with 'hot spots-sources' in suitable environment patches, that could change location/size according to the season and associated with the intensity of transmission. The microscale it is not an appropriate scale to analyze spread; however, at this scale many alleged urbanization-spread reports could be understood as outbreak foci (common source transmission) in ruralized-periurban habitats (micro/macrohabitat), but in cadastral urban areas (mesoscale) in new urbanized regions (macroscale). Further, the results obtained on dispersal at microscale allows to evaluate the feasibility of the means of spread at greater scales (passive/active, adult/preimaginal stages), and the eventual effect of global environmental and climatic changes.

microRNAs in *Echinococcus* spp.: characterization and future applications

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BACKGROUND: *Echinococcus* spp. platyhelminth parasites are ethiological agents of hydatid disease affecting human and animal health worldwide. Understanding the mechanisms that regulate their particular characteristics of development may allow identifying new therapeutic targets. MicroRNAs are small silencing RNAs that impact eukaryotic development and are receiving growing attention as novel therapeutic and diagnosis targets.
METHODS: We have employed high throughput small RNA sequencing to characterize the small RNAomes of *Echinococcus* spp. species/stages using available genomic information including the recently sequenced draft genome of *Echinococcus canadensis* G7.
RESULTS: We have obtained up to 40 million reads per library with high percentage of genome mapping. Significant proportions of small RNA reads corresponded to microRNAs. The previously reported *Echinococcus* spp. microRNA catalog consisting of 20 miRNAs (Cucher et al, 2011) was expanded to 38 conserved and 3 new candidate microRNAs. Interestingly, one microRNA was present in the closely related *Echinococcus granulosus* G1 and *E. canadensis* G7 but absent from *Echinococcus multilocularis*. Expression analyses showed that some miRNAs were highly expressed in all the stages and species analysed. MicroRNAs differentially expressed between stages and species were also identified. *Echinococcus* spp. microRNA biogenesis showed particularities that could impact on targeted genes. No evidence of other canonical small silencing RNAs like piRNAs has been obtained so far.
CONCLUSIONS: MicroRNAs are the principal small RNA silencing molecules in *Echinococcus* spp. The *Echinococcus* spp. small RNAome will be deposited in the FlatDB, a recently launched database of flatworm genetic data. The differential expression of microRNAs during life cycle suggests important roles in development. Highly expressed parasite microRNAs absent or divergent in mammal hosts were indentified and could be candidates for drug and diagnosis targeting.

Inhibiting drug transporters to increase anthelmintic efficacy

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BACKGROUND: Many pathogens evade drug toxicity by inducing xenobiotic detoxification systems, and induction or selection of active drug efflux transporters such as P-glycoprotein (P-gp) homologues occurs in response to ivermectin exposure in free-living and parasitic nematodes. Given that the products of the latter genes contribute to drug efflux out of the target parasite, transporter gene induction may contribute to macrocyclic lactone (ML)-based therapy failure in parasites such as *Haemonchus contortus*. In this context, identification of safe and effective inhibitors of these transporters to increase ML treatment efficiency is of interest. We have tested the ML derivative ivermectin aglycone as a P-gp inhibitor, as it is not a commercial anthelmintic.
METHODS: We evaluate the affinity of ivermectin-aglycone to ABC transporters in cells ovrexpressing human P-glycoprotein (P-gp), and its ability to reverse drug resistance in vitro in vinblastin-resistant cells and in ivermectin-resistant nematodes, *in vitro* and *in vivo* in parasitized sheep. Neurotoxicity was evaluated *in vitro* by measuring capacity to open rat GABA-gated chloride chanels expressed in recombinant *Xenopus laevis* oocytes and *in vivo* in P-gp deficient mice.
RESULTS: Ivermectin-aglycone inhibits human P-gp transport activity. It also increased drug sensitivity in vinblastin-resistant cells and in ivermectin-resistant *Caenorhabditis elegans* and *Teladorsagian circumcincta, in vitro* and *in vivo* in infected sheep. Moreover, unlike ivermectin, ivermectin-aglycone had low affinity for rat GABA receptors and had no observable neurotoxicity in P-gp-deficient mice.
CONCLUSON: Co-administration of MLs and ivermectin aglycone is presumed to lead to an increase of ML concentration in the parasite, thus offering an increased anthelmintic efficacy. Such an approach offers a safe means of delaying ML resistance and new hope for sustaining anthelmintic control after ML resistance has arisen.

A role for rhomboid proteases in *Trichomonas vaginalis* pathogenesis?

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Trichomonas vaginalis is an extracellular parasite that is the causative agent of trichomoniasis, the most common non-viral sexually transmitted infection in the world. Although the disease burden is high, little is known about the pathogenic mechanisms utilized by *T. vaginalis*. We hypothesize that *T. vaginalis* has intramembrane serine proteases called rhomboids that contribute to the parasite's virulence. Through bioinformatics, we identified four rhomboids that contain the defining catalytic dyad required for activity, composed of a serine and a histidine. We exogenously expressed three of these with N-terminal hemagglutinin tags and localized the proteins using indirect immunofluorescence assays. We found that two rhomboids localize near but not within the nucleus and one rhomboid, rhomboid 1 (ROM1), localized on the cell surface and intracellular vesicle-like structures. Using heterologous cell cleavage assays we have demonstrated that ROM1 has protease activity. Furthermore, cell viability assays were used to measure lactate dehydrogenase release from damaged host ectocervical cells and showed that ROM1-overexpressing parasites are significantly more cytolytic compared to control parasites. Conversely, parasites overexpressing a mutant form of ROM1 in which the catalytic residues have been mutated to alanines display a reduction in cytolysis. To confirm the specificity of the observed phenotypes, we expressed the ROM1 catalytic mutant fused to the FKBP destabilization domain (dd). The destabilization domain causes the fusion protein to be degraded in the absence of the dd-ligand Shield. When Shield is present to stabilize the mutant protein, the significant decrease in cytolysis compared to vehicle-treated parasites is recapitulated. Furthermore, we have used a quantitative proteomics approach to identify substrates cleaved by the *T. vaginalis* rhomboids, and we are in the process of validating their interactions. Results from these studies will help elucidate the biological functions of rhomboid proteases in this widespread human pathogen.

Urogenital schistosomiasis among *Fulani pastoralists* in Rivers state, Nigeria

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BACKGROUND: Schistosomiasis is water borne parasitic disease; it is a disease of poverty that leads to chronic ill-health. The disease is characterized by haematuria and transmitted by particular fresh water snails. Schistosomiasis is considered by the World Health Organization as the second most socio-economically devastating parasitic disease, next only to malaria.
METHODS: Urine samples were collected from 593 Fulani pastoralists from six bush encampments and examined for schistosome eggs using centrifugation method. The number of eggs obtained per 10ml of urine specimen was counted and quantified as intensity of infection. Egg counts were reported according to the following categories. Light infection ≤50eggs/10ml of urine, moderate infection- ≥50≤100 eggs/10ml of urine, heavy infection- ≥100 eggs/10ml of urine of urine. Urine samples were tested for proteinuria and haematuria using commercial reagent strips capable of detecting urinary blood, protein and other parameters.
RESULTS: Of the 593 Fulani pastoralists who were investigated, 394(66.4%) were infected with a mean overall intensity of 83.3±2.0 eggs/10ml of urine. The herdsmen in Eleme and Oyigbo bush encampments had the highest prevalence of 91(81.3%) and 77(76.2%) respectively with a mean intensity of 96.1±4.0 and 93.1±5.1 eggs/10ml urine. There were significantly more infected males than the females (p<0.05). The subjects aged 21-30 years had the highest prevalence 92 (76.0%) with heavy intensity of infection (25.0%). About 169(42.9%) of infected Fulani's excreted ≥50≤100 eggs/10ml of urine while 132(33.5%) excreted ≥100 eggs/10ml of urine. Haematuria was recorded in 444(74.9%) with majority observed in 31-40 years age group while 427(72.0%) tested positive for proteinuria.
CONCLUSIONS: The results revealed high prevalence and intensity rates of *Schistosoma haematobium* among the Fulani herdsmen. Since such herdsmen are always on the move in search of greener pastures they would always pollute water bodies and thus serve as source of transmission to neighbouring communities. Health education campaigns by health workers as well as intensified integrated control measures are advocated.

Identification of two Cullin-like proteins and its behavior during the intraerythrocytic cycle of *Plasmodium chabaudi*

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BACKGROUND: Malaria is a mosquito-borne disease caused by members of the genus *Plasmodium spp*. Rodent malaria species have proven being useful to investigate diverse aspects of the biology of human malaria. One of the strategies used to deal with this issue is to study the post-translational modifications in order to understand key regulatory elements throughout the *Plasmodium* life cycle. Among these protein modifications, ubiquitination is one of the most important, regulating protein turnover, transcriptional regulation, cell cycle progression, differentiation and signal transduction. When proteins are degraded by the ubiquitin-proteasome system, the ubiquitin could be transferred by the cullin-RING E3 ligase (CRLs) superfamily which includes the SCF (Skp1-Cullin-F box protein) or SCF-like complexes.
METHODS: We worked with *Plasmodium chabaudi* clone AS which exhibits a synchronous intraerythrocytic cycle. Bioinfomatic analyses were performed using Plasmodb databank. We obtained RNA samples at different times corresponding to ring, trophozoite and schizont stages. qRT-PCR was performed. The carboxyl terminal domain of both Cullin-like genes were expressed to produce the recombinant protein and to immunize rabbits. With these antibodies the proteins were used to perform Western-blot.
RESULTS: With this work, we identified two Cullin-like genes (PCHAS_142830 and PCHAS_112810) in the *P. chabaudi* genome. Both proteins posses a similar structure as the Cullin protein family (amino and carboxyl terminal domains); however, PCHAS_112810 does not posses a Neddylation site. These two genes are transcribed during the intraerythrocytic cycle, and the peak of expression of both is during the schizont phase.
CONCLUSIONS: Our results suggest that Cullin-like scaffold proteins are active during the intraerythrocytic cycle of *P. chabaudi* regulating cell activities by mean of proteins ubiquitination and further destruction by the proteasome system.

Expression analysis and tissue distribution of 14-3-3 isoforms in *Aedes aegypti*

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BACKGROUND: The 14-3-3 proteins are 30kDa acidic proteins evolutionary conserved that forms a family which in mammalian cells is composed of seven isoforms (β, Υ, Ɛ, ζ, η, θ, and σ); 13 in Arabidopsis, and two in Drosophila. Homodimeric or heterodimeric complexes, act as molecular adaptors mediating a phosphorylation-dependent interaction with signaling molecules involved in cell differentiation, control of cell cycle, proliferation, apoptosis and cancer. 14-3-3 has been identified in diptera (*Anopheles*, *Aedes*, *Culex* and *Drosophila*), lepidoptera (*Spodoptera*, *Plutella* and *Pieris*). By proteomic methods it was observed in *Anopheles gambiae* mosquitoes, infected with *Plasmodium berghei* sporozoites, an increase in the expression of 14-3-3 protein in the head. Similarly, in *Aedes aegypti* 14-3-3 was identified in isolated brush border membrane vesicles from midgut.

METHODS: Here, we found, by bioinformatic analysis, two possible 14-3-3 isoforms in the genome of *Ae. aegypti,* which have high homology (85%) to human ζ and Ɛ isoforms. According to RT-PCR analysis with primers designed to discriminate the isoforms messengers. By Western blot and confocal microscopy, using a rabbit polyclonal antibody directed against all members of the human 14-3-3 protein family.

RESULTS: We found expression two 14-3-3 isoforms, *Ae14-3-3ζ* and *Ae14-3-3Ɛ* are expressed in all phases of *Ae aegypti* life cycle and adult dissected tissues (head, midgut, fat body and ovary). By Western blot just one band was detected, although, due to the two predicted 14-3-3 proteins have the similar molecular weight, it was impossible to discriminate among them and by confocal microscopy it was observed that 14-3-3 protein(s) are localized in the cytoplasm of cells near the plasmatic membrane possibly associated to cytoskeleton elements.

CONCLUSIONS: This is the first time that expression of the two 14-3-3 isoforms is observed in *Ae. aegypti*. 14-3-3 can interact with diverse cellular signaling components regulating their functions and at present we are investigating the possible role during development of the mosquito and their possible functional redundancy.

A household latrine to prevent soil transmitted helminth infection in rural villages in Central Java: the BALatrine intervention

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BACKGROUND: Open defecation contributes to Indonesia's high concentrations of soil-transmitted helminth (STH) infection in village environments. This paper reports on a pilot experimental randomised control trial, funded by the UBS-Optimus Foundation, to determine the impact of a household latrine, on the incidence of STH infections in C. Java, Indonesia.
METHODS: The BALatrine intervention, designed for local people and materials in resource poor settings, is a low cost, locally constructed squat toilet that works in both wet and dry conditions. The test site was in two rural villages: one where the BALatrines were introduced and one as a control village with no BALatrine. A baseline and follow-up questionnaire was administered to all village residents (5-65 yrs), who also provided 2 stool samples for parasitological examination. Following the baseline survey all residents were treated with a single oral dose of Albendazole (400mg) according to WHO guidelines, so the incidence of STH could be assessed at follow-up 8 months later. Those found positive at follow-up were treated. The efficacy of the BALatrine on reducing STH infection was measured by comparing human STH incidence between the two villages.
RESULTS: Dependent variables analysed were (i) the presence/absence of worm eggs in stool samples, as confirmed by laboratory analysis; (ii) absence from work/school in the previous 3 months caused by bowel illness. The independent variables were the village of residence; demographic characteristics; and certain behaviours associated with the risk of helminthiasis such as spending time in the paddy fields, not using eating utensils and not having clean hands or fingernails. A significant reduction in human infection rates ($p < .001$, odds ratio = 2.31 (1.42 to 3.79)) in the intervention village and lower infection intensity provided proof of principle.
CONCLUSIONS: Village households with BALatrines have lower STH infection and less school/work absenteeism. Our research confirms earlier findings that the BALatrine is flexible, easy to make and install, and appropriate to the total village environment. As a household rather than a community-based facility, it is 'owned' by family members and thus kept clean and operational. The BALatrine offers an important, culturally appropriate intervention in the fight against helminthiasis. A scaled up project funded through the UBS-Optimus Foundation and a National Health and Medical Research Council (Australia) Partnership Grant is currently under way across 16 villages.

Evaluation of four purification methods of the antigen of cysticerci from *Taenia solium* for human diagnostic

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BACKGROUND: imaging studies and the clinical compatible with cysticercosis are complemented by inmunoserologic diagnosis. Crude antigens extracted from the vesicular fluid of *T.solium* cysticerci (*T.sol*.CVF.Ag) have been used for this purpose; however, the availability of technology and cost required to purify is limited in developing countries such as Peru. We compared and evaluated four purification methods of the cysticercus antigens for human cysticercosis serodiagnostic.
METHODS: Cysticerci were collected from different endemic areas of Peru to obtain antigenic material (*T.sol*.CVF.Ag). The antigenic glycoproteins were purified using the following methods: i) ammonium sulphate, ii) lentil-lectin sepharose (affinity chromatography), iii) sephadex G-75, and iv) electro-elution. The analytic sensitivity and specificity were evaluated using purified glycoproteins, patient serum from individuals with NCC and other parasitosis.
RESULTS: The protein of crude native antigen was quantified and we found 3.6µg/µL, concentration considered by the CDC. SDS-PAGE electrophoresis revealed seven glycoproteins (GP50, GP42-39, GP24, GP21, GP18, GP14 and GP13kDa) with affinity/lentil-lectin method. Our study identified eight bands diagnostics with the same affinity/lentil-lectin method (GPs.35, 31, 24, 23, 18, 17, 14 and 13kDa) and the other three methods evaluated detected different fractions of these proteins (ammonium sulphate: no band, electro-elution: 6 bands (GPs.97, 31, 25, 21, 13 y 12 KDa), and sephadex G-75: 17 bands (6, 10, 12, 13, 14, 17, 18, 21, 23, 24, 31,35, 42-45, 66, 95, 97 y 100 KDa). Western blotting, using native antigenic glycoprotein (*T.sol*.CVF.Ag) Purified (affinity/lentil-lectin method) identified 50/50 patients with NCC (100% sensitivity), 50/50 healthy control individuals (100% specificity), 20 individuals with other parasitosis (*Hymenolepis nana, Echinococcus granulosus, Fasciola hepatica*) give cross-reacted with the GP42-39Kda protein.
CONCLUSIONS: Affinity chromatography (lentil-lectin sepharose) is the method of choice to purify antigenic glycoprotein *T.solium* cysticerci (*T.sol*.CVF.Ag) for the development of a reliable and affordable immunodiagnostic kit for NCC and which can be applied in endemic countries of human cysticercosis.

Evaluation of urinary antibody detection for diagnosis of human strongyloidiasis

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BACKGROUND: Serodiagnosis of strongyloidiasis has higher sensitivity than conventional parasitological method but it usually requires blood sample for the analyses. Here we established a protocol for *Strongyloides*-specific IgG detection in urine specimens by enzyme-linked immunosorbent assays (ELISA) and assesses its value in diagnosis of strongyloidiasis in comparison with serum antibody.
METHODS: Indirect enzyme-linked immunosorbent assay protocol was developed to detect *Strongyloides*-specific IgG in urine specimens. The conventional serum-based ELISA system was performed on matched specimens. Standard faecal examination for parasitic infection was performed using agar plate culture technique (APCT) and formalin-ethyl acetate concentration techniques (FECT). Performances of urine and serum-based ELISA were evaluated using the parasitological methods as a reference.
RESULTS: The urine-based ELISA for diagnosis of strongyloidiasis had sensitivity and specificity of 82.9% and 100%, respectively. The sensitivity and specificity for serum-based ELISA were 92.7% and 100%, respectively. The seroprevalence of strongyloidiasis by ELISA was 62.4% for urine and 65.8% for serum compared with 27.5% (n=149) by parasitological methods. The urine-based ELISA showed no cross reaction with common parasites in the region i.e. *Opisthorchis viverrini*, *Taenia*, *Trichuris* and hookworms.
CONCLUSIONS: Urinary antibody detection by ELISA for serodiagnosis of strongyloidiasis had similar efficacy to serum-based ELISA and both methods are more sensitive than standard parasitological diagnoses. Since collection of urine is non-invasive and easy to do, the urine ELISA is suitable for diagnosis as well as mass screening of strongyloidiasis prior to a standard confirmatory test

Increase in length of *Toxocara canis* larvae induced by progesterone and prolactin stimulation and identification of progesterone and prolactin-like receptors in larvae cells from *Toxocara canis*.

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BACKGROUND: Sexual hormones affect parasite infection´s course. *Toxocara canis* larvae is encysted in different tissues from adult dogs. During the pregnancy, these larvae are activated and transmitted by transplacentary and lactogenic ways to its progeny. An important event in this stage is the hormone variation; which suggest that larvae are able to recognize this variation and used it for their development. In this study we evaluate the *in vitro* effect of progesterone and prolactin on the size of *Toxocara canis* larvae and identify, by flow cytometry, the presence of progesterone and prolactin binding proteins resembling receptors in *T. canis* larvae cells.
METHODS: Larvae were cultivated during 20 days in RPMI-1640 environment with 1% of Glucose and 37°C and 5% of $C0_2$ (2000 larvae/ml). Every 48 hours, progesterone and prolactin were added to different cultures (0, 20, 40, 80, 400, 800 ng/ml and 0, 2, 20, 40, 400 ng/ml respectively). To assess larval length larvae were measured on days 0, 5, 10, 15 and 20. In flow cytometry, the intracellular labeling was made with primary antibodies which recognize prolactin and progesterone receptors. As secondary antibodies were used anti-mouse IgG and anti-rabbit IgG coupled with APC and Alexa 647 respectively.
RESULTS: Stimulated larvae with 80 ng/mL of progesterone increased their size ($P<0.05$) compared with non-stimulated larvae (53.17± 2.8 and 22.8 ± 2.9 µm respectively) or stimulated with other concentrations. Stimulated larvae with 40 ng/mL prolactin, also increased their size ($P<0.05$) when was compared with non-stimulated larvae (27.9 ± 2.1 and 19.9 ± 2.0 µm respectively). Flow cytometry results showed that larval cells have molecules similar to hormone receptors (13.4% positive to prolactin like-receptor and 13.6% positive to progesterone like-receptor).
CONCLUSIONS: *Toxocara canis larvae* are able to recognize and use the host´s progesterone and prolactin for their development. The *Toxocara canis* larvae have progesterone and prolactin receptors.

Genotyping and phylogenetic relationships of keratitis and environmental *Acanthamoeba* isolates from Vitória, Espírito Santo, Brazil

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BACKGROUND: Molecular analysis of 18S rDNA gene is currently a widely used tool for identification, genotyping and phylogenetic studies among *Acanthamoeba* isolates. In Brazil, few studies focused the diversity of genotypes of *Acanthamoeba* in the ambient and from clinical cases. In this study, we identify the genotypes and infer epidemiological relationships between isolates obtained from corneal scrapings, contact lenses and a variety of domestic and natural ambient in Vitória, Espírito Santo, Brazil.
METHODS: Twenty-six isolates of *Acanthamoeba* were obtained by isolation in NNA-soy agar. The samples were collected from corneal scrapings (three), contact lens (one), domestic dust (three), soil (two), swimming pool (four), tap water (five), sea water (four) and flood water (four). Each isolate was cloned and after the total DNA extraction, ASA.S1 fragment from 18S rDNA was amplified by PCR and sequenced. Alignments, phylogenetic reconstructions and evolutionary distances were done with MEGA5. Sequences were compared to the ones available in GenBank.
RESULTS: Seven environmental isolates were identified as T1, T3, T5 and T11 genotypes. The remaining samples were classified as T4. The isolates were distributed in genotypic clusters, corroborating early studies. Similar sequences were noted in isolates from right eye corneal scraping and right lens of a same patient. Phylogenetic analysis showed that these samples are strictly related.
CONCLUSIONS: T4 is the predominant genotype in clinical and nonclinical isolates, which is in agreement with previously reported *Acanthamoeba* studies. Phylogenetic analysis suggested a correlation between contact lens contamination and infection in one patient, although the possibility of a direct eye infection cannot be discarded. Our perspectives include the addition of new environmental isolates from different Brazilian regions and from recent cases of *Acanthamoeba* keratitis, as well as the identification of intra-genotype variations. Financial support: UFES-NDI/CAPES/FAPES.

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Attitude and knowledge of primary health care physicians and local inhabitants about leishmaniasis and sandfly in west Alexandria, Egypt

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Background: Leishmaniasis is a worldwide disease, affecting 88 countries, it is estimated that about 350 million people are at risk of leishmaniasis. Overall prevalence is 12 million people with annual mortality of about 60,000. Annual incidence is 1,500,000 cases of cutaneous leishmaniasis (CL) worldwide and half million cases of visceral Leishmaniasis (VL).
Objectives: The objective of this study was to assess primary health care physicians knowledge (PHP) and attitude about leishmaniasis and to assess awareness of local inhabitants about the disease and its vector in four areas in west Alexandria, Egypt.
Methods: This study was a cross sectional survey that was conducted in four PHC units in west Alexandria. All physicians currently working in these units during the study period were invited to participate in the study, only 20 PHP completed the questionnaire. 60 local inhabitant were selected randomly from the four areas of the study, 15 from each area; Data was collected through two different specially designed questionnaires.
Results: 11(55%) percent of the physicians had satisfactory knowledge, they answered more than 9 (60%) questions out of a total 14 questions about leishmaniasis and sandfly. The second part of the questionnaire is concerned with attitude of the primary health care physicians about leishmaniasis, 17 (85%) had good attitude and 3 (15%) had poor attitude. The second questionnaire showed that the awareness of local inhabitants about leishmaniasis and sandly as a vector of the disease is poor and needs to be corrected. Most of the respondents (90%) had not heard about leishmaniasis, Only 3 (5%) of the interviewed inhabitants said they know sandfly and its role in transmission of leishmaniasis.
Conclusions: knowledge and attitudes of physicians are acceptable. However, there is, room for improvement and could be done through formal training courses and distribution of guidelines. In addition to raising the awareness of primary health care physicians about the importance of early detection and notification of cases of lesihmaniasis. Moreover, health education for raising awareness of the public regarding the vector and the disease is necessary because related studies have demonstrated that if the inhabitants do not perceive mosquitoes to be responsible for diseases such as malaria they do not take enough measures to protect themselves against the vector.
Key words: leishmaniasis, PHP, knowledge, attitude, local inhabitants.

Natural Infection by *Trypanosoma cruzi* in small wild mammals and bats in in Northeast the Brazil

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BACKGROUND:*Trypanosoma cruzi*, etiologic agent of Chagas Disease, is a multi-host parasite immersed in complex transmission networks that include a hundred species of mammals and dozens species of triatomine insect vectors. Aiming understands the ecological aspects of *T. cruzi* transmission cycles, small mammals and bats were examined in the six municipalities in the Maranhão state- Brazil: São Bento (Low lands), Cururupu (Mangrove), Caxias (Forest Cocais), Açailândia (Amazon), São Domingos (Savannah) and Barreirinhas (Sandbank).
METHODS: It were used the traps cages to capture the small mammals and mist nets to capture bats. For the isolation of *Trypanosoma* species, animal's blood were collected and seeded in a biphasic medium.
RESULTS: A total of 133 animals between rodents, marsupials and bats were examined. The overall rate of infection wild mammals, as evaluated by blood culture was 8.27% (11/133), but only 6.77% (9/133) for established cultures. The positive blood cultures were of the order Chiroptera (*Artibeus lituratus*, *Phyllostomus hastatus*) and Didelphiamorpha (*Philander opossum*, *Gracilinanus* sp., *Didelphis albiventris*) with a prevalence of 44.44% (4/9) and 55.6% (5/9) positive cultures and established. The established cultures were cryopreserved in Brazilian Collection of Trypanosomatid and two positive blood cultures *Pteronotus parnellii* have not been established. We obtained 09 isolates of Trypanosoma of mammals terrestrial and bats. The phylogenetic position through SSUrDNA V7V8 region, demonstrated the existence of two species in specimens captured in the area: *T. cruzi marinkellei* and *T. cruzi*. T. cruzi was the most prevalent species, 78% of isolates from bats and marsupials and only two isolates of T. c. marinkellei (22%). All isolates of *T. cruzi* obtained are belonging by TCI Group (100% boostrap / 100% a posteriori probability).
CONCLUSIONS:The knowledge about the complexity of the populations of T. cruzi is essential to evaluate the risk of emergence of wild lineages as important agents of human infection.

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Localization and alternative splicing the *farnesyl pyrophosphate synthase* (FPPs) involved in the isoprenoid pathway during intra-erythrocytic cycle of *Plasmodium falciparum*

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BACKGROUND: Malaria is one of the leading and most widespread of human parasite. The search for new biological targets has also focused in the understanding of metabolic pathways. In *P. falciparum* has been characterized the bifunctional enzyme FPPs, able to form intermediates of the MEP pathway. In this work we observed different localizations of the FPPs during intra-erythrocytic cycle and several isoforms of this protein were found.
METHODS: The localization was done with live parasites expressing GFP by confocal microscopy. The amplification of FPPs coding region from cDNA of intra-erythrocytic from three stages of *P. falciparum*, was cloned in pGEM-T easy and sequenced. We designed oligonucleotide pairs that amplify product only if one of the alternative splicing events occur and perform quantitative analysis by RT-PCR.
RESULTS: We showing that the young stages of the intra-erythrocytic cycle this enzyme is prepared in the cytoplasm, however, in mature stages focused in different points, as well as in mitochondria. We found the alternative splicing event with the presence of isoforms of this protein. One specific isoform is present in all stages of the parasite cycle, it has a deletion of the important domain by the addition of premature stop codon and it is transcribe about 100 times less compared to primary protein.
CONCLUSIONS: Our results suggest a different localization of this protein during intra-erythrocytic cycle of the *P. falciparum* and the presence of isoforms that may be interfering in its localization and/or function during the cycle of the parasite.

First report of natural infection with filariidae nematodes in Tamandúa (*Tamandua tetradactyla linnaeus*) from Venezuela

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BACKGROUND: The lesser ant eater or "Tamandúa" (*Tamandua tetradactyla* linnaeus 1758) is a Southamerican mammal belonging to Order Xenarthra, family Myrmecophagidae. A few is known about parasites affecting these animals. Some helminths, like *Gygantorhynchus ungriai* (Acantocephala), *Methaevotaenia* spp, (Cestoda), several Trichostrongylidae and *Orihelia anticlava* (Superfilarioidea: Onchocercidae) have been mentioned parasitizing Tamandúa.
METHODS: A young female of Tamandúa was recovered from a private property for national guards in Caicaguan, El Hatillo Municipality, (Miranda, Venezuela). The animal had poor body condition and sowed depression and anorexia. Special diet for edentate with vitamin supplementation was given before trying to release the animal in nature. Eventhough the young tamandúa initially showed some improving, finally dead seven days later. Necropsy findings included haemorragy of upper respiratory system, lung congestion, haemopericardium and whitish spots on spleen. It was noteworthy the presence of several filariform nematodes on peritoneum, mesentery and muscle fascias. Parasites were collected, conserved in alcohol 70% and sent to Parasitology Laboratory (CIPV MARA, FCV-UCV) for identification.
RESULTS: Specimens were filaroid nematodes, males and females with lenght between 12 and 14, 3 cm long, both sexs presenting transversal striations specially noted at middle half of body. Male: 10-12 caudal conspicuous digitiform papillae, spicules unequal; female: vulva located very close to mouth, uterus occupying half of the body, with thick shell eggs, embryo inside.
CONCLUSIONS: according with characteristic features nematodes found were classified as order Spirurida, Superfamily Filarioidea, Family Filariidae, Genus *Filaria*. This is the first report in Venezuela of this genus on *T. tetradactyla.*

Development of experimental model of schistosomal myeloradiculopathy

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BACKGROUND: The schistosomal myeloradiculopathy (SMR) is a severe presentation form of schistosomiasis, in which the nervous system is compromised by *Schistosoma mansoni*. This study aimed to develop an animal model of SMR, besides characterizing the sensory and motor changes caused by *S. mansoni* eggs in the spinal cord and correlate sensory, motor and histological aspects in animals along time.
METHODS: Two experimental series divided into six groups of five male Wistar rats each were made, according to the days when the tests of mechanical, behavioral and subsequent euthanasia in the 5th, 10th, 20th and 30th days. The experimental groups were anaesthetized with halothane and injected a *S. mansoni* suspension eggsat a concentration of 25,000/ml in the subarachnoid space. Control animals underwent the same procedure but with administration of phosphate buffer solution (PBS). The spinal cord was removed from the C1 level of L5 and stored in vials containing 4% formalin. Medullary segment of each rat was subjected to histological sections in a cryostat in the transverse plane with an interval of 20 microns fixed on glass slides and stained by hematoxylin-eosin. Histological changes were evaluated for the presence of eggs and / or granulomas as well as aggregates of inflammatory cells. The data relating to behavioral tests and sensitivity were analyzed by ANOVA two-way repeated measures followed by post hoc Bonferroni test. The critical level was set at 5 % for admitting a mean difference as statistically significant. The results were expressed as mean ± standard error of the mean.
RESULTS: The model is useful for the study of pathophysiological SMR; It was possible to identify changes in surface mechanical sensitivity, thermal sensitivity and muscle strength caused by the eggs of *S. mansoni* in the spinal cord; We identified histological changes in nerve tissue after infection with *S. mansoni* eggs.

Lipophilic Bisphosphonium compounds: potent antiprotozoal compounds with mitochondrial targets

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BACKGROUND: Bisphosphonium analogues have been extensively investigated in recent times as potential lead compounds against bloodstream form *T. b. brucei*. Here, the effects of bisphosphonium analogues *in vitro* on bloodstream form *T. b. brucei* were investigated to identify the target of these compounds.
METHODS: Flow cytometry was used to assess cellular DNA content and mitochondrial membrane potential. ATP content was assessed using luminescence, and intracellular calcium levels were determined using the fluorescent probe Fluo-8. Cell cycle was investigated microscopically after DAPI staining.
RESULTS: We found that all bisphosphonium analogues tested in this study led to an almost immediate growth arrest in trypanosomes, and to a rapid reduction of *T. b. brucei* Ψ_m after only half an hour of incubation, which lead to decreased cellular ATP levels within one hour. Intracellular Ca^{2+} levels were also increased gradually over 8 hrs of incubation and we interpret this as possible evidence that the damaged mitochondria are unable to retain stored Ca^{2+} as their membrane potential dissipates. The trypanosome cell cycle was also studied after incubation with bisphosphonium compounds. After 8 hrs of incubation with different compounds, cell cycle defects became apparent, as DNA synthesis could not be initiated (G1 arrest), leading to a dramatic reduction of cells in S phase. TUNEL assay showed progressive DNA damage at this point. Mitochondrial damage was observed in transmission electron microscopy images taken after 12 h of exposure of the cells.
CONCLUSIONS: Our results suggest that the mitochondrion is the primary target of bisphosphonium compounds.

Reduction of activated phosphorylated signal transducer and activator of transcription (STAT)-3 molecules and increase STAT-5 are associated with active cutaneous leishmaniasis

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BACKGROUND: Cellular immune responses directed against the protozoan parasite, *Leishmania*, are key for controlling pathogen replication and disease resolution. However, an uncontrolled response can be deleterious to the host, leading to the establishment of pathology. In this current study we have focused on studying the cellular response to cytokines as determined by the expression of phospho-STAT molecules.
METHODS: Using multi-parameter flow cytometry to measure the frequency and intensity of expression of specific STATs in lymphocyte subpopulations (memory/experienced cells and naive cells), we have aimed to determine which phospho-STATs are up or down modulated in active cutaneous leishmaniasis (LC) caused by infection with *L. braziliensis*. Thus, we examined the expression of a panel of STATs, some which are activated in response to inflammatory cytokines and Th1 type responses (STAT-1, STAT-4, STAT-5, NFkB,), and others activated by anti-inflammatory often associated with Th2 type responses (STAT-3, STAT-6). Through the comparison of phospho-STATs between non-infected controls (CTL) and active LC patients we have examined the expression profile of lymphocytes for both cultures with media alone and after stimulation with Soluble Leishmania Antigen (SLA).
RESULTS: Our studies found that after stimulation with SLA there was a decrease percent of STAT-3 in experienced cells in CTL and LC patients. In contrast, LC patients demonstrated an increase in the percent of STAT-5 produced by both experienced and naive cells as compared to CTL individuals. The other STATs did not show any statistically significant.
CONCLUSION: These data show an active role for STATs in establishing an inflammatory environment in LC indicated by an increase in the inflammation related (STAT-5) produced by experienced lymphocytes in LC patients stimulated with SLA, and a decrease in a phospho-STAT related to an anti-inflammatory environment induced by IL-10 (STAT-3).

Course of chronic Trypanosoma cruzi infection after treatment based on parasitological and serological tests: a systematic review of follow-up studies(in course)

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BACKGROUND: The current criteria for cure of chronic Trypanosoma cruzi infection consist of a negative parasitological test and seronegativization of two different serological tests. The primary is to summarize the patterns of response to treatment (success or failure) of parasitological and serological tests during the chronic *T. cruzi* infection.
METHOD: systematic review of cohort studies and RCTs.
The *exclusion criteria* are: subjects with diagnosis of acute *T. cruzi* infection, treatment with allopurinol or itraconazole, immunodepresion, pregnancy.
The outcome measures are: results of parasitological (xenodiagnosis, PCR, hemoculture) and serological (ELISA, IFI, IHA) tests. The following databases were searched: Cochrane Library, MEDLINE, EMBASE, LILACS, Clinicaltrials.gov and WHO ICTRP.
Two review authors independently screened abstracts to decide about eligibility and extracted data from included studies. The qualitative analysis is based on basic domains: selection, detection, attrition, and confounding risk of bias. The quantitative analysis of data is currently under development:
a) Scatter plots of positive percentages for parasitological outcomes and negative percentages for serological outcomes after treatments smoothed by lowess curve, b) Meta-analysis of aggregated data, and c) Meta-analysis of individual participant data. Heterogeneity was measured through I^2 statistics.
RESULTS (preliminary): A total of 2.134 cites were screened, 61 full text were assessed for eligibility and 50 studies were included (45 cohort studies and five RCTs).
We present Ors in the treated group compare to controls: OR positive xenodiagnosis=0.03 (CI95% 0.01 to 0.09; I²65%, twelve studies); OR positive PCR=0.33 (0.14 to 0.76; I²28%, three studies); OR negative ELISA=16.40 (3.28 to 82.05; I²76%, eight studies); OR negative IFI=7.73 (3.54 to 16.87; I²62%, sixteen studies); OR negative HAI=8.22 (3.46 to 19.53; I² 73%, fifteen studies).
CONCLUSIONS (preliminary): Trypanocide effect are being demonstrated in chronic patients treated with nifurtimox or benznidazole

'The Magic Glasses' – Cartoons for Worms

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A third of the global population, mainly in developing countries, is infected with soil-transmitted helminths (STHs). Infection with these intestinal parasitic worms is associated with poverty, inadequate sanitation and waste disposal, a lack of clean water and poor hygiene. STHs are among the most prevalent human infections globally.

Our recent article published in the *New England Journal of Medicine* reported major success in preventing STH infections in Chinese schoolchildren as the result of a health education package we implemented incorporating a cartoon video [1]. The study, undertaken in Hunan Province, showed a 50% efficacy in preventing the incidence of STH infection after a cluster randomized controlled trial in 38 schools involving 1718 schoolchildren.

We are now evaluating the efficacy of the educational package in two additional cluster-randomized controlled trials in China and in the Philippines. In China, we evaluate the package in a high STH prevalence setting in Yunnan Province. For the Philippines, the educational package will be culturally adapted so as to assess its efficacy in another Southeast Asian setting. The overall research objectives, study design and the main results of the Hunan Province trial will be described briefly, we will show baseline results of the new trial in Yunnan and selected scenes of the animated narrative cartoon video, which forms the basis of the education package.

New STH control strategies are urgently needed, since current control efforts focusing on mass drug administration (MDA) have been shown to be unsustainable due to rapid reinfection. The video-based educational package we have developed and trialled provides a promising new tool for integrated 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.

Prevalence and identification of microsporidian parasites in monkfish (*Lophius piscatorius)*

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BACKGROUND: Protozoan and Metazoan parasites frequently infest edible fish worldwide. Some of them are recognized as important zoonotic agents with a high public health impact whereas others are able to alter the organoleptic properties of fish products, having therefore a negative impact on fish industry. In spite of these facts and despite the European legislation, that imposes the withdrawal from the market of any products obviously contaminated with parasites, they are quite often encountered in sold products in Europe. In France, the consumption of raw or slightly cooked seafood is rising. For these reasons, the Fish-Parasites project (ANR-10-ALIA-004) was implemented to target fish parasites with impact on consumers' health (Anisakidae, Diphyllobothriidae, *Cryptosporidium*) and/or seafood quality. *Microsporidia* belonging to the genus *Spraguea* are parasites of the nervous tissue of most monkfish species from the genus *Lophius*. These parasites develop large, visible whitish cyst-like structures called xenomas that are localized along the central fishbone. Their prevalence is high and observing them on sold monkfish tails is quite frequent.
METHODS: Two batches of common monkfish, *Lophius piscatorius*, that were eviscerated and coming from North-East Atlantic (FAO zone 27) were bought from a stakeholder in Boulogne sur Mer (France). Fish were caught in November 2012 and in May 2013. In total, 58 fresh fish of 2-4.4 kg in weight and 55-79 cm in length were examined. Numerous cysts were seen and sampled along the central fishbone. Fresh microscopic smears of *Microsporidia* xenomas were prepared to visualize the parasites and some cysts were kept in absolute ethanol for subsequent molecular studies. DNA was extracted from several samples from both batches. A portion of the SSU rRNA gene was targeted using primers SF4m (5'-CACCAGGTTGATYCTGCCTRD-3') and SR1147m (5'-TGTRGTRAICYTCCCGYCAATY-3') described by Mansour *et al.* (2013). PCR products were sequenced.
RESULTS: The prevalence of microsporidian cysts was of 80 % for the first batch and of 64 % for second batch. Sequences were identified from cysts isolated from 39 fish in total. All 39 sequences were perfectly identical. The comparison of these sequences to reference sequences deposited in GenBank led to the identification of *Spraguea lophii*.
CONCLUSIONS: *Microsporidia* are parasites of insects, fish, birds or mammals. Some species have been described as human opportunistic pathogens. The members of the genus *Spraguea* are phylogenetically far from the genera pathogenic for humans but, no data are available, so far, on their potential infectivity in humans. Moreover, their prevalence may be very high in the main monkfish species, *L. piscatorius* and *L. budegassa*, which are of high commercial value. Besides the potential risk in terms of public health, the visible presence of these parasites may induce a decrease in the market value of these fish as well as a rejection of the product by the consumer.

Epidemiology and Control of Malaria in India with special emphasis on forest areas of Odisha state

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BACKGROUND**:** Malaria continues to be a major vector borne disease in India. Its epidemiology is complex and the endemicity varies distinctly in diverse ecosystems of the country with varying proportions of two predominant malaria parasites *Plasmodium falciparum* and *P. vivax*, and six major *Anopheles* vector species along with one or two local vectors transmitting malaria. The objective of this review is to analyze the complex malaria epidemiology and control in the country in general and in forest areas inhabited predominantly by tribal populations in the villages of district Sundergarh in Odisha state in particular.

MATERIALS AND METHODS: Materials used from: (i) Malaria data from the NVBDCP directorate and its web site www.nvbdcp.gov.in and Odisha state health directorate, and (ii) main references used: Nanda et al. 2000. J AmMosq Control Assoc 16: 199–205; Shah et al. 2013 PLoS ONE 8(2): e56740; Sharma et al. 2004. Am J Trop Med Hyg 71: 457–465, Sharma et al. 2006 Trans R Soc Trop Med Hyg 100: 917–925

RESULTS: The major ecosystems where malaria is endemic in the country are forest, rural plains, urban and coastal. In hilly forests and forest fringe areas in central Indian states *A. fluviatilis* and *A. culicifacies*, and in north-eastern states *A. dirus* and *A. minimus* are the major vectors along with *A. annularis*, *A. varuna* and *A. philippinensis*/*A. nivipes* as secondary vectors transmitting malaria. In many of these areas *P. falciparum* malaria is predominant followed by that of *P. vivax*. A small percentage of cases is of *P. malariae.* Resistance to chloroquine and sulphadoxine plus pyrmethamine in *P. falciparum* is widespread. *A. culicifacies* is resistant to DDT, malathion and in a few areas to synthetic pyrethroids, while other species are susceptible to the insecticides in use.

The state of Odisha contributes more malaria cases than any other state in the country. This state contributed 24.6% of malaria cases, 45.7% *P. falciparum* cases and 13.29 deaths reported in 2012 in the country. In this state too as in the rest of the country malaria has declined over the years. In Sundergarh district, in forest villages *An. fluviatilis* species S and *A. culicifacies* species C were identified as vectors transmitting malaria.

CONCLUSIONS: There is a need for detailed situation analyses of biology and bionomics of vectors, resistance status of parasites to drugs and of vectors to insecticides, control strategies, and operational and technical challenges that are faced in these areas for further improving malaria scenario.

Regulation of nuclear translocation of the Myb1 transcription factor by *Tv*Cyclophilin 1 in *Trichomonas vaginalis*

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BACKGROUND: In *Trichomonas vaginalis*, a human cyclophilin A homologue, *Tv*Cyclophilin 1 (*Tv*CyP1), which represses transcription of an iron-inducible *ap65-1* gene, was identified as a Myb1-binding protein using a bacterial two-hybrid library screening system.
METHODS: Bacterial two-hybrid system, GST-pull down and immunoprecipitation were employed to study the protein-protein interaction. Enzyme activity of *Tv*CyP1 was examined spectroscopically using a chromogenic tetrapeptide as substrate. Site-directed mutagenesis was used to identify the dominant negative mutants of *Tv*CyP1 and Myb1 for biochemical and functional studies. Subcellular localization of *Tv*CyP1 was studied by an immunofluorescence assay and differential and gradient centrifugations.
RESULTS: The recombinant *Tv*CyP1 (r*Tv*CyP1) exhibited typical peptidyl-prolyl isomerase activity, with a k_{cat}/K_M of ~7.1 $\mu M^{-1}s^{-1}$. In a pull-down assay, the His-tagged Myb1 interacted with a GST-*Tv*CyP1 fusion protein, which had an enzyme activity half that of r*Tv*CyP1. Both the enzyme activity of GST-*Tv*CyP1 and its binding to His-Myb1 were eliminated by mutation of R63 in the catalytic motif, or inhibited by cyclosporine A. *Tv*CyP1 was primarily localized to the hydrogenosomes by an immunofluorescence assay, but it was also co-purified with Myb1 in certain vesicle fractions from differential and gradient centrifugations. Transgenic cells overexpressing HA-*Tv*CyP1 had a higher level of nuclear Myb1, but a much lower level of Myb1 associated with the vesicles, than control and those overexpressing HA-*Tv*CyP1(R63A). Myb1 was detected to a much higher level in the protein complex of HA-*Tv*CyP1 than that of HA-*Tv*CyP1(R63A) immunoprecipitated from P15 and P100, but not S100, fractions of postnuclear lysates. A *Tv*CyP1-binding motif, 105YGPKWNK111, was identified in Myb1, in which G106 and P107 were essential for its binding to *Tv*CyP1. Mutation of G106 or P107 in HA-Myb1 respectively resulted in cytoplasmic retention and elevated nuclear translocation of the overexpressed protein.
CONCLUSIONS: *Tv*CyP1 may induce the release of Myb1 restrained on certain cytoplasmic vesicles prior to its nuclear translocation.

Prevalence of *T. gondii* in pigs from Yucatan

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BACKGROUND: Toxoplasmosis is a worldwide distributed zoonotic parasitic disease caused by *Toxoplasma gondii.* Humans can become infected mainly by ingestion of infected meat and by contaminated water and vegetables. As pork is one of the most highly consumed meats, pigs are an important source of human infection.
METHODS: The prevalence of *T. gondii* was assessed on two intensive farms located in Yucatan, in Mexico. Serum IgG antibodies levels were measured in 53 fattening pigs with an ELISA kit (Human-GmbH, Wiesbaden, Germany) and the target B1 gene was amplified by PCR in tissues samples for 11 individuals. Isolation of *T. gondii* also was attempted from tongues and/or blood samples of 13 animals. Questionnaire information was collected of the farm and animals characteristics and they were studied as a possible risk factors of *T. gondii* infection.
RESULTS: Results of this preliminary survey showed a high prevalence (85%) of *T. gondii* antibodies and some evidence of parasites in tissue. Both farms were positive for *T. gondii* infection and prevalence increased with the age. In terms of possible transmission networks the number of cats was high on both farms and both also had bird and rodents access, and the feeders were open to the environment. Later sampling of cats from one farm led to isolate *T. gondii* by mice bioassay.
CONCLUSIONS: This suggests that pork could be an important risk of infection in human toxoplasmosis in Yucatan. The increase of *T. gondii* prevalence with the age confirmed the importance of horizontal transmission in pigs. The isolation of *T. gondii* from a cat on farm 2 from supports the concept that open feeders and the presence of mice, birds and cats could be a risk of infection for *T. gondii.*

Detection of *Entamoeba histolytica*, *Giardia lamblia*, *Dientamoeba fragilis* and *Cryptosporidium spp*. in clinical stool samples by using Multiplex Tandem Real-Time PCR

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BACKGROUND: Diagnosis of intestinal parasites is usually provided by microscopy. But this method is usually known to have a low sensitivity and is unable to discriminate some protozoa. To overcome all of these limitations, a Multiplex Tandem Real-Time PCR was evaluated as an alternative method for diagnosis of *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium spp* and *Dientamoeba fragilis* in stool samples. Our study evaluated the frequency of detection and identification of four common intestinal pathogenic protozoon parasites in human clinical samples.
METHODS: Stool samples were collected at Eskisehir Osmangazi University Parasitology Laboratory. We analyzed 1500 stool samples by microscopy (Saline solution and formalin ethyl acetate methods). We selected suspicious 450 stool samples after the microscopic examination. Selected samples were studied by Multiplex Tandem Real Time PCR. DNA extraction was performed from all unfixed stool samples by using the QIAamp minikit (Qiagen). Additionally, permanent smears stained with modified Ziehl-Nelsen and Trichrome were prepared for all samples.
RESULTS: A total of 78/450samples were positive by MT-PCR. The parasites defined were as 59 cases of *Dientamoeba fragilis, 7* cases of *Giardia lamblia,* 5 cases of *Entamoeba histolytica,* 2 cases of *Cryptosporidium spp.* in clinical samples. In 5 patients two different parasites were detected. *Entamoeba histolytica* was identified with PCR test cases, 4 of 5 were identified by direct microscopy and Trichrom staining. PCR results were consistent when permanently stained by direct microscopy used together.
CONCLUSIONS: We found that Multiplex Tandem Real-Time PCR for the detection of *E. histolytica*, *G. lamblia*, *Cryptosporidium spp*. and *D. fragilis* presented in this study is a useful alternative method for these individual additional methods. There was no difference in the performance of the amplification of the specific targets in the individual assays compared with the multiplex PCR. The presence of intestinal parasites in the population indicates that more epidemiological studies are needed.

Human antibody responses to surface antigens of *Plasmodium falciparum* gametocyte-infected erythrocytes and their relation with gametocytaemia

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BACKGROUND**:** In *Plasmodium falciparum*, only mature gametocytes are found in peripheral circulation and available to uptake by the mosquito vector. Efficient transmission of gametocytes from the human host to mosquitoes contributes to the persistent burden posed by malaria. There is little evidence to suggest that natural immune responses to circulating gametocytes play a role in clearing gametocytes.
METHODS: Here, magnet-purified and enriched mature gametocytes obtained from a laboratory parasite, 3D7, and a clinical isolate from Kenya, HL1204, were used to test for plasma antibody recognition to the surface of gametocyte-infected erythrocytes (GSA) and to measure longitudinal antibody responses in a cohort of Ghanaian school children using flow cytometry.
RESULTS: Analysis of plasma antibodies in three sequential weekly samples from 113 asymptomatic *Plasmodium falciparum*-infected individuals showed that a proportion of the children (50%) exhibited marked antibody responses that recognized GSA on both 3D7 and HL1204. Some responsive individuals maintained their antibody levels during the study period, irrespective of concurrent gametocyte carriage status. Children with GSA antibodies present at enrolment, were less likely to develop new gametocytaemia at subsequent visits (odds ratio = 0.29, 95% CI 0.06 - 1.05; P = 0.034).
CONCLUSION: Our data support the hypothesis that conserved antigens exist on the surface of gametocyte-infected erythrocytes in malaria parasites which elicit natural human antibody responses, and that these play a role in reducing gametocyte carriage. The identification of these novel gametocyte surface antigens and their evaluation as potential anti-gametocyte vaccines candidates and/or biomarkers of gametocytes are imminent tasks.

The elimination of Onchocerciasis in Mexico and the Americas.

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Onchocerciasis, a chronic, debilitating, poverty-promoting parasitic disease, is one of the five most common of the officially designated neglected tropical diseases. It has been found in 13 discrete foci distributed among six countries (Brazil, Colombia, Ecuador, Guatemala, Mexico, and Venezuela) in Latin America (LA). Onchocerciasis was brought to the Americas through the slave trade in the 16th century, was transmitted to the indigenous American population once introduced and then spread through migration. Since its discovery in LA, numerous efforts have been put forth to understand the epidemiology of the disease to control and eventually eliminate the disease. The establishment of public-private partnerships and the development of community wide mass distribution programs of Mectizan® (ivermectin, donated by Merck, Sharpe, and Dohme) have resulted in dramatic progress against onchocerciasis in all of the endemic foci of LA. Transmission has been interrupted in 11 of 13 foci in LA, including all foci in Colombia, Ecuador, Guatemala, and Mexico as well as in two of the three foci in Venezuela. Transmission remains active only in the two foci straddling the border between Brazil and Venezuela. This area is inhabited by the Yanomami tribe indigenous to the Amazonian forest, and evidence suggests that transmission has been suppressed in some Yanomami communities. Interruption of transmission in these Amazonian foci, the last active foci in LA, will require intensified efforts and cross-border collaboration, but once successful, will culminate in the complete elimination of this scourge from the Americas.

Analysis of the participation of UAP56 homologue (RNA helicase) in the mRNA export in the hemocyte-like cell line C6/36 derived from *Aedes albopictus*

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BACKGROUND: Insect blood cells (hemocytes) play an essential role in defense against parasites and other pathogenic organisms. The hemocytes have diverse mechanisms of action including phagocytosis, melanization, encapsulation and coagulation. To accomplish these functions the hemocytes synthesize and secrete molecules that are necessary to activate the cells of the fat body and produce antimicrobial peptides needed to confront invading parasites. In several models have been demonstrated that splicing of pre-messenger RNA and export of mRNA are highly coordinated *in vivo*. The transcription-export complex (TREX) is a highly conserved apparatus that is essential for mRNA capping, splicing and export from the nucleus, all these functions are necessary for secretory molecules synthesis.
METHODS: In this work we are studying in the hemocyte-like cell line C6/36, derived from *Aedes albopictus* (Aealb), the expression of the components of TREX. To reach this goal we designed oligonucleotides based on *Ae. aegypti* genome, in order to identify the expression of TREX proteins messengers. In addition, immunoprecipitation and 2-dimensional electrophoresis followed by LC-MS/MS as well as confocal microcopy are conducted to identify and follow to TREX.
RESULTS: We found UAP56 expression both, as messenger and protein. It is located in the cytoplasm and by immunoprecipitation assays and mass spectrometry we look for the UAP56 partners forming the AealbTREX complex.
CONCLUSIONS: In the hemocyte-like cell line C6/36 we found some components of the TREX complex, showing that these are conserved in mosquito vectors. The characterization of hemocyte-like C6/36 cell line TREX is a first step to understand mechanisms by which mRNA is exported from the nucleus to the cytoplasm and its participation in the secretory pathways of mosquito cells.

Characterization of the TATA binding protein (TBP) of *Taenia solium* and analysis of core promoter elements in *Taenidae* family genes.

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BACKGROUND: Little is known about the transcriptional system in cestodes, therefore we want study and found the differences with the human and pig (host) transcription system. The aim of this study was the identification of the elements present in the core promoter that regulates the formation of the pre initiation complex (PIC) in *Taenidae* family genes and the analysis of TATA Binding Protein of *Taenia solium* (TsTBP).
METHODS: We analyze the core promoter of different genes and identify the cis-elements presents. We have cloned and characterized a TBP in *T. solium*. We produce an *in silico* model from TsTBP, produce nuclear extracts from cysts and identified TsTBP by western blot. Sothern and northern blot was carried out. We perform EMSA and Super Shift to TATA Box present in proximal promoters of *T. solium* genes.
RESULTS: We identified putative core promoter elements present in *Taenidae* family. We isolate the gene and cDNA that encodes TsTBP. The amino acid sequence reveal a 238-residues protein with an expected weight of 26.7 kDa. Southern and northern blot suggests the presence of one gene and two mRNA for TsTBP. EMSA and supershift shows a specific interaction between the TATA box of different promoters and nuclear extracts from *T. solium*.
CONCLUSIONS: Our results indicate that we have isolated the gen encode a TBP and showed it interaction with TATA box from different genes. We identified INR and putative DPE in some genes from *Taenidae* family.

Expression of glucosamine-6-phosphate isomerase and Jacob glycoprotein during culture of isolates of *Entamoeba histolytica*

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Background: *E. histolytica* has two-stage life cycle, consisting of the infective cyst and colon invasive trophozoite forms. The metabolic pathways that determine the conversion from one state to the other are still unknown. *E. histolytica* cysts have an extracellular rigid wall mainly formed by chitin, a homopolymer of N-acetyl-D-glucosamine of ß-(1.4) linkages. The purpose of this study was to determine the expression of genes, glucosamine-6-phosphate isomerase and Jacob glycoprotein during the excystation at 24, 48 and 96 h.

Methods**:** Glucosamine-6-phosphate isomerase and Jacob glycoprotein gene expression was assessed during *E. histolytica* excystation from the feces of five patients with gastrointestinal disorders. Excystation was performed at 0, 24, 48 and 96 h in Robinson medium and RNA was extracted from cysts and trophozoites. cDNA was synthesized to subsequently amplify the genes using real-time RT-PCR.

Results: Results of the expression of the genes showed differences for each of the isolates according to the incubation time and/or conversion of the cystic phase to the trophozoite where the most important expression in the genes was observed at 96 h compared with the reference strain in the trophozoite stage. The trophozoite was observed after 24 h of culture in 2/5 clinical isolates.

Conclusions: Expression of the genes was different in each isolate due to the influence of the characteristics of the parasite such as strain kind and host relationship that induce the expression of the genes studied to form the chitin polymer forming the cyst wall.

Cellular and genetic immunological mechanisms associated with distinct clinical outcomes in human Chagas disease

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BACKGROUND: Chagas disease, discovered by the Brazilian scientist Carlos Chagas in 1909, is caused by the infection with the protozoan *Trypanosoma cruzi*. One of the challenges in the study of Chagas disease is to identify markers of progression and risk factors for disease susceptibility, which will lead to better clinical management, decreasing or preventing pathology. Several studies have shown an association between host's immune response and the outcome of human infection with *T. cruzi*, pointing to possible biomarkers of susceptibility to development of severe disease. Our working hypothesis is that expression of immunoregulatory cytokines, coordinated by gene polymorphisms, are associated with mild and severe clinical forms of Chagas disease.
METHODS: We have been studying the expression of inflammatory and anti-inflammatory cytokines by different cell populations in patients with indeterminate or cardiac clinical forms of Chagas disease, and evaluating the association of candidate gene polymorphisms and the establishment of cardiac disease. We have evaluated the expression of TNF-alpha, IFN-gamma, IL-17 and IL-10 by monocytes and activated T cells from indeterminate and cardiac patients, and functional polymorphisms in the genes that code for these molecules.
RESULTS: Briefly, our results have shown that: (1) monocytes and CD4-CD8- T cells are critical sources of IL-10 in chronic Chagas patients; (2) CD4+ and CD4-CD8- are the main sources of IL-17; (3) TNF-alpha is mainly expressed by monocytes in individuals with indeterminate and cardiac clinical forms; (4) expression of IL-10 and TNF-alpha, by different cells, is associated with indeterminate and cardiac forms, respectively; (5) IL-10 gene polymorphism is associated with the occurrence of cardiac disease while IL-17 gene polymorphism is associated with the indeterminate clinical form.
CONCLUSIONS: Differential expression of immunoregulatory cytokines, as well as gene polymorphisms, is associated with distinct clinical outcomes of human Chagas disease, suggesting their possible use as biomarkers.

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Biogeography of the genus *Triatoma*: Origin, diversification, and distribution

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BACKGROUND: Several species responsible for transmitting *Trypanosoma cruzi* belong to the genus *Triatoma*. Of the 82 described species in that genus, 73 occur in the Americas, seven in Asia/Oceania, one is pantropical, and one is represented by a fossil from the Dominican Republic. The search for biogeographic patterns is an important step in the identification of primary homologies, which, when confronted with phylogenetic hypotheses, evidences diversification areas and supports the reconstruction of the evolutionary history of the clade.
METHODS: We performed a comprehensive bibliographic review and compiled occurrence records for *Triatoma* species in order to obtain high-resolution and updated distributions, by using modeling techniques (Maxent). For a multimethod-multiscale (1–6º) approach in panbiogeographic analysis, we used geometric and parsimony track analysis, as well as parsimony analysis of endemicity. We then challenged the formulated biogeographic hypotheses with phylogenetic hypotheses proposed for the group.
RESULTS: We obtained more than 10,000 occurrence points for the analysis. Nine generalized tracks and five endemism areas were identified. The most consistent diversification areas identified were located in: south-central Mexico (Rubrofasciata group); the extreme northwest of the Andes and South America, Colombia and Ecuador (Dispar group); meridional and south-central Andes, north of Argentina (Infestans group); and northeast Brazil (Infestans group).
CONCLUSIONS: The geological events compatible with the clade age (32 Ma) and its distribution were as follows: rise of the Andes, Sierra Madre Occidental/Oriental, and Trans-Mexican Volcanic Belt, as well as their subsequent tectonic processes; marine transgressions that formed the Pebasian, Amazon, Paranense, and Paranan seas; and forest connections that occurred between dry or humid forests. The northeast Brazil diversification area is the most probable origin area of the group, since this area has the highest species richness, several generalist and widely distributed species, and some biogeographic and phylogenetic inconsistences among some species associated with this area.

Cargo selection in the early secretory pathway of *Trypanosoma brucei*

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BACKGROUND: In African trypanosomes trafficking of GPI-anchored **V**ariant **S**urface **G**lycoproteins (VSG) to the cell surface, and digestive hydrolases to the lysosome, are critical to parasite survival and pathogenesis. In the first step of trafficking nascent secretory proteins are selected into COPII vesicles departing from **ER E**xit **S**ites (ERES). In other eukaryotes, the p24 family of Type I transmembrane proteins form heteromeric complexes that interact simultaneously with COPII components (Sec23/24 heterodimer) on the cytoplasmic side, and secretory cargos (soluble & GPI-anchored) on the lumenal side, of budding vesicles. In trypanosomes, ER exit of VSG is GPI-dependent and mediated by a subset of COPII components (TbSec23.2/TbSec24.1). We hypothesize that GPI-dependent selection of VSG in the ERES is mediated by trypanosomal p24 orthologues.

METHODS/RESULTS: Querying the *T. brucei* genome with yeast *EMP24* identified 8 putative p24 genes (*TbERP1-8*, **E**MP-**R**elated **P**rotein). Thus far, we have evaluated *TbERP1,2,3,8* by RNAi silencing. None appear to be essential; only *TbERP2* silencing caused a moderate growth defect. None delayed VSG trafficking or bulk secretion. However, silencing of *ERP1*, *ERP2* or *ERP8*, but not *TbERP3*, significantly delayed (2-4 fold reduction) trafficking of both TbCatL (soluble hydrolase) and p67 (transmembrane protein) to the lysosome. Immunolocalization of chromosomally epitope-tagged proteins confirm that TbERP1 and TbERP2 localize to ERES (TbERP3/8 localization in progress). Specific RNAi silencing of TbERP2 results in coordinate loss of epitope-tagged TbERP1 protein without impacting message levels (other combinations in progress).

CONCLUSIONS: The shared RNAi phenotypes, colocalization, and coordinate expression of TbERP1, TbERP2 and TbERP8 suggest that these p24s comprise a complex(s) with novel specificity(s) for lysosomal cargo. Current efforts are focused on completing these analyses and developing in vivo and in vitro pull down assays to confirm the proposed physical interactions. Work is also ongoing to evaluate the role(s) of the remaining TbERPs in GPI-dependent trafficking of VSG.

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Palms infestation with Rhodnius prolixus in the Orinoco region, Colombia

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Background. In Colombia, the ability of the insect *Rhodnius prolixus*, the main vector of Chagas disease in, to migrate from one sylvatic cycle to another domestic cycle hinders the vector control and it makes the imminent need to design new control strategies. These strategies require a deep understanding of the eco-epidemiology of the disease, especially in the sylvatic cycle, which unfortunately there are still many questions.
Methods. The study was conducted in “la vereda El Amparo” of the municipality of Maní, Casanare. The area was selected considering the high rates of infection with *Trypanosoma cruzi* in *R. prolixus* both in the sylvatic environment as in the domiciled environment. The capture of *R. prolixus* in palms (*Attalea butyracea*) was conducted using two techniques: manual search and traps baited with chicken. Captured insects were stored and labeled with geographical coordinates obtained by GPS. All triatomines collected in the field were taken to the laboratory for the detection of infection with *T. cruzi*.
Results. 158 palms were examined finding an infestation of 90.8% and a density of 9 insects per palm. 1525 triatomines were captured of which 285 insects (fifth-instar nymphs and adults) were reviewed to obtain 57.9% of natural infection.
Conclusions. A high rate of infestation was found in palms corroborating the distribution of this species of palm as a risk factor in the transmission of the parasite to the human and in processes of domiciliary reinfestation. The high prevalence of natural infection in triatomines calls for a study of the population that is at risk of contracting the disease.

Gene-cloning, Expression and Structural Modeling of Rhoptry Protein 39 of *Toxoplasma gondii*

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BACKGROUND: The ROP2-superfamily is the largest superfamily of proteins that have been known to be secreted by *Toxoplasma* into the host cell. Two virulence factors ROP16 and ROP18 have been defined as the members of this superfamily. *Toxoplasma gondii* ROP39 have a similar expression pattern to these virulence factors. The objective of this work was to clone, to express and to perform the structural modeling of protein TgROP39. METHODS: Total RNA was extracted from tachyzoites of RH strain of *T. gondii.* The open reading frame of TgROP39 gene was amplified with a pair of specific primers which was designed according to the coding sequence of the TgROP39 gene (ToxoDB accession No. TGGT1_262050). The product of RT-PCR was ligated to the pEXP5-CT TOPO vector. The recombinant pEXP5-CT-TgROP39 plasmid was transferred into *E. coli* DH5α and the positive clones were selected through the colony-PCR and confirmed by sequencing. The construct pEXP5-CT-TgROP39 was transformed into *E. Coli* (DE3) and induced with IPTG for expression. The expression products were analyzed through SDS-PAGE followed by Coomasie blue staining, ELISA and western blot with anti 6x- Histidine-Peroxidase antibody. The structural model of kinase domain of TgROP39 was performed with server I-TASSER. The template selected for homology modeling was TgROP18 (PDB code: 4JRN).
RESULTS: The product of RT-PCR had 1683 bp length. The recombinant pEXP5-CT-TgROP39 plasmid was confirmed by colony-PCR and sequencing. A recombinant protein with relative molecular weight of 65 DA was analyzed by SDS-PAGE, followed by coomassie blue staining. The 6xHis tag in 6XHis-TgROP39 was detected efficiently by western blot with the 6Xhis antibody. The C-Score of TgROP39 Model was -0.11 and Tm-Score was 0.74. Arg 283, Asp406 and Asp424, form the cysteine protease catalytic triad in the active site.
CONCLUSIONS: We performed the first gene-cloning, expression and structural modeling of TgROP39 protein. At this point the rTgROP39 protein provides the foundation for the future protein-protein interaction study.

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Molecular Ecoepidemiology of Chagas Disease in Colombia

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American trypanosomiasis is a very complex zoonosis which is present throughout South-America, Central-America, and Mexico and continues to represent a serious threat to the health of countries in the region.

The parasite infects 150 species from 24 families of domestic and wild animals and shows remarkable genetic variability evinced in at least seven near-clades named TcI-TcVI with the presence of a novel genotype associated to bats named as TcBat. These genotypes show a wide-range geographical and host distribution.

Our work aims to establish the relationship between the genetic diversity of T.cruzi with the diverse clinical manifestations of infected patients and also to unravel the molecular eco-epidemiology in the epizootic and enzootic scenarios in Colombia. We undertook intensive sampling in 17 departments of Colombia among 11 triatomine species, 9 mammalian reservoir species and humans obtaining 637 biological clones that were subsequently analysed by nuclear and mitochondrial molecular markers.

Hence, we observed the presence of TcI (80,7%) followed by TcII (7,2%), TcIII (3,9%), TcIV (5%), TcV (0,8%), TcVI (1,6%) and TcBat (0,8%); and the occurrence in the domestic foci of TcI (70%), TcII (20%), TcIII (1,6%), TcIV (3,6%), TcV (2,6%) and TcVI (2,6%); and for the sylvatic foci of TcI (85%), TcII (0,3%), TcIII (5%), TcIV (7%), TcVI (1,1%) and TcBat (1,1%). The results suggest the ocurrence of the seven genotypes and strict associations of independent DTU´s with host and environment. The implications are discussed.

Proteomic approach to identify virulence factors linked to stage-specific forms in *Leishmania infantum* strains

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BACKGROUND: *Leishmania infantum* is the agent of visceral leishmaniasis (VL) in zoonotic disease. In humans the disease present high morbidity and mortality with dogs being the main reservoir. Human and canine VL is a severe disease in which the symptoms are absent in almost 50% of infected dogs and unknown number in human. Clinical variability suggests that parasite factors are involved in virulence.
METHODS: We used amastigotes and promastigotes forms from two *L. infantum* strains MHOM/BR/1972/BH46 (BH46) and MCAN/BR/2000/BH400 (BH400) with low and high virulence respectively. To select and identify proteins differentially expressed that may be involved in virulence, we used Differential Gel Electrophoresis (DIGE), images were analyzed by DeCyder® software (GE Healthcare, USA) and proteins identified by Mass Spectrometry (MALDI/Tof-ToF).
RESULTS: The analysis between promastigotes forms from each strain showed 66 spots with higher expression in BH400, and 64 spots overexpressed in BH46. Comparing Axenic Amastigotes forms, we observed 32 spots with higher expression in BH400 and 38 spots overexpressed in BH46. We compared also splenic Amastigotes, and showed 41 spots overexpressed in BH400 and only 8 overexpressed in BH46. Another analysis was the Stage-Specific proteomic profile, in which we compare the three forms in each strain. Those differential expressed spots were submitted to Mass Spectrometry.
CONCLUSIONS: A total of 76 spots were identified from promastigotes form in BH400 strain and 61 in BH46 strain. For axenic amastigotes form we identified a total of 144 spots being 53 from BH400 strain and 91 from BH 46 strain. While in splenic amastigotes, the total spots identified were 40, being 17 from BH400 and 23 from BH46. Thus, a total of 321 spots with differential expression were successfully identified. The virulence factor studies may contribute to the discovery of new targets with therapeutic potential against leishmaniasis and in parasite-host interaction.

Proteins diferenttially expressed between *Leishmania (Leishmania) infantum, Leishmamia (Leishmania) amazonensis* and *Leishmania (Viannia) braziliensis*

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BACKGROUND: Leishmaniasis is caused by flagellate parasite of the *Leishmania* genus. The leishmaniasis can be subdivided, according parasite's species and host immune response, into five main clinical forms: cutaneous, diffuse cutaneous, disseminated cutaneous, mucocutaneous and visceral. The genomes sequencing of representative parasite species provides a global framework allowing investigating the contribution of these and other parasite factors to the diverse forms of leishmaniasis. The genome between *Leishmania* species is not very much different, it's possible that expression levels of some genes differ both between and within species.
METHODS: Considering proteomic a good tool for identification of differentially expressed proteins, we used Differential Gel Electrophoresis (DIGE) followed by mass spectrometry to compare proteomic profile in *Leishmania (Leishmania) infantum, Leishmania (L.) amazonensis* and *L. (Viannia) braziliensis*; whereas they are the three most important and widely distributed species in Brazil.
RESULTS: Comparing *L. (V.) amazonensis* and *L. (L.) brazileinsis* had shown 9.3% spots overexpreesed in *L. amazonensis* and 7.2% spots overexpreesed in *L. (L.) brazileinsis*. The comparison between *L. (V.) amazonensis* and *L. (L.) infantum* shown in these species 8.6% of spots overexpressed in *L. (V.) amazonensis* and 7.1% of spots overexpressed in *L. (L.) infantum.* The most similar proteomic profile was observed comparing *L. (L.) brazileinsis* and *L. (L.) infantum,* 5.5% overexpressed in *L. (L.) brazileinsis* and 5.6% overexpressed in *L. (L.) infantum.* The identified spots resulted in 152 different expressed proteins.
CONCLUSIONS: These proteins could be evaluated to antigenicity, virulence and parasite-host interaction by *in silico* analysis and subsequently confirmed by *in vitro* assays. This study may contribute for better understanding regarding the mechanisms of pathogenesis and clinical diversity of the disease.

Synthesis of a modified recombinant Chimeric Protein using tes26, tes30, tes120 and Myosin heavy chain antigens of *Toxocara canis*.

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BACKGROUND: Laboratory diagnosis of human Toxocarosis is carried out based on serological tests (ELISA and inmunoblot) that commonly use excretion/secretion antigens from *Toxocara canis* L2 Larvae (TES). Such tests have difficulties in Tropical countries related to sensitivity and specificity performance, due to cross-reactivity with antigens from other prevalent helminthes. Implementation of recombinant proteins as antigens in serological tests has already been validated as a successful strategy to improve diagnostic efficiency.
METHODS: We designed a system to express in *E. coli BL21DE3* 18 recombinant eGFP-fused peptides derived from the TES26, TES30, TES120 and Myosin antigens from *Toxocara canis*. The peptides were purified by affinity chromatography using Ni^{2+}-IDA columns and then tested for cross reactivity by inmunoblot. DNA sequences from non-cross-reactive peptides were used to construct a 2193bp gene coding for a recombinant chimeric protein (Q1). The Q1 prototype was expressed in *E. coli BL21DE3* and purified by FPLC.
RESULTS: we have identified five cross-reactive peptides inside the TES26, TES30, TES120 and Myosine heavy chain recombinant antigens. A chimeric 80,2 kDa protein containing the remaining 13 non cross-reactive peptides from these 4 antigens was successfully expressed in *BL21DE3 E. coli cells*. Purification (75% purity) was carried out from inclusion bodies with urea under denaturing conditions.
CONCLUSIONS: Our methodology using purified recombinant eGFP-fused peptides and inmunoblot demonstrated to be a successful technique that allows detection of antigenic peptides. Recombinant Chimeric protein Q1 could be a potential candidate for human toxocarosis diagnosis and epidemiological studies in Colombia and around the world.

Genotype Identification of Echinococcus Granulosus from Paraffin-Embedded Tissues of Hydatid cysts Isolated from Human by PCR-RFLP

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BACKGROUND: Identifying the various genotypes of *Echinococcus granulosus* as the agent of hydatid cysts in endemic areas can influence the disease control programs, particularly in humans. Therefore, this study was conducted to identify the different genotypes of *E. granulosus* from paraffin-embedded tissues of hydatid cysts isolated from human by PCR-RFLP
METHODS: To identify the molecular characteristics of *E. granulosus*, tissue samples from 30 human patients infected with hydatid cysts were collected from hospitals across the province of Golestan. DNA was extracted and characterized by PCR-RFLP method. In this study, 3 restriction endonuclease enzymes were used.
RESULTS: PCR product obtained from amplification of *E. granulosus* rDNA-ITS1 from human hydatid cysts showed two different patterns of DNA bands in human isolates. In spite of the difference between human isolates in the size of DNA bands (1000 base pairs), the use of BD1/4S and EGF1/EGR2 primers showed that these isolates are to some extent similar in the size of band (391 base pairs). PCR products by RFLP method showed a different pattern of genotype or strain with Taq1 restriction enzyme in human isolates. No change in the size of DNA bands was observed with Msp1 and Alu1 restriction enzyme in human isolates.
CONCLUSIONS: Genotypic differences and similarities between the size of DNA bands of *E. granulosus* from human isolates with PCR-RFLP method indicated the occurrence of different genotypes of *E. granulosus* in different parts of Golestan Province

Role of Inflammatory monocytes in pathogenesis of visceral leishmaniasis

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Monocyte recruitment to the infection site is one of the earliest steps of the immune response and it is generally associated with bacterial and parasitic elimination. During visceral leishmaniasis the role of inflammatory monocytes is completely unknown. In this study we found a detrimental role for inflammatory monocytes during *Leishmania donovani* infection. Infected mice showed preferential and continuous recruitment of Ly6Chi monocytes into the spleen and liver. Inflammatory monocytes harbored parasites during early infection and displayed an unconventional activation state. Interestingly, blockade of inflammatory monocyte recruitment using a CCR2 antagonist during acute or chronic infection reduced parasitic loads in infected organs and reduced the frequency of IFNy/IL10 double-producing CD4+ T cells. Our findings reveal an unexpected role for inflammatory monocytes in promoting parasite survival and open the possibility of targeting this cell population during visceral leishmaniasis.

Present situation of congenital Chagas disease in endemic and non-endemic countries

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BACKGROUND and METHODS. Although maternal-fetal transmission of *T. cruzi* occurs in a low percentage of infected mothers, major differences seems to occur among the different countries. Parasitological (microhematocrit, hemoculture)-, molecular (PCR)- and/or serological-methods (these last used after eight months of age, when maternal transferred antibodies disappeared) may be used for detecting congenital Chagas disease.
RESULTS. The largest surveys have been performed in endemic countries located in the South cone of Latin America. *T. cruzi* infection was detected in 431 from 7,188 children in Argentina (6%), from 292 on 7,086 in Bolivia (4.1%) and 292 from 2,691 in Paraguay (4.3%). Studies from Brazil and other endemic countries include shorter series. Nevertheless, in a sero-epidemiological study of 104,813 children below 5 years old, living in vector-free areas of all Brazil, only 20 were infected, 14 of them coming from a single state (Rio Grande do Sul), border to Argentina and Paraguay. Transmission has been also recorded from non-endemic countries, as a consequence of migration of infected pregnant women from Latin America (mainly Bolivia), in USA, Spain, Switzerland and Sweden. From the 18 studies performed in Spain, a total of 32 children from the 743 born to infected mothers were found infected (4.3%), and, in another study from Switzerland two children were infected from eight.
CONCLUSIONS. Congenital transmission is a global problem, occurring on average in 5% of children born from infected mothers, with variations depending on the region, the method used and likely other factors still not clearly understood. Correct diagnosis of infection in pregnant women (born in endemic regions) and their newborns is mandatory, considering that etiological treatment of the child is always effective if performed before one year of age.

Cellular immune response in dairy cattle naturally infected with *Fasciola hepatica* in Cajamarca, Peru

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BACKGROUND: Fasciolosis produced by *Fasciola hepatica* is an important parasitic disease of livestock in many countries. In Cajamarca, Peru, prevalence rates above 80% are reported in dairy cattle. The cellular immune responses is poorly understood in natural infection, hence the present work studied the proliferative capacity and expression of cytokines (IFN-γ and IL-4) in peripheral blood mononuclear cells (PBMC) from cows and calves against nonspecific Phytohemagglutinin (PHA) and specific Excretory/Secretory antigens from immature (FhE/S-I) and mature stages (FhE/S-M) of *F. hepatica*.
METHODS: The proliferative capacity was determined by *in vitro* lymphocyte proliferation, and cytokine expression was evaluated in cell culture supernatants by ELISA.
RESULTS: Cows infected with *F. hepatica* showed a decreased capacity to respond against nonspecific and specific proliferative stimulus. When the cytokine profile was studied, IFN-γ expression was low with high IL-4 against specific stimulus indicating that the response remains polarized towards a TH2 type response. Naturally infected calves expressed higher IFN-γ levels compared to cows, with a similar expression of IL-4 between both groups of animals. These results demonstrate an immunomodulatory response in adult animals as the disease progresses. No differences were observed regards the immune response to antigens of immature and mature stages of the parasite.
CONCLUSIONS: Our results suggest that cows infected with *F. hepatica* respond with a polarized TH2 immune response with low levels of IFN-γ and a high IL-4 response. On the other hand, infected calves expressed significantly higher levels of IFN-γ compared with cows, but both groups expressed similar quantities of IL-4.

Symptomatic prenatal and congenital toxoplasmosis is associated with unregulated inflammatory immune response

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BACKGROUND. Toxoplasmosis is a worldwide distribution infection; if the infection is acquired during pregnancy it can cross the placenta and cause congenital toxoplasmosis. The pathology that occurs depends on the infective dose, parasite virulence and immune response type. In adults, a Th1 profile is protective while a Th2 profile is not. Paradoxical observations on the immune response shown that the "protective" type must be regulated to prevent damage caused by inflammation in adults or spiral artery apoptosis with concomitant abortion.
OBJECTIVE: To determine the profile of immune response associated with symptomatic congenital and acquired toxoplasmosis.
METHODS: Proliferation assays of PBMCs from pregnant women with acute toxoplasmosis and congenitally newborns were performed. Cytokines produced were measured by flow cytometry. Cell proliferation was assessed at 120 h post incubation by CFSE dilution.
RESULTS: A CD4+ T cell specific proliferation to *T. gondii* crude extract was the main response observed both in mothers and newborns. A mixed Th1/Th2 unregulated response was observed in mothers with obstetric problems and in congenital infected newborns which showed a relation with clinical problems, including chorioretinitis and hepato-splenomegaly. A mixed response was also observed in women without obstetric problems, however it was stronger but with concomitant production of regulatory cytokines. CONCLUSIONS: Results found support the notion that a non-regulated inflammatory response is responsible for clinical problems observed in congenital toxoplasmosis. Conversely, obstetric problems seem related to a relatively weak immune response.

Strongyloides stercoralis infection in patients with Systemic Lupus Erythematosus (SLE)

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BACKGROUND: The infection caused by *S. stercoralis* usually is chronic and asymptomatic and may persist for decades undiagnosed. However, in immunocompromised individuals, the infection can cause hyperinfection and/or dissemination. Therefore, early diagnosis is essential to prevent the severe forms of strongyloidiasis. The aims of this study were: (1) to evaluate the frequency of *S. stercoralis* infection in patients with Systemic Lupus Erythematous (SLE) and (2) to estimate specific IgG and IgE production using enzyme linked immunosorbent assay (ELISA) method.
METHODS: There were evaluated 75 patients with SLE by spontaneous sedimentation (SS), Baermann-Moraes (BM) and agar plates culture (APC) methods. Serum antibody responses were measured by ELISA for IgG and IGE anti-*S. stercoralis.*
RESULTS: The frequency of intestinal parasites using parasitological methods was 10.7%, whereas the frequency of *S. stercoralis* infection was 1.3%. The sensitivity of the ELISA to detect IgG and IgE anti-*S. stercoralis* was 80% and 76.9%, respectively. Both assays presented the same specificity, 96.7%. The frequency of IgG and IgE anti-*S. stercoralis* was 16% and 28%, respectively. Six patients were positive for both ELISAs.
CONCLUSIONS: A diagnostical approach using high-sensitivity parasitological methods and the detection of specific antibodies is essential for the diagnosis of immunocompromised patients infected with *S. stercoralis*. Early detection of the infection can alter the course of the disease, after appropriate treatment, preventing the occurrence of severe strongyloidiasis.

Antimalarial Drug Development of Synthetic N-251 Compound as New Drug

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BACKGROUND: Multi-drug resistant *Plasmodium falciparum has been spread out* world-wide and artemisinin-based combination therapy (ACT)–resistant *P. facliparum* was reported in southern area of Cambodia, recently. A new antimalarial drug urgently desired for malaria control. We have started to develop a new antimalarial drug to overcome the drug-resistant *P. falciparum* 18 years ago. New antimalarial drug candidates should be required that (1) easy to prepare, (2) less toxic, and (3) highly selective antimalarial action.
METHODS: Over 8,000 compounds from natural and synthetic sources were tested on their antimalarial activities using *P. falciparum* and their toxicities on mammalian cells *in vitro.* The selected compounds were then tested on their antimalarial activities using *P. berghei* infected-mice *in vivo*. The target molecule for endoperxode candidates were analyzed by omics tecniques, analysis of pharmacokinetics of it and safety test also.
RESULTS: Fully synthesized N-251 compound showed anti-malarial activity for CQ-resistant *P. falciparum in vitro* (EC_{50} = 23 nM), and N-251 for oral administration has cure effect using 1% parasitemia in mice with out any side effect. To analyze the drug action of N-251 in malaria parasites, possible target molecules of N-251, PfERC, in malaria parasites have been selected after the series of studies of affinity chromatography and omics. These are parasite specific proteins with N-251 treatment but not with artemisinin. This indicates that both N-251 and artemisinin has peroxide structure in the body but the mechanism of their drug actions is different. In addition, we didn't found specific toxic data for N-251 in a series of general safety test.
CONCLUSIONS: Our new antimalarial candidate, N-251, has high antimalarial activity *in vitro* and *in vivo* test with cure effect and low cytotoxicity. To toxicity and safety tests of N-251, N-251 shows safe with no any side effects in the animal. Also, the synthetic method of N-251 was established to conform of GMP standards and the validation of pharmaco-kinetics of it. Together above results, N-251 has high potency of new generation of antimalarial drug to overcome the drug-resistant *P. falciparum*.

Contemporary cryptic sexuality in *Trypanosoma cruzi*

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BACKGROUND: 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*, the causative agent of Chagas disease, is known to undergo non-Mendelian recombination *in vitro*, while two of the six major circulating genetic lineages (TcV and TcVI) resemble meiotic F1 progeny. Despite the existence of natural hybrid strains, a pervasive view is that recombination was an evolutionarily ancient phenomenon and contemporary genetic exchange is of little epidemiological relevance.
METHODS: We undertook high resolution molecular genotyping (48 nuclear and 10 mitochondrial loci) of field isolates belonging to TcI (n=300) and TcIV (n=80), the principal lineages responsible for human Chagas disease in northern South America.
RESULTS: Gross nuclear-mitochondrial phylogenetic incongruence was observed at multiple levels, including among disparate populations as well as major lineages. In all cases, hybrids had undergone mitochondrial introgression without apparent reciprocal nuclear recombination between parental genotypes, implying additional, as yet uncharacterized, cellular mechanisms may facilitate natural hybridization in *T. cruzi*.
CONCLUSIONS: These results indicate that genetic exchange is geographically widespread and continues to influence parasite population structures, driving the emergence of novel strains with epidemiologically important phenotypes, and challenging the traditional paradigm of clonality in *T. cruzi.* We describe current work elucidating the frequency of recombination within an endemic disease foci in Colombia and along an ecological cline in Bolivia and discuss the implications of parasite sexuality for Chagas disease transmission in sylvatic and domestic settings.

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Human migration drives the dispersal of epizootic Chagas disease: the case of highland Bolivia

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BACKGROUND: *Trypanosoma cruzi*, the aetiological agent of Chagas disease, is an ancient and widespread zoonosis distributed throughout the Americas. TcI is the most abundant genetic lineage; it is the principal cause of human chagasic cardiomyopathy in Colombia and Venezuela and is ubiquitous among sylvatic transmission cycles. Multiple molecular markers consistently identify high levels of genetic variation within sylvatic TcI populations, and divergent, but genetically homogeneous, strains isolated from human infections. However, current understanding of the genetic determinants that drive natural *T. cruzi* diversification is incomplete.
METHODS: We performed high resolution nuclear and mitochondrial genotyping of contemporaneous sylvatic TcI (n=199 biological clones), isolated from a range of triatomine and mammalian hosts across Bolivia.
RESULTS: We detected two distinct sylvatic transmission cycles in adjacent highland and lowland areas. Highland Bolivian strains were characterized by reduced genetic diversity and heterozygosity (A_r= 1.92-2.22, F_{IS}=-0.241-0.026) compared to lowland areas (A_r = 3.40-3.93, F_{IS}=0.176). We observed equivalent levels of subdivision among highland areas spanning >465 km (F_{ST} = 0.084) and between lowland populations across 155 km (F_{ST} = 0.084). Measurements of isolation by distance detected greater parasite dispersal among geographically disparate highland areas (R_{XY}= 0.053, p=0.142) than between proximate lowland foci (R_{XY}= 0.209, p<0.001).
CONCLUSIONS: The most parsimonious explanation for our results is a founder event in highland Bolivia with long-range anthropogenic dispersal of parasites across an ecological cline. We discuss the role of humans as an abundant, but often neglected, vector of *T. cruzi* and consider their implications for the epidemiological risk of emergent epizootic Chagas disease.

The influence of the drug *Tri-V-Plus* with hydatid pathology in experimental rats

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BACKGROUND:The aim of this study was to investigate the free radical oxidation of lipids, oxidative degradation of proteins, the content of nitric oxide in experimental echinococcosis and the introduction of the drug three *Tri-V-Plus* as a therapeutic agent.
METHODS:When modeling experimental *echinococcosis*, we chose the method of intraperitoneal inoculation of animals protoskoleks *E.granulosus*, which were obtained from spontaneously invaded donors. Their owners were white males inbred rats weighing 80-100 g.
As an antioxidant and vitamin- mineral complex , we chose the three - drug *Tri-V-Plus*. One coated tablet contains 5000 MU of beta carotene , alpha -tocopherol acetate, 30 MU , 60 mg of ascorbic acid , 2 mg of Cu oxide , 40 g of selenium and 40 mg zinc . The drug is a powerful antioxidant and is known metebolit .
The LPO activity was estimated by the measurement of the amount of malondialdehyde (MDA) . MDA was determined by reaction with thiobarbituric acid. The amount of protein determined by Lowry.
Nitrogen oxide content was determined using the Griess reagent and the absorbance of the solution was measured at 546 nm. The level of oxidative modification of proteins in blood serum was estimated by the content of carbonyl derivatives of amino acids in proteins.
RESULTS: Thus, the results of the study showed that the introduction of Tri-V-Plus 10 days before infection and for 50 days after infection tended to decrease levels of MDA , carbonyl derivatives and nitrogen oxide content.
CONCLUSIONS: Thus, it can be concluded that hydatid disease of the liver is accompanied by activation of free radical processes, and the preparate regulates these processes as powerful antioxidant.

Purification and kinetic analysis of cytosolic and mitochondrial Thioredoxin glutathione reductase extracted from *Taenia solium* cysticerci

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BACKGROUND: *Taenia solium*, the agent that produces neurocisticercosis, one of the major central nervous system (CNS) parasitic diseases in humans, lacks both of thioredoxin reductase (TrxR) and glutathione reductase (GR), two major enzymes associated to detoxification mechanisms. Instead of those proteins some platyhelminthes, exhibit a GR and TrxR molecular link with the fusion of glutaredoxin (Grx) and thioredoxin reductase (TrxR) domains into a single protein selenocysteine-containing enzyme thioredoxin glutathione reductase (TGR).
METHODS: In order to establish the presence of TGRs in *T. solium* tissues, protein purification was performed on cytoplasm and mitochondrial from cysticerci. Protein purification chromatography procedures were developed to purify the enzymes and enzyme kinetic techniques were performed to characterize the cytoplasm (cTGR*Ts*) and mitochondrial (mTGR*Ts*) proteins from *T. solium.* Both enzymes are dimers (132,000 kDa) and were more catalytically active towards glutaredoxin, followed by thioredoxin reductase and lastly by glutathione reductase. cTGR*Ts* also showed hydroperoxide reductase activity with a specific activity of 0.21 U mg^{-1} using hydroperoxide as substrate.
RESULTS: The $K_{m(DTNB)}$ and $K_{cat(DTNB)}$ values for cTGR*Ts* and mTGR*Ts* (88 μM and 1.9 s^{-1}, 45 μM and 0.04 s^{-1}, respectively) and the $K_{m(GSSG)}$ and $K_{cat(GSSG)}$ values for cTGR*Ts* and mTGR*Ts* (6.3 μM and 0.96 s^{-1}, 4 μM and 0.024 s^{-1}, respectively) were similar or lower than those reported for mammalian TGR. The second to the twelve st amino acids from cTGR*Ts* and mTGR*Ts* N-terminal group were APIGGSAEQVEK indicating that both enzymes are coded by the same gene and this region is identical to *Echinococcus granulosus* TGR. cTGR*Ts* was inhibited by auranofin, a selective inhibitor of thiol-dependent flavoreductases (I_{50} = 3.25 nM, 2.29 nM for DTNB and GSSG substrates respectively). Glutathione reductase activity of cTGR*Ts* and mTGR*Ts* exhibited hysteretic behavior as is observed with TGRs.
CONCLUSIONS: The data suggest the existence of an effective substitute accounting for the lack of glutathione reductase and thioredoxin reductase in *T. solium.*
Support: Universidad Nacional Autonoma de Mexico, DGAPA (PAPIIT IN211313).

Evaluation of the non-catalytic binding function of Ts26GST a glutathione transferase isoform of *Taenia solium*

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BACKGROUND: *Taenia solium*, the agent that produces neurocisticercosis, one of the major central nervous system (CNS) parasitic diseases in humans, presents several isoforms of glutathione transferase (GST). These proteins belong at major phase II detoxification enzymes that catalyze the conjugation of xenobiotics to glutathione. But GSTs have also a ligandin activity, referred to a detoxification mechanism characterized by an absence of catalytic enzyme function. Because there is not information about ligandin activity exhibited by any *T. solium* GST isoforms we decided to evaluate this function in Ts26GST a GST isoform of this cestode.
METHODS: Protein purification chromatography procedures were developed to purify the enzyme and assessed by inhibition kinetics, fluorescence spectroscopy and competitive fluorescence assays with 8-anilino-1-naphthalene sulphonate (ANS). Ts26GST was observed to bind non-catalytically to porphyrins, trans-trans-dienals, bile acids and fatty acids, as assessed by the techniques described above. The quenching of Ts26GST intrinsic fluorescence allowed for the determination of the dissociation constants (K_D) for the twelve ligands employed.
RESULTS: THE data obtained indicate that Ts26GST binds to all ligands but with different affinity. Porphyrins and lipid peroxide products inhibited Ts26GST catalytic activity up to 100% in contrast with only 20-30% inhibition observed for bile acids and two saturated fatty acids. Non-competitive type inhibition was observed for all enzyme inhibitor ligands except for trans-trans-2,4-decadienal, which exhibited uncompetitive type inhibition. The dissociation constant value K_D= 0.7 µM for the hematin ligand, determined by competitive fluorescence assays with ANS, was in good agreement with its inhibition kinetic value Ki = 0.3 µM and its intrinsic fluorescence quenching K_D = 0.7 µM. The remaining ligands did not displace ANS from the enzyme suggesting the existence of different binding sites.
CONCLUSIONS: In addition to the catalytic activity of Ts26GST the results obtained suggest that the enzyme exhibits a ligandin function with broad specificity towards nonsubstrate ligands.
Support: Universidad Nacional Autonoma de Mexico, DGPA (PAPIIT IN211313).

Acaricide resistance and strategies to mitigate economic impact of the southern cattle fever tick (*Rhipicephalus microplus*) on livestock production systems in the Americas

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BACKGROUND: The southern cattle fever tick (SCFT), *Rhipicephalus microplus*, is considered the most economically important external parasite of livestock worldwide. SCFT populations resistant to acaricides complicate efforts to enhance the productivity of livestock. Here, acaricide resistance is summarized and integrated approaches in the Americas to mitigate the impact of the SCFT on livestock production systems are updated.
METHODS: Trends in acaricide resistance among SCFT populations across the continent were analyzed. Adaptive strategies in the context of recent trends in livestock production systems that involve ranching of cattle with wild ruminant ungulate species was summarized. Economic impact data on livestock production were compiled.
RESULTS: An increase in the frequency of resistance to multiple classes of acaricides is alarming. Multiple resistance in the SCFT has been recorded across the Americas. A population of SCFT was shown to be resistant to 6 classes of acaricides in Brazil where there are ~212 million cattle and the economic impact of the SCFT is estimated to be USD 3.2 billion. Efforts underway in several countries aim to integrate diverse technologies to manage the problem of multiple acaricide resistance.
CONCLUSIONS: Acaricide resistance in the SCFT is a problem that has grown in complexity. Biological and epidemiological aspects of multiple acaricide resistance remain to be fully understood. Research and development of integrated SCFT management strategies in the Americas is facilitated by the commercialization of non-chemical technologies. This approach is required to mitigate the economic impact of the SCFT and for sustainable livestock production.
* USDA is an equal opportunity provider and employer.

Post-genomic analysis of genetic resistance to gastrointestinal nematodes

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BACKGROUND: The major histocompatibility complex (MHC) is the most variable region of the genome and this variation is a consequence of its role in resistance to parasitic and infectious diseases. However the precise mechanisms that maintain variation are unknown. Consequently, we cannot use MHC diversity to maximize resistance to disease. The aim of this project was to determine how natural and deliberate selection influence MHC diversity.
METHODS: We examined faecal egg counts in over 1300 naturally infected lambs from two breeds of sheep. Worm burdens were obtained by standard parasitological methods from over 500 lambs at the end of the grazing season. Direct DNA sequencing using locus specific primers for DRB1, DQA1, DQB1, DQA2 and DQB2 was used to determine variation in the MHC class II region. Associations were examined with mixed model analysis of variance. Mathematical models were used to explore the effects of different forms of natural selection on MHC variation.
RESULTS: Each locus contained from 8-33 alleles in different breeds. There was very strong linkage disequilibrium within each breed. There were differences among alleles in faecal egg count and worm burden. There was also evidence for heterozygote advantage. Mathematical modelling indicated that a special form of heterozygote advantage (divergent allele advantage) was the main driver of MHC polymorphism.
CONCLUSIONS: MHC variation is primarily a consequence of divergent allele advantage. Genomic selection can mimic natural selection if alleles are appropriately weighted by their divergence and their frequency. These results allow us to utilize the most important region for parasite resistance to maximize resistance to disease in managed populations.

The metabolic regulation of alternative macrophage activation in immunity to helminths

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Macrophages are crucial for immunity and can adopt different activation states depending on context. Interferon-γ (IFNγ) in combination with TLR agonists promotes M1 (or classical) activation, whereas the cytokines IL-4 and IL-13 promote M2 (or alternative) activation. Whereas M1 macrophages are inflammatory and are important for immunity to microbial pathogens, M2 macrophages are generally considered to play a more anti-inflammatory role and to be important for immunity to parasitic helminths. We are interested in the metabolic differences between M1 macrophages, which rely on aerobic glycolysis, and M2 macrophages, which utilize fatty acid oxidation (FAO) to fuel mitochondrial oxidative phosphorylation, and whether or not these metabolic states can be manipulated to promote or inhibit cell function to change disease outcome. The presentation will focus on our recent findings on the role of regulated lipolysis in the generation of fatty acids for FAO in M2 macrophages.

On the evolution of salivary gland genes in Anophelines

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BACKGROUND: Saliva of adult female mosquitoes assists blood feeding by impairing host hemostasis and affecting inflammation and immunity. The salivary potion of mosquitoes is complex and in *Anopheles gambiae* it is estimated to contain the product of at least 75 genes, most being expressed solely in the adult female salivary glands. The sequencing of 16 anopheline genomes, in addition to those of *An. gambiae* and *An. darlingi* allows an unprecedented opportunity to obtain insights into the evolution of salivary genes associated with blood feeding in mosquitoes.
METHODS: Using Artemis we manually re-annotated and analyzed the salivary gland genes of the 18 species, and proceeded with their phylogenetical analysis. SnIPRE and the HYPHY suite FEL and MEME programs were used to investigate the evolutionary signatures.
RESULTS: A scenario of high plasticity of these genes emerges following annotation of the existing 18 anopheline genomes, revealing a relatively large number of gene gains/losses involving both individual salivary genes and multigene families. SnIPRE analysis indicated that salivary genes show the shortest average divergence times, the smallest negative constraint effect, and highest proportion of non-synonymous mutations that are non-lethal of all gene classes. Both FEL and MEME analysis showed that salivary gland genes are among those having the highest rate of positively selected codons among seven gene classes.
CONCLUSIONS: Results support the estimates that salivary gland genes associated to blood feeding are at an accelerated pace of evolution and are remarkably driven by positive selection.

Identification of endogenous substrates for protein tyrosine phosphatases EhPTPA and EhPTPB of *Entamoeba histolytica*

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BACKGROUND: Protein tyrosine phosphatases (PTPases) and protein tyrosine kinases (PTKs) regulate the reversible phosphorylation of tyrosine residues in proteins, controlling processes such as: growth, differentiation, cell cycle, metabolism and regulation of cytoskeletal dynamics. The genome of *Entamoeba histolytica*, the causative agent of amoebiasis, has two genes coding for classic protein tyrosine phosphatases *EhPTPA* and *EhPTPB* the function of which is unknown. So it is important to identify substrates for these PTPases to understand the role that these proteins play in parasite biology and pathogenicity mechanisms.

METHODS: *EhPTPA* and *EhPTPB* PTPases were cloned in the pCRTOPO-TA cohesive-Ended plasmid, subcloned in-frame in the expression vector pGEX-5X-1 and pGEX-3X-1 respectively and subjected to directed mutagenesis of amino acids involved in the catalytic mechanism to obtain the EhPTPAD177A, EhPTPAQ294A, EhPTPBD167A and EhPTPBC203S mutant proteins, protein phosphatase activity was measured using 30 mM p-nitrophenylphosphate as substrate. Endogenous substrates were captured by these mutants, purified by affinity chromatography and identified by mass spectrometry (MS).

RESULTS: we have determined the kinetic parameters of proteins EhPTPA and EhPTPB. The mutant proteins obtained showed decreased or nule enzymatic activity compared to the enzymatic activity of native proteins. In *E. histolytica* have not been described the function of the substrates identified by MS yet, however these have been described in eukaryotic cells to regulate processes such as rearrangement of the cytoskeleton, adhesion, cytokinesis and regulation cell cycle among others.

CONCLUSION: The identification of the substrates of these PTPases represent an important step in understanding the function of this family of proteins in *E. histolytica*, revealing important insight into their possible role *in vivo*.

Strongyloides hyperinfection as a sign of immunosuppression: A case report

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BACKGROUND: Asymptomatic strongyloidiasis is the most common form of the *Strongyloides stercoralis* infection. However, in patients with immunosuppressive disorders, this infection may develop into a hyperinfection syndrome or disseminated disease. The present study describes a case of *Strongyloides* hyperinfection in a patient without previous diagnosis of immunocompromising conditions, presenting resistance to anthelmintic treatment

RESULTS: In December 2010, a 47-year-old woman, reported cough, abdominal pain, nausea and vomiting. The stool examination showed 2,500 larvae of *S. stercoralis*/g of feces. Once the patient had no previous reports of any immunosuppression, an investigation was conducted and diagnosis of HTLV-1 infection was made. The patient was treated for *Strongyloides* hyperinfection with albendazole 400 mg daily for three days. After one month, new parasitological examinations indicated that the patient was still infected with *S. stercoralis* (200 larvae/g of feces). ELISA for IgG anti-*S. stercoralis* was positive. At this moment, the patient was treated again with albendazole in the same dose as before. About three months after the last treatment the patient still presented *S. stercoralis* infection. Then, ivermectin (6 mg, single dose) was prescribed. After one month, stool examinations were negative for larvae of *S. stercoralis*. The follow-up treatment was carried out for about one year. After this period, the patient still had negative parasitological examinations and the ELISA for IgG anti- *S. stercoralis* was negative.

CONCLUSIONS: This case report suggests that patients with *Strongyloides* hyperinfection without a previous diagnose of immunosuppression can have an undiagnosed disease. Therefore, regularly parasitological examination and follow-up for strongyloidiasis is necessary in regions where *S. stercoralis* infection is endemic even in patients apparently healthy.

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O-deGlcNAcylation via OGT down-regulation is required for cell death of human hepatocytes induced by cysteine protease of pathogenic *Entamoeba histolytica*

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BACKGROUND: *Entamoeba histolytica* is an enteric tissue-invasion protozoan parasite that causes amoebic colitis and occasionally liver abscess in humans. *E. histolytica* can induce host cell apoptosis by the induction of various intracellular signal mechanisms. These modulations triggered by *E. histolytica* are closely associated with tissue pathogenesis and parasitic immune evasion mechanism. O-GlcNAcyation, similar to phosphorylation, has been thought to contribute the various cellular signal processes including apoptosis and proliferation. O-GlcNAc addition and removal are regulated by O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), respectively. However, it is unknown whether the alteration of O-GlcNAc level in host cells can affect *Entamoeba*-induced cell death and what signal molecules are participated in *Entamoeba*-induced deglycosylation.

METHODS: In this study, co-incubation of HepG2 cells with HM-1 strain remarkably increased DNA fragmentation and LDH release compared to cells incubated with Rahman strain or medium alone. In addition, *Entamoeba* HM-1 induced tyrosine dephosphorylation, and activation of caspases-3 and calpain in HepG2 cells.

RESULTS: Co-incubation of live HM-1 trophozoites with HepG2 cells induced the decrease of O-GlcNAcylated protein levels and OGT down-regulation in HepG2 cells within 2 min. In addition, pretreated host cells with O-GlcNAcase inhibitors (PUGNAc or Thi-Met G) or OGA siRNA prevented the *E. histolytica*-induced deGlcNAcylation in HepG2 cells. Moreover, DNA fragmentation and LDH release triggered by *E. histolytica* HM-1 were strongly inhibited by pretreatment of host cells with OGA inhibitors. Interestingly, we found that pretreatment of *E. histolytica* with cysteine protease (CP) inhibitor E64 or ICP1 (endogenous inhibitor of CP) overexpressing amoeba strain was reduced *Entamoeba*-induced deglycosylation in HepG2 cells.

CONCLUSIONS: These results suggest that amoebic cysteine protease is involved in the decrease of O-GlcNAcylated proteins in HepG2 cells and this process is an important process required for hepatocyte cell death induced by *E. histolytica*.

An iron-triggered signal transduction pathway in *Trichomonas vaginalis*

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BACKGROUND: Iron was previously shown to induce transient nuclear translocation of a Myb3 transcription factor, which up regulates iron-inducible transcription of an adhesion protein, *ap65-1*, gene, in *Trichomonas vaginalis*..
METHODS: Nuclear translocation of Myb3 was studied by an immunofluorescence assay. The level of cellular cAMP was determined by a radioimmunoassay. Posttranslation modifications of Myb3 and a protein kinase A were identified by mass spectrometry. Site-directed mutagenesis was used to identify the dominant negative mutants of Myb3 in nuclear translocation. Interaction of protein kinase A and Myb3 and consequences of this interaction were studied by immunoprecipitation coupled Western blotting.
RESULTS: Iron was shown to induce transient increase of cellular cAMP, while 8-bromo-cAMP alone could also induce prolonged nuclear influx of Myb3. Iron-inducible cAMP production and Myb3 nuclear influx were inhibited by suramin and SQ22536, respective inhibitors of Gαs and adenylyl cyclases, but iron-inducible Myb3 nuclear influx was only delayed by H89, an inhibitor of protein kinase A. Phosphorylation and ubiquitination of Myb3 were respectively detected at T156 and K143. These modifications, which were iron-inducible, were inhibited by H89. Iron-inducible ubiquitination and nuclear influx were aborted in T156A and K143R, while T156D was constitutively ubiquitinated and localized to nucleus. A putative *Tv*PKA with a conserved autophosphorylation site was shown to phosphorylate Myb3 in an *in vitro* protein kinase assay. Interaction of *Tv*PKA and Myb3 was induced in *T. vaginalis* upon iron repletion, with concomitant phosphorylation of each protein and nuclear influx of Myb3.
CONCLUSIONS: Our results suggest that iron probably triggers a Gαs-mediated signal transduction pathway that relays the upstream signal to PKA and induces sequential phosphorylation and ubiquitination of Myb3 for its nuclear influx.

Identification and characterization of enolase as a plasminogen-binding protein from *Taenia solium*

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BACKGROUND: Cysticercus cellulosae is a larval stage of *Taenia solium*, which can cause porcine and human cysticercosis, affecting the development of pig-raising sectors and ubiquitously present in a wide range of living organisms. It is not only as a glycolytic enzyme affecting substance and energy metabolism of cells, but also has a variety of functions such as participates in pathogen invasion. In this study, we have cloned, expressed the enolase gene and studied the characterization of enolase.
METHODS: *Taenia solium* enolase gene was amplified and detected the enolase expression levels at different developmental stages by fluorescence quantitative PCR. Recombinant protein of *Taenia solium* enolase, rTsENO, was expressed in prokaryotic expression system. Immunohistochemistry assays were performed in adult worms using rabbit anti-rTsENO polyclonal antibody. Immunoreactivity, enzyme activity and plasminogen binding property of the rTsENO were also analyzed.
RESULTS: The result of real-time PCR showed that the enolase gene was highly expressed at adult worms of the life cycle. Immunohistochemical results showed that rTsENO mainly expressed in the adult tegument and reproductive organs. Western blot analysis showed that the rTsENO was recognized by the serum of swine infected with Cysticercosis. It was confirmed that rTsENO possesses good enzymatic activity, could bind to human plasminogen as its receptor, and had the capacity to generate plasmin and to enhance the plasmin generation by the tissue-type plasminogen activator. In addition, 6-ACA could inhibit the combinding of plasminogen with rTsENO.
CONCLUSIONS: In this paper, enolase of *Taenia solium* was assessed by enzyme activity and plasminogen binding property, and could be considered as a immunodiagnostic agents, promising drug target and vaccine candidate against Cysticercosis cellulosae.

Evaluation of the efficacy of chloroquine chemoprophylaxis for vivax malaria among Republic of Korea military personnel

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BACKGROUND: Chloroquine has been used massively for vivax malaria prophylaxis and treatment in the Republic of Korea (ROK, South Korea) military personnel from 1997. Although prophylaxis is generally regarded as successful among ROK military, prophylaxis failure has been repeatedly reported. Thus, continuous monitoring of rates of prophylaxis failure is required to identify the emergence of chloroquine resistant *P. vivax*. In the present study, we re-examined the efficiency of chemoprophylaxis in ROK military soldiers during 2011.

METHODS: This study was conducted at Armed Forces Hospitals that treats soldiers stationed near the Demilitarized Zone (DMZ), a malaria high-risk area that separates the ROK from the Democratic People's Republic of Korea (DPRK, North Korea). During the prophylaxis program by the ROK military was started on July 4th 2011, which was completed on October 16th 2011, we examined the efficiency of chloroquine chemoprophylaxis in ROK military by measuring compliance and whole blood chloroquine levels in 41 malaria patients immediately before instituting antimalarial therapy.

RESULTS: Before the prophylaxis program was started, more than 60% of malaria cases were attributed to new infection or long-latency relapse. Three patients (7.3%) showed good compliance, and had whole blood total chloroquine levels above the minimally inhibitory concentration (100 ng/mL). However, 28 (69.3%) of 41 malaria patients when admitted to hospital showed poor or no compliance with prophylaxis; 4 of the 28 (14.3%) were stationed outside the mass prophylaxis region, and 5 (17.9%) subjects were infected after the prophylaxis program had finished.

CONCLUSIONS: These findings indicate that the current malaria control program should be carefully reconsidered, in terms of, individual instruction, current chemoprophylaxis program regimens, and schedules to improve the efficacy of prophylaxis in the ROK military.

Anti-tick vaccines: Current situation and perspectives

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BACKGROUND: Ticks are haematophagous ectoparasites of domestic, wild animals, and humans. Control of ticks is performed by the use of chemical acaricides. However, chemical control produces serious problems such as elimination of acaricide residues in milk and meat, environmental contamination, and selection of acaricides resistant ticks. Currently, a vaccine based on the Bm86 recombinant protein from the cattle tick *Rhipicephalus (Boophilus) microplus*, is available. This vaccine protects partially against *R. microplus* and has great efficacy against *R. annulatus.* However, a wide variation of efficacy among geographical tick strains has been observed, and it does not protect against other tick species, like *Amblyomma* spp. Current research goals are focused towards the identification of new antigens that alone or combined may improve the existent vaccines; to avoid transmission of tick-borne pathogens and increase the spectrum of tick protection.
METHODS: RNA interference (RNAi), a research tool for functional analysis, became the most widely used technique for antigen identification and gene function, but experimental trials of immunization and tick infestations are the final steps to test for vaccine efficacy. RNAi, experimental trials of immunization and tick infestation, immunological response and evaluation of tick parameters were used to evaluate the protection of Bm86, Ba86, Bm95-msp1a and subolesin against the cattle ticks *R. microplus* and *R. annulatus.*
RESULTS AND CONCLUSIONS: The efficacy of evaluated proteins ranks from 60 to 99%, according to the tick species and strain. These experiments allowed us to conclude that tick vaccines are cost-effective by reducing tick infestations, oviposition and fertility. However, improved vaccines should protect against multi-tick species, and avoid transmission of tick-borne pathogens. As future perspectives to improve tick vaccines, the development of algorithms for analysis and validation of data produced by biological approaches like RNAi, and evaluation of selected candidates in natural and exposed hosts are proposed herein.

Immuno-mimetics of *Plasmodium* antigens as antimalarial vaccine candidates

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BACKGROUND: The molecular basis for obtaining new generations of anti-malarial vaccine candidates depends on selecting non-polymorphic antigenic peptides. Since such native molecules are poorly immunogenic when tested as vaccine components, these have to be rationally modified to modulate their immunological profile. Peptide-bond processing by peptidases follows a multi-step pathway including unstable high energy molecular complexes termed transition-states. Given peptide-bond isosteres would mimic these possible transition-states. Therefore stabilizing these specific molecular states by employing immuno-mimetics could become a strategy for inducing antibodies exhibiting neutralizing and catalytic properties. Hence, isostere-bond surrogates, represent a rational choice for obtaining site-directed designed immuno-mimetics from selected malarial targets.
METHODS: Peptido-mimetic chemistry and malarial functional tests, allowed us to explore a variety of peptide-bond chemical modifications to produce structurally-modulated immunogical probes.
RESULTS: Selected merozoite and sporozoite *Plasmodium* antigens were specifically chosen as potential targets to be modified based on either, a high-binding motif or a potential HLA-reading frame presence.
CONCLUSIONS: This new family of immuno-mimetics is proving to be efficient neutralizing antibody inducers when tested in controlled experiments, thus representing potential vaccine components.

Haplotypic resolution of parental and hybrid *Trypasosoma cruzi* lineages from the Chaco region

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BACKGROUND: *Trypanosoma cruzi*, the aetiological agent of Chagas disease, is currently divided into six lineages (TcI to TcVI). In the Chaco region, where this disease is hyperendemic, parental lineages (TcII/TcIII) and its natural hybrids (TcV/TcVI) circulate sympatrically. In this study we investigated the associations of these *T. cruzi* genotypes using a sequencing approach.

METHODS: Eighty three cloned *T. cruzi* isolates were genotyped by sequencing two single copy nuclear genes: ascorbate-dependent haemoperoxidase (Tc*APX*) and galactonolactone oxidase (Tc*GAL*). Isolates were derived from hosts and vectors of different localities within Argentina, Paraguay and Bolivia. Reference strains from Brazil, Colombia and Chile were included for comparison. Haplotypes were reconstructed using PHASE analysis.

RESULTS: Across the panel of isolates analyzed, 14 and 47 distinct haplotypes were identified for Tc*APX* and Tc*GAL* genes respectively. Different TcII-like and TcIII-like haplotype combinations were observed in the hybrid TcV and TcVI isolates, for both genes. Thus, within TcV isolates, for the Tc*APX* gene, two haplotype combinations were found (hap8/hap9 and hap8/hap12), whereas TcVI isolates possessed three haplotypic combinations (hap6/hap9, hap6/hap12, and hap7/hap9). Haplotypes 6 and 8 were observed in TcII strains from Paraguay and Chile and haplotype 9 was present in TcIII strains from Paraguay and Bolivia. For the second gene, Tc*GAL*, there was an apparent loss of heterozygosity for the TcII haplotype in all TcV isolates, each possessing the TcIII- like haplotype (hap24). Haplotype 24 was found only in TcIII strains from Paraguay. In contrast hybrid TcVI had two haplotypic combinations (hap22/hap26 and hap22/42). In terms of single nucleotide polymorphism (SNP) profiles, haplotype 22 was very similar to one present in TcIII strains from Paraguay, whereas haplotypes 26 and 42 were related with one haplotype found in TcII strains from Paraguay. TcIII haplotypes from Colombia and Brazil strains were not observed in any TcV and TcVI isolates, for either targets.

CONCLUSIONS: Sequence analysis of nuclear genes Tc*APX* and Tc*GAL*, revealed that TcII and TcIII isolates possessed haplotypes found in hybrid TcV and TcVI isolates but with observable SNP differences. Haplotypes observed in hybrids (TcV/TcVI) were more similar with those present in TcII and TcIII strains from Chile, Paraguay and Bolivia and less related with haplotypes from Brazil and Colombia strains. Further sampling may give insight to the likely geographical origins of the hybrid lineages.

Key words: *Trypanosoma cruzi* - Chaco region - sequence analysis - haplotypes

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Moving towards the elimination of urogenital schistosomiasis in Zanzibar (Unguja and Pemba Islands): design and implementation of an integrated multidisciplinary research programme

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BACKGROUND: Driven by the 2020 targets set by the World Health Organization and the projected increase in the supply and availability of praziquantel many African countries are stepping up their efforts to control and in some cases eliminate schistosomiasis. In Zanzibar, control of urogenital schistosomiasis has been on-going for decades and is now focusing on the possibility of elimination. As part of a larger endeavour (Zanzibar Elimination of Schistosomiasis Transmission) a research study is on going to determine the impact of interventions in addition to chemotherapy, namely behavioural change and snail control.
METHODS: The study is a randomized intervention trial involving 3 different study arms on the islands of Pemba and Unguja. Communities (shehias) in the first study arm are restricted to biannual mass drug administration (MDA) of praziquantel to the whole at-risk population, the second study arm, includes MDA plus snail control interventions and the third arm involves MDA plus behaviour change interventions. A total of 45 communities (shehias) are involved on both Unguja and Pemba islands and there are 15 communities in each study arm.
RESULTS: Since the onset of the project in November 2011, four praziquantel treatment rounds have been conducted with a reported coverage of around 80%. Snail control started in August 2012 and almost 100 natural freshwater bodies in the second study arms are treated regularly with niclosamide when intermediate host snails (*Bulinus globosus*) are present. Behaviour change interventions were designed together with the communities in the third study arms. Implementation of urinals, teacher's packages, safe play for children, and laundry areas commenced in October 2012. The results from the annual parasitological surveys will be discussed.
CONCLUSIONS: While progress can be seen at the mid-point of the study it is clear that there are many challenges for elimination programmes including treatment strategies, community mobilisation, infection hot-spots, diagnosis, migration, and suboptimal adherence to drug intake.

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The TNF-alpha expression by CD8 is associated with cytotoxicity after infection with *T. cruzi* Y strain but not Col cl1.7

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BACKGROUND: According to the World Health Organization, there are about 10 million individuals infected with *Trypanosoma cruzi* worldwide. *T. cruzi* population can be classified into six *discrete typing units*, named Tc I-VI. Is known that infection with Colcl1.7 (TcI) and Y strain (TcII) lead to differential development in experimental models, with Y strain associated with higher virulence. Our hypothesis is that these strains infect human peripheral blood cells differently, causing distinct immunological effects. Therefore, we evaluate the infectivity of Colcl.1.7 and Y strain, and the immunological profile of the host cell populations.
METHODS: *T. cruzi* trypomastigotes were stained with CFSE, and infection performed on cells from peripheral blood of healthy donors for 3 or 72 hours. After these periods, cells were lysed and incubated with monoclonal antibodies to determine the expression of TNF and IL-10 by CD14+ cells, as well as CD69, TNF, IL-10 and granzyme by CD4+ and CD8+ T cells. Data were obtained by multiparametric flow cytometry and analyzed by FlowJo.
RESULTS: Our results showed that Y and Colcl1.7 infected monocytes similarly. We observed a increased expression of IL-10 by monocytes infected Colcl1.7 compared to Y strain. Moreover, we observed an increased expression of CD69 IL-10 and TNF in CD4 and CD8 lymphocytes when infected with both lineages. Interestingly, we observed a positive correlation between the expression of TNF-alpha and granzyme by CD8 T cells after infection with Y strain, but not with Colcl.1.7.
CONCLUSION: This results suggests that infection with Colcl.1.7 led to the establishment of an anti-inflammatory profile, shown by the higher expression of IL-10 by CD14+ cells, while Y strain led to a more inflammatory profile, with TNF expression by CD8 T cells related to increased cytotoxicity evidenced by the positive correlation with the expression of granzyme which does not occur after infection with Colcl.1.7.

Indicators of gastrointestinal nematodes infection in sheep

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BACKGROUND: Gastrointestinal nematodes cause health problems and economic losses in grazing systems for sheep production Uruguay. *Haemonchus* and *Trichostrongylus* were the most prevalent and pathogenic nematodes that are involved in anthelmintic resistance to different active principles. In studies of production, reproduction and control of nematodes is essential to have an indicator that reflects the evolution of the parasitic infection and the effect caused by the parasitic load, as early as possible, and that suits the production system running. The objective of this work was to assess the indicators of infection by gastrointestinal nematodes in Australian Merino sheep in the region of crystalline basalt of the Uruguay.
METHODS: In a natural parasite challenge, monitoring of parasitic infection was performed by counting eggs per gram of feces (EPG), the color of the ocular mucosa (FAMACHA) and hematimetric values (hematocrit and hemoglobin).
RESULTS: The reported parasitic genera were mainly *Haemonchus* spp. and *Trichostrongylus* spp. The level of parasitism by gastrointestinal nematodes had an average of 273 EPG and less than 7% of the samples had values above 1000 EPG. That level of afectation do not allowed us to appreciate the effect of parasites by determining the FAMACHA ($p = 0, 42$). The EPG relationship with hematocrit has a borderline significance ($p = 0.05$), however, the relationship between EPG and hemoglobin concentration was significant ($p = 0.025$).
CONCLUSIONS: It can be concluded that, for these relatively low levels of parasitism, the FAMACHA was not a good marker of infection, being hemoglobin a better correlated indicator with parasite load, followed by the hematocrit.

The quest for a recombinant vaccine against liver fluke in ruminants

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BACKGROUND: Fasciolosis caused by the liver fluke *Fasciola hepatica* represents a global constraint to cattle and sheep production particularly in heavily affected agriculturally-based countries. Peptidases and antioxidant enzymes are among the groups of molecules tested as vaccines against liver fluke infection.
METHODS: Following different approaches we selected two enzymes as vaccine candidates, a digestive exopeptidase named leucine aminopeptidase (*Fh*LAP) and the antioxidant enzyme thioredoxin glutathione reductase (*Fh*TGR). *Fh*LAP was selected based on its histochemical localization at the gastrodermis and relevant participation in late stages of host protein degradation. The antioxidant *Fh*TGR was selected based on its unique central role in flatworm thiol-based redox pathways, its tegumental localization, and its validation as a drug target in juvenile forms.
REULTS: Native *Fh*LAP induced a high protective response in sheep, and more recently the recombinant functional multimeric enzyme demonstrated protective levels between 39% in cattle and 84% in sheep using different adjuvant and vaccination schemes. On the contrary two different vaccine trials in cattle using recombinant *Fh*TGR failed to demonstrate any significant protective response against metacercarial challenge.
CONCLUSIONS: Total and subclass IgG responses against both antigens will be presented, and possible explanations for these different outcomes will be discussed.

Preliminary studies of biosynthesis of heme O and heme A in intraerythrocytic stages of *P. falciparum*

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Background: Intermediates of the isoprenoid metabolism can bind to heme groups, or take part in the biosynthesis of gibberellin in plants. Gibberillin-precursor inhibitors, Inabenfide and Uniconazole-P, reduce *P. falciparum* growth in vitro. Since a number of plant-exclusive isoprenic metabolites have also been found in *P. falciparum*, we set out to characterize the biosynthesis of gibberellins. Like others antifungals they can interfere in the Heme biosynthetic pathway. For this, it is important to characterize this pathway in *P. falciparum* entirely, starting with farnesylation of the heme group by "heme O synthase" (HOS or Cox10) and "heme A synthase" (HAS or Cox15).
Method: We monitored gibberellin synthesis by metabolic labeling of parasites with [^{3}H]GGPP, organic extraction and analyses by RP-HPLC. Growth inhibition assays were conducted using Inabenfide or Uniconazole-P and recovered with giberellins. The plasmodial orthologs of HOS and HAS genes were identified by their homology HOS and HAS enzymes form other organisms. We then genetically tagged both genes with GFP and HA coding sequences and visualized transfectants by fluorescence microscopy.
Results: We observed no production of gibberellin in blood stage parasites and supplementation with giberellins did not recover growth in drug treated parasites. Visualization of tagged of PfCox10 and PfCox15 revealed that they were most probably localized in the mitochondria although PfCox10 presented an unusual extension at its N-terminus.
Conclusion: We conclude that the parasites do not produce significant amounts of gibberellins. Given that the two drugs affect parasite growth, it would be interesting to see if these like others antifungals will interfere in the heme pathway, since azoles can remove heme from histidine rich peptide-heme complex. PfCox10 PfCox15 are fairly similar to HOS and HAS from other organisms, showing several conserved residues reported essential for these type of enzymes in *S. cerevisae.*

A novel crowd-sourcing technique for predicting densities and distribution of disease-transmitting mosquitoes in rural Tanzania

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BACKGROUND: Lack of reliable techniques that can be used for large-scale programmatic monitoring of distribution and densities of disease-transmitting mosquitoes is a major challenge to public health authorities, especially in rural and remote communities where high-tech GIS and remote sensing facilities are not readily applicable for regular use. We developed and evaluated a new community-based participatory mapping approach that relies simply on the knowledge and experiences of residents to rapidly identify areas where disease-transmitting mosquitoes are most abundant. The method is proposed for use in spatial targeting of mosquito control interventions. Such simplified methodologies for mapping vector densities will be particularly necessary for optimal placement of new interventions to complement existing ones such as LLINs for malaria control.
METHODS: This new crowd-sourcing technique consisted of 5 steps. We initially mapped three test villages comprehensively identify major land marks. We then selected 60 community members monthly, taught them basic map-reading and offered them gridded maps of their own villages so they could identify locations where they think mosquitoes are most abundant, by simply ranking the grids on scale of 1-5. The data generated was interpolated in ArcGIS using IDW method and classified to show places where people thought there were high, medium and low mosquito densities .Finally, mosquito sampling was done using an effective odor-baited sampling tool, to verify outdoor mosquito densities in locations pre-identified by community members as mosquito densities, and to validate this crowd-based prediction method.
RESULTS: Maps were derived from community knowledge and opinions on the mosquito density distributions. For twelve months in three villages, entomological surveys depicted the same vector densities and distribution pattern as the crowd-sourcing technique.
CONCLUSIONS: This study thus provides evidence that we can rely on community knowledge and experience to identify suitable areas where mosquitoes are most abundant and where to locate outdoor complementary interventions. Such a method will be cheaper, quicker and easier even for planning and implementing large-scale vector control operations.

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EhRabB and actin cytoskeleton transport the EhCPADH112 complex during the erythrophagocytosis of *Entamoeba histolytica*

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Background: *Entamoeba histolytica* is an invasive organism that requires the participation of multiple proteins to cause damage to host cells. A protein complex (EhCPADH), formed by a cysteine proteinase (EhCP112) and an adhesin (EhADH112), is involved in pathogenicity EhADH one of the proteins forming this complex, shares similar characteristics to Alix proteins, which in higher eukaryotes are implicated in receptor endocytosis, endosomal protein sorting, and cell adhesion. A Rab protein (EhRabB) encoding gene is located closely, but in the complementary strand, to the EhCP112 and EhADH112 encoding genes, suggesting that these proteins are functionally related. Thus, it is possible that EhRabB, together with the cytoskeleton, participates in the transport of the EhCPADH complex. In this work we describe how EhRabB, and actin cytoskeleton interact during the mobilization of the EhCPADH complex. **Methods:** To analyze the trafficking process of the EhCPADH complex during erythrophagocytosis we performed transmission electron microscopy (TEM), confocal microscopy and FRET techniques, using specific antibodies against the EhCPADH complex, the EhRabB protein, and actin, on trophozoites incubated at different times with human erythrocytes. Additionally, we carried out molecular dynamics and molecular docking assays to investigate the protein motives implicated in their interaction, and immunoprecipitation and cellular fractioning assays to investigate protein association and cellular location of EhCPADH, EhRAbB and actin. **Results:** Confocal microscopy assays show that at early times of phagocytosis the EhCPADH complex, EhRabB and actin co-localize in various cellular compartments, mainly in the ingested erythrocytes. At longer times, the EhCPADH complex returned to the plasma membrane, while EhRabB proteins decorated the phagocytosed erythrocytes. Using TEM images of the process at different times, we design the route of the EhCPADH complex mobilization directed by EhRabB protein. FRET analysis confirmed the association between the EhCPADH complex and EhRabB during erythrophagocytosis**. Conclusions:** We conclude that the EhRabB and the actin cytoskeleton play a major function in the vesicular trafficking of EhCPADH during erythrophagocytosis. These results will allow us to better understand the processes by which pathogenicity *E. histolytica* causes damage to the host.

Prevalence of *Cryptosporidium* spp. and *Giardia* spp. In dogs and kindergarten children in Boca del Rio, Veracruz, Mexico.

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BACKGROUND: *Cryptosporidium* spp. and *Giardia* spp. are protozoan parasites that cause zoonotic acute gastroenteritis. Infected dogs, children, and immunocompromised patients can suffer severe clinical disease. Dogs with diarrhea are a risk factor for young children. Here, we evaluated the prevalence of infection with the two zoonotic waterborne protozoan parasites in kindergarten children and dogs in the urban center of Boca del Rio in the state of Veracruz, México.
METHODS: A cross-sectional epidemiological study was conducted with an "n" of 356 children and 96 dogs; this sample size was calculated using the Win Episcope Ver 2.0 program. Stool samples collected from February-December 2013 were processed for testing using a commercially available direct immunofluorescence kit to detect *Cryptosporidium* oocysts and *Giardia* cysts (MERIFLUOR® C/G, Meridian Bioscience, Inc., Cincinnati, OH, U.S.A.). Survey data were captured in an Excel spreadsheet and analyzed with descriptive statistics using STATA version 11.0 program.
RESULTS: A stool sample was collected from 356 children. There were 169 children positive for *Giardia* spp., which yielded a prevalence of 47.5% (95 CI%: 41.1- 51.6). Eighty-six of the children were positive for *Cryptosporidium* spp. so the prevalence for this parasite was 24.15% (95% CI: 19.9- 29). Of the 117 dogs sampled, 92 were positive for *Giardia* spp. and thus had a prevalence of 78.6% (95% CI: 68.9-85.4). Seventy-four of the dogs sampled were positive for *Cryptosporidium* spp., which resulted in a prevalence of 63.24% (95% CI: 53.7- 71.8%).
CONCLUSIONS: The prevalence for *Giardia* spp. and *Cryptosporidium* spp. was higher in dogs than in children. Exposure to dogs appears to place children at risk of infection with these zoonotic parasites. Education on hygiene practices to interact with dogs will help mitigate the risk of zoonotic infection with *Giardia* spp. and *Cryptosporidium* spp. among children in Boca del Rio, Veracruz, Mexico.

Learning and forgetting Parasitology after formal medical teaching

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BACKGROUND. The medical career is structured and oriented so that progressive and increased knowledge, skills, attitudes and values are obtained. The basic cycles contain several biomedical disciplines, while in clinical cycles students participate in hospital environments, so that teaching practices develop in academic and practical ways. The first phase is performed during the first and second years of the career, the second phase in the third and fourth years. During the second year Parasitology is studied. The third phase takes place in the fifth year (Internship). During the sixth year students undertake their Social Service after obtaining their title. The time between the course of Parasitology and its application in clinical clerkship, Internship and social service is 2 to 4 years. The course of Parasitology in the second year is based on academic construct and declarative learning, immersed in a sea of information with no link to the rest of the career. In this presentation, the permanence of the knowledge of Parasitology gained during the second year of the career is investigated and later during specialty training.
METHODS. The same assessment for students who completed the course of Parasitology in the 2nd year, students in 4th year of the career, in the internship, in medical specialty of Internal Medicine and Pediatrics and students performing a subspecialty in infectious diseases was applied.
RESULTS. The results showed differences in knowledge ranging from 72% after the 2nd year, and decreasing to 36%, 24%, 27% and 23%, respectively, thereafter; knowledge increased to 49% at the sub specialization in Infectious Diseases.
CONCLUSIONS. The study showed that knowledge of Parasitology has little permanence and forgetting is high; possibly the information and teaching methods have low practical correlation with the practice of medicine; and are of little use and hardly motivate students, who do not understand the medical and social importance and relevance of acquiring and retaining knowledge of Parasitology, until they are fully trained.

Comparative study of learning Parasitology in two educational systems

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BACKGROUND. Teaching Parasitology to medical students takes place in the second year of the curriculum in schools, colleges and faculties in Mexico, but include different systems of organization and schoolwork. Like any social structure, teaching must be accepted by the students involved in it, and is destined to fail from the time the individual is not convinced of its validity. The fundamental learning takes place successfully in very different conditions from those provided by the school; students develop skills often without the help of a teacher, and there are lessons that are achieved in very different ways in the classroom class. In this paper a comparison of learning outcomes between students in the course of Parasitology, in two school systems, one university and one from a military school is presented.
METHODS. Learning outcomes of students in two schools were assessed after the completion of a course of Parasitology for medical students in the second year of the school with the same program and taught by the same teachers. As an assessment tool, the routine examination method utilized during the course was used. The numerical results obtained by the students of the two educational systems were compared, statistical tests were performed and a table of comparison for oral responses to specific questions of parasitological knowledge given by the students was analyzed.
RESULTS. Clear differences between the two groups of students were identified. In general a better learning of students in the university system, with respect to the students of the military school system, was identified.
CONCLUSIONS. This educational research demonstrated that the university school life induces better learning, possibly due to increased openness of the system and the students to teaching procedures and resources that are used in it, as well as the way of life makes student engagement different, as are their interests and needs.

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Protein phosphorylation during gametogenesis of *Plasmodium berghei*

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BACKGROUND: Malaria is caused by protozoan parasites of the genus *Plasmodium* and is a major cause of mortality and morbidity worldwide. The sexual development of the parasites initiate when mosquitoes feed on an infected vertebrate host carrying sexual blood stages (gametocytes), which reach the mosquito midgut, where gametocytes rapidly exit erythrocytes and transform into gametes (gametogenesis). Male gametocyte goes through three rounds of genome replication and mitotic division, and release eight highly motile flagellated gametes (exflagellation). Female gametocyte differentiates into a macrogamete that is fertilized by one microgamete to develop into zygote. Protein phosphorylation is a post-translational modification that play key role in many cellular processes, which occur in response to extracellular signals, and participate in signal transduction, metabolism, differentiation and cell cycle regulation.
METHODS: The *P. berghei*, gametocyte-producing ANKA strain, clone 2.34 was used to produce sexual development *in vitro*. Proteins samples were analyzed in 2D electrophoresis and immunoblotting; Antibodies that specifically recognize phosphorylated residues in serine, threonine or tyrosine were identified by mass spectrometry.
RESULTS: Approximately 70 proteins exhibited phosphorylation changes, 18 of which were identified. These proteins included components of the cytoskeleton, others involved in DNA synthesis and signaling pathways.
CONCLUSIONS: Phosphorylation changes were observed mainly in threonine residues followed by tyrosine and serine. Also, novel phosphorylation events during gametogenesis were identified.

Helminth parasites in reptiles from the Biological Reserve Foz do Rio Aguapeí, Southeast of Brazil

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BACKGROUND: Host-parasite specificity, geographical distance and phylogenetic relationships among host populations are factors which contribute to the composition and structure of helminth communities. The objective of this study is to characterize the component helminth parasite communities associated with reptiles in a region of biome transition Cerrado-Atlantic Forest in a conservation area (Biological Reserve Foz do Rio Aguapeí, Southeast of Brazil).
METHODS: Specimens of lizards (n=40), snakes (n=33), turtles (n=11) and crocodilians (n=4) were collected, euthanized and evaluated for the presence of helminths, which were collected and prepared in accordance with classical methodologies in Parasitology.
RESULTS: Among the lizards, prevalence [P] of 42.5%, mean intensity of infection [MII] of 18.2±13.6, and mean abundance [MA] of 7.8±5.9 were observed and *Physaloptera* sp., *Rhabdias* sp., *Thelandros alvarengai*, *Oochoristica* sp. were found infecting several host species. The snakes showed P=63.6%, MII=48.1±14.6, and MA=30.6±10.1, and *Infidum infidum*, *Infidum similis*, *Travtrema stenocotyle*, *Crepidobothrium* sp., *Ophiotaenia* sp., *Brevimulticaecum* sp., *Hastospiculum digiticaudatum*, *Spiroxys figueiredoi*, *Physaloptera* sp., *Rhabdias* sp., *Strongyloides* sp., and acanthocephalan cysthacants were found in various host species of the families Anomalepididae, Boidae, Colubridae, Dipsadidae, and Viperidae. For turtles, P=90.9%, MII=12.0±2.9 and MA=10.9±2.9 were observed, and the helminthes found were *Cheloniodiplostomum testudinis*, *Nematophila grande*, *Polystomoides brasiliensis*, *Physaloptera* sp., *Serpinema* sp., and *Spiroxys figueireidoi* infecting *Phrynops geoffroanus*. Among the crocodilians, host specimens of *Paleosuchus palpebrosus* and *Cayman yacare* were infected with *Brevimulticaecum* sp.
CONCLUSIONS: The data include the occurrence of new hosts and new geographical location of some helminth species, broadening the knowledge about the distribution of parasites of reptiles in Brazil (FAPESP 2011/20186-6).

Molecular detection of bacterial and protozoan pathogens in *Ixodes ricinus* from Italy

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BACKGROUND: In Europe, a wide range of tick-borne pathogens, including viruses, bacteria, and protozoa can cause diseases in both animals and humans. In Italy, available data about the species present in the country and their distribution on the territory are few and fragmentary. In this study, *I. ricinus* collected in Northeastern, Central and insular areas of Italy were analyzed by molecular methods to determine the presence of *Rickettsia spp*., *Borrelia spp*., and *Babesia spp*.
METHODS: A total of 447 ticks from 5 regions (Pianosa, Belluno, Perugia, Rieti, and Capri) were screened for DNA of the *gltA* gene of *Rickettsia spp.*, the *18S* rRNA gene of *Babesia spp*. and the flagellin gene (*Fla*) of *Borrelia spp.,* using primers previously described. Positive samples were compared with reference sequences from GenBank.
RESULTS: Altogether 86 ticks (19%) were positive for *Rickettsia spp*. Samples showed 98-100% blast identity to four rickettsiae of the spotted fever group of zoonotic concern: *Rickettsia monacensis*, *Rickettsia raoultii*, *Rickettsia massiliae*, and *Rickettsia helvetica*. None of the samples from Capri were positive. DNA of *Babesia spp.* was detected only in one (0,22%) tick from Belluno, whereas 17 (3,8%) ticks from the Belluno and Perugia areas were infected with *Borrelia burgdorferi* s. l.
CONCLUSIONS: The results of the present study show a wide distribution of bacterial and protozoan pathogens in Italy. *I. ricinus*, one of the most abundant tick species in the country, confirms to be a Pandora's vase and, having a large record of attacking humans, a fearful possible source of infection.

Soil-Transmitted Helminths from Malaysian Landscape - a Never Ending Story

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BACKGROUND: Soil-transmitted helminth (STH) is a public health problem worldwide. This prospective study was carried out to determine the prevalence of STH infections in humans and animals, also to investigate the egg counting capacity between Kato-Katz and McMaster techniques.

METHODS: A total of 402 (200 from humans and 202 from animals) fecal samples were screened for STHs by using direct smear and formal-ether sedimentation techniques. Subsequently, these samples were compared by using Kato-Katz thick smear and McMaster techniques for fecal egg count.

RESULTS: In humans, the overall prevalence of STHs was 54.5% (109/200) which was found in all recruited Aborigines communities. Of STHs, *Trichuris trichiura* was shown the highest prevalent (49.5%), followed by Hookworm (19.5%) and *Ascaris lumbricoides* (12%). In univariate analysis, gender, race, occupation, education, residency, no toilet, location of toilet, barefoot, eating raw vegetables, and drinking river water were shown significant association with STHs (p<0.05). Multivariate analysis showed that eating raw vegetables (OR=3.391, CI=1.160-9.910, p=0.026) was the only identified factor associated with STHs. The overall prevalence of STHs was 45.5% (98/202) in animals. Hookworm was the most common STH infection (45.1%), followed by *Toxocara* spp. (11.4%) and *Trichuris vulpis* (3%) found in animal fecal samples. For fecal egg count, Kato-Katz was shown higher detectable rate (51%) compared to McMaster (47.5%) techniques in human STH infections. Contrarily, McMaster was shown better counting capacity (50%) than Kato-Katz (45%) techniques for animal samples.

CONCLUSIONS: Based on the result obtained, the level of STH contaminating in our environment is worrying. This suggests health education for implementing primary behavioral practices to curve the incidence of STH infections in humans. A proper management (veterinary care, vaccination and parasite control) is recommended to prevent zoonotic transmission from animals to humans. Kato-Katz and McMaster techniques can be complimentarily used in routine screening of STHs in cross-fecal samples.

Taxonomy and Diversity of monogenean parasites of clupeoid fishes of Visakhapatnam coast, Bay of Bengal

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BACKGROUND: Clupeoid fishes constitute one of the dominant groups of fishes in the coastal waters of Visakhapatnam, Bay of Bengal. These fishes are also known to serve as suitable hosts for monogeneans, by virtue of their shoaling behaviour which facilitates easy transmission of parasites from one host to another. But no monogenean has so far been recorded from clupeoid fishes of Visakhapatnam coast. An investigation has therefore been undertaken on monogenean parasites of common species of clupeoid fishes, covering taxonomy, faunal diversity, host specificity and endemicity.
METHODS: A total of 1071 individuals of clupeoid fishes belonging to 11 species spread over 8 genera and 4 families were examined for monogeneans parasites. All the monogeneans found were identified up to specific level. The molecular and morphometric methods were employed as supplementary to traditional morphological methods for identification of the species.
RESULTS: Altogether 20 species of monogeneans belonging to 5 families: Mazocraeidae (14 spp.), Gastrocotylidae (3 spp.), Axinidae (1 sp.), Microcotylidae (1 sp.) and Diclidophoridae (1 sp.) were identified. The fauna included 9 new species and 1 new genus. The number of parasite species in a host species ranged from 1 to 7. The role of each species of clupeoid fish as a host for monogeneans is determined. The maximum number of species are found in *Thryssa* sp.(Engraulidae). On the other hand sardines and anchovies are found to be poor hosts for monogeneans. The various monogeneans species exhibited high degree of host specificity and endemicity. The results of the phylogenetic analysis provided support to the conclusions drawn from traditional taxonomic studies regarding identification of various species and their phylogenetic relationships.
CONCLUSIONS: Clupeoid fishes are found to be most suitable hosts for mazocraeid monogeneans. A close phylogenetic relationship is also noted between the clupeoids and mazocraeid monogeneans. It is presumed that mazocraeids had their origin in clupeids, colonized this group of fishes and gradually spread to other groups of fishes through host switching.

Metazoan Parasite communities of the frigate tuna *Auxis thazard* (Bloch) from the coast of Visakhapatnam, Bay of Bengal

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BACKGROUND: The frigate tuna *Auxis thazard* (Bloch) (Family Scombridae) is an epipelagic neritic as well as oceanic migratory fish that inhabits warm waters in tropical and subtropical waters. It is known to serve as a host for many species of metazoan parasites. Although several authors have reported parasite species from *A. thazard* from the Indian and other oceans, to date detailed studies dealing with analysis of its parasite communities are lacking. Along the coast of Visakhapatnam, where there is intensive tuna fishing activity almost throughout the year, the frigate tuna occurs commonly in the catches. An investigation was therefore, undertaken to analyse the species composition and the diversity of metazoan parasite communities of the tuna.
METHODS: Specimens of *A. thazard* were collected from line catches along the coast of Visakhapatnam and examined for metazoan parasites. Details of the species of parasites found, their numbers and site of infection were recorded. Parasite community was characterized from an estimation of mean total parasite individuals, species richness and species diversity calculated by Shannon-Wiener diversity index (H'). Dominance index was calculated by employing Berger-Parker Dominance index.
RESULTS: Twenty three species of metazoan parasites were found infecting *A. thazard* including 4 species of Monogenea, 13 spp. of Digenea (including 8 species of Didymozoids), 2 varieties of cestode larvae (Scolex pleuronectes) 1 species of Nematoda, 2 of Acanthocephala and 1 of Copepoda The mean parasite burden was estimated as 55.2±62.8. (Range 4-270).The number of parasite species in a host ranged from 1 to 7 with a mean of 3.8±1.46. Species diversity is moderately high. Majority of the metazoan parasite species showed a high degree of host specialization.
CONCLUSIONS: Tunas are known for their large size, high vagility capable of extensive migrations across world oceans and rich diet composed of fish, crustaceans and squids. All these traits favour infection with wide range of parasites and this explains the richness and diversity of parasite fauna found in *A. thazard*.

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Evaluation, analysis, and characterizion of the *Trypanosoma vivax* immunogenic proteins with double dimension Western Blots and nano LC-MS/MS.

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Trypanosomosis caused by *Trypanosoma vivax* (*T. vivax*) is a problem in cattle, sheep, goat, and buffalo in Latin America and in Africa due to the great economic losses caused. However, *T. vivax* has been scarcely studied with new technologies, perhaps because of maintenance in laboratory animals and purification difficulties. Because of this, there are few serological tests for diagnosing specific species, and there is not a lot of knowledge of the basic biological aspects of this hemoflagelate. With the aim of evaluation, analysis, and characterization of the *Trypanosoma vivax* immunogenic proteins, positive and negative control sera from field animals infected with *T. vivax* and *T. evansi* according to Woo criteria, ELISA and PCR were selected. Also, in this study, sera of experimentally infected sheep and cattle were used. The 2D WB allowed a better characterization of the recognition of *T. vivax* antigens by homologous sera, finding immunodominant proteins of 50, 55, 60, 70 and 83 kDa. None of these proteins were identified by sera anti *T. evansi*. These antigenic polypeptides were assessed by nano LC-MS/MS and twelve proteins were identified with the aid of the database transcriptome *T. vivax* and every protein was analyzed by bioinformatics, describing the possible structure and function. Among the twelve immunodominant proteins, five are promising candidates for species specific diagnosis of *T. vivax*. Within this group, two Paraflagelar Rod Proteins (PFR69, PFR73) and three Heat Shock Proteins (HSP60, HSP70, HSP83) were found. In the identified proteins there were no VSG, which does not seem to be antigenic in *T. vivax*. These results, together with the recently published transcriptome of *T. vivax* (BMC Genomics 2013 , 14:149), allowed us to define, for the first time, specific antigens of *T. vivax* (strain LIEM -176) which significantly opens new ways towards development of diagnostic methods as well as potential immunogens for the development of immunoprophylaxis.

Key Words: *Trypanosoma vivax*, Westen blot 2D, immunone, *Trypanosoma evansi*.

Exogenous expression of the EhADH *Entamoeba histolytica* adhesin in MDCK cells facilitates parasite invasion

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BACKGROUND: *Entamoeba histolytica* is the causative agent of human amoebiasis. Infection is initiated by interaction of this pathogen with the intestine. This interaction leads to disruption of intercellular structures such as tight junctions (TJ). We recently showed that the *E. histolytica* complex EhCPADH formed by the EhADH adhesin and the EhCP112 cysteine protease, participates during epithelial invasion. To elucidate the particular role of EhADH in this process, we overexpressed this adhesion molecule in epithelial cells.
METHODS: MDCK epithelial cells were transfected with the complete gene of *ehadh* and its exogenous expression was proven by RT-PCR and immunofluorescence assays. To evaluate the effect of EhADH in MDCK adhesive properties, we performed aggregation and adhesion experiments. Furthermore, the effect on TJ was analyzed by transepithelial electrical resistance (TER) assays and the alteration of TJ proteins was determined by immunofluorescence and western blot.
RESULTS: EhADH localized at the plasma membrane of epithelial cells, similar to its location in trophozoites. MDCK cells expressing EhADH improved their adhesion to erythrocytes and between them. This augmentation in adhesive properties was probably induced by an increase in TER and by an up-regulation of TJ proteins. Surprisingly, infection with trophozoites of MDCK cells expressing EhADH caused a faster drop in TER and more epithelial damage compared to control cells.
CONCLUSIONS: Our results suggest that EHADH confers more adhesive properties to epithelial cells and in these cells EhADH serves as a ligand for the parasite, facilitating trophozoite entrance into the epithelium. Combined with our previous results that EhCPADH localizes to TJ after secretion by trophozoites, EhADH may be a promising new target to prevent amoebiasis.

Use of old/new genetics to identify a novel Toxoplasma effector that up-regulates host c-Myc.

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BACKGROUND: *Toxoplasma* introduces a number of effectors into the host cell to manipulate the host to its advantage. We have shown that changes in the abundance of specific host miRNAs are among the changes mediated by infection with *Toxoplasma* tachyzoites but the mechanism responsible for this has not been known. Given the role of c-Myc in regulating some of the affected miRNAs, we hypothesized that this important transcription factor might be involved and have now shown that c-Myc is indeed up-regulated by tachyzoite infection. To address the mechanism used to drive this up-regulation, we have taken advantage of a key property of *Toxoplasma* as a genetic system, its haploid genome. As pioneered by Pfefferkorn and colleagues, this allows the easy generation of mutants but, until recently, identifying the causal mutation has been a challenge. Here I will discuss how genetics and next-generation sequencing rapidly revealed a gene responsible for the up-regulation of c-Myc.
METHODS: We used a reporter mouse where GFP is fused to the endogenous c-Myc gene and selected mutant tachyzoites unable to induce the c-Myc-GFP fusion by FACS of reporter cells infected *in vitro*. The resulting mutants were sequenced to identify the *Toxoplasma* gene mediating c-Myc up-regulation and their phenotype was characterized *in vivo* and *in vitro*.
RESULTS: We obtained several mutants that fail to induce up-regulation of c-Myc in cells from our c-Myc-GFP reporter mice. Three of these mutants have now been sequenced and found to contain a total of just ~5-7 coding changes each, relative to the parental strain. Of these 5-7 changes, each mutant was found to have precisely one nonsense mutation (the other 4-6 coding mutations are all missense) at a different, unique position within a single gene. No other gene harbored a missense or even a silent mutation in all three mutants. This shows that these are three independent mutants and that the altered gene is almost certainly involved in the up-regulation of c-Myc in cells infected with *Toxoplasma*. This gene encodes a previously uncharacterized protein but, consistent with its role as an effector, it has a predicted signal peptide and its expression pattern strongly predicts it is a dense granule protein. *In vivo* analysis indicated that the affected gene is necessary for full virulence following intraperitoneal infection of mice.
CONCLUSIONS: Chemical mutagenesis and next-generation sequencing of the resulting mutants is a powerful method for identification of *Toxoplasma* genes involved in important biological functions. The novel effector identified here adds another to the list of proteins used by *Toxoplasma* to profoundly alter the infected host cell.

Polymorphic Effector MAF1 Mediates Toxoplasma's Recruitment of Host Mitochondria and Impacts the Host Response

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BACKGROUND: Toxoplasma strain types have been shown to differ in many remarkable ways, including many key interactions with the host cell. These differences are the result of polymorphic effectors that the parasite introduces into the host cell. Their activities range from tryrosine kinases that mimic JAKs to proteins whose biochemical activity is unknown but that impact other host transcription factors in a strain-specific way. It has also been long known that *Toxoplasma* tachyzoites can be found within vacuoles surrounded by host mitochondria. Although many hypotheses have been proposed for the existence of host mitochondrial association (HMA), which has also been reported for several bacterial pathogens, the causes and biological consequences of HMA have remained unanswered.

METHODS: We asked whether HMA is a trait of all major Toxoplasma strains and, if not, whether we could use genetics to identify the molecular basis for this phenomenon and its consequences to the infection.

RESULTS: We show that, surprisingly, HMA is present in type I and III strains of *Toxoplasma* but missing in type II strains, both *in vitro* and *in vivo*. Analysis of F1 progeny from a type II × III cross revealed that HMA is a Mendelian trait that we could map. Using bioinformatics, we identified potential candidates and experimentally identified the polymorphic parasite protein involved, mitochondrial association factor 1 (MAF1). We show that introducing the type I (HMA^+) *MAF1* allele into type II (HMA^-) parasites results in conversion to HMA^+ and deletion of *MAF1* in type I parasites results in a loss of HMA. We observe that the loss and gain of HMA are associated with alterations in the transcription of host cell immune genes and the *in vivo* cytokine response during murine infection. Lastly, we use exogenous expression of MAF1 to show that it binds host mitochondria and thus MAF1 is the parasite protein directly responsible for HMA.

CONCLUSIONS: Our findings suggest that association with host mitochondria may represent a novel means by which *Toxoplasma* tachyzoites manipulate the host. The existence of naturally occurring HMA^+ and HMA^- strains of *Toxoplasma* indicates the existence of evolutionary niches where HMA is either advantageous or disadvantageous, likely reflecting tradeoffs in metabolism, immune regulation, and other functions of mitochondria.

Activity of neem tree (*Azadirachta indica*) seed extracts from Veracruz, México against *Rhipicephalus microplus*

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BACKGROUND: The neem tree (*Azadirachta indica*) is known to contain components that affect the biology of *R. microplus*. This tick species is regarded as the most economically important ectoparasite of cattle globally because of direct impacts associated with blood feeding and its role as vector of the pathogens causing bovine babesiosis and anaplasmosis. Extracts from seeds of local neem trees were tested for ixodicidal activity and other effects against *R. microplus*.
METHODS: Neem seeds harvested in 2013 were processed using an industrial blender. Different extracts were obtained by leaching using hexane, ethyl acetate, ethanol, and methanol. An oily extract was obtained by cold extrusion, and an aqueous extract was produced by maceration. Bioassays were conducted with adult female ticks of the "San Alfonso" strain according to the immersion technique. The concentrations tested were: 100, 50, 25, and 12.5%. Probit analysis was performed to assess the ixodicidal activity of extracts at 6, 24, 48 and 72 hours post-treatment.
RESULTS: All the adult female ticks exposed to all the concentrations of the oily extract perished (100% efficacy) by 48 hr after treatment. One-hundred % efficacy was observed with all the test concentrations of the ethyl acetate extract too, but at 72 hr post-treatment. Oviposition was inhibited 100% with the highest concentration of the ethanol extract. Larval hatching was affected 60% with the highest concentration of the aqueous extract.
CONCLUSIONS: Our findings confirmed previous reports of activity against *R. microplus* by extracts from neem seeds. Effects were noted on the survival of fully-engorged adult females, oviposition, and larval hatching. Neem seed extracts could be formulated to control *R. microplus* populations in Veracruz, México that are resistant to synthetic acaricides.

Environmental Temperature Affects Prevalence of Blood Parasites of Birds on an Elevation Gradient: Implications for Disease in a Warming Climate

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BACKGROUND: The rising global temperature is predicted to expand the distribution of vector-borne diseases both in latitude and altitude. Many host communities could be affected by increased prevalence of disease, heightening the risk of extinction for many already threatened species. To understand how host communities could be affected by changing parasite distributions, we need information on the distribution of parasites in relation to variables like temperature and rainfall that are predicted to be affected by climate change.
METHODS: We determined relations between prevalence of blood parasites, temperature, and seasonal rainfall in a bird community of the Australian Wet Tropics along an elevation gradient. We used PCR screening to investigate the prevalence and lineage diversity of four genera of blood parasites (*Plasmodium*, *Haemoproteus*, *Leucocytozoon* and *Trypanosoma*) in 403 birds.
RESULTS: The overall prevalence of the four genera of blood parasites was 32.3%, with *Haemoproteus* the predominant genus. A total of 48 unique lineages were detected. Independent of elevation, parasite prevalence was positively and strongly associated with annual temperature. Parasite prevalence was elevated during the dry season.
CONCLUSIONS: Low temperatures of the higher elevations can help to reduce both the development of avian haematozoa and the abundance of parasite vectors, and hence parasite prevalence. In contrast, high temperatures of the lowland areas provide an excellent environment for the development and transmission of haematozoa. We showed that rising temperatures are likely to lead to increased prevalence of parasites in birds, and may force shifts of bird distribution to higher elevations. We found that upland tropical areas are currently a low-disease habitat and their conservation should be given high priority in management plans under climate change.

Antiprotozoal Potential of *Anethum graveolens* and *Punica granatum* plant extracts against *Giardia lamblia*

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Giardiasis is worldwide spread disease caused by the protozoan several *Giardia* species. Its treatment includes drugs which may produce side effects and, in addition, onset of chemical resistance of this pathogenic protozoan. Thus, with the purpose of searching for new natural anti-protozoal chemotherapy, *Anethum graveolens* and *Punica granatum* two plants of the family *Apiaceae*, and Lythraceae respectively, used in traditional medicine against intestinal disorders were selected to evaluate their petroleum ether, ethyl acetate, methanol and aqueous leaves extracts activity against *G. lamblia* trophozoite. Pet. ether extract from *A. graveolens* was strongly active against *G. lamblia* (IC_{50} = 30.807 mg ml^{-1}) and no activity of *P. granatum*. Ethyl acetate extract from *A. graveolens* and *P. granatum* showed no activity against *G. lamblia*. Methanol extract from *A. graveolens* was good activity against *G. lamblia* (IC_{50} = 11.248 mg ml^{-1}) and strongly active of *P. granatum* (IC_{50} = 0.719 mg ml^{-1}). The aqueous extract from *A. graveolens* showed no activity against *G. lamblia* and strongly active of *P. granatum* (IC_{50} = 0.12 mg ml^{-1}). Such results indicate *A. graveolens* methanol extract and the aqueous extract from *P. granatum* as possible candidates for further investigations to isolate and characterize their active principles as possible new natural anti-protozoal agents.

Keywords: *Anethum graveolens*; *Punica granatum; Giardia lamblia.*

Study of copper oxide wire particles effect on *Haemonchus contortus* infection

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BACKGROUND: The main control method against gastrointestinal nematodes (GN) has been the treatment with conventional drugs; unfortunately, their indiscriminate use has led to the development of resistance. Current control strategies are focused to avoid or delay the emergency of anthelmintic resistance. The use of copper oxide wire particles (COWP) has demonstrated to be effective against the abomasal nematode *Haemonchus contortus*. Nowadays, the mechanism of COWP has still unknown, therefore, the aim of this project is to study how the presence of COWP on sheep abomasal tissues will affect *H. contortus.*
METHODS: To determine the direct effect of copper oxide (CuO) on *H. contortus* L3, were exposed to the compound at different concentrations, to assess their viability. On the other hand, to prove whether COWP are involved in the *H. contortus* L3 establishment, an *in vitro* test with L3 and abomasal tissue directly exposed to CuO, was made. Finally, 36 naturally infected sheep with GN, among which *H. contortus* was identified, were treated with COWP.
RESULTS: CuO applied on L3 did not affect larval viability significantly ($p>0.05$). Moreover, a less association of L3 to tissue with CuO treatment was observed compared with its control. In relation to *in vivo* assay, the decrease of fecal egg counts ($p<0.05$) started through 72 hours after being applied the COWP (3081±1108; 3856±2211), continued until the fourth week post-treatment (PT) (683±176.38) and the higher effect was observed during the second week PT (91% effectiveness; $p<0.05$).
CONCLUSIONS: These results suggest an effect of CuO on abomasal tissues more than a direct toxic effect on L3. However, other studies to determine this are required.
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Epidemiological approach for alveolar echinococcosis, Mongolia

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BACKGROUND: After the former Soviet Union collapsed in 1991, there are a few published data on echinococcoses in Mongolia (Davaatseren et al. 1995; Galtsog et al. 2002; Ebright et al. 2003; Gurbadam et al. 2010; Ito et al. 2010, 2013, 2014; Jabbar et al. 2011). Evidence-based epidemiology is essential towards control of echinococcoses. Bretagne et al. (1996) reported three genotypes of *Echinococcus multilocularis*. Then, Nakao et al. (2009) added Mongolia genotype in addition to North America, Asia, and Europe genotypes. Mongolian genotype is new. It was reported as a new species, *Echinococcus russicensis* from Inner Mongolia, China, and the definitive host was *Vulpes corsac* (Tang et al. 2007). However, it was confirmed to be an intra-species variation of *E. multilocularis* and this variant has been found wider regions including Mongolia and Russia and called Mongolian genotype (Ito et al. 2010).

PRESENT SITUATION: So far we know, there are only 5 AE cases confirmed in Mongolia. We analyzed mitochondrial DNA of recent three AE cases and confirmed two Mongolian genotypes and one Asian genotype. Serum sample from one each showed crucially different antibody responses to both Em18 and Antigen B (Ito et al. 2010). Similar approaches have been done on human CE cases (Jabbar et al. 2011; Ito et al. 2014). There is no animal data until our recent work (Ito et al. 2013). Recent studies of the small intestines of wild canids, *Canis lupus*, *Vulpes vulpes*, *Vulpes corsac*, have shown that both *C. lupus*, *V. vulpes* are at least the definitive host of *E. multilocularis* in Mongolia. All *E. multilocularis* confirmed from these canids were Mongolian genotype. A single vole, *Microtus limnophilus* was found to be infected with *E. multilocularis* (Gardner et al. 2013).

Advances in serodiagnosis of echinococcoses

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Alveolar and cystic echinococcoses (AE and CE) are known to be misdiagnosed as hepatic cancer and hepatic cyst, respectively. Recent studies for serodiagnosis have revealed that recombinant antigens such as rEm18 and rAgB8/1 are better in sensitivity and specificity than crude antigens. The usefulness of rEm18 by ELISA, Immunoblot and Immunochromatography (IC) for pre-operative diagnosis and monitoring of progression after surgery of AE cases are overviewed. Approximately 94% of active AE cases have easily been detected by rEm18 serology under blind test. There was no difference in specificity and sensitivity between ELISA and Immunoblot or IC. Hepatic AE cases with bigger than 3 cm diameter cysts have been detected. The rapid decrease in antibody responses after curative surgery started within a few days of surgery. The usefulness of rAgB8/1 by ELISA, immunoblot and IC for pre-operative diagnosis of CE cases either by *Echinococcus granulosus* sensu stricto (G1) or *Echinococcus canadensis* (G6/7) are also overviewed. More than 90% of CE cases of CE1-2 stages caused by *E. granulosus* s.s. become sero-positive. We were recommended to produce commercially available IC kits for AE (ADAMU-AE), CE (ADAMU-CE) and cysticercosis (ADAMU-CC) using the Translational Research Fund (2007-2011) and Special Coordination Fund for Promoting Science and Technology (2010-2012) from the Ministry of Education, Japan. IC kits for the three diseases have been commercially available from March 2013 (ICST Co. Ltd., Saitama, Japan). Our serological studies have revealed that rEm18 serology is highly useful for detection of the majority of active AE cases and highly useful for monitoring the progression. As ICT is simple and reliable, we recommend application of IC for screening and identification of AE and monitoring the progression of AE after surgery. This IC test, ADAMU-AE, is expected to be useful for detection of gorillas and monkeys in zoos in endemic countries.

Development of a sensitive sandwich ELISA assay for detecting *Leishmania* ribosomal protein S12 antigen

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BACKGROUND: Visceral leishmaniasis (VL) caused by *Leishmania donovani* complex parasites is one of the major human parasitic diseases, which are fatal if left untreated. Although assays based on detection of parasite-specific antibodies (such as rK39 test) have proven to be extremely efficient for VL diagnosis, these tests cannot distinguish active from past infections and are less sensitive in patients co-infected with HIV. PCR, the most sensitive method to detect the presence of parasites in clinical samples, is currently restricted to referral hospitals and research centers. Definitive diagnosis of VL still mainly relies on the visualization of the parasite in spleen, liver or bone marrow aspirates, an invasive and dangerous process with varied sensitivity (53-99%). Therefore, development of an assay that can sensitively detect *Leishmania* antigen in aspirates and blood (if possible) would be helpful for quick and definitive VL diagnosis.
METHODS: Based on the assumption that abundant *Leishmania* proteins might be the easier antigen targets for detection, rabbit polyclonal antibodies were raised against eight recombinant *Leishmania* proteins that are highly abundant in *Leishmania* lysate, as shown from proteomics' studies. The specificity of these antisera was verified by Western blot analysis. The antibodies were purified and labeled with Biotin for developing prototype Sandwich/Capture ELISA assays. The sensitivity and specificity of these ELISA assays were determined and compared with purified *Leishmania* proteins, *Leishmania* promastigote lysates, and human PBMC lysates.
RESULTS: All eight rabbit antisera specifically recognized the corresponding *Leishmania* proteins (antigens) with low cross reaction to human proteins in Western blot analysis. Among the eight Sandwich ELISA assays set up, the assay for *Leishmania* ribosomal protein S12 antigen detected target antigen with the highest sensitivity and specificity and was able to detect 1 pg of purified ribosomal protein S12, lysate from 50-150 *Leishmania donovani* parasites, and the lysate of 250-500 parasites mixed with 200,000 PBMCs, which equals 250-500 *Leishmania* amastigotes in 300 µl blood.
CONCLUSIONS: Our results suggest that this ribosomal protein S12 Sandwich ELISA could become a useful tool for confirming VL diagnosis and for monitoring treatment progress. Validation of this novel capture ELISA assay is underway with clinical PBMC samples of VL patients in India.

The present situation of human taeniases and cysticercosis in Asia

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BACKGROUND: Neurocysticercosis due to accidental uptake of eggs of *Taenia solium* is common in developing countries where people eat pork. It is transmitted from humans (taeniasis carriers) to both humans and pigs (cysticercosis), and emerging and reemerging worldwide. Therefore, this disease is based on consumption of pork full of cysticerci under poverty and expected to be a local disease in rural and remote areas of developing countries where meat-inspection was not introduced, and was rare or not distributed in Muslim or Jewish societies in the 20 century.

PRESENT SITUATION: However, globalization with huge number of immigrants or refugees as labor and tourists has high risk to introduce cysticercosis everywhere even in Muslim or Jewish societies or developed countries including Japan. Nonetheless, we have almost no data on the real situation in any countries due to the lack of reliable tools to detect cysticercosis or identify the parasite. In Asia-Pacific, we have many other parasitic diseases including schistosomiasis, food- or fish-borne trematodiases, soil-transmitted helminthiases and fish- or meat-borne cestodiases. In any areas where we are facing these parasitic diseases, we are simultaneously facing unexpected outbreaks of cysticercosis. In this presentation, the present situation of taeniases caused by three species, *Taenia saginata*, *Taenia asiatica*, and *T. solium*, and cysticercosis of *T. solium* in Asia are overviewed. The background information of the reason why it is neglected is stressed. Molecular identification of the species and serology for detection of cysticercosis in humans and pigs are discussed. Detection of circulating antigen(s) of *T. saginata* cysticerci might be useful for detection of human cysticercosis by cross responses among the three human *Taenia* species, but perhaps no use in coprophagy and scavenger pigs. Therefore, it is urgent to establish highly reliable serology for detection of pigs infected with *T. solium*.

Molecular comparison of *Dactylogyrus lamellatus* Achmerow, 1952 found in Assam with the species reported from China, UK and Iran

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The present communication deals with the molecular comparison of *Dactylogyrus lamellatus* Achmerow, 1952 (collected from Assam, India) and same species reported from different continents using 28S rDNA. Since, they are morphologically more or less similar, comparative study for conservedness in sequences has been made for Indian and others using ExpaRNA (online software). Besides this, the study is also supported by motif prediction using MEME online software that can be considered as a promising tool for monogenean species identification. Phylogenetic relationships have also been inferred using Neighbor-Joining (NJ) and Maximum Parsimony (MP) methods.
Since they are of same species, they should have conserved regions (motifs) in the sequence, number of motifs, their frequency and position must be similar. During the study, an attempt has been made to evaluate validation of species, *D. lamellatus* using molecular biological sequences. Therefore, secondary structure for each sequence has been generated separately using ExpaRNA software to compare the conservedness. The conserved sequences are found in all species but at distinct positions. Probable reasons for this genetic variation in the sequences of *D. lamellatus* from India and abroad are discussed in detail and it is also believed that these changes might be responsible for the gene shifting

In vivo anticoccidial activity of Berberine [18, 5,6-dihydro-9,10-dimethoxybenzo(*g*)-1,3-benzodioxolo(5,6-*a*) quinolizinium]-an isoquinoline alkaloid present in the root bark of *Berberis lycium*

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BACKGROUND: Coccidiosis, caused by various Eimeria species, is a major parasitic disease in chicken. However increasing resistance of these parasites to currently used anticoccidial drugs has stimulated the search for new methods of control. As part of this effort we investigated the root bark of *Berberis lycium* (barberry) as a potential source of compounds with anticoccidial activity.
METHODS: In the present study anticoccidial activity of different solvent extracts of the root bark of *B. lycium* and berberine was evaluated *in vivo* using broiler chicken. The results were evaluated on the basis of reduction in coccidian oocyst output, body weight gain of chicken and feed conversion ratio. For oocyst counting Mc-Masters technique was used.
RESULTS: Results of the study demonstrated equipotent efficacy of pure berberine in comparison to that of standard drug amprolium, but the key highlight of our study was the synergistic anticoccidial effects of berberine and amprolium. Among the extracts crude methanolic extract showed highest anticoccidial activity tested at 300 mg/kg body weight which could be due to the presence of alcohol-soluble active ingredients in root bark of *B. lycium*. Toxicological studies revealed that *B. lycium* extracts as well as berberine were not lethal up to dosage of 2,000 mg/kg body weight. LD^{50} was not determined as mortalities were not recorded in any of the five groups of chicken.
CONCLUSIONS: From the present study it can be concluded that root bark of *B. lycium* has the immense potential to contribute to the control of coccidian parasites of chicken. Our results corroborate the use of berberine for treatment of severe diarrhoea, amoebiasis and intestinal infections and could justify its use in folk medicine for treatment of haemorrhagic dysentery.

The role of DNA in deciphering freshwater fish parasite diversity: opening Pandoras´s box

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BACKGROUND: Parasite systematists dedicate a huge effort to survey, identify, and describe parasite diversity. Freshwater fish helminths are probably the best well-know parasitic group worldwide from a taxonomic perspective. Mexico has been actually catalogued as a *biodiversity hotspot* for freshwater fish helminth parasites. We recently posed that in Mexico the inventory was nearing completion from a traditional view based on morphology, predicting that a modern view of the survey work, following an integrative taxonomy that use DNA sequences, ultrastructure, distribution and host-association, might change our estimates of extant diversity and urged for the generation of such data.
METHODS: I used the most up-to-date data we have gathered on the freshwater fish helminth inventory in Mexico to demonstrate the large progress we have made in more than 80 years of taxonomic reasearch. Then, I describe the novel molecular evidence we have obtained in terms of species discovery and delimitation in selected host groups (Goodeidae, Ictaluridae) and parasite groups (Allocreadiidae, Gorgoderidae), and how this data modified our species richness estimates.
RESULTS AND CONCLUSIONS: Mexico possess one of the most complete inventories of freshwater fish helminth parasites in the world. However, new sequence data, along with other sources of information, has dramatically changed our expectations on describing the diversity of this parasitic group in a timely manner. Our data show that a re-evaluatuation of our entire research strategy is neccesary, not to trying to complete the inventory, but to conduct a molecular prospecting approach of selected host-parasite systems to define more precise species limits, and to better understand the processess that have shaped the intricate evolutionary and biogeographical history promoting the current diversity and distribution patterns of hosts and parasites. I stress the need for a collaborative effort to study parasite diversity in this context in areas of North, Central and South America.

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Some secrets are revealed:

Parasitic keratitis amoebae as vectors of the scarcely described Pandoraviruses to humans

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Background: Free-living amoebae (FLA) belonging to the genus *Acanthamoeba* occur ubiquitously in many aquatic habitats and humid soils. In addition to their role as facultative pathogens, Acanthamoebae are known as vehicles for and hosts of various intracellular organisms. They serve as Trojan horses for diverse microorganisms (including bacteria, fungi or viruses) and as training grounds for intracellularly replicating microorganisms in terms of the development of human pathogenicity by these endocytobionts during their amoebal passage.

The host: An *Acanthamoeba* strain was recently isolated from the contact lens storage cases of a female patient with keratitis. The Acanthamoebae were classified morphologically as *Acanthamoeba* sp. group II and were identified genetically as T4-genotype (sequence type), which is the most common genotype in keratitis associated Acanthamoebiasis cases.

The endocytobiont: These Acanthamoebae harboured endocytobionts, proliferating intracellularly and subsequently leading to the lysis of the *Acanthamoeba* trophozoites. Sequence analysis using several recently published primers and proteomic profiles proved them as members of the new *Pandoravirus* genus. The spore-like *Pandoravirus* particles, 1,1µm in length, possess a massive electron-dense outer wall. The morphology of the present *Pandoravirus* isolate differs from any other megaviral endocytobionts reported as yet. Other Pandoraviruses were found several years later within the sediments of rivers. Although the identification on the genetic level is partially done, there are a lot of open questions starting with the exact phylogenetic position, the evolutionary significance and the potential extension of the microbial diversity.

Conclusion: These were the first Pandoraviruses described, and because this is the first documented association with humans as well, we have clearly demonstrated how easily such endocytobionts can be transferred to humans. The Pandoraviruses were initially described and published correctly as “extraordinary microorganisms” or endocytobionts without having to make a phylogenetic or taxonomic predefinition at the time of their discovery in 2008.
This case counts as another example of parasites acting as vectors of phylogenetically different microorganisms especially when living sympatric within their biocoenosis of biofilms.

Mapping cutaneous leishmaniasis and its vectors in municipalities of the Coffee-Triangle Region of Colombia using Geographic information system (GIS)

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BACKGROUND: Use of GIS for development of epidemiological and entomological maps in cutaneous leishmaniasis has not been extensively used in Colombia, particularly at the Coffee-Triangle region of Colombia, an area of three departments and 53 municipalities with endemic areas of disease. Then, we developed such maps.
METHODS: Surveillance cases data (2007-2011) were used to estimate annual incidence rates using reference population data, on cutaneous leishmaniasis (cases/100,000 pop) to develop the first maps in the municipalities of the region (departments Caldas, Quindio, Risaralda). In addition sandflies reported presence was also mapped. Eight species (*Lutzomyia hartmanni, Lu. trapidoi, Lu. panamensis, Lu. yuilli, Lu. gomezi, Lu. colombiana, Lu. youngi, Lu. lichyi*) and their combinations were mapped. Also a map linking disease incidence with reported *Lutzomyia spp.* presence was generated. GIS used was Kosmo® 3.1. Fifteen thematic maps were developed according municipalities, years and disease incidence and vectors presence.
RESULTS: Between 2007-2011, 2471 cases were reported (1440 Caldas, 895 Risaralda and 136 Quindio) for a cumulated rate of 101.16 cases/100,000pop. At Caldas, Victoria municipality reached the highest incidence (1947.46 cases/100,000pop, 2010), followed by Norcasia 1762.05 cases/100,000pop). Victoria was the municipality with more vectors (5 different species). Among vectors, *Lu. hartamanni* was present at 5 municipalities (including Victoria and Norcasia), followed by *Lu. trapidoi* (4), *Lu. panamensis* (3) and *Lu. yuilli* (3). There were 7 municipalities with leishmaniasis incidence and entomological studies identifying their vectors, but there are 36 municipalities (of 53) that presented cases and there are not yet such entomological studies. Disease was not reported in the rest 10 municipalities.
CONCLUSIONS: Burden of disease is concentrated in one department (over 55% of the cases of the whole region). Use of GIS-based epidemiological maps allow to identification of zones requiring entomological studies to characterize the sandflies species related to transmission of the parasite.

Evaluation of antimalarial activity of cyclopentanone and cyclohexanone analogues of curcumin in *Plasmodium berghei* infected mice

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BACKGROUND: Malaria is one of the world's most common and serious tropical diseases. Resistance of *Plasmodium* to available antimalarial agents has necessitated the need to develop new antimalarial drugs which would be efficacious against drug resistant strains of malaria parasites.
METHOD: The *in vivo* antimalarial activity of cyclopentanone (CP) and cyclohexanone (CH) analogues of curcumin as potential antimalarial agents was evaluated using *Plasmodium berghei* mouse model. Female Swiss albino mice (n=55) were infected with standard inoculum ($1x10^7$) of chloroquine resistant strain of *Plasmodium berghei* (ANKA) intravenously. Infected animals were randomly distributed into eleven groups of five animals each. Once daily dose of chloroquine (10 mg/kg),twice daily graded doses (50, 100, 200 and 400 mg/kg) of each of the curcumin analogues and artemether/lumefantrine (4 mg/kg artemether) were administered to the infected animals while the control animals (infected but not treated) received polyethylene glycol (PEG, 100%), the vehicle for drug delivery, twice daily for three days. All treatments were orally administered for three days starting from 24 hours post infection. Thin blood smears were prepared from tail snips of the mice daily between days 4 and 7 and subsequently on days 9, 12, 14 and 21, and parasite count was estimated by microscopic examination of Giemsa-stained thin smears.
RESULTS: The result of this studyshowed that there was no significant difference in the antimalarial activity of both analogues of curcumin ($p>0.05$) on day four. However, the cyclopentanone analogue of curcumin at a dose of 200mg/kg demonstrated a recordable suppressive antimalarial activity (75% suppression).
CONCLUSION: Cyclopentanone and cyclohexanone analogues of curcumin appeared to have weak suppressive antimalarial activity. Further research into the pharmacological properties of these analogues is recommended.

Age, gender and body distributions of cutaneous lesions by *Leishmania mexicana* in cocoa plantations in Tabasco, Mexico

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Background: In Mexico most cases of human cutaneous leishmaniasis are caused by *Leismania mexicana*. The leishmaniases comprise a group of diseases that display a wide range of clinical manifestations in humans, depending in part on the parasite species initiating infection on various factors relating to the general health and genetic background of the host. The incidence and severity of these diseases are different between males and females. Throughout most of its history, cutaneous leishmaniasis was considered a professional disease, as most of the affected individuals were adult males exposed to forested areas. However, its epidemiology has changed considerably and has infected a growing number of women and children. The differences in clinical presentation are generally associated with individual variations in immunological response and with different species of parasites.
Were analyzed 28 patients with localized cutaneous leishmaniasis (LCL) from La Chontalpa (Tabasco State), an endemic area in southeastern Mexico. All patients were informed and signed a written consent to participate in the study.
A total of 61% individuals were male and 39% were female. 53% of patients presented only one lesion (< 1cm). The distribution of lesions were more frequently observed in lower limbs in female (46%) and lower in pinna (9%). In male face (41%) and pinna (17%). The most commonly reported occupations were student (39%), followed by homemaker (32%). We found differences between gender and age which had a mean age of 33 years and a disease duration 3 months in female. while in male patients was 25 years and 6 months disease duration.
In conclusion, resistance and susceptibility to infection may be altered with age and gender. Besides, the differences in exposure to various species or strains of the parasite.