Raw sewage as breeding sites to *Aedes (Stegomyia) aegypti* (Diptera, Culicidae)

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BACKGROUND: The selection of sites for oviposition by females of *Aedes aegypti* is a key factor for the survival of their immature and has direct influence in vector control programs. In this study we evaluated the oviposition rate of *Ae. aegypti* and aspects of reproductive physiology in sewage .
METHODS: The *Ae. aegypti* females were used in oviposition bioassays according to two methodologies: (i) choice: in the same cage three oviposition substrates were offered – treatment (sewer), positive control (distilled water) and negative control (1% sodium hypochlorite) and (ii) no choice: only one substrate was available. The solutions were changed and clockwise rotated every 24 hours. After 72 hours the eggs were counted and the females dissected to check egg retention. Physicochemical and microbiological analysis of sewage was conducted.
RESULTS: No oviposition was detected in the negative control. For the oviposition rate no statistical difference ($p>0.05$) was observed between sewage and positive control, for both methodologies. Regarding the eggs retention in the ovaries, positive and negative control where different ($p<0.05$), and comparing the sewer with the negative control we observed a $p=0.06$, an indicative of possible statistical difference. The oviposition attractiveness index deemed the sewer as not attractive, however the positive values found OAI=+0.1246 (choice) and OAI=+0.1048 (no choice) are indicative that this solution can be attractive for the specie. Physicochemical and microbiological analysis showed a low level of dissolved oxygen and chlorine and, high levels of nitrogenous compounds, *E. coli* and total fecal coliforms, which are conducive conditions to oviposition and development of the specie.
CONCLUSIONS: We showed that *Ae. aegypti* can adapt to new sites and lay eggs in polluted water, which implies the importance of re-think control and surveillance programs given the conditions of poor infrastructure and lack of basic sanitation found in many regions of the world.

Characterization of an area endemic for Chagas disease in the state of Veracruz, Mexico.

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BACKGROUND: Chagas disease is caused by *Trypanosoma cruzi*, and is widespread throughout Latin America with endemic areas in 21 countries where there are seropositive cases in humans and vectors carrying the parasite. The main way to control the disease is vector control. Studies to control the disease require the molecular characterization of *T. cruzi* and its vector, as well as epidemiological and serological studies for each study area. In this study we characterize an area endemic for Chagas disease in the state of Veracruz, Mexico.
METHODS: Blood samples of inhabitants of the endemic area were screened by 3 different kits and a homemade ELISA. Homes in the endemic area were georeferenced. Vectors captured were analyzed molecularly for natural infection, *T. cruzi* lineage, sources of alimentation and typing of subspecies.
RESULTS: 196 samples were obtained, 6.09% were positive for two of four ELISA tests, the correlation between the different kits and a homemade ELISA was low, with differences in antibody recognition. 47 vectors were captured of the species *T. dimidiata*, 57% were positive for T. cruzi infection, 29.5% correspond to lineage TCI, 23.5% to no-TcI and 47% were positive for TCI-TcVI. Heteroduplex PCR assays showed that the sources of alimentation in vectors are humans and rodents. Vectors of the captured species *T. dimidiata* belong to group 2 (32.1%) and 67.9% to groups 1 and 2.
CONCLUSIONS: The results demonstrate the presence of seropositive cases and vectors infected with *T. cruzi* TCI, no-Tc1 and both. Two feeding sources were identified in the area, as well as hybrid vectors of the species *T. dimidiata*. This study shows the status of an endemic area, providing data plans for vector control.

Evaluation of mice experimentally infected with a strain of *Trypanosoma cruzi* (SC2005) by intragastric and intraperitoneal route.

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BACKGROUND: Chagas disease is a worldwide public health problem. Although the vectorial transmission of Chagas disease has been controlled in Brazil there are other ways of transmission, such as the ingestion of *T. cruzi* contaminated food, which ensures the continuation of this zoonosis. Recent outbreaks of Chagas disease from the consumption of foods and beverages contaminated with *T. cruzi* have emphasized the importance of this transmission route in humans. This study aims to shed some light on the mechanisms involved in oral infection by *T. cruzi*.
METHODS: Groups of Swiss mice were infected intragastrically (IG) or intraperitoneally (IP) with the *T. cruzi* SC2005 strain derived from an outbreak of oral Chagas disease. The present study is an attempt to compare the results obtained from mice infected by IG or IP route, using parasitemia, mortality, histological analyses and molecular approaches.
RESULTS: The results show that the parasitemia peaks were later and less intense in the IG infected mice. Mortality of the IP infected animals was more intense and earlier when compared to the IG infected mice. Histopathological analyses revealed a myotropic pattern of the SC2005 strain with the presence of inflammatory infiltrates and parasites in different organs of the animals infected by both routes as well as fibrosis foci and collagen redistribution. *T. cruzi* DNA associated with the presence of inflammatory infiltrates was detected by PCR in the esophagus, stomach and intestine of all infected mice.
CONCLUSION: Our results show an influence of the inoculation route on the establishment and development of the SC2005 *T. cruzi* strain infection in mice.

The PNOC gene as a tool for detection of blood meal source of sandflies (Diptera: Psychodidae: Phlebotominae).

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BACKGROUND: The feeding behavior of sandflies provides valuable information about the vector/host interactions and elucidates the epidemiological patterns of American cutaneous leishmaniasis (ACL) transmission. The aim of this study was to identify the blood meal sources of *Lutzomyia (Nyssomyia) intermedia s.l.* in endemic area of leishmaniasis in Paraná State by sequencing prepronociceptin (PNOC) gene.
METHODS: A total of 1,263 female sandflies was catched in the rural locality of Epitácio Pessoa, Adrianópolis municipality, Paraná State, southern of Brazil. 96 specimens were engorged but only 27 showed amplification by polymerase chain reaction (PCR) to prepronociceptin (PNOC) gene. The PCR products were purified using the enzyme ExoSAP-IT (USB®) and sequencing reaction products were sequenced using an Applied Biosystems® 3500 automated sequencer. The sequences of both strands were edited using the BioEdit and MEGA 5.0 (Molecular Evolutionary Genetics Analysis) programs.
RESULTS: Of 1,263 female sandflies collected in the field, 93 (3.6%) specimens were engorged and 27 allowed efficient amplification of the PNOC gene. These flies had fed on equine (*Equus caballus*), porcine (*Sus scrofa*) and canine (*Canis lupus familiaris*) species.
CONCLUSIONS: The efficiency to identification of blood feeding in the sandflies by molecular method is directly associated to the level of digestion of blood instead the amount of blood ingested. Investigations on the feeding behavior of the sandflies have great ecological and epidemiological significance because it enables the correct identification of the mammalian reservoirs and the vector feeding preferences.

Histone H3 N-terminal tail proteolytic clipping: a new type of epigenetic mechanism linked to the control of DNA replication in *Plasmodium falciparum.*

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BACKGROUND: A prominent role of histone modifications and nuclear architecture in the coordinated control of gene expression in the human Malaria parasite *Plasmodium falciparum* has been shown recently. However, the existence of other epigenetic mechanism that governs the gene expression in this parasite such as proteolytic processing of histone H3, remain unknown.
METHODS: Histones from *P. falciparum* were obtained and analyzed by LC-MS/MS. WB assays were carried out using commercial antibodies against post-traductional modifications. Immunofluorescence assays were used to analyze the nuclear architecture. Mononucleosome preparation and co-immunoprecipitation assays were performed to determine that histone H3 processed forms part of the nucleosomes of *P. falciparum*. Edman reaction was performed to identifying in which amino acid the N-terminal cleavage of the parasite's histone H3 occurs. ChIP assays were performed to identify the association of histone H3 processed form in upstream regions of some genes that are expressed mainly in schizont stage.
RESULTS: In this work, we demonstrate for first time the proteolytic cleavage of histone H3 of *P. falciparum* at its N-terminus carried out by a cathepsin C protease during the schizont stage. Furthermore, ChIP assays suggested that processed histone H3 was associated with genes involved in DNA replication in this parasite. Thus, we propose the proteolysis of the N-terminus of histone H3 as novel epigenetic mechanism in *P. falciparum* that can be employed to control gene expression involved in replication by altering chromatin structure.
CONCLUSIONS: Our data suggest that the proteolysis of H3 histone is a new mechanism controlling replication and trophozoite/schizont-stage differentiation/transition in *Plasmodium falciparum*.

Quinoxaline derivatives as leishmanicidal compounds

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BACKGROUND: Leishmaniasis has been classified by the WHO as a neglected tropical disease. In Mexico, cutaneous leishmaniasis caused by *L. (L.) mexicana* is the most common. Currently, drugs available for leishmaniasis treatment are toxic, expensive and frequently ineffective, so it is necessary to find new therapeutic alternatives. Recent research shows that quinoxalines have good antiprotozoal activity. The aim of this study was to evaluate the *in vitro* biological activity of 39 quinoxaline 1,4di-*N*-oxide derivatives (Q1,4dNO) on *L. (L.) mexicana* promastigotes by flow cytometry.
METHODS: The *in vitro* leshmanicidal effect of Q1,4dNO derivatives was assayed against *L. (L.) mexicana* promastigotes in stationary phase; analysis was performed by flow cytometry evaluating cell death markers including changes in both forward and 90° side scatter, decrease in mitochondrial membrane potential and loss of membrane integrity; data acquisition and analysis was realized in a BD FACSCalibur flow cytometer using the CellQuestPro software. Additionally, cytotoxic activity (CC50) of active compounds was evaluated on J774 cell line. Amphotericin B was employed as reference drug.
RESULTS: Nine Q1,4dNO derivatives showed important leishmanicidal effect at 5µg/mL; active compounds were then tested at different concentrations and the 50% inhibitory concentration (IC50) was determined. The most effective compound was T-073 with an IC50=7.15µg/mL and a CC50=26.886µg/mL.
CONCLUSION: Results provide interesting data about structural features on Q1,4dNO derivatives that favors leishmanicidal activity.

Evaluation of the effectiveness of a Hazard Communication Program as shown on intestinal parasitosis, applied community Dam Organito Villa de Reyes, San Luis Potosi, Mexico.

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BACKGROUND: Communication Risk (CR) process is a plural and collective awareness, through which it is to persuade, inform and influence the population, all those factors and threats that endanger health; among them, are intestinal parasites, which are now a major health problem worldwide. METHODS: The study included 47 people from the community, Dam Organito Villa de Reyes, SLP, Mexico. The Hazard Communication Program (PCR) was applied; Analysis of Risk Perception, determination of intestinal parasitosis by a sedimentation method coproparasitoscopic with Brij-35 (at baseline, after administration of albendazole and after PCR). Chi square test (x2), Calculate Relative Risk (RR) with 95% CI was used; using JMP ® 9.0.2 Programme (2010). RESULTS: The parasitized population at baseline was 100%; after administration of albendazole, the percentage decreased to 34.04%; statistically significant ($p < 0.0001$). The value of the relative risk (RR) shows that the population underwent a deworming was less likely, ie 0.340426 times the risk of contracting a parasitic disease at baseline. After worming campaign to Hazard Communication Program, the parasitized population fell from a 34.04% to a 25.53%, although this decrease was not statistically significant ($p > 0.05$), the value of the relative risk indicates the probability of acquire a parasitic disease decreased by participating in the PCR only when the drug was taken. CONCLUSIONS: With the intervention program (PCR) was achieved that people knew the risks they are exposed to in their environment and expand their perception to thereby positively change their habits in improving their quality of life.

Evaluating three types of immunoassays for the seroprevalence of Chagas' disease in the endemic and non-endemic zones of Peru

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BACKGROUND: A likely factor in the transmission of *T. cruzi* and the presence of Chagas' disease in Peru is believed to be from the presence of a vector in endemic areas and migration into non-endemic areas. The objective of this study was to evaluate the seroprevalence of Chagas' disease in the endemic and other non-endemic zones of Peru. METHODS: Three seroimmunological methods of disease detection, immunofluorescence, ELISA (Chagatek), and Western blot (using secretory-excretory trypomastigote antigens) were evaluated for concordance. To achieve this objective, 430 serum samples from an endemic zone population (Quequeña -Arequipa, Perú) were evaluated.
RESULTS: Of this sampling, 12 (2.8%) were positive for at least 2 tests, 35 (8.14%) were positive for one test, and 383 (89%) were negative in all three tests, giving a kappa index (I.C. 95%) of 0.51 for ELISA and Western blot, 0.68 for ELISA and immunofluorescence, and 0.44 for immunofluorescence and Western blot, which was interpreted as a concordance of *moderate, good, and moderate*, respectively. Of the 455 serum samples provided from the non-endemic zone (district San Juan de Miraflores –Lima, Peru), 2 (0.44%) tested positive with two tests, 11 (2.4%) tested positive with only one test, and 442 (97%) were negative in all tests. The kappa index (I.C.) determined for immunofluorescence and Western blot (0.28) was interpreted as *weak*. Additionally, 231 serum samples from Bolivia (an endemic population) were tested in the same manner. The kappa index (I.C. 95%) of 0.97, equal in all comparisons, was interpreted as a concordance of *very good*.
CONCLUSION: it is important to consider that Chagas' disease is not limited to endemic zones and that not all types of diagnostic testing can be reliably utilized in all populations. Therefore, one must take into account both epidemiological information and the genetics of the host, including the parasite strain circulating among the population.
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Identification of anti-IgG antibodies against *Trypanosoma cruzi* in a population of low human development in Yucatan, Mexico.

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BACKGROUND: Chagas disease (ChD) or American tripanosomiasis, is a zoonosis ranked as one the seventeen neglected tropical diseases in the world. It is caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*) whose principal route of transmission to human is by feces of infected triatomine bugs. World Health Organization (WHO) an estimates that 10 million people are infected with *T. cruzi* worldwide, mainly in Latin America and more than 25 million people are at risk of infection. In Mexico, the disease is endemic and in Yucatan have been reported prevalence of 0.5 to 10%, both in rural and urban areas. The purpose of this study was to determine the prevalence of IgG antibodies against *T. cruzi* in an adult population of the municipality Mayapán, Yucatán, with low human development.
METHODS: Random sampling was performed to 360 people attended routine visit to the clinic of the local health system (IMSS). Previous informed consent, a blood sample was taken to obtain serum. Samples were evaluated with indirect ELISA (Chagas microELISA Test, Accutrack) in duplicate and according to the diagnostic algorithm ChD recommended by the WHO. Samples identified as positive or indeterminate, were confirmed by Western Blot
RESULTS: Forty-six samples were found positive; this represents a 12.77 % prevalence of IgG antibodies against *T. cruzi.*
CONCLUSIONS: Our results suggest that this population has been in contact with the causal agent of ChD, so more studies focused on the finding and implementing measures for control and eradication of the vector in this area is necessary in order prevent transmission of *T. cruzi*.

Cytokine production during *Leishmania amazonensis* infection in mice with different genetic backgrounds

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BACKGROUND: The inflammation caused by *Leishmania* infection is modulated by various cytokines, which may determine the resolution of the lesions or their progression. Cytokines are the key elements of the host immune response against this pathogen and for decades many authors have described the cytokine profile in murine leishmaniasis, but the results concerning the mouse strain and the specie of *Leishmania* used are controversial. In *L. amazonensis* infection a low and mixed Th1/Th2 response is observed both in mice model and in humans. Even though *L. amazonensis* promastigotes can, transiently, active dendrict cells and macrophages, the cell activation intensity and cytokine production are sometimes lower than the activation observed by *L. major and L. braziliensis* promastigotes.
METHODS: Groups of C3H, C57BL/10, CBA and DBA2 mice were subcutaneously infected by injecting 10^6 L. amazonensis promastigotes in the right hind footpad. The present study is an attempt to evaluate the cytokine production in terms of disease progression, and the patterns of resistance and susceptibility, using detection of cytokines in serum and cell culture supernatants.
RESULTS: The analysis of TGF-β, TNF-α, IFN-γ, IL-4, IL-10 and IL-12 production showed that, independent of the mouse strains studied, there was no predominance in the pattern of expressed cytokines having a mixed pattern of Th1 and Th2 cytokines.
CONCLUSION: These data allowed us to infer that the host genetic background does not influence the cytokine pattern expressed in *L. amazonensis* infection. These results may contribute to a clearer understanding of the immune response in mice infected with *L. amazonensis* since the immunopathological aspects of this infection, in the murine model, are different from those observed in *L. major* infection, where the susceptible and resistant mice show a polarized immune response.

Clinical and ophthalmological alterations in dogs (*Canis familiaris*) (Linnaeus, 1758) naturally infected with *Leishmania infantum*.

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BACKGROUND: The clinical features of canine visceral leishmaniasis (CVL), as well as human, are variable and nonspecific. Despite the LVC is a serious and fatal systemic disease, there are few studies describing the ophthalmological and clinical alterations during the infection. Therefore, the present work aims to study the ophthalmological and clinical alterations in dogs with visceral leishmaniasis arising from of CCZ São Luís, Maranhão.
METHODS: The trial of animals was realized by the rapid test for canine visceral leishmaniasis DPP® (BIOMANGUINHOS, RJ). Reagents dogs were clinically evaluated and classified as symptomatic and asymptomatic. Shimer test and fluorescein test were used to evaluate the occurrence of ceratoconjuntive and corneal ulcers respectively. Furthermore, hemogram, biochemical and ELISA tests . Puncture biopsy of the bone marrow and spleen are held to the isolation and characterization of Leishmania species involved in the infection.
RESULTS: Seven animals were reactive to *Leishmania* antigens in serological tests and were classified as symptomatic. Two animals were positive in Schimer test and one in fluorescein test. Parasites were isolated from bone marrow and spleen of five animals and characterized by isoenzimes technics such as *Leishmania infantum*. Hematological evaluation showed hematocrit lower, featuring the anemia; occurrence of thrombocytopenia with or without lymphopenia. Changes in leukocyte counts were rare. No changes were found by biochemical tests.
CONCLUSION: Ophthalmologic manifestations are present in animals with LV and may occur concomitantly with other systemic signs of the disease. Routine hematological tests serve as a diagnostic tool of this disease.

Activity and stability of the amoebic chitinase (*Eh*CHIT1) as function of pH, temperature and guanidine hydrochloride

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BACKGROUND: Chitinases are hydrolases that break glycosidic bonds β-(1-4) of chitin. These enzymes are found in several species, including the human parasite *Entamoeba histolytica* (causative agent of amoebiasis). The life cycle of this parasite comprises two main stages: cyst and trophozoite. Interestingly, the cyst is protected by a wall of chitin that helps the parasite to withstand harsh environmental conditions. The amoebic chitinase (*Eh*CHIT1) has been associated with the structural arrangement of the chitin wall during both encystation and excystation processes, so this enzyme represents a putative target for drug development.
METHODS: Recombinant enzyme, soluble and active, was expressed in bacteria *E. coli* and purified from bacterial lysates. The endoquitinolytic activity was determined using a standard fluorometric assay. Effect of pH, temperature (T) or denaturant (GuHCl) on the activity was obtained by repeating the assay at singular conditions. Effect of pH or denaturant on protein stability was determined by analyzing the residual activity after 18 hours of treatment at specific conditions. Thermal inactivation was determined by evaluating the residual activity after 60 min of incubation at different temperatures.
RESULTS: *Eh*CHIT1 showed maximum activity at pH = 4.5–5.0 and T = 50 ºC. Moreover, *Eh*CHIT1 is stable at a wide range of pH and temperature values, since it preserves more than 80% of maximum activity after treatment at pH = 4.0-9.5 and T = 37-45 ºC. Interestingly, *Eh*CHIT1 is sensitive to low concentrations of GuHCl, losing more than 50% of maximum activity after treatment at concentrations >0.5 M.
CONCLUSIONS: *Eh*CHIT1 is highly stable to a wide range of pH and temperature values. However, both enzyme activity and protein stability were affected by increasing concentrations of GuHCl, suggesting a close relationship between its structure and function.

Leishmanicidal activity of mixtures of lupenone and caryophyllene oxide

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BACKGROUND: Leishmaniasis is a disease caused by parasitic protozoa of the genus *Leishmania*, which affects over 12 million people and it is endemic to 88 countries on four continents. The disease is transmitted to humans by sandflies causing a wide spectrum of symptoms. To date the chemotherapeutic agents most commonly used for treating leishmaniasis, are toxic, costly, require long periods of treatment, and cause serious side effects. Recently, resistance of *Leishmania* parasites has been reported to some of these drugs, and this fact emphasizes the importance of searching for new effective pharmaceuticals to treat this disease. Recently, was reported the presence of trypanocidal activity in the leaf extract of *Serjania yucatanensis* and also was demonstrated the synergism of a 1:4 mixture of lupenone and caryophyllene oxide against *Trypanosoma cruzi in vitro* and *in vivo*. In the present work, we have evaluated the leishmanicidal activity of mixtures of lupenone and caryophyllene oxide against different *Leishmania* spp.
METHODS: In this work, the leishmanicidal activity of mixture of lupenone and caryophyllene oxide in different proportions (1:0, 1:4, 2:3, 1:1, 3:2, 4:1 and 0:1; w/w) was performed against promastigotes of *L. amazonensis*, *L. braziliensis*, *L. mexicana*, *L. tropica* and L*. aethiopica*. The inhibition of THP-1 cells infected with fluorescent parasites and treated with the mixture of terpenoids and amphotericin B as positive control was analyzed via flow cytometry.
RESULTS: The results obtained confirm the synergistic effect of the mixture of terpenoids in a 1:4 proportion against *L. amazonensis* and *L. braziliensis* and that the pure caryophyllene oxide is the most active against *L. tropica*, *L. mexicana* and *L aethiopica*.

Sociality in trematode parthenitae – how common is it?

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BACKGROUND: Animal societies where some individuals relinquish the ability to reproduce and exhibit extreme behavioral specialization, polymorphism and caste formation are not uncommon. Well known examples include the social insects and naked mole rats. However, recent findings extend such complex sociality to a new class - Trematoda. A reproductive division of labor involving a caste of non-reproducing soldiers has now been documented for intramolluscan stages of five trematode species from different geographic regions and host species, suggesting that this phenomenon is widespread. Here we present the extent of our evidence for trematode social organization.
METHOD: First, we systematically examine the nature of sociality in trematodes that infect the California horn snail, *Cerithidea californica.* Morphological descriptions (individual size and productivity) and behavioral assessments (attack rates) are obtained for an additional 15 species infecting *C. californica*. To further explore the evolution of sociality in the Trematoda, we search the literature for evidence of caste formation in various trematode families.
RESULTS: Seven of the trematode species infecting *C. californica* have a division of labor involving a soldier caste, while the rest provide information on colony structure when soldiers are lacking. Literature descriptions of the life history of several species suggest that this phenomenon is widespread.
CONCLUSION: Because there are roughly 18,000 species of digenean trematodes and many may have such a social organization, trematodes may exhibit the most substantial radiation of sociality other than among the social insects.

Binding and endocytosis of Bovine Hololactoferrin by the parasite *Entamoeba histolytica*

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BACKGROUND: *Entamoeba histolytica* is an enteric protozoan that exclusively infects humans. This parasite requires iron (Fe) for its metabolic functions and virulence. Bovine lactoferrin (BLf) and its peptides can be found in the digestive tract after ingesting milk and dairy products. The aim of this study was to compare virulent trophozoites recently isolated from hamster liver abscesses with non-virulent trophozoites that have been maintained for more than 30 years in cultures *in vitro*, in their interaction with iron-charged BLf (BholoLf).
METHODS: We performed growth kinetics of trophozoites for 96 h in different concentration of BholoLf, and growth throughout several consecutive transfers. Binding maximal (B_{max}), and velocity maximal of endocytosis (V_{max}) of FITC-BholoLf were determined, as well as the K_d and number of BholoLf binding proteins per amoeba; these determinations were done by flow cytometry and confocal laser microscopy. Also, inhibitors of endocytosis were used to determine the route of BholoLf internalization.
RESULTS: A concentration of 100 µM Fe from BholoLf supported growth of both variants of amoeba but virulent parasites showed higher growth and tolerance to ferric-iron than non-virulent parasites. An average of 945,000 and 6.65 x 10^6 binding sites/cell were found for this glycoprotein in non-virulent and virulent amoebae, respectively. Virulent amoebae bound more efficiently human and bovine holoLf, human holo-transferrin, and human and bovine hemoglobin than non-virulent amoebae. In addition, virulent amoebae showed two types of proteins for BholoLf internalization and although both amoebae endocytosed BholoLf through clathrin-coated vesicles, the virulent amoebae also endocytosed this glycoprotein through a lipid-rafts-dependent mechanism.
CONCLUSIONS: These data allow us to understand that endocytosis of iron-containing proteins from the human host as well as from bovine milk products could be important for the parasite during colonization and invasion of the intestinal mucosa and liver.
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Clinical and ophthalmological alterations in dogs (*Canis familiaris*) (Linnaeus, 1758) naturally infected with *Leishmania infantum*.

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BACKGROUND: The clinical features of canine visceral leishmaniasis (CVL), as well as human, are variable and nonspecific. Despite the LVC is a serious and fatal systemic disease, there are few studies describing the ophthalmological and clinical alterations during the infection. Therefore, the present work aims to study the ophthalmological and clinical alterations in dogs with visceral leishmaniasis arising from of CCZ São Luís, Maranhão.
METHODS: The trial of animals was realized by the rapid test for canine visceral leishmaniasis DPP® (BIOMANGUINHOS, RJ). Reagents dogs were clinically evaluated and classified as symptomatic and asymptomatic. Shimer test and fluorescein test were used to evaluate the occurrence of ceratoconjuntive and corneal ulcers respectively. Furthermore, hemogram, biochemical and ELISA tests were realized in whole blood and serum. Puncture of the bone marrow and spleen were realized for isolation and characterization of *Leishmania* species involved in the infection.
RESULTS: All animals were reactive to *Leishmania* antigens in serological tests (DPP and ELISA). Six animals were classified as symptomatic and one as asymptomatic in accordance with clinical signs. Two animals were positive in Schimer test and one in fluorescein test. Parasites were isolated from bone marrow and spleen of five animals and characterized by isoenzimes technics such as *Leishmania infantum*. Hematological evaluation showed low hematocrit levels, featuring the anaemia; occurrence of thrombocytopenia with or without lymphopenia. Changes in leukocyte counts were rare. No changes were found by biochemical tests.
CONCLUSION: Ophthalmologic manifestations are present in animals with LVC and may occur concomitantly with other systemic signs of the disease. Routine hematological tests serve as a diagnostic tool of this disease. The concentration of parasites in bone marrow and spleen favors the isolation of *Leishmania*, allowing the confirmation of diagnosis. The DPP ® test proved effective to detect dogs with LV.

Use of fish parasites and EROD activity in the silver croaker *Plagioscion squamosissimus* to indicate environmental impact in the Tietê River, Brazil

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BACKGROUND: Fish parasites and ethoxyresorufin-*O*-deethylase (EROD) activity have been considered useful tools to indicate environmental impact in aquatic ecosystems. Although parasite burden and EROD activity in fish have been used as bioindicator and biomarker of environmental impact respectively, their values can be affected by seasonality, and host related factors. We therefore examined whether the parasite burden and EROD activity of the silver croacker *Plagioscion squamosissimus* were affected in a pollution gradient, considering host related-factors, and seasonality.
METHODS: We collected fish and parasites and determined EROD levels from three localities with different pollution levels (Barra Bonita = polluted; Bariri = slightly polluted; Promissão = unpolluted) on the early-rainy (November 2012), rainy (March 2013), and dry (July to August 2013) seasons from Tietê River, Southeast Brazil.
RESULTS: The monogenean *Diplectanum piscinarius* had the highest abundance in Barra Bonita on the early-rainy (Mean abundance = 79.36 ± 30.14; $F_{2, 83}$ = 5,89; p = 0,004) and rainy (Mean abundance = 59,10 ± 21.53; $F_{2, 72}$ = 5,36; p = 0,007) seasons, with a significant decrease of this species on the dry season in all localities. *Austrodiplostomum* sp., abundance was very variable with high abundance on the early-rainy season at Barra Bonita and Bariri, but only in the dry season at Bariri. No significant statistical effect on EROD activity was found with respect to the abundance of *D. piscinarius* (ANCOVA, $F_{1,202}$ = 0.01; p = 0.91) or *Austrodiplostomum* sp. (ANCOVA, $F_{1, 202}$ = 0.01; p = 0.91). In contrast, we observed an influence of the higher pollution levels on the abundance of *D. piscinarius* which had higher abundance in the polluted place (Barra Bonita), with respect to the slightly polluted and unpolluted places (H = 6,03; df = 2; p = 0,04). We also found that sex had a significant positive effect on the seasonal variability of EROD levels in the most polluted locality (near São Paulo) (ANCOVA, $F_{1, 178}$ = 4.44; p = 0.036).
CONCLUSIONS: Our results suggest that low parasite abundance is not enough to interfere with biomarker levels in the silver croacker. A potential explanation for the high abundance of *D. piscinarius* in the polluted localities is that the pollutants could be acting as immunosuppressors in *P. squamosissimus,* enhancing parasitism. The statistical association between EROD activity and sex could be due to the reproductive status of females that downregulated the EROD levels. Our results revealed that, the reproductive status of females can be a significant confounding factor to determine EROD activity in freshwater ecosystems when compared to males (FAPESP 2012/00561-0; CAPES/PDSE 13836/2013-07).

Seroprevalence of antibodies against *Trypanosoma cruzi* in blood donors in the Center Region of the State of Guerrero

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BACKGROUND: In Chagas disease, the main transmission mechanism is the vector, followed by blood transfusions. In order to determine the prevalence of anti-*Trypanosoma cruzi*, and relate positivity with age, sex, occupation, education, residence, ethnicity. A study was performed on blood donors who attended the Regional Blood Bank of the city of Chilpancingo, Gro.
METHODS: By phlebotomy, to each of the patients, the blood were extracted in a pilot tube which is used to perform serology. In HexaBank software, data and results of serological tests and the clinical history of each donor are introduced. For frequency measurements and analysis of risk, the data were processed in the statistical programs SPSS 15.0 and Stata v. 8, respectively. In the period are presented to donate 2,485, most often 89.8% (2,232) were males and 10.2% (253) females.
RESULTS: In the study, the seroprevalence was 0.6% (16) reactive to *T. cruzi*. The largest number of reactive donors were those from Chilpancingo [31.25% (5)] and those from lower Tixtla, San Marcos, Quechultenango, Mochitlán, Martyr Cuilapa and City Altamirano with a reagent (6.25%). Of the total studied males (0.56%, 14) predominated in terms of reactive antibodies against *T. cruzi* compared with female 0.08% (2). The age group of 21 to 30 years, which was attended donate the most frequent (51.3%, 1,275), the same group presented the most reactive cases [0.32% (8)].
CONCLUSIONS: Some blood donors who attended the Blood Bank were seropositive for *T. cruzi* exposed the risk of contamination by transfusion. These results show that it is extremely important to run the appropriate screening when donations to prevent the spread of the parasite and thus to prevent new infections.

Identification of a SERCA-like protein and analysis of the effect of SERCA specific blockers during *Entamoeba histolytica* in vitro virulence.

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BACKGROUND: In the protozoa parasite *Entamoeba histolytica* calcium has an important role on signaling of different cellular processes, including development and pathogenesis, suggesting that there is a fine regulatory mechanism that maintains calcium homeostasis in this amoeba. Calcium-ATPases (Ca^{2+}-ATPases) are proteins that play an important role in calcium homeostasis by catalyzing the active efflux or influx of this ion from cytoplasm and are essential to the correct functioning of the cell machinery.
METHODS: For the subcellular localization of EhSERCA by electron microscopy and confocal microscopy *E. histolytica* trophozoites were used under culture conditions. For in vitro virulence assays trophozoites were treated with Thapsigargin and Cyclopiazonic acid, specific inhibitors of SERCA
RESULTS: We identified one gen in *E. histolytica* that possibly encodes an organellar ATPase. Specific antibodies against the SERCA-like member located this protein in a continuous cytoplasmic network, this structure is similar to that described as the endoplasmic reticulum of *E. histolytica*. Also trophozoites treated with specific SERCA blockers showed an alteration in the in vitro virulence.
CONCLUSIONS: Our results support that this SERCA-like protein corresponds to the calcium ATPase responsible to sequester calcium in the endoplasmic reticulum of this parasite.

Gastrointestinal parasites infecting water buffalo *(Bubalus bubalis)* in Veracruz, Mexico.

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BACKGROUND: Following its introduction to Mexico at the end of the last century, water buffalo herds have expanded in tropical and subtropical parts of the country. Water buffalo is an alternative to cattle for livestock production in areas prone to flooding in the state of Veracruz. However, little is known about the parasites associated with water buffaloes, some of which could affect cattle or be of veterinary public health importance.
METHODS: A prospective longitudinal study lasting 12 months was conducted with a meat production herd of buffalo in the municipality of Ciudad Isla, which is located in the central part of Veracruz state. The study covered three distinct seasons: wet (August-September), cold (December-February), and dry (April-May).
Out of the 98 buffaloes in the herd, 50 were selected randomly and identified individually regardless of sex, age, and reproductive stage. A fecal sample was obtained from each animal in the study at the beginning and the end of each season. The coprological diagnostic techniques used included: McMaster, sedimentation, flotation, and Baermann.
RESULTS: Gastrointestinal parasites were detected more frequently during the rainy season when 42% of the animals were shown to be infected. The most commonly found endoparasites across seasons were: *Ostertagia ostertagi* (7.0%), *Fasciola hepatica* (6.7%), *Chabertia* spp. (5.0%). Less frequently detected parasites included *Trichostringylus* spp. (1.3%), *Moniezia expansa*, and *Haemonchus contortus* (0.7%).
CONCLUSION: Information on the gastrointestinal parasites infecting water buffaloes in Veracruz is presented here for the first time. Most of the endoparasites infecting buffaloes are known to be pathogenic in cattle. The impact of endoparasitic infections on the productivity of water buffaloes in Veracruz remains to be determined.
* USDA is an equal opportunity provider and employer.

Risk analysis of population and geographical distribution of *Trypanosoma cruzi* vectors Tecalpulco, Taxco, Guerrero

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BACKGROUND: A descriptive study was conducted based on the geographic distribution of households with presence of triatomine vectors of *Trypanosoma cruzi* and entomological indices, in order to assess the risk of infection of Chagas disease in the population.
METHODS: For socio-economic, environmental and knowledge of data vectors and disease, after raising awareness, a questionnaire was applied to the homeowners. By the hour/man method was conducted the search and capture of triatomines. For keys Lent and Wygodzinsky, the identification was performed. The *T. cruzi* parasite was identified by parasitoscopic methods. 15% (59) of the houses was revised and distribution maps were developed with the georeferencing of positive houses triatomine.
RESULTS: *Meccus pallidipennis* was the only triatomine species identified. In 88.1% (52/59) of the homes, the presence of bugs was observed. In 48% (25/52), triatomines were positive for the presence of the parasite *T. cruzi*. Were collected mainly outdoors (86.1%), houses were located near and in the vegetation.
CONCLUSIONS*:* Immediate action is required in Tecalpulco community in vector control, such as fumigation and health education, because these results combined with high entomological indices (46.1% colonization, 88.1% infestation, natural infection 21.52%) placed this community emergency. Also, in this study described the adaptation of the *M. pallidipennis* species to peridomiciliary conditions of the houses

Identification and subcellular localization of a putative sodium-calcium exchanger of *entamoeba histolytica*.

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BACKGROUND: In *Entamoeba histolytica*, the protozoan parasite responsible of human amoebiasis, calcium has an important role on signaling of different cellular processes, including development and pathogenesis. However, few is known about the proteins that are involved in calcium regulation. Sodium calcium exchangers (NCX) are proteins that play an important role in calcium homeostasis by catalyzing the active efflux of this ion by using the energy stored in the electrochemical gradient of sodium. These proteins allow sodium to flow down its gradient across the plasma membrane in exchange for the counter transport of calcium ions.
METHODS: *E. histolytica* trophozoites were grown, extracted RNA and proceeded to cDNA synthesis for RT-PCR. Specific antibodies against this protein were generated for testing are confocal and electron microscopy to localize the protein in the plasma membrane.
RESULTS: We identified a gene that possibly encodes a plasma membrane NCX of *E. histolytica*, this gene is expressed in basal conditions. Specific antibodies were generated against this protein located this putative NCX in the plasma membrane.
CONCLUSIONS: Our results suggest that it corresponds to the exchanger responsible for calcium efflux in *E. histolytica*. Specific antibodies against this protein located this putative NCX in the plasma membrane. Therefore this protein could be the proteins that are involved in calcium regulation

Use of quantitative PCR to determine the *T. cruzi* parasite load in patients with Chagas disease in blood clot samples

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BACKGROUND: Quantitative real-time PCR (qPCR) is used to follow-up parasitemia in chronic cases or in immunocompromised patients with Chagas disease (CD). The use clinical sample other than blood (buffy coat, blood clot, etc.) to detect parasites by qPCR is being performed with good results for sensitivity and specificity. In this study, qPCR using TaqMan probes for quantification of *T. cruzi* minicircle DNA (kDNA) was evaluate for its ability to detect parasites in clot samples.
METHODS: qPCR were performed on 104 patients from Santa Cruz-Bolivia, consisting in 74 seropositive and 30 seronegative samples to CD by two different serological assays. Additionally, 30 seronegative samples to CD obtained from individuals from Lima-Peru were also included as negative controls. PCR amplifications were performed using the primers 32F/148R that amplifies a 188 bp segment of *T. cruzi* kDNA.
RESULTS: The detection limit (DL) was 0.01 parasite equivalents/ml (eq-p/ml), with an efficiency of 96.64% and a coefficient of determination (R^2) of 0.998. The median parasitemia found in patients seropositive was 0.382 eq-p/ml. Also, minimum and maximum values were 0.0104 and 81.252 eq-p/ml respectively. The sensitivity was 60.8 % and the specificity was 93.3%.
CONCLUSIONS: The results presented here show the use of blood clot as an alternative source of DNA for quantification of *T. cruzi* parasite load by qPCR. In addition, its sensitivity and reproducibility proved to be a suitable technique to detect low parasite burden in patients with Chagas disease.

Molecular diagnosis of polycystic hydatid disease in humans and *Cuniculus paca* samples from República Bolivariana de Venezuela.

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BACKGROUND: The polycystic echinococcosis by *E. vogeli* represents an emerging disease in South America. The parasite involved is considered very aggressive and there is a lack of clinical pathology recognition. The aim of this work is to perform molecular diagnosis in human and lapa samples from Venezuela, previously confirmed by morphological studies.
METHODS: Four samples of polycystic hydatid localized in human liver (n = 3) and Cuniculus paca (n: 1) were isolated. The samples were preserved in alcohol 70° and sent to INEI - ANLIS "Carlos G. Malbran" Argentina, for molecular analysis. The extraction of genomic DNA from protoscoleces, was performed by an automated method (Kit MagNA Pure Compact Nucleic Acid Isolation I- ROCHE). Specific primers for *E. vogeli* were designed and tested (fragments of 324 and 188 bp of the mitochondrial gene cox1). PCR reactions were performed using two sets of primers. The products obtained were sequenced and obtained sequences were compared with the Genebank database.
RESULTS: Two of 3 human samples and lapa sample could be amplified and sequenced. The obtained sequences were 99 and 100% identical to the reference sequence of *E. vogeli* in Genebank.
CONCLUSIONS: This is the first molecular study in human and lapa samples of *E. vogeli*, involving these specific primer pairs. Being the hooks morphology, confirmatory for this species, the specific molecular diagnosis would identify parasite genetic material even in samples where no rostellar hooks can be found.

Triatomines distribution and climatic factors in Taxco, Guerrero, Mexico

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BACKGROUND: The distribution areas triatomine provide information about the risk of infection in the population. A study to have an approximation of the diversity and distribution of triatomines in relation to climatic factors was performed.
METHODS: By the hour/man method of was conducted the search and capture of triatomines. For keys Wygodzinsky Lent, the identification was performed. Were estimated entomological indices (natural infection, colonization and infestation). A household heads were interviewed about disease-related, vector, sociodemographic and environmental data.
RESULTS: A total of 1,224 were collected triatomines of the genus *Triatoma* and *Meccus* in 28 of 31 sampled localities. *M. pallidipennis* (98.9 %) was the most abundant and distributed species, followed by *T. dimidiata* (1.1 %). The largest number 82.0 % (1004) of specimens were collected in peridomiciliary area and the lowest 18.0 % (220) within the households. The average rate of natural infection (IIN) for the species *M. pallidipennis* was 24.6 %. By location, Atzala had the highest (86%) and Teacalco, Texcaltitla and Icatepec had the lowest (0%). In the majority (57.14 %) of localities with presence of triatomines was observed that colonization rates were above 50%, regardless of whether it was a rural or urban area. *M. pallidipennis* is distributed between 900 and 1800msnm and *T. dimidiata* at of 1180 to 1430msnm. *M. pallidipennis* is the species with greater abundance and geographic distribution in warm humid climates and altitudes between 900 - 1800msnm.
CONCLUSIONS: *M. pallidipennis* is the species of greatest epidemiological importance because of its abundance and distribution in the study area and probably responsible for the transmission of the parasite *T. cruzi.*

Use of a Novel Chagas Urine Nanoparticle Test (Chunap) for Diagnosis of Congenital Chagas Disease

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BACKGROUND: Detection of congenital *T. cruzi* transmission is considered one of the pillars of control programs of Chagas disease. Congenital transmission accounts for 25% of new infections with an estimated 15,000 infected infants per year in Latin America. Current programs to detect congenital Chagas disease in Latin America utilize microscopy early in life and serology after 6 months. These programs suffer from low sensitivity by microscopy and high loss to follow-up later in infancy. We developed a Chagas urine nanoparticle test (Chunap) to concentrate, preserve and detect *T. cruzi* antigens in urine for early, non-invasive diagnosis of congenital Chagas disease.
METHODS AND RESULTS: This is a proof-of-concept study of Chunap for the early diagnosis of congenital Chagas disease. Poly N-isopropylacrylamide nano-particles functionalized with trypan blue were synthesized by precipitation polymerization and characterized with photon correlation spectroscopy. We evaluated the ability of the nanoparticles to capture, concentrate and preserve *T. cruzi* antigens. Urine samples from congenitally infected and uninfected infants were then concentrated using these nanoparticles. The antigens were eluted and detected by Western Blot using a monoclonal antibody against *T. cruzi* lipophosphoglycan. The nanoparticles concentrated *T. cruzi* antigens by 100 fold (western blot detection limit decreased from 50 ng/ml to 0.5 ng/ml). The sensitivity of Chunap in a single specimen at one month of age was 91.3% (21/23, 95% CI: 71.92%-98.68%), comparable to PCR in two specimens at 0 and 1 month (91.3%) and significantly higher than microscopy in two specimens (34.8%, 95% CI: 16.42%-57.26%). Chunap specificity was 96.5% (71/74 endemic, 12/12 non-endemic specimens). Particle-sequestered *T. cruzi* antigens were protected from trypsin digestion.
CONCLUSION: Chunap has the potential to be developed into a simple and sensitive test for the early diagnosis of congenital Chagas disease.

Seroprevalence of Chagas disease in rural dogs from La Antigua, Veracruz, Mexico.

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BACKGROUND: Chagas disease is an insect-borne zoonotic malady caused by *Trypanosoma cruzi*. The kissing bug *Triatoma dimidiata* is considered to be the main vector in Veracruz. Because dogs in rural communities of endemic areas are reservoir hosts, they can serve as sentinels and in active surveillance disease programs. This study was conducted to determine the seroprevalence and risk factors associated with *T. cruzi* infection in rural dogs in the state of Veracruz, Mexico.
METHODS: The program Win Episcope Ver 2.0 was used to determine a sample size of 340 according to these parameters: 50% seroprevalence, 95% confidence level, and 5% margin of error. Dogs from 10 localities in the municipality of La Antigua, Veracruz were sampled between February and October 2013. Serum was obtained from individual blood samples for testing by ELISA to detect antibodies against *T. cruzi*. Data were analyzed with descriptive statistics using the STATA program, version 11.0.
RESULTS: Overall seroprevalence was 19.5% (66/340), CI 95% 15.4-24.1. The localities with the highest seroprevalence were La Posta with 52.9% (18 /34), CI 95% 35.4-69.8, followed by José Ingenieros and San Pancho with 38.2% (13 /34) IC 95% 22.6-56.3. Localities with the lowest prevalence were Hatillo and Playa Oriente with 2.9% (1 /34), CI 95% 0.1-17.0.
CONCLUSION: Rural dogs are infected with *Trypanosoma cruzi* in La Antigua. The seroprevalence reported in this reservoir host indicates that humans in this municipality of Veracruz are at risk of exposure to infected vectors inhabiting peridomestic or domestic sites.
* USDA is an equal opportunity provider and employer.

Trichomonas hominis: persevering to engulf _Blastocystis_ sp. conduct in two and three dimensional images.

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BACKGROUND: The videos are very important tools in science for both research and teaching and this is an example.
METHODS: Submitted video and photomicrographs from culture to *Trichomonas hominis*,
RESULTS: protozoan commensal of the intestinal tract, with persevering to engulf *Blastocystis* sp conduct and use it as food, not yet reported in the literature and illustrated with two and three dimensional images in a new vision.
CONCLUSIONS: The observation of persistent behaviour of *Trichomonas hominis*, may be useful for better understanding of the biology of parasites.

Genotipification of *Trichomonas vaginalis.* Review

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BACKGROUND: *Trichomonas vaginalis* is a flagellated protozoan parasite from the urogenital region. It is the cause of trichomoniasis, the mechanism of infection is by sexual contact, being of hight prevalence. The re is famale and male infection, however male infection is short-term and commonly asymptomatic, which makes it difficult the study epidemiological and genetic variability.
METHODS: This work is a review of articles about the use of the PCR-RFLP molecular technique for studies of variability of *Trichomonas vaginalis*, wich provides higher sensitivity and specificity than PCR fingerprinting by RAPD and RFLP analysis that are techniques that have been used to study variation in *T. vaginalis.* I searched the PubMed database articles with the topic, specifically using the words: *Trichomonas vaginalis,* PCR-RFLP, and actin gene.
RESULTS*:* Using PCR-RFLP technique and actin gene eight genotypes are obteined. I found four relevant articles on the topic, indicating little studied this subject is. There's no studies in Mexico of genotipification of *Trichomonas vaginalis.*
CONCLUSIONS: Trichomoniasis is a parasitic disease that exists in our society, without however little is known about its epidemiology and therefore variability genetics of trichomonas vaginalis. Using the PCR-RFLP technique eight genotypes can be obtained, the next step is to carry out epidemiological studies and virulence in the country. In Mexico there are no case studies of *Trichomonas vaginalis* in men, so very little is known of its prevalence.

MDR genes over expression in *Giardia intestinalis* trophozoites treated with albendazole, nitazoxanide and an albendazole derivative.

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BACKGROUND: The ABC (ATP-binding cassette) transporters are a phylogenetic conserved family of proteins involved in drug resistance. It has been reported the participation of ABC transporters in drug resistance in parasites such as *Entamoeba histolytica*, *Leishmania Trypanosoma cruzi*, and *Plasmodium*. In this work, we searched for P-glycoproteins orthologs in GiardiaDB and found more than 50 open reading frames. Thirteen genes were selected for further analysis.
METHODS: The trophozoites were treated with 1µg /ml nitazoanide, albendazole and JG9 (albendazole derivative) and incubated at 37º C for one or two hours. Then RNA was extracted with Trizol, treated with DNAse and real time RT-PCR was performed. From the ABC sequence a 3D model was obtained using the structures 4FAC and 3G5U (*Caenorhabditis elegans* and *Mus musculus*) and Modeller program. The best built-in model generated was selected and evaluated using PROCHECK.
RESULTS: We observed the over expression of six genes (ABC1, ABC2, ABC3, ABC4, ABC6 y ABC10). The ABC1 gene expression was increased in trophozoites treated with the three drugs, meanwhile the ABC2 gene increased only in cells incubated with nitazoxanide and JVG9. Likewise, ABC3 and ABC4 genes increased their expression only in cells treated with nitazoxanide. On the other hand, the best 3D model was obtained using the *Caenorhabditis elegans* structure 4F4C as a reference.
CONCLUSIONS: The protein ABC1 has p-glycoprotein architecture and is over expressed in trophozoites treated with aldendazole, nitazoxanide and JVG9.

Leishmanicidal activity of the ethyl and benzyl esters of N-isopropyl oxamate on promastigotes and amastigotes of *Leishmania Mexicana*

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BACKGROUND: Cutaneous Leishmaniasis (CL) is the most important form of Leishmaniasis in Mexico. It is distributed in several States, and the main endemic areas are located in the South-East of the country. In Mexico CL can be produced by *L. mexicana* and *L braziliensis* Complex members, and the clinical forms produced are primary localized skin lesions (LCL) some of which can self-heal, mucocutaneous leishmaniasis (MCL), when parasites disseminate to the nasopharyngeal mucosa or diffuse cutaneous leishmaniasis (DCL) when parasites disseminate to the entire body as nodular lesions. Several drugs are available for the treatment of leishmaniasis, but most available drugs are very toxic and require long treatment regimes. Meglumine antimoniate (Glucantime) is the principal drug used for treatment of CL.
METHODS: In this study, the leishmanicidal activity of the ethyl and benzyl esters of N-isopropyl oxamate (NIPOX-B AND NIPOX-Et) was evaluated on promastigotes and amastigotes of *L. mexicana*.RESULTS: A leishmanicidal activity with LD_{50} of 16 mM for NIPOX-B and 8 mM for NIPOX-ET was found for promastigotes. In axenic extracellular amastigotes the leishmanicidal activity was found with a LD_{50} of 2 mM for NIPOX-B and 8 mM for NIPOX-ET. In *L. mexicana*-infected macrophages (J774) a 100% of parasite elimination was found with a dose of 64 mM for NIPOX-B, 32 mM for NIPOX-ET, and 32 mM for Amph B I; whereas only 80% of parasite elimination was found with Glucantime at 64 mM. The more toxic drugs for macrophages were Amph B and Glucantime; NIPOXs showed low toxicity.
CONCLUSIONS: Our results suggest that NIPOXs are good candidates for CL therapeutic treatment.

Molecular characterization of the Peptidyl tRNA hydrolases of *Entamoeba histolytica*

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BACKGROUND: The Peptidyl-tRNA hydrolase (Pth) is an esterase that hydrolyses the bond between tRNA and the attached peptide in peptidyl-tRNAs prematurely released from ribosomes. The mutant gene *pth*(Ts) encodes an enzyme with a substitution at a residue of 100 (Gly to Asp) resulting in temperature sensitive phenotype (strain AA7852). *Entamoeba histolytica* has 3 Pths with a high homology to Pth2 from eukaryotes and *Archaea* orthologs.
Methods: The plasmids to express Pths were constructed by cloning *E. histolytica* PCR fragments in pPROEX-1.The strain AA7852 was transformed with plasmids encoding the wild-type or *E. histolytica* Pths. AA7852 fresh cultures (IPTG 1 mM) were adjusted to 0.1 optical density at 600 nm and incubated at 42°C for 6 h or 20 min. The peptidyl tRNAs were extracted and the levels of aminoacylated and peptidylated tRNA were determined by northern blot assay. *E. histolytica* trophozoites were incubated with puromycin 2.5 or 1.25 mM, DTT 2.5 or 1.25 mM for 10 min at 37º C. The total RNA was extracted with Trizol and a RT-PCR was performed. A 3D model was obtained for each protein using several structures provided by Modeller, Swiss-Model and I-Tasser.
RESULTS: *E. histolytica* Pth-B suppresses the Pth(Ts) phenotype but in a lower level than the *E. coli* Pth wildtype. Pth-A and Pth-C exhibited an activity lower than Pth-B; the cells transformed with the mock die after 180 min. The relative concentrations of $tRNA^{Arg4}$ were higher in AA7852 cells transformed with *E. coli* Pth wildtype than *E. histolytica* constructions. The RT-PCR analysis revealed that Pth-A is only expressed in trophozoites treated with puromycin 2.5 mM. Pth-B and Pth-C were expressed in all the conditions without any modification. The 3D models obtained show a high similarity with the human Pth2.
CONCLUSIONS:The *E. histolytica* Pths suppresses the *Escherichia coli* termosensitive mutation.

Description of inflammatory reaction to saliva of *Triatoma dimidiata* at the inoculation site in a murine model

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BACKGROUND: Salivary components of hematophagous triatomines have different compounds in order to counteract the vertebrate's hemostasis, inflammation, and immunity. *Triatoma dimidiata* is abundant in some parts of México and in some rural towns almost 50% of inhabitants refer insect`s bite. However, the inflammatory reaction to *Triatoma dimidiata´*saliva is poor studied yet.
METHODS: Four group of Balb/c mice received *T. dimidiata´*bite into the hindpad for 10 minutes. Group A, B and C was mono-exposed and sacrificed at 1, 4 and 24 h later respectively (innate response). Group D received multiple exposures for four months and sacrificed at 1, 24 and 48 h later (chronic immune response). The inoculation site was excised and processed for HE and Toluidine blue stain. Infiltrated cells and type of inflammation was recorded.
RESULTS: The inflammatory reaction at 1, 4 and 24 h to saliva components in mono-exposed mice was composed of neutrophils and mast cells basically. The infiltrate was focalized and delimited to dermis. In multi-exposed mice to triatomine´s bite, the neutrophils continued the most abundant infiltrating cell, followed by mast cells, but now lymphocytes and eosinophils were evident although at low level.
CONCLUSIONS: The inflammatory reaction to *T. dimidiata´*saliva at the inoculation site is reach in neutrophils and mast cells independently of times to exposure. This finding may suggest that T. dimidiata´saliva is poor allergenic.

Trypanosoma cruzi evades the protective role of interferon-γ-signaling in parasite-infected cells

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The protozoan parasite *Trypanosoma cruzi* is responsible for the zoonotic Chagas disease, a chronic and systemic infection in humans and warm-blooded animals typically leading to progressive dilated cardiomyopathy and gastrointestinal manifestations. In the present study, we report that the transcription factor STAT1 (signal *t*ransducer and *a*ctivator of *t*ranscription *1*) reduces the susceptibility of human cells to infection with *T. cruzi*. Our data demonstrate that IFN© treatment causes *T. cruzi*-infected cells to enter an anti-parasitic state through the activation of the STAT1 transcription factor. Whereas stimulation of STAT1-expressing cells with IFN© significantly impaired replication of parasites, no protective effect of IFN© was observed in STAT1-deficient U3A cells. The gene encoding indoleamine 2,3- dioxygenase (*ido*) was identified as a STAT1-regulated target gene engaged in parasite clearance. Exposure of cells to *T. cruzi* trypomastigotes itself resulted in a sustained tyrosine and serine phosphorylation of STAT1 and was associated with an increased binding to STAT-specific binding elements on DNA. Furthermore, we found that in response to *T. cruzi* the total amount of intracellular STAT1 increased in an infectious dose-dependent manner, both at the mRNA and protein level. However, we also revealed that amastigotes replicating intracellularly antagonize STAT1 signaling by specifically promoting the dephosphorylation of serine-, but not tyrosine- phosphorylated STAT1 molecule, thereby partially circumventing its protective effects. These findings point to the crucial role of the IFN©/STAT1 signal pathway in the evolutionary combat between *T. cruzi* parasites and their host

Lipid composition of 9 strains of *Leishmania infantum*, *L tropica*, and *L. major* isolated in Tunisia.

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BACKGROUND: Visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) are endemic in Tunisia VL is caused by *Leishmania infantum*, whereas CL is due to *L. infantum, L. major and L. tropica*. These diseases evolve in individual epidemiological and ecological contexts and their geographical distribution is often more or less based on bioclimatic specificities.
METHODS: Nine human strains and one canine strain originating from various regions of Tunisia were isolated in order to analyze the variations in lipid composition from the *Leishmania* species endemic in Tunisia. Total lipids and cholesterol were quantified in the promastigote forms of *L. tropica*, *L. major* and *L. infantum*, cultivated axenically and obtained in great amounts for analysis. Lipid extraction was performed using the method of Bligh and Dyer followed by thin-layer chromatography. The samples are analyzed by gas chromatography or GC-MS.
RESULTS: The total lipids composition was the same in the *L. infantum* and *L. tropica* strains tested, while the *L. major* strains exhibited some variations in their lipid composition. No major difference in the saturated fatty acids of the tested parasite strains was evidenced, as well as in their mono-unsaturated fatty acids. The fatty acid precursors of the family n-6 (18:2 n-6) and n-3 (18:3 n-3) were present in a significant proportion, which suggests that the elongation and desaturation reactions have a fairly low rate in the parasites. In addition, n-3 were in greater amounts than n-6 fatty acids, as well as 22:6 n-3 were far more present than 20:4 n-6 fatty acids, a fact that seems to differentiate *Leishmania* promastigotes from most eukaryotic cells.
CONCLUSIONS: We intend to further investigate the role of these lipids in the infectivity of *Leishmania* and their potential use as therapeutic targets.

Predicting the global distribution of multiple diseases

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BACKGROUND: The primary aim of the review presented here was to evaluate the state of knowledge of the geographical distribution of all infectious diseases of clinical significance to humans.
METHODS: A systematic review was conducted to enumerate cartographic progress, with respect to the data available for mapping and the methods currently applied. The results helped define the minimum information requirements for mapping infectious disease occurrence, and a quantitative framework for assessing the mapping opportunities for all infectious diseases.
RESULTS: The methodology used revealed that of 352 infectious diseases identified, 176 (50%) have a strong rationale for mapping and of these only 7 (4%) had been comprehensively mapped. A variety of ambitions, such as the quantification of the global burden of infectious disease, international bio-surveillance, assessing the likelihood of infectious disease outbreaks and exploring the propensity for infectious disease evolution and emergence, are limited by these omissions.
CONCLUSIONS: An overview of the factors hindering progress in disease cartography is provided. It is argued that rapid improvement in the landscape of infectious diseases mapping can be made by embracing non-conventional data sources, automation of geo-positioning and mapping procedures enabled by machine learning and information technology, respectively, in addition to harnessing labour of the volunteer 'cognitive surplus' through crowdsourcing.

Predicting the global distribution and population at risk of *Plasmodium vivax*

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BACKGROUND: Current understanding of the spatial epidemiology and geographical distribution of *Plasmodium vivax* is far less developed than that for *P. falciparum*, representing a barrier to rational strategies for control and elimination. Here we review the first systematic effort to map the global endemicity of this hitherto neglected parasite.
METHODOLOGY AND FINDINGS: We first updated to the year 2010 our earlier estimate of the geographical limits of *P. vivax* transmission. Within areas of stable transmission, an assembly of 9,970 geopositioned *P. vivax* parasite rate (*Pv*PR) surveys collected from 1985 to 2010 were used with a spatiotemporal Bayesian model-based geostatistical approach to estimate endemicity age-standardised to the 1–99 year age range ($PvPR_{1-99}$) within every 5x5 km resolution grid square. The model incorporated data on Duffy negative phenotype frequency to suppress endemicity predictions, particularly in Africa. Endemicity was predicted within a relatively narrow range throughout the endemic world, with the point estimate rarely exceeding 7% $PvPR_{1-99}$. The Americas contributed 22% of the global area at risk of *P. vivax* transmission, but high endemic areas were generally sparsely populated and the region contributed only 6% of the 2.5 billion people at risk (PAR) globally. In Africa, Duffy negativity meant stable transmission was constrained to Madagascar and parts of the Horn, contributing 3.5% of global PAR. Central Asia was home to 82% of global PAR with important high endemic areas coinciding with dense populations particularly in India and Myanmar. South East Asia contained areas of the highest endemicity in Indonesia and Papua New Guinea and contributed 9% of global PAR.
CONCLUSIONS: This detailed depiction of spatially varying endemicity is intended to contribute to a much needed paradigm shift towards geographically stratified and evidence-based planning for *P. vivax* control and elimination.

Targeting outdoor malaria vectors using odor-baited Mosquito Landing Box (MLB) equipped with low- cost electrocuting grids

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BACKGROUND: Residual malaria transmission, especially the proportion that occurs outdoors, is now among the major obstacles in achieving malaria elimination goal. This outdoor transmission is attributed to outdoor mosquito bites by mosquitoes that are behaviourally resilient or resistant to existing indoor insecticidal interventions.
METHODS: Field experiments were conducted against free-flying wild mosquitoes, to evaluate an improved version of the recently developed odour-baited mosquito landing box (MLB), fitted with solar-powered low-cost electrocuting grids on its sides to rapidly kill even mosquitoes that only make very short contacts with the devices. Three MLBs were equipped with EC grids made from locally purchased mosquito racket zappers. One MLB was fitted with the EC grids on only one of its sides; another MLB had EC grids on two sides while the third MLB had EC grids on three of its sides. The three were comparatively evaluated using a 3 by 3 Latin square experiment, with outcome measure being average number of mosquitoes of different species.
RESULTS: A total of 4986 dead mosquitoes were collected from the 3 odor-baited MLBs equipped with EC grids, 29% (1432) of which were from MLB with grid on one side, 35% (1738) from the MLB with EC grids on 2 sides while 36% (1816) were from the MLB with EC grids on 3 sides.
DISCUSSION AND CONCLUSION: Our results showed that more mosquitoes were caught from the MLB with more than one grid, which might be due to in the higher surface area of contact for mosquitoes with the EC grids. As targeting host-seeking mosquitoes with insecticide-based methods is increasingly challenging due to either behavioural or adaptive resistance of the mosquito vectors after prolonged use, MLBs equipped with low-cost EC grids regularly charged by solar energy, could have significant advantages, as it would also effectively control even those species that are behaviourally resilient or physiologically resistant to insecticidal interventions like long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS) after prolonged use.

Changing epidemiology of leishmaniasis in the Old World: Focus on Israel and the Palestinian Authority.

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Cutaneous (CL) and visceral leishmaniasis (VL) is endemic in the Middle East and the Mediterranean Basin. Changes in environmental, ecological, demographic, and political conditions can result in outbreaks, re-emergence and/or spread of disease. In Israel and the Palestinian Authority, *Leishmania infantum, L. tropica* and *L. major* are responsible for the majority of disease. Travelers, migrant laborers and immigrants introduce additional species, like *L. Viannia braziliensis* and *L. donovani*, from outside this region. HIV - VL co-infections while rare are also seen. In the last two decades dramatic changes in the epidemiology of VL and CL have occurred in Israel and the Palestinian Authority. Canine and human VL, once restricted to the Galilee, Northern Israel are now found throughout much of central Israel and the Palestinian Authority from the Lebanese border to Hebron in the south. CL caused by *L. tropica* was almost unknown before the year 2000. Since then outbreaks have occurred in several cities, and the disease continues to spread in Israel and the Palestinian Authority with new foci reported yearly. Recently a new reservoir host, voles (*Microtus guentheri),* was identified, and implicated in outbreaks of CL caused by *L. major* in an emerging focus of this disease. Factors affecting emergence and spread of leishmaniasis will be discussed.
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Comparison of moxidectin+praziquantel, ivermectin and febantel+metriphonate efficacy against horse parasites in three Mexican regions.

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BACKGROUND: The aim of this study was to compare the nematodicidal efficacy of three anthelmintics in horses located in three different regions of Mexico.
METHODS: 300 horses were assigned to 3 treatment groups. Group A (South-Veracruz), group B (North-Nuevo León and Tamaulipas) and group C (Central-Edo. de México). All animals received moxidectin 2%+praziquantel 12.5%; ivermectin 1.87%, and febantel 7.83%+metriphonate 37.35% orally. Fecal samples were collected on days -7, 0, 30, 60 and 90, and analyzed by McMaster, Baermann and fecal culture techniques. Mean, standard deviation, decrease in number of nematode eggs/g (efficacy), percent of nematode genus, species and time of reinfection were calculated.
RESULTS: Efficacy on day 30 was of 96.67%, 99.89%, and 98.06% for Veracruz horses treated with moxidectin+praziquantel, ivermectin, and febantel+metriphonate, respectively. Nuevo Leon's efficacy was of 97.15%, 98.81% and 89.36% for moxidectin+praziquantel, ivermectin and febantel+metriphonate, respectively. Tamaulipas efficacy was of 100% for moxidectin+praziquantel and ivermectin, but of 92.12% for febantel+metriphonate. Efficacy in group C was of 90.27%, 88.34%, and 88.09% for moxidectin+praziquantel, ivermectin, and febantel+metriphonate, respectively. Detected nematode species were: Cyathostomun spp, Strongylus equinus, S. edentatus and S. vulgaris. Reinfection occurred after treatment in Veracruz and Tamaulipas with febantel+metriphonate and praziquantel on day 30 and ivermectin on day 60; in Nuevo León with ivermectin and febantel+metriphonate (day 30); and moxidectin+praziquantel (day 90); and with the 3 drugs (day 30) in edo. de México.
CONCLUSIONS: It is concluded that moxidectin+praziquantel significantly decreased nematode eggs/g in contrast to ivermectin and febantel+metriphonate in group C. Moreover, moxidectin+praziquantel and ivermectin showed a similar reduction of eggs/g in Tamaulipas horses.

Importance of the Microbiome in *E. histolytica* Intestinal Infections

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BACKGROUND: While *Entamoeba histolytica* trophozoites can become invasive in the human intestine and cause severe damage to the host tissues, *Entamoeba dispar* trophozoites, also present in the intestine, only colonize the intestine as commensals Search for expression of genes that could be related with the different behavior of these amebas has mainly revealed quantitative differences in genes and proteins considered responsible for the aggressive behavior of *E. histolytica* trophozoites. For many years, it has been thought that *E. histolytica* pathogenicity can be modulated and even induced by the presence and/or ingestion of bacteria. Epidemiological studies have shown that in areas in which Amebiasis is endemic, pathogenic bacteria and other intestinal parasites are present in mixed infections.
METHODS: The intestinal environment is a complex system to study as many organisms co-exist, however it is in these conditions where we could start to identify the factors that could influence the behavior of the parasite. We utilized an in vitro cell model in which colonic human cells were cultured with different species of intestinal bacteria, both non-pathogenic and pathogenic, and tested different conditions in which the amebas were induced to become invasive.
RESULTS: This model revealed that amebas could induce an inflammatory response in the colonic cells through an amebic protein that binds to Toll-like receptors, initiating an inflammatory response that was synergized by the bacteria in different degrees. Furthermore, trophozoites could act as opportunists and invade the cell layer, taking advantage of the inflammatory environment created by bacteria.
CONCLUSIONS: These findings showed the importance of the intestinal microbiota in *Entamoeba histolytica* Infections and the consideration of these factors in the treatment of patients with diarrhea, as indiscriminate use of antibiotics could increase the inflammatory environment and select for invasive pathogens, leading to severe disease.

Perspectives for the control of triatomines in Mexico

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BACKGROUND: In Mexico thirty-two species of *Trypanosoma.cruzi* vectors have been reported, parasite associated with Chagas disease, they have been divided into domiciled, peridomiciled and wild. Health educational programs, housing improvement and the use of pyrethroid have been implemented to control the domiciled species (*Triatoma dimidiata y Triatoma barberi*). In Veracruz, a state with *Triatoma dimidiata*, this initiative has not been successful among peridomiciled *(Triatoma pallidipennis)*, therefore not only the use of mosquito nets, pavilion and cementing fences, but also biological control-oriented actions using entomopathogenic fungi are necessary to control various insects considered agricultural pests.
METHODS: Insecticide method: Three different interventions to control *Triatoma dimidiata* in Veracruz state were implemented: X-1= whole dwelling spraying, X-2= middle wall spraying, X-3= household cleaning. Cyfluthrin was sprayed three times at intervals of eight months. Biological control method: Two strains of entomopathogenic fungi were used on the egg stage of *Meccus pallidipennis: Isaria fumosorosea and Metarhizium anisopliae.* For CL50 two µL of *Isaria fumosorosea* conidia suspension were applied. They were incubated at a temperature of 28°C for ten days. For TL50 2ul of *Isaria fumosorosea* and *Metarhizium anisopliae* conidia suspension were applied to the surface of the egg and were incubated at a temperature of 28°C for ten days.
RESULTS: Insecticide method: With X-1, the infestation, colonization and natural infection indexes were reduced to 0% in the three localities, with respect to t0. With X-2, the infestation index was reduced to 10% at t3 in three localities; the colonization index was reduced to 0% in only one locality at t3, and the natural infection index was reduced to 0% at t3. With X-3 the three indexes were not effectively reduced but they decreased with respect to the baseline study. Insecticide application to the whole dwelling is a more efficient intervention than its application to only the lower half of the walls and to the house cleaning.
Biological control method: CL50: 100% of mortality was achieved with concentrations of 20,000 and 6000 conidia within 108 hours (4.5 days) and 132 hours (5.5 days) after the infection, respectively. TL50 reported 100% of mortality within 66.685 hours (2.77 days). Data obtained suggest a high virulence of the fungus towards *Meccus pallidipennis.*
CONCLUSIONS: Insecticide effectiveness for the control of domiciled insects was observed, and there is a need for biological control in peridomiciled insects since the eggs are not affected by chemical insecticides used to control them. The results of this first work with entomopathogenic fungi for the control of the vectors *Meccus pallidipennis* are promising because they can help in the development of new strategies for the control of the vectors in Chagas disease, specifically in the peridomiciled level, where the current actions (chemical insecticides) have failed. This simple, sustainable and inexpensive methodology may be applied alone or in combination with other control measures.

The Road from Concept to Proof of Principle to Deployment of the PfSPZ Vaccine for Elimination of *Plasmodium falciparum* Malaria

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The term, Vaccine that Interrupts Malaria Transmission (VIMT) was introduced by the Malaria Eradication Research Agenda (malERA) initiative [malERA Consultative Group on Vaccines. A research agenda for malaria eradication: vaccines. *PLoS Med.* **8**, e1000398 (2011)]. An ideal VIMT would induce protective immune responses against all stages of the parasite life cycle. However, the ideal single stage VIMT would prevent infection at the pre-erythrocytic stage of the parasite life cycle, thereby preventing erythrocytic stage infection and all parasite-caused disease and transmission of the parasite from humans to mosquitoes. PfSPZ Vaccine, composed of radiation attenuated, aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ) is a pre-erythrocytic stage vaccine and the malaria vaccine closest to being able to be used as a VIMT. We recently reported PfSPZ Vaccine to be safe and to completely protect from Pf infection six of six volunteers who received the highest dosage administered in a clinical trial (Seder et al., *Science* 341:1359-65, 2013). Follow-on clinical trials at 3 sites in the U.S., and in Mali, Tanzania, Equatorial Guinea, and Germany have begun or will soon begin. These trials are designed to establish reproducibility of results of the first clinical trial, establish an immunization regimen that provides durable protection against all Pf strains with the least number of doses and least quantity of vaccine, identify an immunological test that predicts protection, and begin the implementation research needed to pave the way for mass administration campaigns. We set an ambitious 4-year timeline for moving through the stages of clinical development to pivotal phase 3 clinical trials and licensure and demonstration of the capacity of the vaccine to eliminate Pf malaria from populations > 200,000 individuals. The plans for doing so, including challenges we face, our strategies for overcoming them, and the roles of the numerous international partners will be discussed.

Proteomic Analysis of Apoptosis induced by the aminoglycoside G418 in *Entamoeba histolytica.*

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BACKGROUND: One of the mechanisms of *Entamoeba histolytica* to evade the immune response is the induction of apoptosis in the host cells, on the other hand has been demonstrated PCD in *E. histolytica* trophozoites induced by drugs *in vitro*. In this study we have used a proteomic analysis to identify differential proteins expressed in *E. histolytica* induced PCD by G418.
METHODS: PCD was induced in trophozoites by incubation with 10 ug / ml G418 for 0, 1 ½, 3, 6, 9 h. To the isoelectrofocusing was used immobilized pH gradient strips (3 to 10), these proteins were separated by their molecular weight in SDS-PAGE gels. The gels were analyzed using the Software: Ludesi REDFIN 3, the differential spots were processed by peptide fingerprinting and MALDI TOF-TOF technique to identify the corresponding peptides.
RESULTS: The results showed proteins expressed specifically in different stages of PCD induction. We identified actin, alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, malate dehydrogenase, adenylate kinase, elongation factor 1-alpha, coronine, aminoacyl-histidine dipeptidase, pyruvate: ferredoxin oxidoreductase, and grainine 2.
CONCLUSIONS: Our results of proteomic analysis of PCD in *E. histolytica in vitro*, allowed us to elucidate and understand the expression of some proteins in different phases of the induced PCD G418. 15 differential spots were observed. Two proteins specifically related to apoptosis were detected: Elongation factor 1-alpha, which could be involved in the control of PCD and Grainin 2, probably functioning as an anti-apoptotic factor, these proteins could be potential candidates as drug targets.

Effect of Dengue Virus infection on *Aedes aegypti's* ovaries and its implication on the reproductive biology of vector.

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INTRODUCTION**:** The mosquito *Aedes aegypti*, due to its importance in Dengue virus transmission cycle, has been studied to understand aspects related to vector competence. Salivary glands, midgut and faty body were previously investigated to show the kinetics of viral infection in these organs/tissues. Ovaries are the major organ involved in the vector´s reproduction and transovarial transmission but have been neglected in viral infection analyses. Thus, the present study aimed to analyze the kinetics of ovarian infection and evaluated how the presence of the virus in this organ changes the vector reproductive biology.
METHODS**:** Oral infection with Dengue virus (DENV) was performed under laboratory conditions. *Aedes aegypti* Petrolina strain (field population), was used to study the kinetics of ovarian infection at 7, 14, 21 and 28 days post infection (dpi). Two experimental groups were analyzed: single feeding (SF) and multiple feeding (MF) females. Fertility and fecundity analyses were conducted in the fed infected mosquitoes. After feeding mosquitos were subsequently kept in individual cages for egg collection. In addition, ovaries were collected and processed for histopathological and ultrastructural analysis.
RESULTS**:** Ovarian tissues were positive for DENV at 14, 21 and 28 dpi in both groups. Viral quantification in positive samples was similar in all samples, except at 28 dpi in the MF group. No difference was observed on reproductive patterns (fecundity and fertility). Invaginations were observed in histopathological analysis of follicular epithelium of oocytes from infected group. No changes at the cellular level were observed by the ultrastructural analyses.
CONCLUSIONS**:** Ovarian infection in mosquitos occurs at low levels compared to other organs. The viral presence in ovarian tissue does not result in losses of vector reproductive biology. Further investigations, like immune gene expression analysis in ovaries and transovarial transmission studies using field populations are necessary.

Plasmodium yoelii GPI8p-transamidase related protein (PyTAM) DNA vaccine formulated with nanoparticle showed a protective effect in mouse model.

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BACKGROUND: **Bioinformatics and DNA vaccine strategy** Following a genome-wide search for a blood stage malaria DNA-based vaccine using web-based bioinformatics tools, we identified PyGPI8p-transamidase related protein (PyTAM) as protein containing GPI-anchor motif with protective immune response (1). GPI8p is one of the catalytic components responsible for cleavage of GPI-attachment signal sequences (2, 3) with 25-28% homology to a family of cysteine proteinase (C13 family), one of which is able to act as a transamidase (4).
METHODS and RESULTS**: Nano-particle as a strong tool for DNA vaccine delivery.** Recently, we reported that PyTAM as DNA vaccine formulated with nanoparticle showed a protective effect in mouse model (5). **T cell immunity looked more important:** the mechanism by which this formulation affects the course of infection still not clear. The only thing we noticed is that the antibody is not enough to protect against infection. High titer antibody just prolonged the onset of the parasitemia but finally all the immune mice died.
CONCLUSIONS**: Nanoparticle formulation with candidate DNA vaccine can be used for fishing the candidate vaccine**: Our Methodology, the selection of antigen candidates based on functional protective studies during the early antigen discovery stage could predict that the human malaria orthologous gene of PyTAM (PF11_0298 and PVX_092055 respectively for P. Falciparum and P. vivax) may, therefore be a good blood stage candidate for malaria vaccine development.

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Nano-ball consisted of inner cationic Dendri-grafted poly L-lysines (DGL) or polyethyleneimine (PEI) and outer anionic γ -polyglutamic acid (γ - PGA) enhanced plasmid based DNA vaccines against experimental malaria and schistosomiasis.

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BACKGROUND**:** Drug and vaccine delivery system (DDS) using new materials has been greatly contributing to various area of medicine. We have already reported one of the DDS called nano-ball (NB) consisted of two layers, inner cationic core materials and outer polyanion. Polyethyleneimine (PEI) as inner and γ-poly glutamic acid (γ-PGA) as outer were found to be effective especially when the DNA or protein was wrapped to deliver to dendritic cells (DC) (1). NB of PEI/γ-PGA is expected to be a strong DDS for DNA vaccines (2).

METHODS and RESULTS: Recently, we reported that Py MSP1C19 or PyTAM as DNA vaccine formulated with NB showed a protective effect in mouse malaria model (2). We also found the significant anti-fecundity effect by vaccination with Glutathione S Transferase (GST) of S. japonicum DNA vaccine with NB. Both malaria and Schistosomiasis mouse model showed a strong antigen specific B cell and T cell response. Especially Interferon-γ response was significantly elevated after recall antigen stimulation. IL-12 production from monocytes is also prominent by NB with or without specific plasmids. NB was suggested to stimulate DC in the antigen non-specific manner. However, conventional as well as plasmacytoid DC in the lymph nodes increased in number after the DNA vaccine with NB compared with blank plasmid with NB suggesting the back stimulation of DC by the antigen stimulated T cells.

CONCLUSIONS: Due to the safety issue, we replaced PEI by bio-degradable dendri-grafted poly L-lysines (DGL) as inner layer and found equivalent activity. Nano-ball formulation with DNA vaccine can be a promising vaccine delivery system for the clinical development.

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Artemisinin derivatives molecular resistance in Democratic Republic of Congo

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BACKGROUND: In 2001, WHO recommended the use of Artemisin-based Combination Therapy (ACT) for first-line treatment of uncomplicated malaria cases because monotherapies has become ineffective in many parts of the world due to apparition and spread of resistant strains of *P. falciparum* to almost all antimalarial drugs used. But in 2009, *P. Falciparum* artemisinin resistant strains appeared in western Cambodia. As for previous antimalarial drugs, the resistant strains have spread from that region to all endemic malaria areas. We conducted this study to evaluate the presence of artemisin resistant strains in Democratic Republic of Congo (DRC), where ACT are in use since 2005.
METHODS : Three hundred blood samples have been collected from asymptomatic people living in three provinces of DRC (Equateur, Kinshasa and Kasai-Occidental). Plasmodium species identification has been done by Real-time PCR. Another classsic PCR was run to apmplify the K13-propeller, recently identified as linked to artemisinin sensibility, on *P. Falciparum* positive samples followed by sequencing of amplicons. The sequences were aligned using the GeneStudioTM® Professional software and were compared with the reference sequence of the Kelch protein.
RESULTS : 144 *P. Falciparum* have been diagnosed by Real-Time PCR. No mutations have been identified on the K13-propeller.
CONCLUSION : This is the first data on artemisinin molecular resistance in Africa using the K13-propeller as molecular marker. Our study suggest that resistance to artemisinine and derivatives has not reached yet the DRC. However, studies on greater samples and in vivo studies should be done to confirm this.

Keywords: Malaria, artemisinin, K13-propeller, Congo

Current status of resistance of the cattle-tick *Rhipicephalus (Boophilus) microplus* to acaricides in Brazil

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BACKGROUND: *R. microplus* is the main hematophagous ectoparasite of cattle in different regions of Brazil. It is the transmitter of cattle tick-borne diseases, and has caused great economic losses due to the spoliation that it causes to the host. The main control method currently used is chemical control, but a growing number of reports indicate an increase in tick populations resistant to various chemical principles present in acaricides. The molecular basis of resistance in *R. microplus* is not yet entirely known, but many studies indicate some mechanisms. This work aims to present an overview of ticks' resistance to acaricides and the current situation in Brazil.
METHODS: Data from resistance of the cattle-tick *R. microplus* to acaricides in Brazil were compiled. A review of research results of tests of tick resistance in Brazil in scientific literature was conducted. The methodology and the results were analyzed.
RESULTS In Brazil, the cattle tick has been studied most in the south, where ranching is based on animals of European, in the southeast, and more recently, in the Midwestern region of the country. There is little information about cattle ticks in the north and northeast. The most definitive method for determining the efficacy of an acaricide is to test it under field conditions following the products label. Acaricide resistance has evolved with each introduction of new acaricides. Resistance to organochlorines, organophosphates, synthetic pyretroides, amidines, macrocyclic lactones, and fipronil has been confirmed. To date, fluozuron resistance has not been reported. Generally results demonstrate the low efficacy of most pesticides used regionally for the control of *R microplus*.
CONCLUSIONS: The diagnosis of tick resistance contributes to the choice of the chemical bases of the acaricide to be employed in the tick population of a given property and its use should be intensified and systematized in a government control policy.

The Immunotherapeutic Role of Regulatory T cells in *Leishmania (Viannia) panamensis* Infection

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BACKGROUND: *Leishmania (Viannia)* parasites are etiological agents of cutaneous leishmaniasis in the New World. Infection is characterized by mixed Th1/Th2 and inflammatory responses, which contribute to disease pathology. However, the role of T regulatory cells (Treg) in *Leishmania (Viannia)* disease pathogenesis is unclear.
METHODS and RESULTS: Using the mouse model of chronic *L. (V.) panamensis* infection, the contribution of Tregs to pathogenesis/healing was examined. Upon infection, the percentage of Tregs ($CD4^+Foxp3+$) was reduced. Further, these cells presented with a dysregulated phenotype (IFN-γ+, Tbet+) with reduced ability to suppress T cell proliferation. Targeted ablation of Tregs resulted in enlarged lesions, increased parasite load and enhanced production of IL-17 and IFN-γ, with no change in IL-10 and IL-13 levels. Thus, decrease in regulatory function and increased inflammatory responses were commensurate with disease exacerbation. Conversely, adoptive transfer of Tregs halted disease progression, lowered parasite burden and reduced cytokine production. As Tregs appeared important for controlling infection, *in vivo* immunotherapeutic expansion of Tregs (using rIL-2-anti-IL-2 antibody complex) was employed in chronically infected mice. Treatment increased the percentage of Tregs, reduced cytokine responses, ameliorated lesions and significantly reduced parasite load (10^5-fold). The use of chemical immunomodulatory compounds for topical treatment represents a potential treatment approach. With the goal of enhancing Tregs , the therapeutic potential of theTLR9 ligand, CpG, which is capable of inducing a counter-regulatory response, was evaluated. In PBMCs from infected patients, CpG treatment reduced the production of IFNγ, IL-10 and IL-13. In the *L. (V.) panamensis* mouse model of chronic infection, local administration of high dose CpG reduced lesion size and parasite load. The healing response corresponded to increased Treg function and TGFβ production with decreased IFNγ, IL-10 and IL-13 levels.
CONCLUSIONS: Thus, local immunotherapy targeting Tregs together with chemotherapy could provide an alternate treatment strategy for leishmaniasis caused by *L. (Viannia)* parasites.

Mosquito vectors are good targets for malaria control: Characterization of the *Anopheles aquasalis* immune response to *Plasmodium vivax*

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

Vaccination for control of cystic echinococcosis

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Numerous small scale experimental vaccine trials have been carried out with the EG95 vaccine in sheep or other hosts of *Echinococcus granulosus* in Argentina, Australia, Chile, China, New Zealand and Romania all of which have found the vaccine to be >90% effective against a challenge infection with *E. granulosus* eggs. Field trials with the vaccine have been undertaken or are underway in Argentina, Australia, China and Italy. More widespread application of the vaccine as part of hydatid control campaigns will require a commercial scale supply of high quality vaccine. To this end, the vaccine has undergone testing and has received registration for commercial production and use in China and Argentina.

While methodologies suitable for commercial-scale production of the EG95 vaccine have been developed and validated, research is continuing towards improving the yield of the EG95 protein from *Escherichia coli* fermentation as well as investigating other expression hosts in order to simplify vaccine production methods and reduce costs. A modification in the original EG95 construct has been found to provide substantial improvement in the expression levels of the protein in *E. coli*, substantially reducing the cost of vaccine production. More recently, expression of secreted EG95 in using a *Pichia* host has achieved very promising results – a vaccination and challenge trial is underway in sheep using *Pichia*-expressed antigen against challenge infection with *E. granulosus* eggs.

One aspect of the EG95 vaccine that warrants attention is that the vaccine prevents infection, it does not cure an existing infection. Hence, in areas where hydatid transmission is hyper-endemic there is a risk that young animals may be exposed to hydatid eggs before the animals reach an age at which it would be practical to vaccinate them. At present there is no suitable anthelmintic treatment for *E. granulosus* infection in livestock animals. Experiments have been initiated to investigate the susceptibility of immature hydatid cysts in sheep to benzimidazole drugs and/or praziquantel with a view to establishing a vaccination plus anthelminitic treatment regime for young livestock which would solve the problem of animals being exposed to hydatid infection prior to vaccination.

While the EG95 vaccine is clearly effective against experimental infections with *E. granulosus,* and a number of field trials are underway with the vaccine, an urgent need remains for the vaccine to be utilized in a carefully monitored hydatid control program such that the cost effectiveness of the vaccine can be determined.

Does use of albendazole for GI nematodes affect ovine echinococcosis?

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Many campaigns seeking to control of cystic echinococcosis have been ineffective or unsustainable. The disease continues to be an important cause of human morbidity and mortality worldwide and is recognised as a neglected disease by the World Health Organisation. Most control campaigns have relied heavily on the treatment of dogs to kill adult tapeworms. More recently, the EG95 vaccine for livestock is providing an additional option for control programs and mathematical models predict that a combination of both vaccination of livestock plus 6-monthly treatment of dogs may provide a sustainable and effective strategy.

During preparations for undertaking a pilot control program for cystic echinococcosis in Sardinia, it was revealed that the prevalence of fertile hydatid cysts seemed to have been declining in Sardinia in the absence of control activities directed specifically at *Echinococcus granulosus*. The use of drenches containing albendazole is relatively common on the island and it has been speculated that this may be affecting the growth and fertility of hydatid cysts. A similar observation was made almost 20 years ago by Perla Cabrera and her colleagues concerning the possible effects of the use of benzimidazole drenches on cystic echinococcosis in sheep in Uruguay. Evidence from treatment of cystic echinococcosis in humans would suggest that the frequency and dosages used to treat gastrointestinal nematode infections in sheep would be unlikely to affect the cysts in sheep. However, no definitive evidence is available to determine the effects of infrequent and low-dose exposure to benzimidazoles in sheep on the development/fertility of hydatid cysts.

In order to investigate the effects of albendazole use in sheep on the development of *E. granulosus* cysts, and experiment has been initiated involving 56 dorper lambs. All animals were experimentally infected with 1000 viable *E. granulosus* eggs at approximately 4 months of age by intra-ruminal injection. Groups of 10 animals are being drenched with 4.75 albendazole (Valbazen) at intervals of either 2, 4 or 6 months, or 9.5mg/kg 2-monthly, over a 30 month period after the experimental infection. Sixteen infected animals are being maintained as controls with no exposure to benzimidazole drugs after the infection with *E. granulosus*. Two control animals were assessed 12 months following the experimental infection and both showed the presence of normally-developing, viable *E. granulosus* cysts. Further details of progress in the experiment will be reported.

Small-scale field trials of the TSOL18 vaccine

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Two small-scale field trials have been completed using TSOL18 or TSOL18+TSOL16 vaccines. Both have achieved significant reductions in the incidence of cysticercosis caused by *Taenia solium* in vaccinated pigs.

The first trial was undertaken in the Mayo-Danay district in the Far North region of Cameroon. One hundred and twenty pairs of 2-3 month old piglets were distributed to households in an area known to have high endemicity for porcine cysticercosis. One member of each pair was vaccinated on three occasions with the TSOL18 vaccine. The vaccinations were given at the start of the trial, and 1 and 4 months subsequently. At the time of the second immunisation, both the vaccinated and control pigs were given a single oral dose of 30mg/kg oxfendazole so as to kill any cysticerci that may have established in the animals early in their lives. When the trial animals were approximately 12 months old, necropsies were performed on all animals that could be recovered, the carcase cut longitudinally into halves and all the striated muscle in one half examined meticulously to enumerate all cysticerci and classify them as viable or not-viable, together with the entire heart and brain. At the completion of the trial, 97 of the control/vaccinated paired pigs had both animals available for necropsy. Eighteen animals which were available for necropsy, comprising five control animals and 13 vaccinated animals, had their partner unavailable for necropsy. No cysticerci were found at necropsy anywhere in any of the vaccinated animals, including both those for which the paired control animals was necropsied as well as the 13 vaccinated animals for which the pair partner was not available for necropsy. McNemar's test was used to compare the proportion of paired vaccinated pigs that were infected with the proportion of control pigs that were infected. Comparison of the number of cysts in vaccinated and control paired pigs was evaluated by Wilcoxon's signed rank test. Statistical comparison of the pairs of control and vaccinated animals showed a significant reduction in vaccinated animals in total cysts ($P < 0.0001$), viable cysts in muscles ($P < 0.0001$), total cysts in the brain ($P = 0.0002$) and viable cysts in the brain ($P = 0.0002$). There was a reduction in the prevalence of infection from 19.6% (19/97) in paired control pigs to 0% (0/97) in paired vaccinated pigs ($P < 0.0001$).

A similar pair-matched vaccination trial field was undertaken in rural villages of Peru using a combination vaccine comprising both TSOL18 together with a second, host-protective antigen, TSOL16. Pairs of pigs ($n = 137$) comprising one vaccinated and one control animal, were allocated to local villagers. Animals received two vaccinations with 200µg of each of TSOL16 and TSOL18, plus 5 mg Quil-A. Necropsies were performed 7 months after the animals were distributed to the farmers. Vaccination reduced 99.7% and 99.9% ($p < 0.01$) the total number of cysts and the number of viable cysts, respectively.

Vaccination was found to be capable of reducing the potential of pigs in endemic areas to transmit *T. solium*. Further field trials are needed in order to determine the minimum number of interventions required to be delivered in order to achieve a high level of disruption of *T. solium* transmission on an on-going basis, rather than in single cohort studies as described here. The absence of a defined breeding season for pigs, and the typically remote and poor locations where *T. solium* is highly endemic, present challenges for the sustainable implementation of cysticercosis control measures. Nevertheless, vaccination of pigs has clear potential for application in to control of *T. solium* transmission and thereby contributing to reducing the global burden of human neurocysticercosis.

PTEX is a nexus for protein export in malaria

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During the blood-stages of malaria, several hundred parasite-encoded proteins are exported beyond the double membrane barrier that separates the parasite from the host cell cytosol. These proteins play a variety of roles that are essential to virulence or parasite growth. There is keen interest in understanding how proteins are exported and whether common machineries are involved in trafficking the different classes of exported proteins. One potential trafficking machine is a protein complex known as the *Plasmodium* Translocon of Exported Proteins (PTEX). While PTEX has been linked to the export of one class of exported proteins, direct evidence for its role and scope in protein translocation has been lacking. To address this we generated parasite lines that are defective for different essential components of the PTEX complex. The results of these experiments and the work that led to this approach will be presented. Essentially, this work proves the role for the PTEX translocon in the export of proteins across the parasitophorous vacuole membrane.

Prevalence of intestinal parasitosis in nursery and primary schools in the municipality of Puebla

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BACKGROUND: Intestinal parasitic infections (PI) are one of the health problems more widespread and prevalent in the world, especially in countries with limited socio-economic development. The harmful effects resulting in physical and mental development especially of children and the negative form with which have an impact on the economy of the population constitute a health and social problem.
METHODS: Participated 391 children, in a kindergarten of the DIF and 252 children of 2 urban primary schools (A) and (B). Report and consent of parents or guardians, study was conducted serial stool (CPS) (method of Faust) and/or direct fresh).
RESULTS: A total of 282 (72.12%) this parasitized with protozoa or geohelminths, being 115 children (82.73%) of the nursery and 167 (66.26%) primary schools. There was no significant difference with respect to sex. The prevalence of PI were: *G. lamblia* 159 (40.66%), *E. histolytica/E. dispar* 101 (25.83%), *E. coli* 97 (24.80%), 45 *H. nana* (11.50%), *A. lumbricoides* 7 (1.79%), *E. nana* 5 (1.27%) and *T. trichiura* 2 (0.51%). The polyparasitism was: with a parasite 154 (39.38%), with two 93 (23.78%), with three 31 (7.92%), with 4 and 5 with 2 (0.51%). The association *E. histolytica/E. dispar-E. coli* is the most frequent in the primary (A) 26 (16.56%) and primary school (B) 19 (20%), *G. lamblia - E. histolytica/E. dispar* care 15 (10.79%).
CONCLUSIONS: The prevalence of PI in the nursery and primary schools is high (72.12%), *G. lamblia* is the protozoan parasite with higher prevalence and helminth *H. nana* the most frequent. The association *E. histolytica/E. dispar-E. coli* was the most prevalent.

Echinococcus multilocularis – is there need for control?

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As a conclusion from recent epidemiological surveys, *E. multilocularis* is endemic in a far larger proportion of Europe and North America than previousy thought. In addition, accidental introductions have taken place into previously non-endemic areas, the frequency in host animals and humans has increased in many countries, and the establishment of synanthropic populations of foxes and coyotes has brought the parasite into urban environments. *E. multilocularis* shows great plasticity in adapting to different environments and host species, including invasive mammals and domestic dogs. Owing to this diversity of environment and host range, any control strategy must be tailored for specific regional conditions.

In principle, there are four options for prevention and control. (1) Information and education has no impact on the life cycle but may reduce the risk for human infection. (2) Regular deworming of domestic carnivores has no or only a minor impact on the life cycle, but the risk of humans is reduced. (3) Culling of host species is only feasible for definitive hosts (e.g. foxes, stray dogs) and appears to be rather inefficient for a variety of reasons (effort, cultural acceptance). (4) Deworming of wild and stray definitive hosts resulted in drastic reductions of prevalence in European and Japanese studies where anthelmintic baits were distributed, so methods for large-scale and focal application of baits are ready for application. However, sustainability and cost–benefit relations of this approach are controversial. As long-term application is necessary, the relevant political and financial commitment must be available from funding bodies (communes, counties, local government). In view of these difficulties, a ‚wait-and-see' approach may be justified, where human cases are rare and parasite frequency is stable. However, this approach requires close monitoring of the parasite frequency in humans and animals, risk information of the public and well developed medical infrastructure.

Characterization of *Lutzomyia longipalpis* interaction with *Leishmania* and virus

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Lutzomyia longipalpis is the major vector for visceral leishmaniasis in Brazil and also transmits some viruses. We are studying immune responses both in the insect vector and in the *L. longipalpis* LL5 embryonic cell line. Insect innate immune response pathways are Toll, IMD and JAK-STAT. We have identified an IMD and Toll response in LL5 cells through the silencing of the respective repressor genes caspar and cactus. This silencing produced the expected effect of increasing the expression of the antimicrobial peptides (AMPs) defensin and cecropin, thus establishing LL5 cells as a valid model to study sandfly immunity. We also investigated de effect of these repressors in female adult insects. We have previously determined a role for the IMD pathway in the vector infection by *Leishmania* through the silencing of the repressor caspar, which decreased infection. We now silenced cactus and, surprisingly, as opposed to LL5 cells, this led to the decrease of AMPs production. As a possible explanation, we verified that WntD, an inhibitor of the Toll pathway identified in *Drosophila,* had increased expression upon cactus silencing. This might be related to the preservation of the insect microbiota, which was actually decreased when cactus was silenced. We have also studied the effects of *Leishmania* infection on *L. longipalpis.* Infection caused an early increase of cactus expression followed by a return to normal levels that was accompanied by a higher expression of AMPs. Interestingly the infection by *Leishmania* also caused an early growth of the microbiota, probably related to the increase of cactus expression. We were intrigued by the apparent capacity of *Leishmania* to increase the expression of cactus and thus inhibit the Toll pathway. It is known that *Leishmania* Gp63 activates a macrophage tyrosine phosphatase (SHP-1), capable of inhibiting the Toll and Jak/STAT pathways. *L. longipalpis* has a homoloque of SHP-1, which has increased expression during *L. longipalpis* infection by *L. i. chagasi,* indicating the possible conservation of a mechanism of *Leishmania* infection control in mammals and insects.
We have previously identified a nonspecific antiviral response in LL5 cells in response to transfection with double stranded RNA (dsRNA). This was the first report of this kind of response in an insect cell line. We are presently identifying the mechanisms by which LL5 cells recognize dsRNA, using various approaches. We have performed deep-sequencing of transcripts from cells transfected with dsRNA or mock-transfected (control). These data are under analysis. We have also investigated the involvement of exosomes in the anti-viral response. Exosomes isolated from dsRNA transfected cells induced an antiviral response in LL5 cells. The exosomes protein composition was determined by mass spectroscopy and these data are under analysis. Among interesting proteins encountered specifically in dsRNA transfected exosomes was vir, a virus induced protein. miRNAs were detected in the exosomal fractions. These are presently being sequenced in search for differential small RNAS expression in cells transfected with dsRNA. We also prepared conditioned media from cells mock or dsRNA transfected and performed proteomics of the secreted proteins. Among candidates we found a scramblase, which in humans is an interferon-stimulated gene with an antiviral function. These and other candidates of interest are presently being validated.

Loss of heterozygosity in African trypanosomes causes metabolic changes leading to altered growth rates.

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BACKGROUND: The protozoan parasite *Trypanosoma brucei* is the causative agent of Human African Trypanosomiasis and Nagana disease in cattle. Together, these diseases present a major socio-economic burden to large areas of sub-Saharan Africa. Recent advancements in 'omics' technologies have enabled the investigation of whole cells systems ranging from genetics to metabolomics, thereby allowing us to dissect the relationship between genome, transcriptome and metabolome. Our laboratory recently discovered spontaneous loss-of-heterozygosity (LOH) events occurring in the genome of several strains of *T. brucei*. Whilst the trigger for this phenomenon is currently unknown, these genetic changes are clustered on the same chromosome and are associated with an increased growth rate phenotype in axenic culture. We are currently using genomics and metabolomics-based approaches to understand the mechanisms underlying these growth changes.
METHODS: The entire genome of wild-type TREU 927 *T. brucei* was sequenced using Illumina sequencing. Changes in the metabolome were investigated using LC/MS-based approaches.
RESULTS: It was established that LOH occurs on, and is limited to, a single chromosome in the *T. brucei* genome. In addition, mass spectrometry analysis of the cells shows that LOH results in diminished TCA cycle activity. Most importantly, the growth phenotype was found to exist only in a glucose-rich environment.
CONCLUSIONS: The data presented here indicates that changes in the genetic composition of one chromosome in *T. brucei* enables laboratory-cultured parasites to thrive in glucose-rich environments. The identification of this LOH phenomenon may also have wider implications for the effect of selection pressure on the evolution of the trypanosome genome in the field.

Host metabolism and intracellular *T. cruzi* growth.

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BACKGROUND: The intracellular amastigote stage of *Trypanosoma cruzi* is a critical target for vaccine and drug development to combat human Chagas' disease. Despite their importance, knowledge of the biology of intracellular *T. cruzi* amastigotes is limited, particularly the functional interactions between amastigotes and their host cells that facilitate growth and survival of these parasites. A recent genome-wide RNA interference screen conducted in HeLa cells identified host metabolic networks centered around energy production, nucleotide metabolism and lipid metabolism as central processes supporting intracellular *T. cruzi* growth. Focusing on host glucose and lipid metabolism, the current study builds on these findings with a view to defining the metabolic dependencies of intracellular *T. cruzi* amastigotes.
METHODS: Differential growth medium, targeted gene knockdown and extracellular flux measurements are exploited to characterize host metabolic phenotypes associated with altered amastigote replication rates in human fibroblasts and in induced pluripotent stem cell-derived human cardiomyocytes.
RESULTS: Intracellular *T. cruzi* amastigotes exhibit flexibility with respect to nutrient requirements for growth in host cells. Exogenous glucose and lipids are largely dispensable if glutamine is supplied. In the absence of glutamine, lipid-dependence emerges where impaired amastigote growth can be rescued with the addition of exogenous palmitate. The metabolic flexibility of the host cell is compromised by decreasing pyruvate dehydrogenase kinase (PDK4) activity. Targeted siRNA-mediated knockdown of PDK4 skews the fuel utilization balance away from fatty acid oxidation (toward glucose utilization) and results in reduced intracellular parasite growth. Experiments are underway to determine how perturbation of fatty acid flux in host cells impacts *T. cruzi* amastigote growth.
CONCLUSIONS: These studies provide new insights into host metabolic pathways that support *T. cruzi* amastigote growth and provide a platform for determining how intracellular amastigotes adapt to changing metabolic conditions in the host, a potential predictor for tissue tropism.

Vaccination with proteins involved in tick–pathogen interactions reduces vector infestations and pathogen infection

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BACKGROUND: Tick-borne pathogens cause diseases that greatly impact animal health and production worldwide. The ultimate goal of tick vaccines is to protect against tick-borne diseases through the control of vector infestations and reducing pathogen infection and transmission. Tick genetic traits are involved in vector–pathogen interactions and some of these molecules such as Subolesin (SUB) have been shown to protect against vector infestations and pathogen infection.
METHODS: Seven-month-old crossbred Anaplasma and Babesia free calves were assigned to 6 experimental groups of 3 animals each: vaccinated with TROSPA, vaccinated with SILK, vaccinated with Q38, vaccinated with SUB (positive control), injected with adjuvant/saline alone (placebo) and untreated (uninfected) controls. Calves were immunized with 3 doses on days 0, 28 and 49. Animals in vaccinated and control groups (placebo and untreated) were infested with *R. microplus* larvae in three separate cells for each animal on days 72, 75 and 77. Animals in vaccinated and placebo groups were then infected with *A. marginale* and *B. bigemina* on days 69 and 92, respectively. This experimental design allowed ticks to feed on vaccinated or placebo control cattle co-infected with both pathogens as well as on untreated and uninfected animals. Calves were evaluated for antibody response to vaccination and pathogen infection. Engorged female ticks dropped from the host on days 98–104 and were collected, counted and evaluated for tick weight, oviposition and pathogen infection levels. The mRNA levels of genes encoding for protective antigens were also characterized in engorged ticks.
RESULTS: The results showed that vaccination with Q38, SILK and SUB reduced tick infestations and oviposition with vaccine efficacies of 75% (Q38), 62% (SILK) and 60% (SUB) with respect to ticks fed on placebo control cattle. Vaccination with TROSPA did not have a significant effect on any of the tick parameters analyzed. The results also showed that vaccination with Q38, TROSPA and SUB reduced *B. bigemina* DNA levels in ticks while vaccination with SILK and SUB resulted in lower *A. marginale* DNA levels when compared to ticks fed on placebo control cattle. The positive correlation between antigen-specific antibody titers and reduction of tick infestations and pathogen infection strongly suggested that the effect of the vaccine was the result of the antibody response in vaccinated cattle. Vaccination and co-infection with A. marginale and B. bigemina also affected the expression of genes encoding for vaccine antigens in ticks fed on cattle. CONCLUSIONS: These results showed that vaccines using tick proteins involved in vector–pathogen interactions could be used for the dual control of tick infestations and pathogen infection.

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Molecular diagnosis of polycystic hydatid disease in humans and *Cuniculus paca* samples from República Bolivariana de Venezuela.

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The polycystic echinococcosis by *E. vogeli* represents an emerging disease in South America. The parasite involved is considered very aggressive and there is a lack of clinical pathology recognition. The aim of this work is to perform molecular diagnosis in human and lapa samples from Venezuela, previously confirmed by morphological studies.

Materials and methods: Four samples of polycystic hydatid localized in human liver (n = 3) and Cuniculus paca (n: 1) were isolated. The samples were preserved in alcohol 70° and sent to INEI - ANLIS "Carlos G. Malbran" Argentina, for molecular analysis. The extraction of genomic DNA from protoscoleces, was performed by an automated method (Kit MagNA Pure Compact Nucleic Acid Isolation I- ROCHE). Specific primers for *E. vogeli* were designed and tested (fragments of 324 and 188 bp of the mitochondrial gene cox1). PCR reactions were performed using two sets of primers. The products obtained were sequenced and obtained sequences were compared with the GENBANK database.

Results: Two of 3 human samples and lapa sample could be amplified and sequenced. The obtained sequences were 99 and 100% identical to the reference sequence of *E. vogeli* in GENEBANK.

Conclusions: This is the first molecular study in human and lapa samples of *E. vogeli*, involving these specific primer pairs. Being the hooks morphology, confirmatory for this species, the specific molecular diagnosis would identify parasite genetic material even in samples where no rostellar hooks can be found.

Immune Response to *Taenia solium* Calreticulin During Experimental Taeniosis in the Golden Hamster

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BACKGROUND: *Taenia solium* is a tapeworm that causes two diseases in humans. Research focuses mainly on the metacestode that causes neurocysticercosis, an important parasitic disease of the central nervous system. However the tapeworm carrier is the main risk factor for acquiring neurocysticercosis and little is known about the immunity induced in the small intestine. Calreticulin is a ubiquitous protein involved in cellular Ca^{2+} homeostasis, present in excretion/secretion products from several helminths and shown to induce predominantly Th2 responses. Our group identified and cloned rTscRT as a functional Ca^{2+}-binding protein and showed that the native protein is a component of ES products from tapeworms.
METHODS: We used a crude tapeworm extract (TsCE) and purified rTsCRT to analyze the humoral and cellular immune responses during experimental taeniosis in the previously standardized golden hamster model for *T. solium* infection. ELISA, lymphocyte proliferation assays and RT-PCR for cytokine determination were performed.
RESULTS: TsCE-specific serum antibodies were present in all infected hamsters while rTsCRT-specific IgG, only in 25-30% of infected animals. Upon *in vitro* stimulation of spleen or mesenteric lymph node cells, TsCE induced a Th2-polarized response characterized by the production of IL-4 and IL-5 during infection. In contrast rTsCRT significantly induced expression of the immune regulatory cytokine IL-10 in cells from uninfected as well as infected hamsters, in both mucosal and systemic lymphoid organs.
CONCLUSIONS: Our data suggest that there is a time- and microenvironment-dependent cytokine expression profile during *T. solium* infection and that rTSCRT might function as an immune evasion strategy by inducing IL-10 production at the local and systemic levels in order for tapeworms to survive and persist in nature.

The Current Status Efficacy of Artesunate/ Sulfadoxine Pyrimethamine Tablets for the Treatment of Uncomplicated Plasmodium falciparum Malaria in Great Wad Medani Locality, Gezira State, Sudan

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INTRODUCTION: In sudan the National Malaria Control Program adopted the use of artesunate + sulphadoxine-pyrimethamine (ASP) as the first line of treatment for uncomplicated malaria since 2004. This study was done in Medani town, Gezira State, Central Sudan to evaluate the current efficacy of ASP among infected patients within the national monitoring of antimalarial drugs.

METHODS: From October to December 2011, 81 Patients with uncomplicated *P. falciparum* malaria who met the study inclusion criteria were enrolled, treated with ASP and monitored for 28 days. The follow-up consisted of a fixed schedule of check-up visits and corresponding clinical and laboratory examinations. On the basis of the results of these assessments, the patients were classified as having therapeutic failure (early or late) or an adequate response to ASP according to the WHO (2005) susceptibility to antimalarial drugs protocol.

Blood samples from each patient were taken on Whatman filter paper (3M) on days 0, 7, 14, 21 and 28 and also the day when the parasite and symptoms reappeared for Polymerase chain reaction (PCR) to distinguish between a true recrudescence due to treatment failure and reinfection.

RESULTS*:* At the end of the follow up period (28 days) and before the PCR correction, 76/80(93.8%) of the 81 patients enrolled in the study were classified as had adequate clinical and parasitological response (ACPR), two (2.5%) as had late clinical failure (LCF) and late parasitological failure (LPF), two (2.5%) as lost to follow-up and only one patient (1.2%) as had early treatment failure.

The two patients whom were considered to have had late clinical failure (LCF) and late parasitological failure (LPF) when subjected to PCR correction. The result revealed that the late clinical failure and late parasitological failure were due to reinfection rather than due to recrudescence. Therefore the parasitological and clinical efficacy of ASP was found to be 98.7% (77/79)*.*

CONCLUSION: The finding of this study concluded that the first line treatment (ASP) for uncomplicated *P. falciparum* is still effective and monitoring its efficacy is recommended annually in all Sudan states .

KEYWORDS: Malaria, monitoring ASP efficacy, Sudan

Trypanosoma cruzi has a functional specie-specific actin variant.

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BACKGROUND: Trypanosomatid parasites are characterized by an atypical cytoskeleton mainly composed by microtubules. The presence of a conventional actin similar to the one found in animals and plants have been demonstrated in these parasites but no microfilaments have been detected by electronic microscopy. Interestingly, the bioinformatic analysis of the *T. cruzi* genome identified the presence of three other genes encoding for putative actins. Of these, Actin 2 (A2) has a 51% identity with conventional actin (A1) and it is only found in *T. cruzi* but not in *T. brucei* or *Leishmania*. In this study, we studied the expression of A2 in epimastigotes of *T. cruzi*.
METHODS: Expression of mRNA was analyzed by PCR using a cDNA library as template. Specific polyclonal antibodies were produced using a recombinant GST-A2 fusion protein and used in Western blot and immunofluorescence assays. Coimmunolocalization with the conventional actin in *T. cruzi* was also performed.
RESULTS: A2 gene is transcribed to mRNA, and its expression as protein was confirmed in parasites lysates by WB assays. The isovariant A2 was detected in epimastigote forms by immunofluorescence assays. Confocal studies demonstrated that subcellular localization of A2 is different from that of A1. While A1 is expressed as patches in the flagellum and as a dense concentration near its base, A2 is observed in a speckle pattern all over the parasite.
CONCLUSIONS: *Trypanosoma cruzi* actin 2 is a functional gene of the parasite. Considering its absence in other trypanosomatids, and the lack of colocalization with A1, A2 may be involved in different functions in the biology of *T. cruzi*.

Effect of oral administration of recombinant *Taenia solium* calreticulin (rTsCRT) in a mouse model of colitis: Histopathological findings

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BACKGROUND: Calreticulin is a highly conserved protein that participates in several cellular functions. This protein has been identified in numerous parasites and in *T. solium*. Oral vaccination with rTsCRT partially protects hamsters for *T. solium* infection and induces a Th2 immune response. The aim of this study was to evaluate the prophylactic effect of rTsCRT in an experimental model of colitis in mice.

METHODS: Female 6-8 week-old Balb/c mice were divided in five groups: groups 1 and 3 were orally immunized with 50 µg of rTsCRT weekly in four occasions, groups 2 and 4 received carbonate buffer alone. One week after the last oral immunization, colitis was induced in groups 3 and 4 by rectal instillation of 4 mg of trinitrobenzene sulfonic acid (TNBS) in 50% ethanol. Group 5 was rectally instilled with 50% ethanol. Three days later mice were necropsied and the large intestines were recovered, washed and a small piece of colon was fixed in 4% formaldehyde for histological processing. Sections of 4 µm were stained with hematoxylin and eosin and analyzed under the microscope. Inflammatory parameters such as epithelium integrity, cellular infiltrate, number of intestinal layers infiltrated and edema were scored.

RESULTS: No signs of inflammation were seen in control mice orally immunized with rTsCRT or buffer. In mice treated with ethanol slight hypersecretion of mucus and goblet cell hyperplasia was observed; while in the group treated with TNBS, loss of epithelium integrity, severe cellular infiltrate reaching the submucosa and high vascular density with edema was seen. In mice immunized with rTsCRT and treated with TNBS, colon inflammation and leukocyte infiltration around crypts were low with moderate vascular density, slight hypersecretion of mucus and goblet cells hyperplasia were observed.

CONCLUSIONS: rTsCRT reduces microscopic inflammatory parameters in experimental colitis induced by TNBS suggesting that rTsCRT has immunomodulatory properties, however more studies are needed to elucidate the mechanisms involved.

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Human fasciolosis cases in Mexico, from 1895 until nowadays

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BACKGROUND: Fasciolosis is a parasitic disease produced by *Fasciola hepatica* and *F. gigantica*. They can be differentiated only by the morphology of the adult parasite or molecular methods. This trematode affects herbivorous animals and occasionally, humans. It is considered a veterinarian important disease with economic loss in cattle. Human fasciolosis produces bile duct pathology. *F. hepatica* is present in all the American continent. Humans are considered important accidental definitive hosts for *F. hepatica*. Human fasciolosis shows serious clinical symptoms; however, there is not much information regarding human infections caused by *F. gigantica*.
In Mexico, Toussaint in 1895 reported the finding in an autopsy on a human, of structures that seemed distoma, found in the lungs, leading him to think of erratic fasciolosis. In 1936, Caballero reported the first human case of native fasciolosis in the country, diagnosed in a boy who had high eosinophilia in whom, in coproparasitoscopic studies, the presence of *F. hepatica* was frequently reported. Since then, a series of human cases of such parasitosis has been reported.
Few cases of human fasciolisis have been reported in Mexico, most diagnosed as surgical findings.
METHODS: An exhaustive search in literature was performed to find cases of human fasciolosis in Mexico, from the second half of the 19th century, the whole 20th century, and the first years of the 21st century.
RESULTS: Since 1895 until the 21st century, 126 cases have been documented, 38.4% diagnosed by surgical finding.
CONCLUSIONS: We consider that fasciolosis in humans is more frequent than it has been reported, since the etiologic diagnosis is not performed, mainly because this parasitosis is not suspected by the attending physician. Hence, it is advisable to include a routine concentration coproparasitoscopic study as part of common medical care in those regions where fasciolosis is an endemic disease in cattle, specially, due to the significant increase of cases worldwide.

Rapid genotyping of Echinococcus granulosus using High Resolution Melting (HRM) analysis by focused on Single Nucleotide polymorphism in locus 3 in Iranian isolates

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3-Research Center of Tropical and Infectious Diseases, Kerman University of Medical Sciences, Kerman, Iran.

BACKGROUND: Echinococcus granulosus, causes hydatid cyst in human and animals, is one of the most important zoonotic parasitic diseases. High resolution melting (HRM) analysis, combines PCR chemistry with fluorescent probes for detection of DNA fragments. HRM is provides rapid, low-cost and sensitive scanning method for detection of variation in DNA sequencing. In this study we have tested a HRM method for discriminating common genotypes of E. granulosus in Isfahan, Iran.
METHODS: One hundred forty-one hydatid cysts were collected from infected slaughtered animals in different parts of Iran during 2013. After DNA was extracted from all of the samples, the mitochondrial cytochrome C oxidase (cox1) was amplified using polymerase chain reaction coupled with high resolution melting curve (HRM)
RESULTS: Out of 141 isolates, 11 isolates were sterile and excluded from further investigation. A result of HRM PCR analysis using the partial sequences of cox1 gene showed that 93, 35, and 2 isolates were identified as G1, G3, and G6 genotypes respectively. We found one SNP polymorphism in critical locus 3. This position is critical loci for differentiation between G6 and G7 genotypes. In phylogenic tree this isolate was located between G6 and G7 genotype, which suggest this isolate may be intermediate strain G6 and G7.
CONCLOUSIONS: As G7 genotype has not been reported before in Iran, this intermediate genotype may be suggested the existence of G7 genotype in this region. However, in this study we have not confirmed G7 genotype but it raises the chance of existence in Iran. Therefore, this variation could suggest a new phenotype in Iran. For that reason we recommended that in future studies, using specific primer for any genotype and finer techniques for detection other latent genotype or other SNP in critical loci in Iran.

Frequency distribution of blood-tissue parasitic infections in patients with multiple sclerosis (MS), as compared to the control group (Isfahan, Iran, 2013)

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BACKGROUND: A general look at the status of health clearly reveals that in today's modern societies compared to the past decades, there has been considerable advances and growth in control of infectious diseases, especially in control of water, food and insect- borne diseases. On the other hand, today diseases that are recognized as autoimmune are on the increase. In this article, efforts have been made to investigate the frequency distribution of blood tissue parasitic infections in patients with multiple sclerosis (MS), in comparison to a control group in Isfahan province in 2013, and also to take another look at the interaction and the link between these diseases. Parasites studied included those capable of long-term stimulation of the immune system, causing chronic diseases.
METHODS: This was specifically an epidemiological study in which case and control groups consisted of 50 patients with multiple sclerosis and control group consisted of 50 family members of these patients (for consistency in socio-economic status). Serum samples were analyzed for anti-*Toxoplasma gondii* IgG and IgM antibodies using a commercially available ELISA kit, and the data was analyzed using SPSS.
RESULTS: :Of the study population,36% in case group and 49% in the control group had positive test in IgG and IgM Toxoplasma gondii were negative .However, the difference between the two groups was insignificant. In terms of other infections (cutaneous leishmaniasis, and malaria), there was no significant difference between the two groups, either. In addition, health education towards avoiding eating undercooked and raw meat or milk, and avoiding contact with cats were recommended, especially during pregnancy.
CONCLUSIONS: In this study, attempts were made to examine the relationship between MS and a few parasitic diseases. The results showed light the relationship between these diseases and a mismatch with hypotheses examined. However, considering that the prevalence of toxoplasmosis, leishmaniasis, and malaria varies with time, and depends on numerous epidemiological factors, hence, at the time of this study, this rate was low in the control group, as well. These results do not discredit the theory investigated. It is recommended that these theories should be verified with a larger sample size, or by infecting animal models with these parasites to examine the clinical changes in MS disease in them.

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Abstract

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Foodborne is not only meatborne. Other foods as *Toxoplasma* infection sources

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With the greatest disease burden of all parasitic infections, toxoplasmosis has recently been established by the WHO and FAO as a foodborne infection of global concern. Transmission of toxoplasmosis occurs by ingesting tissue cysts from undercooked meat and meat products, and oocysts from the environment with contaminated fresh produce or water. The meat route has been intensively explored, in parallel with the development of serological and molecular tools that allowed for the analysis of meat/meat products. The lack of such tools has left the oocyst stage much less studied, and until recently it was virtually impossible to distinguish if a case of toxoplasmosis was caused with the tissue cyst or the oocyst. Several lines of evidence have linked oocyst infection to cases of acute toxoplasmosis, including hydric epidemics, case-control studies which showed consumption of fresh produce as in infection risk factor, and experimental studies showing the adherence of oocysts to fruits such as berries that produced infection in mice, (although the latter has yet to be confirmed in natural settings). Only the recent development of a sporocyst-specific antibody allowed for clinical studies which showed that 43% of infections in pregnant women in Chile and even 78% in mothers giving birth to congenitally infected children in the USA, were caused by oocysts. Importantly, oocyst-induced infections appear to be clinically more serious than those caused by tissue cysts, and in fact most often induce clinical infection. Thus, alteration of the concept that *T. gondii* infections are mostly subclinical has even been suggested. Oocyst sources for human infection include soil and water, directly or indirectly through contamination of produce. Additional sources increasingly gaining importance include marine mammals, as well as filter-feeding invertebrates such as mussels and oysters, that do not get infected but can concentrate viable oocysts and serve as an infection reservoir for marine predators and humans. Continuous climate and man-made environmental changes favor an increase in oocyst-induced infections in both humans and animals, calling for the development of commercial technologies to detect oocysts in produce as well as for strategies for large-scale detection of oocysts in terrestrial and aquatic environments.

Neuroimmunomodulation of IL-10 and IFN-Ɣ production by macrophages and neutrophils in the presence of a 220 kDa lectin from *Entamoeba histolytica in vitro*.

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BACKGROUND: Neuroinmunorregulation of inflammation is performed by adrenergic and cholinergic receptors in macrophages and neutrophils. On macrophages, Nicotine acts on alpha 7 nAChR inhibiting inflammatory signals; on neutrophils, it alters superoxide anion production, chemotaxis, cytokine production, and apoptosis. Norepinephrine through the adrenergic alpha-1R exerts pro-inflammatory effects and inhibits the β1 and β2 adrenergic receptors. Macrophages and neutrophils are important cells during an immune response against *E. histolytica*. A 220 KDa lectin (L220) from *E. histolytica* is capable of down-regulating certain inflammatory cytokines such as IL-5, IL-6, INF-γ, TNF-α in macrophages, therefore the aim of this work was to determine if L220 may exert a neuroimmunomodulation of inflammatory cytokines in immune cells.
METHODS: Cells were purified by ficoll/histopaque and Percoll, from healthy donors, and adjusted to 5 X 10^5 cells/ml; neutrophils were placed in culture dishes with RPMI, and macrophages/monocytes were separated with magnetic beads and left to mature into macrophages with 0.3 µl of MCSF IL-3 for 48 h. Then, both cell types were incubated with: (M) RPMI, (ML) RPMI + lectin; (MLE) RPMI + lectin + epinephrine; (MLES) RPMI + lectin + esmolol; (MLB) RPMI + lectin + Vecuronium Bromide; (MLN) RPMI + lectin + nicotine. Supernatants were obtained at 0, 15, and, 30 min; 1, 2, 4, 8, and 16 h, in the presence of 2 µl of *E. histolytica* L220 and the different neurotransmitters were added as follow: E = 5.49, B = 4.7, ES = 1, N = 15.20 X 10^{-4}M, and Quantification of IL-10 and IFN-Ɣ was performed by immunoassay Kit (IL-10 Invitrogen KHC0104, Carlsbad, CA, USA, and IFN-Ɣ EASIA KAC1232 BIOSURCE, Nivelles, Belgium).
RESULTS: IL-10 production by macrophages peaked at 30 min, producing 12 pg/ml. This production was inhibited in 50% by L220; 45% by epinephrine; 60% by vecuronium and 70% by nicotine. Neutrophils did not produce IL-10, however, they produced an average of 1 pg/ml in the presence of vecuronium and nicotine. This occurred at all-times interactions.
CONCLUSIONS: L220 inhibits the production of IL-10 and IFN-Ɣ by macrophages, whereas neutrophils produce IL-10 in the presence of a nicotinic antagonist, and increased production of IFN-Ɣ by esmolol.

Mucus and immunity to *Trichinella spiralis*: A systemic affair.

Grencis Richard, Campbell Laura, Whittingham-Dowde Jayde, Gallagher Amanda, Hussell Tracy, Roberts Ian & Thornton Dave.

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BACKGROUND: *Trichinella spiralis* induces a strong Type 2 cytokine response to infection. Type 2 cytokines drive expulsion of the parasite from the intestine by generating a broad inflammatory response that includes intestinal mast cell and goblet hyperplasia. *T. spiralis* also has a prolonged parenteral phase in the muscle. The present work focuses on the mucosal goblet cell response both within the intestine and in the lung as an example of a non-parasitised epithelium to explore the broader systemic response to infection. METHODS: C57BL/6 mice were infected with *T. spiralis* and goblet cell responses and mucin analysis investigated in the small intestine and lungs. In addition the mucin and goblet cell response was investigated in immunodeficient SCID mice.
RESULTS: The data demonstrates that in addition to a profound goblet cell hyperplasia in the small intestine, *T. spiralis* induces a strong goblet cell hyperplasia within the lungs (characterized by elevation of Muc5B and Muc5Ac). Muc5Ac was found to be up-regulated at both sites in immunocompetent and immunodeficient animals mice even after treatment to remove innate lymphoid cells, which are known to be potent producers of cytokines controlling goblet cell production such as interleukin 13.
CONCLUSIONS: The results suggest that *Trichinella* drives a systemic mucosal response characterised by the production of Muc5ac producing goblet cells at sites distinct from the infection site. This response can occur in the absence of adaptive immunity and does not appear to require innate lymphoid cells although further investigation is required to conclusively show this.

Chronic infection by *Trichuris muris* profoundly changes the microflora and metabolome of the infected caecum.

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BACKGROUND: Intestinal nematode infection typically presents as a chronic infection. This is associated with modulation of the host immune response to the parasite preventing worm expulsion and modifying intestinal pathology. Furthermore using mouse whipworm (*Trichuris muris*) as a model of infection we have shown that a dynamic relationship exists between the host microflora and the parasite. The present study was designed to define the effect of long term infection upon the host intestinal microbiome together with the effect of antihelmintic treatment and worm clearance upon changes in intestinal microflora and host immune response.
METHODS: C57BL/6 mice were administered a low dose infection *T.muris* infection. Animals were sampled throughout infection for changes in caecal and faecal microflora populations together with deep sequencing. Changes in immune populations of the caecum were also monitored at selected time points. Moreover, some animals were treated with albendazole after patency to examine the effect of parasite removal on the microbial populations.
RESULTS: Low dose infection by *T. muris* has a clear effect upon the microbial populations of the large intestine, with a clear reduction in diversity of species over time. Significant changes only become apparent after three weeks of infection but persist up to three months (when the study was ended). Anthelminitc treatment not only removes parasites but also induces changes in intestinal microflora with microbial populations in worm free animals gradually returning to those found in naïve animals.
CONCLUSIONS: Our results suggest that chronic intestinal nematode infection can significantly modify gut microflora over prolonged periods. As a consequence host metabolism and the host immune response will operate significantly different to that in naïve animals. The functional consequences for host and parasite remain to be defined.

A prospective clinical study evaluating the role of colchicine or vitamin E in combination with praziquantel in treatment of human S. *mansoni*

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BACKGROUND: Schistosomiasis is an endemic disease representing a public health problem in Egypt. This study was carried out to evaluate the efficacy of colchicine and vitamin E in combination with praziquantel (PZQ) in controlling *S. mansoni* infection and complications. METHODS: The study included 72 schistosomiasis infected patients classified into three groups: I- 24 patients with men serum procollagen type III amino terminal propeptide (PIII NP) concentration 8.28 ± 9.66 µg/L, treated with with PZQ 60 mg/kg body weight in two divided doses at 6 hours interval. II- 24 patients with mean serum PIII NP concentration 13.9± 33.10 µg/L, treated with PZQ (60 mg / kg bwt) in two divided doses at 6 hours interval and colchicine 500 µg twice daily for two months. III- 24 patients with mean serum PIII NP concentration 5.09±4.01 µg/L, treated with PZQ (60 mg/kg bwt after treatment) in two divided doses at 6 hours interval plus Vitamin E 400 mg twice daily for two months. In addition to 24 healthy volunteers (control group) received no treatment. Blood samples were taken from all individuals before the start of treatment, two hours after treatment and two months after treatment for measuring serum PIII NP by radioimmunoassay (RIA); PZQ by high performance liquid chromatography (HPLC); and serum circulating S. *mansoni* antigen by fast-dot enzyme linked immuonosorbent assay (ELISA).
RESULTS: Serum circulating schistosome antigen exhibited a reduction of 95.8%, 91.7% and 95.8% respectively two months after treatment compared to before treatment levels. However there was a non-significant reduction in serum PIII NP. Group II (PZQ plus colchicine) and group III (PZQ plus Vitamin E) showed a higher reduction rate of serum PIII NP compared with group I (PZQ alone). The study suggests the use of serum circulating *S. mansoni* antigen is necessary for confirming diagnosis especially in patients with negative stool for assessment of drug therapy.
CONCLUSIONS: The use of PZQ in a dose of 60mg/kg bwt in two divided doses at 6 hr interval is considered a highly effective and safer dosage regimen. The use of colchicine and vitamin E as adjuvants to PZQ can improve schistosomal hepatic and intestinal complications and clinical picture.

Molecular tracking of Chagas disease outbreaks by possible oral transmission route in Colombia

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BACKGROUND: Orally transmitted Chagas disease has been widely informed in Latin America. In Colombia, there have been annually reported several human oral outbreaks since 2008, representing a new epidemiological scenario. *Trypanosoma cruzi* presents significant genetic variability evidenced through six Discrete Typing Units (DTUs), TcI-TcVI.
METHODS: We obtained GEB samples of symptomatic patients (33), asymptomatic contacts (11), post-mortem tissues (1 patient), reservoirs (32 dogs and 5 opossum *D. Marsupialis*) and vectors (12) involved in two Chagas disease outbreaks of possible oral transmission that occurred between February and April of 2014 in the Orinoquía Region (Eastern Colombia, departments of Meta and Casanare). The diagnosis was performed by direct parasitological methods, conventional serology, conventional PCR, qPCR, and typing by using SL-IR, 24Sα and 18S regions.
RESULTS: In symptomatic patients, direct parasitological methods were positive in 40%, hemoculture in 12%, conventional serology in 84% and qPCR in 80% of patients with parasitic loads ranging from 1,7 to 21,3 equiv. parasites/mL. The DTUs detected in these patients were $TcI_{sylvatic\text{-}like}$. The 54.5% of reservoirs were positive by qPCR and simultaneously infected with $TcI_{sylvatic}$, TcIII and TcIV. The vectors collected, *Rhodnius pictipes* and *Pastrongylus geniculatus,* were positive by qPCR and were infected with$TcI_{sylvatic.}$
CONCLUSIONS: Our results evidenced the incrimination of sylvatic populations of *T.cruzi* associated with sylvatic vectors and reservoirs in these two oral human outbreaks. These findings evidence the need of implementing new initiatives of control and prevention to face new transmission scenarios of Chagas disease.

Total IgE and IgG levels in cases of co-infection with malaria and geohelminths

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BACKGROUND: In tropical regions, co-infection with various parasites, including *Plasmodium* spp. and geohelminths, can occur. Interactions between these parasites can influence the human immune system. This study sought to measure total immunoglobulin G (*IgG*) and immunoglobulin E (*IgE*) concentrations in patients with uncomplicated *P. falciparum* malaria and in control subjects without malaria and to compare the concentrations observed in the absence and presence of co-infection with geohelminths.
METHODS: Serum and stool samples were obtained from a previous study that examined cases and controls matched for age, sex, and location. Cases refer to patients with uncomplicated P. falciparum malaria, and controls refer to patients without malaria. Total IgG and IgE concentrations and the prevalence of infection by geohelminths were determined for both the case group and the control group. Conditional logistic regression was performed for high/normal IgG levels (with 700-1600 mg/dL and >1600 mg/dL regarded as normal and high IgG levels, respectively) and for high/normal IgE levels (with <280 IU/mL and >280 IU/mL regarded as normal and high IgE levels, respectively, for subjects from 3 to 16 years of age and with <200 IU/mL and >200 IU/mL regarded as normal and high IgE levels, respectively, for subjects who were >16 years of age). The regressions accounted for the matching of cases and controls and were adjusted for the presence of geohelminths and for multiplicative interactions between geohelminths and malaria parasites.
RESULTS: A total of 224 subjects, including 161 controls and 63 cases, were examined in this study. Geohelminth infection was present in 52.38% of cases and 44.72% of controls. The overall prevalences of *Ascaris lumbricoides*, *Trichuris trichiura*, and *Uncinaria* sp. were 29.02%, 20.98%, and 22.77%, respectively. The associations with high/normal total *IgG* levels were an odds ratio (OR) of 2.19 (95% confidence interval (CI): 0.81 to 5.90) for infection with *P. falciparum* alone, an OR of 1.51 (95% CI: 0.65 to 3.51) for infection with geohelminths alone, and an OR of 0.53 (95% CI: 0.11 to 2.58) for the malaria-geohelminths interaction. The associations with high/normal *IgE* levels were an OR of 0.71 (95% CI: 0.15 to 3.29) for infection with *P. falciparum* alone, an OR of 1.66 (95% CI: 0.39 to 6.97) for infection with geohelminths alone, and an OR of 1.70 (95% CI: 0.10 to 27.1) for the malaria-geohelminths interaction.
CONCLUSIONS: Neither total *IgE* nor total *IgG* levels were significantly correlated with uncomplicated *P. falciparum* malaria, the presence of geohelminths, or concurrent infection with both *P. falciparum* and geohelminths.

Search of helminthes in undernourished cattle of Catazajá, Chiapas (Mexico)

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BACKGROUND: Catazajá is located at northeastern of Chiapas State in Mexico. The warm and humid climate enhance the growth of tropical forest, which is gradually substitute by farmland and livestock. Cattle are important to maintain the productivity conditions in the region; however, there are ranches with undernourished bovines. The weight loss of cows could be caused by nutrient deficiency or, by the presence of parasites. The aim of this work was to search for helminthes in cattle of Catazajá, Chiapas by means of the Kato Katz technique and by antibody detection.
METHODS: A total of 134 fecal samples and 295 sera from bovines of 5 herds in Catazajá ranches were collected. Stool samples were processed by Kato Katz technique to identify the presence of helminthes eggs and antibodies against Trematodes, Cestodes and Nematodes were determined by ELISA
RESULTS: In general, the stool samples showed the presence of yeast, pollen and vegetable fibers; regarding to parasites, only in one stool sample *Trichiuris* eggs were find. Currently, an ELISA is validated to determine to the frequency of antibodies against the tissue helminthes.
CONCLUSIONS: Contrary to the expected, Catazajá is a region of high prevalence of parasites; however, no geohelminthes were found, thus problems of malnutrition in cattle could be due to other causes than parasites. Further studies are in progress to determine the presence of tissue helminths such as *Fasciola hepatica, Taenia saginata and Trichostrongylus*.

Teaching Parasitology at the Faculty of Medicine, UNAM

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BACKGROUND: The Faculty of Medicine of the National Autonomous University of Mexico (UNAM) offers a bachelor degree in medicine, which is organized in assignment focused by competencies. During the second year, Microbiology and Parasitology is taught, which consists of four units: Bacteriology, Virology, Mycology and Parasitology. The purpose of this presentation is to share the teaching experience in Parasitology at the Faculty of Medicine.
METHODS: The yearly enrollment is around 1200 students divided into 42 groups; each group has 2 teachers with ages ranging from 25 to 75 years old. In order to uniform the learning in the course, teachers and students receive manual and digital tools that include theoretical and practical information to support the curriculum. During Parasitology unit, biological aspects of parasites, pathogenesis, signs, symptoms, laboratory techniques, treatment and prevention are reviewed. A new teaching system was recently implemented during the practical session; which consists of reviewing a clinical case, where the teacher together with the students, answer a series of questions, ranging from the identification of signs and symptoms in order to establish a possible diagnosis, to the discussion of the clinical approach and laboratory assays for its confirmation; afterwards, students perform parasitological techniques related to the previous discussed case.
RESULTS: Preliminary results have shown that our new system has strengthened Parasitology teaching among students. A questionnaire applied to graduated students of the third year and who are engaged in clinical practices in hospitals, showed that their performance in clinical aspects of Parasitology was better, particularly regarding the approach of their patients.
CONCLUSIONS: One of the main objectives of this new system was to reduce traditional teaching as conference-based to stimulate student participation. So far, our results on the implementation of a novel education system suggest that it is useful for transmission, retention and application of knowledge in the field of Parasitology among students.

Studies on the elimination of Onchocerciasis in southeastern Nigeria: epidemiological evaluation of transmission foci on the Imo river basin

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BACKGROUND: Community directed treatment with Ivermectin (CDTI) has been on - going for close to two decades in Onchocerciasis hyper-endemic communities of Imo and Abia States Nigeria. This study evaluates the current status of Onchocerciasis in some pre-CDTI (1994) hyper- endemic communities located on the Onchocerciasis transmission zone of the Imo River Basin.
METHODS: Institutional ethical approval was given; written consents were obtained from the State Ministry of Health, the Public Health Department of the study Local Government Areas (LGAs) and Traditional rulers. Participants gave their consent. Eight communities from Isiukwuato and Umunneochi LGAs of Abia State and nine communities from Okigwe LGA of Imo State on the Imo river basin foci, that were hyperendemic pre-CDTI for onchocerciasis with 1994 survey, were selected for the study. The selected communities had ≥65% therapeutic coverage. Consenting villagers were physically examined for onchocercal nodules. Thereafter, skin snips were collected from the iliac crest using corneoscleral punch to determine the community microfilarial load (CMFL). At the end, nodulectomy was carried out on consenting positives for palpable onchocercal nodules; excised nodules were subjected to histological preparations and examination to determine the impact of repeated treatment with ivermectin.
RESULTS: Seven out of the nine communities examined from Abia State part of the Imo River Basin focus, had 0.0 mf/mg CMFL while the remaining two communities had 0.28 mf/mg CMFL. Results from Imo State part of the transmission focus also showed that three out of the nine communities had zero CMFL while the remaining six communities had between 0.71mf/mg and 1.33 mf/mg CMFL. Results interpreted epidemiologically in line with WHO ONCHOSIM model trend in prevalence after ivermectin treatment are satisfactory since the observed CMFL is equal or lower than the predicted CMFL. Severe dermatological conditions of Onchocerciasis were not observed. Sections of adult worms on histological slides showed degenerated worms throughout while 63.4 % of villager studied claimed they had nodules but their nodules had disappeared
CONCLUSIONS: CDTI in the Imo river basin transmission focus has significant impact on the community microfilarial load and adult females of *Onchocerca volvulus*. This effect causes the elimination of skin microfilariae thereby ensuring the difficulty for the vector flies, *Simulium,* to pick-up skin microfilariae during blood meal. It is therefore the strong view that the on-going large-scale ivermectin distribution through CDTI in the Imo river basin of Abia and Imo States has lead to the interruption of transmission and that elimination of the disease is achievable. To confirm that the breakpoint has been reached and that treatment can be safely stopped in this focus, a detailed entomological evaluation exercise needs to be conducted to assess the residual infection and transmission levels. This should be based on pool screening of biting black flies collected throughout the breeding season from these high-risk locations along the Imo river basin near major breeding sites of the vector.

TRYPANOSOMA CRUZI INFECTION IN RESIDENTS WHOSE HOUSES HAD POSITIVE TRIATOMINES

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INTRODUCTION: In Peru, Chagas disease is a public health problem, especially in the Arequipa Region where *Triatoma infestans* is the only vector. *Trypanosoma cruzi* infection is not exclusively present in rural areas, and the presence of infected triatomines has also been reported in urban areas. In the present work, sera from 676 individuals, in whose houses positive *T. cruzi* triatomines were found, were analyzed; by origin, these individuals were distributed as follow: Hunter 25, Tiabaya 184, Sachaca 127, Uchumayo 81, Socabaya 58, JL Bustamante y Rivero 25, Paucarpata 40, Mariano Melgar 72, Cayma 36, Yura 28.
METHODS: A sample of 3 ml of blood was taken from each participant and sera were obtained to perform serological studies using Elisa kit Chagatek (valuing qualitative and quantitative) and indirect immunofluorescence (IIF) for Chagas disease. Finally, seropositive individuals underwent xenodiagnosis using 20 third instar nymphs; after 30, 60, and 90 days, droppings nymphs xenodiagnoses applied were reviewed.
RESULTS: Serology was considered positive when both tests were positive, yielding 115 seropositive cases: Hunter 32.0 % (8 /25), Tiabaya 16.8 % (31/184), Sachaca 22.1 % (28 /127), 21.0 % Uchumayo (17/81), Socabaya 6.9 % (4/58), JL Bustamante y Rivero 4.0 % (1 /25), Paucarpata 7.5 % (3 /40), Mariano Melgar 19.4 % (14 /72), Cayma 13.9 % (5 /36), Yura 14.3 % (4 /28). Xenodiagnosis applied to 115 seropositive participants resulted in 16.5 % of positive cases for *Trypanosoma cruzi*.
CONCLUSIONS: We conclude that these districts were active in the transmission dynamics of *Trypanosoma cruzi* from *Triatoma infestans*.

Parasite antioxidant mechanisms and immunomodulation

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The host-parasite interface interaction has been matter of study since the discovery of the fact that the events and conditions occuring at this level and around it such as the microbiota, the secreted parasite molecules and the immune competence of the host altogether could determine the course of the infection. Thus, understanding the mechanisms that are intereacting acquires relevance in the matter of preventing or reversing the inflammatory effects from diverse sources that could even gradually lead to immune chronic diseases. Of particular interest is the redox state at the host-parasite interface and the effects of the parasite antioxidant elements as immunoregulatory elements. It is well known that ROS and other molecules of the redox homeostasis maintenance are important signaling molecules. Dietary antioxidants research has not proved the total effectiveness of these as therapies for some inflammatory diseases. However there is recent evidence that parasite infections can reduce the damage in inflammatory diseases such as multiple sclerosis, where oxidative stress plays a major role. The antioxidants role as possible immunomodulators at this level of host-parasite interface is not known at all. The present review aim is to gather the evidence suggesting the latter hypothesis, especially of the antioxidant molecules immunological activity through the symbiosis interaction. Finally, it is important to expose the future perspectives of the understanding and characterizing the antioxidant molecules as future possible vaccines or therapies.

A single fish species but how many *Gyrodactylus* species? An African case of *Clarias gariepinus*

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BACKGROUND: In natural habitat sharptooth catfish, *C. gariepinus* (Burchell, 1822) is widely distributed in Africa. Currently African catfishes are known to be hosts to 12 *Gyrodactylus* von Nordmann, 1832 species, of which seven have been described from *C. gariepinus*.
METHODS: During the period August 2011 - May 2013, several localities were sampled to establish gyrodactylid parasites diversity in the southern African region. In total 28 specimens of *C. gariepinus* were collected on several spots in South Africa (Flag-Bushielo Dam and Loskop Sand River, Limpopo Province) and Zimbabwe (Zambezi River and Lake Kariba). Parasites were removed from fins and gills, fixed on the slides in glicerin ammonium picrate for morphometric analyses and in 96% ethanol for molecular characterization.
RESULTS:The *Gyrodactylus* prevalence was noted to be 43%. Species identification based on hard parts morphometry and nuclear rDNA internal transcribed spacer (ITS) sequences identified the presence of 8 different *Gyrodactylus* species, of which three are currently known, *G. gelnari* Přikrylová, Blažek & Vanhove, 2012, *G. rysavyi* Ergens, 1973 and *G. transvaalensis* Prudhoe &Hussey, 1977. Detailed morphological analyses revealed clear differences in the shape and size of taxonomically important structures between the species examined. Phylogenetic analyses, the maximum likelihood, neighbor joining and Bayesian inference analyses, assisted in revealing interspecific relationships.
CONCLUSIONS: Present finding reveals unexpected *Gyrodactylus* species diversity and supports probable intensive parasite speciation among one host genus.
This study was supported by grant GBP505/12/G112 of the Academy of Sciences of the Czech Republic and the research done in South Africa was founded by the University of Limpopo and the National Research Foundation (NRF)

The funtional role of IL-4 and IL-13 target cells in cutaneous leishmaniasis

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BACKGROUND: Murine models of Leishmania major infection in the 1980s revealed two distinct, counter-regulatory populations of $CD4^+$ T helper (Th) cells, delineated Th1 and Th2, and their archetypal cytokines, interferon gamma (IFN-γ) and interleukin (IL)-4/IL-13, which promoted resistance/susceptibility to infection, respectively. However, the introduction of global cytokine-deficient mice in the 1990s revealed pleiotropic immune-regulatory mechanisms of IL-4 and IL-13 that either controlled or exacerbated disease. This undermined the basic premise that IL-4/IL-13 played paramount roles in facilitating a non-healing Th2 response to Leishmania infection and instead suggested that both IL-4 and IL-13-dependent and IL-4/IL-13-independent factors orchestrate disease outcome.
METHODS: The recent characterization of cell-type specific IL-4Rα deficient mice was initiated to help reconcile these observations and dissect the cell-specific effects of IL-4/IL-13 during infection
RESULTS: I will summarize original and recent findings with regard to the role of IL-4 and IL-13 in cutaneous Leishmaniasis. Using the information discerned from various studies and our conditional IL-4Rα gene-deficient mice, we particularly discuss the double-edged sword IL-4 (and in some Leishmania disease models IL-13) in driving a susceptible Th2 response, their immune cell targets that support healing or non-healing responses and their role in mediating a Th1 response during disease. Moreover, new results on the role of B cells will be presented.
CONSLUSIONS: Taken together, the immunobiology of *Leishmania* infection seems to be a labyrinth of interconnected pathways rather than a rigid dichotomy between Th1 and Th2 as originally proposed.

Ultrastructural study on diplozoid monogeneans – new revelations and perspectives

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BACKGROUND: Diplozoids (Monogenea) are blood-feeding fish ectoparasites with specific cross body arrangement of two permanently fused adult individuals. Digestive tract (two large buccal suckers, muscular pharynx, branched gut) and vitellaria are situated in the anterior part of body. The reproductive organs and terminal part of the gut are situated in the posterior part. The attachment apparatus of adults consist of 4 pairs of clamps and a pair of small central hooks situated on the ventral side of the opisthaptor of each worm. Digestive tract constitutes a highly specialised system for hematophagous feeding life style. Whereas these conspicuous morphological structures have been frequently studied, details of internal morphology are little known.
METHODS: Parasite specimens collected were fixed with 2% osmium tetroxide, dehydrated with anhydrous acetone, infiltrated and embedded in Spurr resin. Semi-thin and thin sections were prepared using a Leica EM UC6 ultramicrotome and examined with a JEOL JEM 1010 transmission electron microscope. Observed cells and fine structures were compared with results on related parasite taxa for better understanding and recognition.
RESULTS: Fine structures of mouth, buccal suckers, pharynx and some other digestive tract details are presented. Also some details of tegument and excretory system are included.
CONCLUSIONS: These results contribute to the understanding of diplozoids life strategy and are helpful for the comprehension of the specific blood digestion strategy. Diplozoid monogeneans are a valuable experimental model for exploring the processes between the structure and function of morphological features in monogeneans.
Financial support: Czech Science Foundation, grant GAP506/12/1258

Molecular Bases of Pyrethroids Resistance in the Cattle Tick *Boophilus microplus.*

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Dialelic crosses and backcrosses of pyrethroid resistant (RR) and susceptible (SS) *Rhipicephalus* (*Boophilus*) *microplus* tick strains were carried out and the substitution (Phe-Ile) within the sodium channel gene was monitored in order to analyze the effects of the genotype on the pyrethroid resistance phenotype as measured by the larval packet test (LPT). Parental strains: susceptible (SS) and Resistant (RR); dialelic crosses: RS (♂RRx♀SS), and SR (♂SSx♀RR); and backcrosses: RSxSS, RSxRR, SRxSS and SRxRR were infested on 280 kg calves. Resistance type (monogenic or polygenic) and effective dominance was determined based on the discriminant concentration (DC) for cypermethrine (0.5%), deltamethrine (0.09%) and flumethrine (0.01%). Allele specific PCR (AS-PCR) was used for genotyping, looking at a sodium channel mutation (Phe-Ile substitution). The mortality rates and allele frequency of susceptible and pyrethroid resistant reference strains were 0% mortality and 90% RR alleles for resistant strain, and 100% mortality and 0% RR alleles as measured by the larval packet test (LPT) and allele specific PCR (AS-PCR) respectively. Backcrossed strain SRxRR showed an effective dominance (DML) of 0.605 for cypermethrin, 0.639 for deltamethrin and 0.498 for flumethrin, while survival of backcrosses RSxSS, RSxRR and SRxSS showed a significant tendency to recesivity. Backcrossed strain SRxRR (69.38) also showed a higher RR genotype frequency with regards to RSxSS (25.53), RSxRR (36.73) and SRxSS (32.00), however susceptible allele was inherited in general as an incomplet dominant trait.

Monogenic inheritance hypothesis was tested and the results showed monogenic inheritance for cypermethrin and flumethrin ($P<0.05$) but not for deltamethrin ($P>0.05$). However, significant correlation was found between RR genotype and the survival rate for all three pyrethroids used ($P<0.05$), suggesting that a single substitution on the sodium channel gene can be responsible of resistance to pyrethroids as a class, due to the high frequency of RR genotypes. Combination with different mutations or metabolic resistance mechanisms cannot be excluded.

Comparative Analysis Of Putative Genes Mediating Invasion Of Vertebrate Hosts By *Plasmodium falciparum* Parasite Of Malaria

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BACKGROUND: Clinical malaria is associated with proliferation of *Plasmodium* parasites within human erythrocytes. Infection of the erythrocytes is a coordinated process of egress of merozoites, recognition, attachment and ultimate invasion into host's erythrocytes. This process is rapid and tightly regulated by proteases from the parasite. Recent and contemporary developments in information and technologies in the host-parasite interactions such as the completion of the *P. falciparum* genome project, the Integrated Eukaryotic Pathogens Database, the Human Genome Project and the High-throughput DNA sequencing technologies has facilitated the identification and profiling of these genes. The objective of the research was to determine the putative genes in merozoites involved in invasive process in *Plasmodium falciparum* in *Apicomplexa* Phylum.
METHODS: Using comparative genomics and bioinformatics' tools, 3985 homologous sequences of putative genes involved in the invasion by *Plasmodium falciparum* were explored. EupathDB, BLAST application, OrthoMCL and InterPro protein signatures/domains were used to identify relatedness of protein sequences of Cysteine, Serine, Aspartyl and Metallo-endopeptidase proteases involved in the invasion.
RESULTS: The study found that serine proteases sequences are important in the attachment and the disengagement to the RBCs and that, inhibitors against these and the Haemoglobin hydrolases will halt the *Plasmodial* propagation since they survive without nutrition.
CONCLUSIONS: These sequences' information from the comparative genomic analysis across the *Apicomplexa* genomes will provide critical information in the future in designing, synthesis and development of viable, successful and broad spectrum inhibitors/drugs which would control the spread of Human Malaria and wide range of other infectious diseases affecting both humans and livestock.

Atomic force microscopic imaging of *Acanthamoeba castellanii* and *Balamuthia mandrillaris* trophozoites and cysts

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BACKGROUND: Light microscopy and electron microscopy have been successfully used in the study of microbes, as well as free-living protists. Light microscopy enables us to observe live organism but at low magnifications whereas electron microscope provides an ultra-structural two-dimensional image of a dead amoebae cell. In contrast, atomic force microscopy provides a three-dimensional ultra-structural surface profile of an organism in a real time and is used in the present study.
METHODS: In the present study, we observed two free-living amoebae, *Acanthamoeba castellanii* and *Balamuthia mandrillaris* under the phase-contrast inverted microscope, transmission electron microscope and atomic force microscope.
RESULTS: Although light microscopy was of lower magnification, it revealed functional biology of live amoebae such as motility and osmoregulation using contractile vacuoles of the trophozoite stage but it is of limited value in defining the cyst stage. In contrast, transmission electron microscopy showed significantly greater magnification and resolution to reveal the ultra-structural features of trophozoites and cysts including intracellular organelles and cyst wall characteristics but it only produced a snapshot in time of a dead amoeba cell. Atomic force microscopy produced three-dimensional images providing detailed topographic description of shape and surface, phase imaging measuring boundary stiffness, and amplitude measurements including width, height and length of *A. castellanii* and *B. mandrillaris* trophozoites and cysts.
CONCLUSION: These results demonstrate the importance of the application of various microscopic methods in the biological and structural characterization of the whole cell, ultra-structural features, as well as surface components and cytoskeleton of protist pathogens.

Silencing of xylose isomerase and cellulose synthase by siRNA inhibits encystation in *Acanthamoeba castellanii*

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BACKGROUND: A key challenge in the successful treatment of *Acanthamoeba* infections is its ability to transform into a dormant cyst form that is resistant to physiological conditions and pharmacological therapies, resulting in recurrent infections. The carbohydrate linkage analysis of cyst walls of *Acanthamoeba castellanii* showed variously linked sugar residues, including xylofuranose/xylopyranose, glucopyranose, mannopyranose, and galactopyranose. Here, using small interfering siRNA, the role of cellulose synthase and xylose isomerase on encystation of *A. castellanii* was hypothesized.
METHODS: We developed three-dimensional structures of cellulose synthase and xylose isomerase of *A. castellanii* using bioinformatics tools. The role of exogenous saccharides on the encystation was determined at various concentrations (10μM, 50μM and 100μM). After this, the role of cellulose synthase and xylose isomerase siRNA on the encystation of *A. castellanii* was evaluated (100nM and 200nM).
RESULTS: Here, it is shown that exogenous xylose significantly reduced *A. castellanii* differentiation in encystation assays ($P<0.05$ using paired t test, one-tailed distribution). Using small interfering RNA (siRNA) probes against xylose isomerase and cellulose synthase, as well as specific inhibitors inhibited encystation in *A. castellanii*. Neither inhibitor nor siRNA probes had any effect on the viability and extracellular proteolytic activities of A. castellanii.
CONCLUSION: The findings revealed that xylose isomerase and cellulose synthase activities are crucial in the differentiation of *A. castellanii*. Inhibition of both enzymes using siRNA against xylose isomerase and cellulose synthase but not scrambled siRNA attenuated *A. castellanii* metamorphosis, as demonstrated by the arrest of encystation of *A. castellanii*.

Exploring natural nematode enemies as possible potential biological tools against ruminant parasitic nematodes

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BACKGROUND: Gastrointestinal parasitic nematodes (GIN) cause enormous economic losses every year in many countries worldwide. Control is based on the use of chemical drugs that help to reduce the parasitic burdens in the animals; however, produce undesirable effects *i.e.,* a harmful effect against non-target organisms in soil and the presence of anthelmintic resistance in the parasites and they can remain in animal products and sub-products for human consumption. Natural-nematode enemies are being studied for a long time back, and some biological systems are gaining the attention of workers who have focused their research to the use of microorganisms that are lethal for nematodes in nature. Nematophagous fungi (NF) is a group of micro-fungi producing trapping devices from their mycelia. Nematodes are caught, penetrated and colonized by fungi and eventually dead. The NF species that has shown to be the best candidate to be used against GIN is *Duddingtonia flagrans*. This specie produces large amounts of chlamydospores (*Chlams*) that are spores of resistance. *Chlams* can be incorporated into the animal food and so consumed. After *chlams* pass through the gastrointestinal tract and they are expelled to the soil into the faeces, they germinate (*in situ*) and colonize faecal material. Such close interaction between *chlams* and recently hatched larvae promote the trap formation and larvae are trapped and destroyed by fungi. On this way, the life cycle of the parasites is interrupted and the re-infections in the grazing animals is also reduced.
CONCLUSIONS: In the present talk, this and other groups of microorganisms attacking nematode parasites are presented in a general way and their potential use in the control of GIN, is discussed.

Nematocidal activity of edible mushroom products

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BACKGROUND: Nematodes affect a wide variety of hosts, including animals, plants, human beings and microorganisms. The continuous use of chemical drugs (ChD) for controlling gastrointestinal parasitic nematodes (GIN) has triggered the anthelmintic resistance (AR) phenomenon. AR lead to an alarming diminishing in the anti-parasitic efficacy of drugs. Additionally, ChD can remain as contaminant residues in animal products for human consumption. On the other hand, some ChD are eliminated as its active form to the soil; provoking a negative effect on non-target microorganisms *ie.,* coprophagous beetles. Such disadvantages in the use of ChD have motivated the search of novel control alternatives against GIN. Edible mushrooms (EM) are an important source of beneficial nutriments and a number of medicinal properties is being identified. Some of the most widely studied EM include *Pleurotus ostreatus, P. eryngii*, *P. djamor*, *Lentinula edodes* and *Coprinus comatus*. These EM possess medicinal properties including: anti-tumoral, anti-oxidant and anti-microbial effects. Additionally, some EM products have shown anti-parasitic (AP) activity. A nematotoxin produced by *P. ostreatus* with an AP effect against bacteriophagous nematodes, has been reported. Some studies about the AP effect of a nematotoxin produced by *P. pulmonarius* against equins and bovins trichostrongylids was published. There is only a few reports about the possible AP effect of EM againt parasites affecting livestock production.
CONCLUSIONS: EM and their products have an important potential for controlling animal parasitic nematodes; although more research has to be performed to identify their practical use against these parasites. A general view of the potential use of EM and their products against GIN affecting ruminants will be described.

Molecular investigation in cestodes from sicilian red foxes

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BACKGROUND: The fox is the most widespread species of wild carnivores in Italy well adapted to environmental changes and have often been referred to be a resevoir of bacterial, viral and parasitic diseases some zoonotic. Traditionally the classification of tapeworms is conducted on the basis of morphological criteria. The optimization of molecular techniques permits a rapid and accurate identification. In this study cestodes collected from hunting Sicilian red foxes during the years 2012-2014 were characterized.
METHODS: A total of 182 foxes coming from hunting were subjected to post mortem examination and all adult gastrointestinal parasites were characterized using mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene sequences. After DNA extraction by parasites the *cox1* was amplified by Polymerase chain reaction (PCR). PCR products were purified and used for sequencing reaction. The sequencing reaction product was purified and analyzed by ABI3130 Genetic Analyzer.
RESULTS: Adult of cestodes were found in 89 foxes and were characterized as:

Samples	Species	%
31	*Mesocestoides* AJ-2012	35,4
14	*Echinococcus canadensis*	16,1
11	*Hydatigera parva*	13
9	*Echinococcus granulosus*	9,6
6	Diphylidium caninum	6,5
3	*Mesocestoides litteratus*	3,22
3	*Mesocestoides corti*	3,22
3	*Echinococcus sp.*	3,22
3	*Echinococcus multilocularis*	3,22
3	Taenia multiceps	3,22
3	*Raillietina sonini*	3,22

CONCLUSIONS: Molecular biology techniques application has also been shown in studies of parasitology an excellent diagnostic aid where traditional techniques fail to provide a conclusive result. Our data are still preliminary and are part of a larger study involving all the regional territory in a more large period addressed to the study of parasitic prevalence in the red foxes population in Sicily.

Recombinant Tsag18 adhesion protein for serological diagnosis of bovine cysticercosis

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BACKGROUND: In Mexico, *post-mortem* examination of carcasses is the only sanitary procedure applied in slaughterhouses to detect bovine cysticercosis. We tested Tsag18 adhesion protein of *Taenia saginata* oncospheres to detect antibody responses in cattle as an indicative of infection.
METHODS: A baculovirus system expressing Tsag18 recombinant protein was developed in Sf9 cell line. Sf9 cultures were infected with recombinant baculovirus and analyzed by SDS-PAGE. Protein bands were identified by Coomassie blue stain and reactivity to antibodies by western blot. Recombinant protein Tsag18 was compared with crude extracts of Cysticercus bovis, *Taenia saginata* gravid proglottids, *Echinococcus granulosus*, *Cysticercus tenuicollis* and *Cysticercus ovis* by SDS-PAGE. Protein bands were identified by Coomassie blue stain and reactivity to antibodies by western blot.
RESULTS: Clear lysates from pellets and cell culture supernatants from infection with Tsag18 recombinant baculovirus and all crude extracts from the listed parasites produced visible bands of expected sizes between 18 to 22 KDa and reactivity to antibodies confirmed were by Western Blot. Additionally, seven proteins of different molecular weight with a similar electrophoretic pattern were identified by Coomassie blue stain and western blot in recombinant Tsag18 extracts as well as in the rest of crude lysates.
CONCLUSIONS: We have successfully developed a system to produce recombinant Tsag18 adhesion protein with potential applications for the serological diagnosis of bovine cysticercosis and other important cestodes parasites. The technology required to produce recombinant proteins in Sf9 cell culture is inexpensive and relatively simple and might be an alternative to current procedures for the *ante-mortem* diagnosis of bovine cysticercosis.

Recovery capacity of direct microscopic examination and sedimentation of Formol -ethyl acetate technique, with respect to *Blastocystis* spp.

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INTRODUCTION: The technique of choice for diagnosis of *Blastocystis* spp, it is usually direct assembly with SSF, since they are sensitive to temperature changes, osmotic shock and exposure to the air. This is the reason for performing a fixing process where proteins are stabilized and promotes the formation of cross-links between protein molecules (soluble proteins bind to the structural and thus it reaches some force), this way the structure stays, the fixative used is Formalin.
The formalin-ethyl acetate technique used by the prevention and control centers of diseases (CDC), avoids problems of flammability of ether, less prone to technical errors and can be used with specimens preserved with formalin, in addition one to the advantages of this technique is that it can concentrate as much eggs as cysts regardless of their weight, as is the case with flotation techniques.
METHODS: 240 stool samples of children between 6 and 7 years of primary school of the zone of Oblatos, Jalisco, Mexico , homogenized with 10% formalin instead of SSF were processed , analysing using the direct microscopic and the formalin-ethyl acetate (CDC) by duplicate both.
RESULTS: From the 240 samples in direct assembly with SSF, 92 samples they were positive for *Blastocystis* spp. accounting for 38 % and 124 positive samples were obtained with the technique, equivalent to 51.6 %.
CONCLUSIONS: The formalin-ethyl acetate technique it proved to be a more sensitive method for *Blastocystis* spp. replacing SSF use to mix the sample by 10% formalin . It has a higher capacity of recovery thanks to the fixation process which carries out the formalin in the structure of *Blastocystis* spp.

Mapping cutaneous leishmaniasis and the potential impacts of macroclimate variability on northeastern departments of Colombia using Geographic information system (GIS), 2007-2011

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BACKGROUND: Previous studies done by us (during 1985-2002) in northeastern Colombia demonstrated influences of El Niño on cutaneous leishmaniasis (CL) incidence. However no further studies in the region regard this and the use of GIS have been published.
METHODS: Surveillance cases data (2007-2011) were used to estimate annual incidence rates using reference population data, on CL (cases/100,000 pop) to develop the first maps in the municipalities of Santander and Norte de Santander (northeastern Colombia). GIS used was Kosmo® 3.1. In addition we assessed with linear regression models (significance $p<0.05$) the impact of El Niño (using ONI) on each of the municipalities of these 2 departments of the country. Epidemiological maps (5) as well a prone-climate CL occurrence map (based on models' coefficients) were developed according municipalities and years.
RESULTS: During this period, 5,452 cases were reported (mean 1090.4 cases/year, min per municipality 0-max 250), ranging from 0 to 2074.78 cases/100,000pop (at municipalities). At regional simple linear regressions, there were associations between ONI and incidence rates at 11 municipalities ($r^2>0.7$; $p<0.05$). These municipalities were not concentrated, were dispersed over the region. At these rates transmission was unstable with rates ranging from 0 to 183.23 cases/100,000 pop (with 29 year-municipalities with 0).
CONCLUSIONS: Previous studies established influences of climate variability on cutaneous leishmaniasis in certain departments of Colombia, but there are not recent studies in northeastern endemic areas. Climate variability highly impact low transmission municipalities. With more available data from disease surveillance incorporating more microclimatic variables improved predictive models would be developed.

The role of IL-4-instructed dendritic cells in *Leishmania major* infection

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BACKGROUND: In BALB/c mice, susceptibility to infection with the intracellular parasite Leishmania major is driven largely by the development of T helper 2 (Th2) responses and the production of interleukin (IL)-4 and IL-13, which share a common receptor subunit, the IL-4 receptor alpha chain (IL-4Ra). While IL-4 is the main inducer of Th2 responses, paradoxically, it has been shown that exogenously administered IL-4 can promote dendritic cell (DC) IL-12 production and enhance Th1 development if given early during infection.
METHODS: To further investigate the relevance of biological quantities of IL-4 acting on DCs during in vivo infection, DC specific IL-4Ra deficient (CD11ccreIL-4Ra-/lox) BALB/c mice were generated by gene targeting and site-specific recombination using the cre/loxP system under control of the cd11c locus.
RESULTS: DNA, protein, and functional characterization of CD11ccreIL-4Ra-/lox mice showed abrogated IL-4Ra expression on dendritic cells and alveolar macrophages in CD11ccreIL-4Ra-/lox mice. Following infection with L. major, CD11ccreIL-4Ra-/lox mice became hypersusceptible to disease, presenting earlier and increased footpad swelling, necrosis and parasite burdens, upregulated Th2 cytokine responses and increased type 2 antibody production as well as impaired classical activation of macrophages. Hypersusceptibility in CD11ccreIL-4Ra-/lox mice was accompanied by a striking increase in parasite burdens in peripheral organs such as the spleen, liver, and even the brain. DCs showed increased parasite loads in CD11ccreIL-4Ra-/lox mice and reduced iNOS production. IL-4Ra-deficient DCs produced reduced IL-12 but increased IL-10 due to impaired DC instruction, with increased mRNA expression of IL-23p19 and activin A, cytokines previously implicated in promoting Th2 responses.
CONSLUSIONS: Together, these data demonstrate that abrogation of IL-4Ra signaling on DCs is severely detrimental to the host, leading to rapid disease progression, and increased survival of parasites in infected DCs due to reduced killing effector functions.

Long-Lasting Microbial Larivicide for Malaria Vector Control: A Field Trial in Kenya

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BACKGROUND: Larval control is a promising intervention for the control of outdoor transmission of malaria. Bio-larvicides are suited to malaria control as they can target both indoor and outdoor biting mosquitoes and do not have negative impact on non-target organisms. However, the currently available bio-larvicide formulations have a short effective duration, and consequently larval control incurs a high operation expense due to requirement for frequent re-treatment of larval habitats. Therefore, formulation of biological larvicides that has long-lasting effects is highly desired. A recently developed fourStar™ slow release *Bacillus thuringiensis israelenis* (Bti) and *Bacillus sphaericus* (Bs) was evaluated under semi-natural and natural conditions to test its effectiveness in reducing mosquito population in western Kenya. This formulation is designed to be effective against mosquito larvae for up to 6 months.
METHODS: In semi-natural habitats containing soil and rain water, second-instar larvae of *Anopheles gambiae* were introduced and FourStar™ briquettes dissolved in rain water with appropriate concentrations were added. The number of pupae produced from the larvae was recorded daily as the outcome. Formulation was also tested in natural productive habitats. Formulation was then tested for its efficiency to reduce mosquito population during the transmission season. Larval control was undertaken in field trials in three sites and with three other sites taken as control.
RESULTS: We found that FourStar briquettes totally inhibited mosquito pupal production in the first 3 months, and then reduced pupal productivity by 87.2- 98.0% 4-6 months after application. In natural habitats, FourStar briquettes reduced malaria vector pupal productivity by 100% in the first 2 months and then by 63.4-90.2% 3-5 months after application. In field randomly cluster trial, FourStar briquettes reduced indoor biting mosquitoes by 68.2-80.6% during the 3-month monitoring period, and reduced outdoor malaria vector abundance by 53.6-63.4%.
CONCLUSION: This study suggest long-lasting microbial larviciding is a promising complementary malaria control tool.

Echinotest technical specific ELISA for determination of *Echinococcus spp* in the intermediate host

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BACKGROUND: This study aims to highlight the etiological agent of hydatidosis in the intermediate host with a modern laboratory technique (ELISA) and be able tested the efficacy of this technique by comparing the results obtained at necropsy.
METHODS: The work was carried out on 42 cattle, 46 sheep, 92 goats, at the slaughterhouse of the province of Djelfa, situated in the center of Algeria, a necropsy showed a prevalence of hydatidosis 42.85% with liver and lung (66.66%, 54.76%) in bovine, 86.95% prevalence with liver and lung (76.08%, 67.40%) in sheep and (52%) with respective prevalence (18.45% - 17.39%) liver and lung for caprine.
RESULT: in terms of comparison between necropsy and convergent ELISA was 24 (57.14%) and 11 (26.19%) cases respectively positive and negative while the results of the test are divergent between 3 (7.14%) and 4 (9.52%) for cattle and 01 (2.17%), 5 (10.87%) cases respectively positive and negative, whereas the divergence rate was 40 (86.95%), 0 (0%) for sheep and finally the percentage compatibility 5 (5.43%) and 30 (32.60%) respectively cases positive and negative, divergence was 47 (51.08%) and 10 (10.86%) in goats.
CONCLUSION: To this effect the liver was the most affected organ as the lung for all the species under study, as well as sensitivity kit used was higher for cattle and goats while the sheep was very sensitive which explains the genetic diversity of the genus *Echinococcus*, while serological tests for the diagnosis of hydatidosis in sheep are complicated by cross-reactions with other species teniide (*Taenia ovis* and *Taenia hydatigena*), the connection not specific antibody sheep especially because the weak immune response and the immune response in animals is low compared with high levels of antibodies specific for human infection. The immunological diagnosis remains a very effective way to demonstrate the hydatid disease.