A novel CDPK inhibitor is effective against cryptosporidiosis in calves

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BACKGROUND: *Cryptosporidium parvum* is a zoonotic agent that infects humans and animals causing heavy, watery diarrhoea. In immunocompetent hosts cryptosporidiosis is self-limiting but it can have a fatal outcome in immunocompromised individuals. *Cryptosporidium* is one of the most common causes of waterborne disease (recreational water and drinking water) in humans. In calves *C. parvum* is one of the main reasons for diarrhoea and leads to high economical losses and in 5-10% of the cases to the death of the animals. So far effective treatment options for either animals or humans are not satisfactory. Here we present for the first time that the novel inhibitor 1294 for the calcium dependent protein kinase 1 (CDPK1) of *Cryptosporidium* is able to drastically reduce the oocyst shedding of *C. parvum* without obvious side effects in its natural host.
METHODS: Calves (n=6) were infected with a single dose of *C. parvum* and treated with Inhibitor 1294 or mock control five times every two days or were left untreated. Oocyst output, health status, dehydration and blood chemistry were assessed.
RESULTS: The single dose infection led only to mild clinical symptoms. Faecal consistency was similar in all groups but the number of days animals experienced dehydration was slightly less in the treated group. Most strikingly oocyst shedding was reduced by up to 75% compared to the control group. Analysis of the blood chemistry revealed no major differences between the groups indicating inhibitor 1294 did not has considerable adverse effects on animal physiology.
CONCLUSIONS: Altogether inhibitor 1294 proved to be an attractive candidate for a new treatment against *C. parvum* in calves and possibly also in humans.

Study and application of real time PCR for detection and characterization of G*iardia spp.* cysts in the wastewater treatment plant, in the city of Campinas, São Paulo, Brazil

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BACKGROUND: Water scarcity in many regions of the world, including southeast of Brazil, has motivated news studies which seek to increase the responsible use of the water and also improve its quality through new technologies that can detect chemical and microbiological contamination in water treatment systems. Outbreaks are frequently caused by contamination of drinking water and a high prevalence of *Giardia* has been detected in surface waters. *Giardia duodenalis* has a great genetic diversity and consists of eight distinct genetic assemblages (A-H), two of them (A-B) responsible for human infections. The aim of this study was to identify *G. duodenalis* in environmental samples and to characterize the main genetic assemblages.
METHODS: Sludge samples from the anaerobic reactor of the Capivari Wastewater Treatment Plant II of Campinas city were monthly collected for six months. *G. duodenalis* genotypes were characterized through amplification followed by melting curve analysis of *β-giardin*, *tpi*, and *orf-c4* genes.
RESULTS: *G. duodenalis* was detected in 67% of samples and genetic assemblages A and B were identified.
CONCLUSIONS: This study revealed anthroponotic assemblages of *G. duodenalis* in the domestic sewage which strengthens the importance for the public health of wastewater treatment associated to the use of molecular tools in the routine of water sanitation companies. Using molecular tools in the routine of water sanitation companies can be extended to monitor the quality of surface waters, drinking waters, effluents of WWTP and reclaimed water production station.

Establishment and standardization of an in vitro assay to test disinfectants against the dispersal stages of coccidia

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BACKGROUND: Coccidian parasites are common threat in animal husbandry and human health. Preventive measures like hygiene and most importantly disinfection are of great importance when combating coccidia associated diarrheal diseases in livestock. Until now, the efficacy of disinfectants to inactivate oocysts is tested in an *in vivo* chicken model recommended by the DVG (German Veterinary Medical Society). We aim to replace the *in vivo* test by a standardized *in vitro* test based on *Cryptosporidium parvum* oocysts.
C. parvum is highly suitable to test disinfectants *in vitro* as the oocysts are available in high amounts, they are highly resistant and *C. parvum* infects cells *in vitro* and replicates within them. We developed an *in vitro* assay to test disinfectants under standardised conditions in suspension as well as on germ carriers.
METHODS: Oocysts are treated with disinfectants and subsequently used to infect cells. The efficacy of the disinfectant is determined by assessing the replication of the parasites within the cells. As germ carriers we used brushed stainless steel disks. Oocysts were dried on these discs and subsequently treated with different disinfectants by overlaying the oocysts with disinfectants. After recovery the oocysts were again used to infect human ileocecal colorectal adenocarcinoma (HCT-8) cells.
RESULTS: Here we present results regarding the standardization and improvement of the suspension assay and the establishment of the germ carrier assay. Excystation time and excystation medium as well as the number of oocysts for infection have been optimized. The germ carrier assay has been optimized to allow recovery of enough oocysts to infect cells and tested to for its reproducibility.
CONCLUSIONS: We developed a reliable suspension and germ carrier *in vitro* assay to test disinfectants against coccidian oocysts that can replace the chicken *in vivo* model.

Subtypes and Virulence of *Cryptosporidium parvum* in Germany

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BACKGROUND: Cryptosporidiosis is zoonotic disease infecting humans but most commonly young calves. In calves *Cryptosporidium* causes enteritis, associated with aqueous, yellow or bloody diarrhea, dehydration, weight loss and a mortality of up to 5-10%. This study was undertaken to evaluate the distribution of subtypes of *Cryptosporidium parvum* in German farms and to determine whether different sub-genotypes respectively field strains have different degrees of virulence.
METHODS: Faecal samples from 479 calves of 99 dairy farms from all German federal states were analyzed for *Cryptosporidium spp.* by ELISA and carbol fuchsin staining and for the presence of other enteropathogens. DNA was extracted and applied in a PCR for the *Cryptosporidium* gp60 gene. PCR-Products were sequenced to detect the sub-genotype. If possible oocysts were purified and used to infect cells to test for cell toxicity (MTT-Test).
RESULTS: 282 (59%) samples from 89 farms were tested positive for *Cryptosporidium spp*. of which 236 samples were identified as *Cryptosporidium parvum*. Of the 12 subgenotypes the IIaA15G2R1 subtype was with 70% the most common once. In most of the farms we detected only a single subgenotype whereas in 7 farms we found multiple subgenotypes.
To evaluate a possible role of co-infections in pathogenesis specimens were regularly checked for viral and bacterial infections. There was a significant correlation between the degree of diarrhoea and the number of enteropathogens found.
Using a viability test (MTT) for cells we analyzed whether different field strains show different cytotoxic effects on HCT-8 cells. Most of the field strains did not show high differences in cell cytotoxicity in comparison to the in house strain. However, some of the strains displayed a 20% higher or lower cytotoxic effect indicating that virulence might differ between various stains.
CONCLUSIONS: *Cryptosporidium* is the most common diarrhoeal pathogen in young calves and seems to be present in nearly every farm in Germany. Pathogenicity depends on infections with further enteropathogens but might also differ between different strains. We created the basis to further investigate potential differences between *C. parvum* field strains concerning their virulence.

Glycosylases and endonucleases of BER pathway: Presence and activity in DNA repair in *Trypanosoma cruzi*

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BACKGROUND: *T. cruzi* survives damage produced by reactive species present in insect vectors and host mammals, probably by repair of its DNA through the Base Excision Repair pathway (BER). In this work we analyzed the importance of BER pathway enzymes TcNTH1, TcAP1 and TcFEN1 in *T. cruzi* submitted to oxidative stress.

METHODS: We generated expression vectors for glycosylase (pTREX-*nth1-gfp*) and endonucleases (pTREX-*tcap1-gfp;* pTREX-*tcfen1-gfp*). We used these vectors to transfect parasites and the expression of the recombinant proteins were evidenced by fluorescence microscopy and western blot using specific antibodies. Proteins were purified and used to determine their activity using a DNA substrate labeled with P^{32}, with specific sites for each one. Proliferation of TcFEN1 transfected parasite was assessed by counting. The viability of the parasites transfected in presence of H_2O_2 was analyzed by MTT assay.

RESULTS: Glycosylase and endonucleases are located in nucleus of *T. cruzi* and they are present in the three cellular form of parasite. Glycosylase but not endonucleases improve survival to oxidative stress in transfected parasites compared to control parasites. TcFEN1 increased proliferation in recombinant epimastigotes. We demonstrate that recombinant proteins TcNTH1, TcAP1 and TcFEN1 present *in vitro* activity.

CONCLUSIONS: Our results suggest that presence of TcNTH1 glycosylase, TcAP1 and TcFEN1 endonucleases activity in the three cellular forms of *T. cruzi* as well as its nuclear location are indicative of the existence of short-patch and long-patch BER pathway in this parasite.

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Cellular growth and mitochondrial ultrastructure of *L. (V.) braziliensis* are affected by the iron chelator 2,2-dipyridyl

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BACKGROUND: *Leishmania* require iron for the generation of infective forms, the colonization of macrophages and the development of lesions in mice. The metal is also important for the energy metabolism and the redox balance in these parasites. *L. (V.) braziliensis* is considered to be the major etiological agent of American Tegumentary Leishmaniasis. However, the role of iron on the biology of this species is still unclear. In the present work, we analyze the effect of 2,2-dipyridyl on growth, iron uptake and ultrastructure of *L. (V.) braziliensis*.

METHODS: Promastigotes were treated with distinct concentrations of 2,2-dipyridyl and cellular density was determined. Transmission electron microscopy was made after treatment with the iron chelator. Flow citometry and fluorescence microscopy of TMRE, PI and TUNEL labeled parasites were conducted. Finally, iron uptake was quantified by the ferrozine method and gene expression of LIT1 and FeSOD was analyzed by qPCR.

RESULTS: Treatment with 2,2-dipyridyl affected proliferation in a dose and time-dependent manner, but growth was restored after inoculation of the parasites in fresh culture medium. Ultrastructural analysis of promastigotes treated for 24 hours revealed severe damage to the mitochondrion. The iron chelator also collapsed the mitochondrial membrane potential (Δψm) after 24 hours. However, after 48 hours, the parasites partially recovered from the dissipation of the Δψm. Plasma membrane and nuclear DNA conserved their integrity after 24 and 48 hours of treatment. The intracellular concentration of iron was higher in promastigotes treated with 2,2-dipyridyl. Accordingly, gene expression of LIT1 and FeSOD increased in parasites treated with the chelator.

CONCLUSIONS: At first, the iron chelator inhibits growth and dissipates the Δψm of *L. (V.) braziliensis*, leading some parasites to death. However, the increased uptake of iron and antioxidant defenses allow part of the population to resist the nutritional stress, survive and resume proliferation.

Characterization of molecules from the mosquito *Anopheles albimanus* midgut necessary for *Plasmodium berghei* ookinete invasion.

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BACKGROUND: Malaria is a parasitic disease caused by *Plasmodium* and transmitted by *Anopheles* mosquitoes. One of the proposed strategies for malaria control is to interrupt the parasite life cycle by blocking *Plasmodium* infection in the vector using transmission blocking vaccines (TBV). Antigens included in TBV could be either parasite molecules or mosquito proteins that participate in the parasite invasion of the insect midgut. Among several such proteins previously identified are glycosaminoglycans, aminopeptidases, carboxipeptidases.

METHODS: To identify new potential molecules from the mosquito midgut important for *Plasmodium* ookinetes invasion, we performed assays using the murine parasite *P. berghei* and the mosquito *An. albimanus*. We used a panel of monoclonal antibodies developed against *An. albimanus* midgut extract and select those that immunoreacted by Western blot with proteins from the brush border of this mosquito midgut. The Mabs were evaluated using standard membrane feeding assays (MFA), we measured the effect on *Plasmodium* development by counting the number of infected mosquitos (prevalence of infection) and the number of oocysts that developed in each mosquito (intensity of infection).

RESULTS: We identified two MAbs A-140 and A-78, the first recognized two proteins of aprox. 80 and 125 kDa. The second recognized two proteins of 18 and 64 kDa. We found that MAb A-140 significantly reduced parasite intensity by 75% and we are currently carrying out the identification of the antigen recognized by the Mab A-140.

CONCLUSIONS: Our results suggest that the Mab A-140 recognized antigens necessaries for the ookinete midgut invasion and could be a potential candidate for a TBV in mosquito *Anopheles albimanus*.

Benzimidazole derivatives as new inhibitors of triosephosphate isomerase from *Trypanosoma brucei*.

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BACKGROUND: Human African Trypanosomiasis (HAT), a disease which affects 36 sub-sahara countries, is caused by *Trypanosoma brucei*. According to the World Health Organization around 300 000 to 500 000 people are infected. Currently available treatments are limited to drugs developed decades ago that are highly toxic and parasites strains resistant to them are emerging. Therefore, there is an urgency to find new drugs against HAT. In this context, *T. brucei* depends on glycolysis as the unique source for ATP supply, so its enzymes are considered excellent targets for drug design. In this work we used the homodimeric glycolytic enzyme triosephosphate isomerase from *T. brucei* (TbTIM), as a target to search new inhibitors.
METHODS: TbTIM activity was determined in an enzyme-coupled reaction. Inhibitors were searched from an in house library of benzimidazole derivatives. A first screening at 200 µM was performed, after that; the two compounds with the best inhibition were selected for further kinetic and flexible docking studies. Assays with human TIM were performed too.
RESULTS. From screening at 200 µM the two most potent compounds were IVM-1 and IVM-4. According to the kinetics analysis this molecules showed an I_{50} value of 115 µM and 73 µM, respectively. Both compounds did not affect the human TIM at least until 1 mM, indicating that these molecules were very selective with respect to parasitic enzyme. Assays at different enzyme concentration indicated that these inhibitors act at the dimer interface. Flexible docking studies showed that inhibitors had more affinity for TbTIM than human TIM. These data could explain in part the selectivity found in kinetics assays.
CONCLUSIONS: These molecules will serve as a guide for the design of more potent inhibitors that could be used to obtain new drugs against HAT.

DNA detection of *T. canis* In brain of rabbits experimentally Iinfected.

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BACKGROUND: *Toxocara canis* performs complex migration and their larvae are distributed to different organs in the host. Migration could enable the uptake of *Toxocara* DNA samples of some host tissues including blood.
METHODS: Adult worms and embryonated eggs of *T. canis*, *Ascaris suum* and *Ascaris lumbricoides* were obtained. *Toxocara* eggs were hatched for *T. canis* excretion-secretion antigens (TES-Ag) obtaining. New-Zeeland rabbits were infected with 5000 embryonated eggs of *T. canis* (n=35), *A. suum* (n=35), *A. lumbricoides* (n=35) or were no infected (n=10 control group). Five rabbits of each infected group were euthanized at days: 1,3,5,10,20,30 and 60 postinfection (p.i.) and at days 1 and 60 in the control group. In all rabbits, samples of blood, liver, lungs, kidney and brain were taken for PCR amplification of the ITS-2 ribosomal DNA of *T. canis* using the primers (F/R) Tcan1/NC-2, YY1/NC-2 and NC-13/NC-2. Serum levels of IgG anti-TES-Ag were measured.
RESULTS: Evidence of larval migration was observed in organs of rabbits infected with the three nematodes. The IgG anti-TES-Ag levels increased ($p<0.05$) in *T. canis*-infected rabbits from day 10 p.i. Primers YY1/NC-2 amplified an expected fragment (330bp) in the brain of *T. canis*-infected rabbits at 30 and 60 days p.i. Neither detection of IgG anti-TES-Ag, nor ITS-2 amplification was observed in rabbits infected with *A. lumbricoides* or *A. suum*. PCR sensibility was 5 larvae/gram of tissue and 25 larvae/ml of blood.
CONCLUSIONS: Results show that primers YY1/NC-2 are the best to molecular detection of *T. canis* in tissues and show that the brain is the most important organ of DNA accumulation of the parasite.

Insight into the role of Cytidine Triphosphate Synthase in Toxoplasma gondii: evaluation of protein-protein interactions

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BACKGROUND: *Toxoplasma gondii* is a cosmopolitan intracellular parasite with worldwide distribution. New antiparasitic drugs with greater effectiveness in controlling *T. gondii* are necessary. The enzymes of *de novo* pyrimidine biosynthetic pathway are considered potential drug targets, because they are required for the parasite's virulence and survival.
Cytidine triphosphate synthase (CTPase) catalyzes the conversion of UTP to CTP. CTP is the precursor of membrane phospholipids and is needed for RNA and DNA synthesis. In many organisms, CTPase also plays a structural role *in vivo* forming filaments together with other proteins. The function of these structures is unknown, and identification of their protein components could aid in elucidating function.
METHODS: The coding sequence of CTPase from *T. gondii* (TgCTPase) was amplified from a cDNA library of RH tachyzoites, cloned, and expressed in bacteria. Electroelution was used to improve purity of the his-tagged recombinant TgCTPase, and preparations were used to immunize rabbits. Serum samples were collected 9 weeks post-immunization and tested by Western blot. Immunoprecipitation experiments are in progress using the antibodies, tachyzoites extracts, and protein A-sepharose.
RESULTS: A yield of 0.47 mg TgCTPase per 1L cell culture was obtained. Rabbits immunized with the purified recombinant protein recognized the recombinant protein and a band migrating at the predicted molecular mass of TgCTPase in parasite extracts. These antibodies are being used to identify TgCTPase-interacting proteins by immunoprecipitation in parasite extracts.
CONCLUSIONS: Purified recombinant TgCTPase was successfully produced, and was used to produce polyclonal antibodies. Immunoprecipitation experiments will permit elucidation of protein-protein interactions in TgCTP-containing filaments in *T. gondii.*

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Significantly Lower Anti-*Leishmania* IgG Responses in Sudanese versus Indian Visceral Leishmaniasis

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BACKGROUND: Visceral leishmaniasis (VL), a widely distributed systemic disease caused by infection with the *Leishmania donovani* complex, is almost always fatal if symptomatic and untreated. A rapid point-of-care diagnostic test for anti-*Leishmania* antibodies, the rK39-immunochromatographic test (rK39-ICT), has high sensitivity and specificity in South Asia but is less sensitive in East Africa. One of the underlying reasons may be continent-specific molecular diversity in the rK39 antigen within *the L. donovani* complex. However, another reason may be differences in specific IgG anti-*Leishmania* levels in patients from different geographical regions, either due to variable antigenicity or immunological response.

METHODS: We determined IgG titres of Indian (n=36) and Sudanese (n=36) VL patients against whole cell lysates of Indian and Sudanese *L. donovani* strains by ELISA. Indian and Sudanese samples were assayed against antigen originating from both countries simultaneously on the same plates.

RESULTS: Indian patients had significantly higher IgG titres against both *L. donovani* strains compared to Sudanese patients ($p<0.0001$). Mean reciprocal $\log_{10}$ 50% end-point titres ($1/\log_{10}t_{50}$) were i) 3.80 and 3.88 for Indian plasma and ii) 2.13 and 2.09 for Sudanese plasma against Indian and Sudanese antigen respectively ($p<0.0001$). Overall, the Indian patients therefore showed a 46.8–61.7 -fold higher mean ELISA titre than the Sudanese patients. The higher IgG titres occurred in children (<16 years old) and adults of either sex from India (mean $1/\log_{10}t_{50}$: 3.60–4.15) versus Sudan (mean $1/\log_{10}t_{50}$: 1.88–2.54). The greatest difference in IgG responses was between male Indian and Sudanese patients of ≥16 years old (mean $1/\log_{10}t_{50}$: 4.15 versus 1.99 = 144-fold ($p<0.0001$).

CONCLUSIONS: Anti-*Leishmania* IgG responses among VL patients in Sudan were significantly lower than in India; this may be due to chronic malnutrition with Zn^{2+} deficiency, or variable antigenicity and capacity to generate IgG responses to *Leishmania* antigens, and may contribute to lower sensitivity of the rK39-ICT in East Africa.

Development of Peptide-based Lineage-specific Serology 1 for Chronic Chagas Disease: Geographical and Clinical Distribution of Epitope Recognition.

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BACKGROUND: Chagas disease, caused by infection with the protozoan *Trypanosoma cruzi*, remains a serious public health issue in Latin America. Genetically diverse, the species is sub-divided into six lineages, known as TcI–TcVI, which have disparate geographical and ecological distributions. TcII, TcV, and TcVI are associated with severe human disease in the Southern Cone countries, whereas TcI is associated with cardiomyopathy north of the Amazon. *T. cruzi* persists as a chronic infection, with cardiac and/or gastrointestinal symptoms developing years or decades after initial infection. Identifying an individual's history of *T. cruzi* lineage infection directly by genotyping of the parasite is complicated by the low parasitaemia and sequestration in the host tissues.

METHODS: We have applied here serology against lineage-specific epitopes of the *T. cruzi* surface antigen TSSA, as an indirect approach to allow identification of infecting lineage. Chagasic sera from chronic patients from a range of endemic countries were tested by ELISA against synthetic peptides representing lineage-specific TSSA epitopes bound to avidin-coated ELISA plates via a biotin labelled polyethylene glycol-glycine spacer to increase rotation and ensure each amino acid side chain could freely interact with their antibodies.

RESULTS: 79/113 (70%) of samples from Brazil, Bolivia, and Argentina recognised the TSSA epitope common to lineages TcII/TcV/TcVI. Comparison with clinical information showed that a higher proportion of Brazilian TSSApep-II/V/VI responders had ECG abnormalities than non-responders (38% vs 17%; $p<0.0001$). Among northern chagasic sera 4/20 (20%) from Ecuador reacted with this peptide; 1/12 Venezuelan and 1/34 Colombian samples reacted with TSSApep-IV. In addition, a proposed TcI-specific epitope, described

elsewhere, was demonstrated here to be highly conserved across lineages and therefore not applicable to lineage-specific serology.

CONCLUSIONS*:* These results demonstrate the considerable potential for synthetic peptide serology to investigate the infection history of individuals, geographical and clinical associations of *T. cruzi* lineages.

Lack of malaria parasitaemia and effect of antimosquito measures among pulmonary tuberculosis (TB) patients in a malaria endemic environment.

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BACKGROUND: Pulmonary tuberculosis (TB) and malaria are infections of major public health concern in Nigeria and other African countries. Significant efforts are still being needed to understand the prevalence of co-infections and reduce the burden of the diseases in Nigeria. This study therefore aimed to assess malaria parasitaemia among TB patients exposed to malaria in an endemic environment.
METHODS: A total of 83 patients attending the Jericho chest hospital in Ibadan, Nigeria were recruited into the study and classified into acid-fast bacilli negative (AFBN), acid-fast bacilli positive (AFBP) and multi-drug resistant TB (MDR-TB) groups using sputum smear microscopy and GeneXpert/Rif test. Structured questionnaire was administered to assess demographic factors, antimosquito preventive measures and antimalarial drug taken. Malaria parasite was screened microscopically using giemsa stained thick smears. Data were analyzed using student's t-test
RESULTS: Of the 83 patients screened, 29(35%), 30(36%) and 24(29%) were AFBN, AFBP and MDR-TB infected respectively. 17(57%) of the AFBP and 13(54.2%) of MDR-TB patients were males. All the patients in the three groups were negative for Plasmodium infection. Significantly lower number of MDR-TB [6(25%)] were anaemic compared with AFBN[16(55.2%)] and AFBP[18(60%)] patients ($p>0.05$). 59(72%) use window and door nets, 11(13%) use insecticide treated bed nets, 4(5%) use aerosol sprays, 1(1%) use mosquito coils, 2(2%) use mosquito repellants while 6(7%) are not practicing any antimosquito preventive measures ($p>0.05$). 48(58%) took antimalarial agents while 35(42%) did not. Of the 58%, 40(83%) use various herbal preparations, 6(13%) use artemether-lumefantrine and 2(4%) use artesunate-camoquine. 6(8%) of all the patients were on opportunistic infection prophylactic therapy using cotrimoxazole ($p>0.05$).
CONCLUSION: The lack of Plasmodium infection among TB patients may be due to the suspected antimalarial activity of anti-TB drugs and cotrimoxazole.

Assessment of anaemia and iron status in pregnant women with co-infections of malaria, intestinal helminthes and HIV in Southwest Nigeria.

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Background. Malaria, HIV and helminth infections are diseases of public health menace in Africa. Pregnant women are a major group at risk of these infections. Iron deficiency and anaemia have been reported among these women. This study was therefore aimed at evaluating the prevalence of malaria, HIV and helminth infections and their effect on haemoglobin concentration and iron status in pregnant women.
Method. Pregnant women (320) were recruited from the ante-natal and HIV clinics of a secondary healthcare facility. Personal details of each participant was documented. Blood samples were obtained for haematocrit and, thick smears for malaria microscopy. Serum samples were used for ferritin and iron level estimation using ELISA and Atomic Absorption Spectrophotometry respectively. Stool was collected and used for identification and quantification of helminth ova by Direct and Kato-Katz methods.
Result. Twenty-three (7.1%) of the women were positive for malaria only, 11 (3.4%) for helminthes only, 65 (20.1%) for HIV only while 175 (54.2%) had no infection. There were 2 (0.6%), 46 (14.2%) and 1 (0.3%) cases of malaria/helminth, malaria/HIV and helminth/HIV co-infections respectively. 190 (60.9%) were not anaemic while 64 (20.5%), 57 (18.3%) and 1 (0.3%) had mild, moderate and severe anaemia respectively. A significantly lower haematocrit value was observed among those positive for HIV, malaria and malaria/HIV (p=0.000) infections relative to those without infections. Women positive for malaria only or coinfection of malaria/helminth had higher ferritin levels compared to those with no infections. There was no significant difference in serum iron levels among the groups.
Conclusion. The burden of malaria and HIV infections are high among the pregnant women. Pregnant women infected with malaria and/or HIV are more prone to anaemia relative to those infected with helminthes only. Effect of helminth coinfection with malaria and/or HIV are discussed.

Serological and molecular evaluation of acute ocular toxoplasmosis: a follow-up of 5 cases

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BACKGROUND: *Toxoplasma gondii* is a cosmopolitan parasite which infects a high number of individuals but just a small percentage of them develop the clinical forms of the disease. Ocular toxoplasmosis (OT) is one of the clinical presentations of the congenital infection or later sequaleae of acquired infection. The frequency of OT in patients attended in the Ophthalmology Outpatient Clinics in our region is 27.0%. The laboratory diagnosis is being worldwide used supporting the clinical diagnosis. The aim of this study is to evaluate the use of serological and molecular methods for the monitoring of acute OT in immunocompetent patients before and after treatment.
METHODS: 05 patients were admitted and treated at the clinics and were clinically diagnosed as having acute OT. The clinical evaluation was performed under fundoscopic examination using indirect binocular ophthalmoscopy, 20D lens (Binocular Ophthalmoscope ID 10, Topcon Corporation, USA). Serology were performed by ELISA (IgA, IgM, IgG Diasorin, Italy) and confirmed by ELFA (IgG, IgM Biomerieux, France). Molecular diagnosis were performed in peripheral blood by cPCR using the *T. gondii B1* gene as marker. The follow-up scheme was performed as D0; D+15; D+45.
RESULTS: All patients were male; mean age 41.2±11.3 y (median 35, min 31, max 54 y). All of them were IgG positive. At D0: ELFA IgM 1-/4+; ELISA IgM 3-/2+; ELISA IgA 4-/1+; cPCR 3-/2+. At D+15: ELFA IgM 5-; ELISA IgM 4-/1+; IgA 4-/1+; cPCR 2-/3+. At D+45: ELFA IgM 5-; ELISA IgM 4-/1+; ELISA IgA 4-/1+; cPCR 3-/2+.
CONCLUSIONS: The presence of IgA and IgM confirm the acute infection and are in agreement to clinical evaluation. Our results shows the adopted treatment modify the serological profile of IgM and PCR results, but not IgG and IgA.

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Prevalence of intestinal parasites in children in the Altos, Chiapas (Mexico)

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BACKGROUND. The region of Altos in the State of Chiapas is located at the mountains of southeastern Mexico and includes 5 urban and 1,002 rural localities. The topography prevents access to health services routinely, so data on the prevalence of infectious agents are scarce. The aim of this study was to determine the prevalence of intestinal parasites in children in the region.
METHODS. A total of 450 stool samples of children under 15 years of age were obtained. Each sample was divided into two parts to determine the presence of geohelminths in a double-blind study. The stools were analyzed by the Kato-Katz technique. In addition, coproantigens were determined for *Entamoeba histolytica, Giardia lamblia* and *Cryptosporidium* sp.
RESULTS. The prevalence of *Ascaris lumbricoides* eggs was of 71%, for *Trichuris trichiura* was of 25% and 5% for hookworms. The kappa index obtained to determine the diagnostic correlation between laboratories was of 0.8235 for *Ascaris* and 0.68 for *Trichiuris*, determining that the Chiapas laboratory identified more eggs than the InDRE; kappa for hookworm was not determined. Prevalence of coproantigens for *E. histolytica* was of 34.7 %, for *G. lamblia* was of 13% and 23.9% for *Cryptosporidium*. No correlation was observed between the presence of geolminthes and protozoa in the same sample.
CONCLUSIONS. Here, we report the presence of three geohelminthes and three protozoa of the most prevalent in the human populations. This data are important since the prevalence of parasites is decreasing in urban areas but not in rural ones. Further studies are required to know the prevalence of parasites in adult persons and peridomestic animals.

ANTIGENICITY AND IMMUNOGENICITY OF THE COOH-TERMINAL EXTENSION OF CYSTEINE PROTEASE B FROM *LEISHMANIA (LEISHMANIA) AMAZONENSIS*

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BACKGROUND: *Leishmania (Leishmania) amazonensis* is a very important etiologic agent of tegumentary leishmaniasis in South America. This parasite presents an array of mechanisms to elude and even subvert the host innate and adaptative immune responses. Such activities are related to the virulence factors, as cysteine proteases (CPs). The aim of the present study is to evaluate the antigenic potential of the COOH-terminal extension of CPB (Cyspep) and to demonstrate the potential immunoregulatory activity of this sequence during experimental infections in BALB/c mice, using a recombinant cyspep protein (rcyspep).
METHODS: The rcyspep was obtained using an *Escherichia coli*-based system and used as antigen in enzyme-linked for detection of IgG in the sera of *L. (L.) amazonensis*-infected BALB/c. Furthermore, BALB/c mice were injected with rcyspep for 4 weeks and, subsequently, challenged with *L. (L.) amazonensis* promastigotes; the lesion development was follwed for 15 weeks.
RESULTS: The results indicate that mice previously inoculated with rcyspep developed larger lesions than animals not inoculated with this protein. The data indicate that during infection the mice elicit a humoral immune response against rcyspep with IgG production, when compared to control animals ($p<0.05$).
CONCLUSIONS: These work shows additional evidence, that cyspep acts on the immune system of mice influencing the clinical manifestation of cutaneous leishmaniasis. This hypothesis is supported by high IgG titer detected throughout infection with *L. (L.) amazonesis* and by increased lesion in animals that were previously inoculated with rcyspep.

Healthy Housing for Healthy Living: Participatory design of a housing prototype to control vectorial transmission of Chagas disease in Loja Province, Ecuador

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BACKGROUND: Chagas disease is caused by the parasite *T. cruzi*, mainly transmitted by the feces of triatomine insects. Triatomines find favorable environments in cracks of walls, floors, and roofs of poorly constructed houses. Therefore, Chagas disease is deeply connected with life conditions of rural areas of Latin America. Entomological research has shown constant triatomine reinfestation in the houses of Loja province in southern Ecuador despite insecticide-based and educational interventions.
METHODS and RESULTS: In order to develop a long term Chagas disease control strategy for this region we developed a *Healthy Homes for Healthy Living (HHHL) Model*. This model considers the physical structure and organization of intra and peri-domiciles, as well as protective practices related to cleaning and housekeeping. In order to guarantee social and cultural acceptability of the model, as well as entomological and epidemiological soundness, the development of the HHHL prototype included: an architectural decay analysis conducted in all the houses of the communities; a KAP study based on positive deviance (PD) methodological framework; participatory design sessions with a partner family and local community members at large; and a participatory construction process that included formal training to community members to ensure technological transferability. The construction of the HHHL prototype house was accompanied by sociological work which is essential for the appropriation of the improved spaces and behavior change.
CONCLUSION: This model is currently been evaluated and is serving as proof of concept prior to the possible scaling up of the intervention as well as to inform Ecuadorian Chagas disease control and housing programs at the national level.

Arginine deprivation induces an RNA stability pathway in *Leishmania*

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BACKGROUND: Arginine is an essential amino acid for *Leishmania*, but not for its mammalian hosts. Parasites import arginine *via* a mono-specific amino acid transporter (LdAAP3) and direct it primarily into the polyamine pathway to provide precursors for trypanothione biosynthesis. For both promastigotes and amastigotes, arginine depletion of the growth medium induces a rapid up-regulation of LdAAP3 expression and activity; along with concomitant increase of mRNA and protein levels for a small group of other genes (encoding mostly metabolic enzymes and RNA binding proteins).
METHODS AND RESULTS: Proteomic analyses show that ~40% of the phosphopeptides that increased in abundance 5-15 minutes after arginine depletion contain a serine-proline (SP) motif, suggesting activation of a protein kinase (PK) pathway involving MAPK2 and MAPK10, which have this substrate specificity. Our preliminary studies also indicate that an external sensor activates the pathway *via* an on/off mechanism. Significantly, this arginine availability pathway is activated during macrophage invasion, suggesting that it plays an important role in adaptation of *Leishmania* amastigotes to intracellular growth.
CONCLUSION: We hypothesize that sensing arginine availability plays a critical role in *Leishmania* virulence by activating a rapid metabolic reaction in response to the lower arginine concentration of the macrophage phagolysosome. This allows the invading amastigote to further deplete the macrophage arginine pool, thereby suppressing host production of cytotoxic nitric oxide, and to increase production of a key parasite anti-oxidant (trypanothione).

Control of Tick Infestations in *Oryctolagus cuniculus* (Lagomorpha: Leporidae) with Spinosad under Field Conditions

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BACKGROUND*: H. lusitanicum* is a three-host tick. Immature stages feed on lagomorphs, mainly on wild rabbits, while adults feed on larger animals. Spinosad is an insecticide-acaricide produced from the fermentation of metabolites of the actinomycete bacterium *Saccharopolyspora spinosa* and a mixture of two components A and D spinosyn. The aim of this study was to test the efficiency of Spinosad by oral administration on wild rabbits as part of an integrated control plan for *H. lusitanicum*.
METHODS: Four distant bus similar wild rabbit habitats were selected, in two of them medicated wheat was administered by hoppers, while the other two were used as control. On Day 0 three rabbits per area were sampled to determine the number of ticks in the ears. On day 16, when all wheat was consumed, five rabbits per area were sampled.
RESULTS: The species of ticks present in rabbits were: *Rhipicephalus pusillus*, *Hyalomma lusitanicum*, *Haemaphysalis hispanica; Ixodes* sp and *Dermacentor marginatus*. After eating all the feed provided, the number of ticks from treated rabbits was significantly reduced compared with those from untreated rabbits in areas that remained parasitized (p= 0.03).
CONCLUSION
Oral spinosad is useful against natural rabbit tick infestation and may be used as a measure in integrated tick control of *H. lusitanicum* population due to its acaricidal activity and because rabbits freely ingest it even when there is an alternative non-medicated food.
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Enzyme immunoassay use in the identification of *Giardia* spp. in *Perna perna* mollusks destined for human consumption

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BACKGROUND: Marine bivalve mollusks are considered bioindicators of environmental pollution especially because they are capable of filtering large volumes of water and can accumulate waterborne pathogens, such as cysts of *Giardia intestinalis*, in their gills and digestive gland. Thus, the ingestion of raw or undercooked mollusks can be a potential source of human infection. This study aimed to use the enzyme-linked immunosorbent assay (ELISA) technique to detect cysts of *Giardia* spp. in tissues of *Perna perna* mussels, destined for human consumption in the coast of the municipality of Mangaratiba, RJ, Brazil.
METHODS: Each sample was prepared from a pool of 10 animals, totaling 72 samples of mussel tissue that were evaluated for the presence of *Giardia* spp. by ELISA. For sampling, only individuals with an average of 6 cm of valve length were analyzed, which is considered the ideal size for consumption. In each sample only the gills and digestive gland were used, which were homogenized with the aid of a mixer and filtered to remove coarse residues. The use of the ELISA followed the recommendations of the manufacturer with minor modifications.
RESULTS: Among the 72 samples, only 22% were positive for the presence of *Giardia* spp. antigens. The obtained results were evaluated by colorimetry and by an ELISA plate reader with a 450/630 nm filter.
CONCLUSIONS: Our results suggest that the use of the immunoassay kit is effective in the diagnosis of *Giardia* spp. and could be considered a screening method prior to analysis by other diagnostic methods.

Frequency of IgG antibodies against *Toxoplasma gondii* in women with recent abortion clinical historial from Yucatan Mexico

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BACKGROUND: Abortion has been historically associated with the parasite Toxoplasma gondii. Although it is not the only causal agent that can cause loss of pregnancy, in Mexico there is evidence that it plays an important role in cases of abortion. However it is not very common conducting diagnostic studies of women who have abortions after the clinical event, but only indicated in patients with a history of recurrent abortion . The objective of this report was to determine the frequency of *T. gondii* IgG antibodies in women with a history of recent abortion, originating in the metropolitan area of Merida Yucatan, Mexico.
METHODS: A convenience sample of 127 women with recent abortion clinical historial who came to receive medical assistance to the hospitals Ignacio Garcia Tellez (T1-IMSS) and Benito Juarez (IMSS) from Merida Yucatan Mexico was conducted. The samples were collected between October 2013 and May 2014. To include voluntary participants should sign a letter of informed consent, no history of abortion by non-infectious causes and also should complete a questionnaire to identify factors associated the presence of antibodies. Serum samples were collected and subsequently were evaluated in duplicate by semi-quantitative indirect ELISA (ToxoIgG, Human).
RESULTS: It was found that 68 of the 127 cases analyzed were positive. Importantly, 14.7% of them showed a high concentration of IgG anti-*T. gondii* , which could be indicating an active infection in these patients.
CONCLUSIONS: The results reported indicates there is a high rate of contact with the parasite, probably the population sampled is in a tropical environment with high circulation of the agent , however should be performed other diagnostic test to determine if abortions could be caused by any other infectious agent.

Malaria and Intestinal Parasites co-infection does not alter plasma cytokines profile in subjects from endemic area of Brazilian Amazon.

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BACKGROUND: The immune response against *Plasmodium* is characterized by a Th1 profile. On the other hand, helminthes infections induce a strong immunoregulatory and Th2 response that can inhibit the inflammatory response against malaria, with possible consequences for clinical disease. The aim of our study was to evaluate the occurrence of alterations in cytokine, chemokine, Nitric Oxide Synthase (NOS) and C-Reactive Protein (CRP) levels in individuals co-infected with malaria and intestinal parasites and in individuals with single infections.
METHODS: We recruited 264 volunteers from Joana D´Arc, a rural settlement in Porto Velho, municipality of Rondonia State, Brazilian Amazon. Blood samples were collected for parasite examination and to obtain the plasma used in cytokine, chemokine, NOS and CRP quantification. Stool samples were collected for detection of intestinal parasites. After parasitological analysis four groups of individuals were formed: Individuals infected with Malaria **(M),** Intestinal Parasites **(IP)**, Co-infected **(CI)** and Uninfected **(UN)**.
RESULTS: A univariate ANOVA showed that there were no significant statistical differences between **M** and **CI** individuals but these groups differed from **IP** and **UN** groups. A Principal Component Analysis showed that the first two principal components explained 57.01% of the total variation of the data (PC1=41.84%, PC2=15.17%) and revealed two main groups of individuals among the studied cytokines. The first group contained the most part of **CI** and **M** individuals, and was characterized by high production of CRP, IL-10, TNF-a, IL-2 and IL-6. The second group, containing **IP** and **UN**, was characterized for high production of IL-17A, IL-12p70, NOS and IL-8 production.
CONCLUSIONS: Our results indicate that in our population the cytokine profile in co-infected individuals is highly influenced by malaria infection and intestinal parasite infection does not influence the cytokine profile induced in the malaria. Moreover, multivariate techniques facilitated the analysis of complex datasets with a great amount of variables.

Evaluation of a targeted selective treatment to control subclinical gastrointestinal nematode infections in small ruminant farms.

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BACKGROUND: Targeted Selective Treatments try to find those animals that could obtain a real benefit from an anthelmintic treatment reducing drug pressure. TST have been successfully applied in areas where clinical signs are evident and/or where Anthelmintic Resistance is developed. In this study we try to determine if they work well in areas where GIN produced subclinical infections.
METHODS: Five flocks with four farm management systems were sampled: Ovine Extensive System; Ovine Semiextensive Semi-irrigated System (two flocks); Ovine Semiextensive System and Caprine Organic Semiextensive System.
During one/two years, animals were monthly sampled and evaluated. Three groups per flock were performed and equally managed excepting the indicator used to apply an anthelmintic: egg output (≥ 200 epg); clinical sign (diarrhea, severe bodyweight loss or anemia); and, finally, live bodyweight (<90% than average weight).
RESULTS: GIN egg elimination was very low in all the flocks with a maximum of 275 epg of average, following a bimodal pattern mainly during the first year. The egg output average and the cumulated egg output was significantly reduced with the tst program but the results were more evident in those flocks sampled for two years.
CONCLUSION: All the three protocols reduced the number of treatments to apply, but it seemed to be that under these circumstances egg output could be the best criteria to control subclinical GIN infections.
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Polymorphism in the genes of IFN-γ, IL10 and nitric oxide synthase and their influence in plasma levels and malaria susceptibility in a Brazilian Amazonian population

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BACKGROUND: The presence of polymorphisms in certain genes has been associated with susceptibility or resistance to various diseases. Some relevant genes such as cytokines and nitric oxide synthase genes play a key role in the regulation of the immune response and to the defense against infectious agents. Therefore, in this study we evaluated the polymorphisms in the genes of interferon gama (IFN-γ) and interleukin 10 (IL-10), and nitric oxide synthase and their influence in serum levels and susceptibility to malaria in a population from Brazilian Amazon exposed to infection.
METHODS: We verified the allelic and genotypic frequencies of the single nucleotide polymorphism (SNP), namely IFNG+874T/A, IL10A-1082G/A, IL10A-592A/C, IL10A-819T/C and NOS2A-954G/C in 267 individuals from rural areas of Porto Velho, Rondonia State. Specific DNA fragments were amplified by polymerase chain reaction (PCR), allowing the detection of the polymorphism genotypes. Plasma was used to measure the levels of IFN-γ and IL-10 cytokines, and nitrogen radicals by Luminex and Griess reaction, respectively
RESULTS: Investigation of IFNG+874T/A and NOS2A-954G/C polymorphisms found no association between groups or parameters of susceptibility. Carriers of IL10A -592 A/ -819 T alleles (genotypes AA/TT + AC/TC) were more frequent among subjects with malaria than in negative subjects that presented a higher frequency of the variant C allele. The presence of allele C was associated with low IL-10 and low parasitemia. IL10A-1082G/A polymorphism showed high frequency of heterozygous AG genotype, but it was not possible to infer an association of the polymorphism due to the representativeness of the sample.
CONCLUSIONS: Investigation of IFNG+874T/A and NOS2A-954G/C polymorphisms found no association between the groups and any of the parameters of susceptibility (diagnosis, IFN-γ or nitrogen radicals levels and parasitemia). The results of our study suggest that IL10A-592A/C and IL10A-819T/C polymorphisms are associated with malaria and influence disease susceptibility.

Role in transmission of human and animal fascioliasis in Chile based on nuclear ribosomal and mitochondrial DNA analysis of the freshwater Lymnaeid snail species

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BACKGROUND: Freshwater lymnaeid snails transmit fascioliasis. Disease distribution depends on the presence of the specific lymnaeid vector species. Despite the importance of fascioliasis in Chile, lymnaeid snails have been only the focus of a very few studies.
METHODS: Nuclear ribosomal DNA, primarily ITS-2 and secondarily ITS-1 sequences are the most useful for classification purposes. The mtDNA *cox*1 gene has also proven to be useful for lymnaeids.
RESULTS: DNA sequences indicates that there are three lymnaeid vector species presenting different geographical distributions and transmission capacities in Chile: (i) *Galba truncatula,* as introduced species, would be the responsible for most of the human infection cases throughout a large area restricted to central regions of the country, (ii) *Lymnaea viator* would be the main vector involved in animal infection, mainly in areas *G. truncatula* has not yet have the time to colonise, and (iii) *Pectinidens diaphana* may play a secondary role in southern latitudes if overlapped with *L. viator* and/or *G. truncatula* or in the most extreme southern areas where it is the only lymnaeid.
CONCLUSIONS: Results obtained change the lymnaeid scenario known so far for that country. The DNA sequencing results here included furnish a new baseline on which to undertake future appropriate studies on transmission, epidemiology and control of both human and animal fascioliasis in Chile. Elucidating the detailed geographical distribution of each one of the aforementioned lymnaeid vectors becomes crucial.

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Larvicidal effects of extracts from *Artemisia assoana* cultivated under different environmental conditions against the tick *Hyalomma lusitanicum*

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BACKGROUND: Hyalomma lusitanicum (Ixodidae) is a hard tick, within the group of exofilic ticks and with a North-African and West-Mediterranean distribution. It is consider being vector of *Theileria annulata* and *Coxiella burnetii*, and carrier of several zoonosic agents. Due to the complexity of his life cycle, an appropriate vector control is difficult. The current trend is an integrated control, which includes the use of natural agents with acaricide activity. The genus *Artemisia* L. (Asteraceae) comprises about 500 species, which some of them has, among others, an acaricide activity. As part of our ongoing taxonomically-oriented bioprospection of *Artemisia* sp we have selected *A. assoana,* a rare plant with an Ibero-Mediterranean distribution that grows in degraded grazed land in continental climate at 900–2000 m.
METHODS: Plant material and cuttings were obtained from a wild population growing in Teruel (Spain). These cuttings have been kept in a greenhouse and further multiplied to establish artificial (Aeroponic and *in vitro* transformed root), greenhouse and field cultivations. Following the bioassay method developed in our laboratory volatile (Clevenger distillation) and organic extracts (Sohxlet ethanolic extraction) obtained from wild aerial, aeroponic aerial and root, *in vitro* transformed root, and field aerial parts have been tested against *Hyalomma lusitanicum* larvae to detect potential botanic acaricides. Essential oil from the wild aerial part of *A. assoana* was analyzed by GC-MS, gass chromatograph coupled to a mass detector.
RESULTS: The essential oils from wild aerial and aeroponic aerial parts were the most active ones against *H. lusitanicum* larvae with a mortality percentage of 95, 24±2, 39 y 96, 67±3, 33, respectively, major compounds were camphor and 1, 8-cineole.

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Evaluation of an ELISA test for the serological diagnosis of human fascioliasis in different epidemiological scenarios

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BACKGROUND: Fascioliasis is a zoonosis caused by two fluke species, *Fasciola hepatica* and *Fasciola gigantica*. To improve diagnosis we evaluated the diagnostic accuracy of an enzyme-linked immunosorbent assay (ELISA), with *Fasciola* antigen from the adult liver fluke, for the detection of IgG against fascioliasis in human sera.
METHODS: The sera of 54 fascioliasis patients, originating from three endemic areas, were used in this evaluation: (i) a hyperendemic *F. hepatica* area where humans usually shed a great number of parasite eggs in faeces (11 sera); (ii) an epidemic *F. hepatica* area where humans usually shed small amounts of parasite eggs (24 sera) and (iii) an overlap area of both *Fasciola* species and where human shedding of parasite eggs in faeces is usually scarce or non-existent (19 sera). One hundred and sixty-eight patients with other parasitic infections and 89 healthy controls were also analysed.
RESULTS: The respective sensitivity and specificity of this assay were 95.3% (95% confidence intervals, 82.9–99.2%) and 95.7% (95% confidence intervals, 92.3–97.5%). No correlation between egg output and the OD450 values of the *F. hepatica* IgG ELISA test was observed.
CONCLUSIONS: This test could be used both as an individual serodiagnostic test for human fascioliasis when backed up by a compatible clinical history together with a second diagnostic technique for other cross-reactive helminth infections, and in large-scale epidemiological studies of human fascioliasis worldwide.

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CD4$^+$ T cells apoptosis in *Plasmodium vivax* infection is mediated by activation of both intrinsic and extrinsic pathways

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BACKGROUND: Reduction in the number of circulating blood lymphocytes (lymphocytopenia) has been reported during clinical episodes of malaria and is normalized after treatment with anti-malaria drugs. While this phenomenon is well established in malaria infection, the underlying mechanisms are still not fully elucidated. In the present study, we investigated the occurrence of apoptosis and its pathways in CD4$^+$ T cells in naturally *Plasmodium vivax*-infected individuals from a Brazilian endemic area (Porto Velho – RO).
METHODS: Blood samples were collected from *Plasmodium vivax*-infected individuals. The apoptosis was characterized by cell staining with Annexin V/FITC and propidium iodide and the apoptosis-associated gene expression profile was carried out using RT2 Profiler PCR Array–Human Apoptosis. The plasma TNF-α level was determined by ELISA.
RESULTS: *Plasmodium vivax*-infected individuals present low number of leukocytes and lymphocytes with a higher percentage of CD4+ T cells in early and/or late apoptosis. We observed increased gene expression for TNFRSF1B and Bid, associated with a reduction of Bcl-2, in individuals with *P. vivax* malaria. Furthermore, these individuals showed increased plasma levels of TNF-α compared to malaria-naive donors.
CONCLUSIONS: Our results suggest that *P. vivax* infection induces apoptosis of CD4$^+$ T cells mediated by two types of signaling: by activation of the TNFR1 death receptor (extrinsic pathway), which is amplified by Bid, and by decreased expression of the anti-apoptotic protein Bcl-2 (intrinsic pathway). The T lymphocytes apoptosis could reflect a strategy of immune evasion triggered by the parasite, enabling their persistence but also limiting the occurrence of immunopathology.

The role of the wild boar (*Sus scrofa* Linnaeus, 1758) as secondary reservoir of *Fasciola hepatica* in Galicia (NW Spain)

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BACKGROUND: Fascioliasis is an emerging or re-emerging human and animal disease in numerous parts of the world. In Galicia (NW, Spain), the wild boar (*Sus scrofa*) is the main wild ungulate in terms of abundance and distribution.
METHODS: Livers from 358 hunted wild boars were analyzed showing that 11.2% were parasitized by *F. hepatica*, with burdens ranging from 1 to 14 flukes (mean=2.3). Fecal analysis demonstrated that 40.0% of parasitized animals shed *F. hepatica* eggs with a mean excretion of 6.1 eggs per gram of feces (epg). The presence of coproantigens analyzed by MM3-COPRO ELISA was positive in 62.9% of infected wild boars. After incubation, the percentage of hatched eggs ranged from 41.0% to 90.0%. Comparative morphometric data were obtained using a computer image analysis system (CIAS) on the basis of standardized measurements.
RESULTS*: F. hepatica* specimens from cattle, sheep and wild boars from the same geographical area present similar body development and gravidity.
CONCLUSIONS: The high prevalence of infection detected in the wild boar, the normal fluke development in the liver, and the possibility of shedding *F. hepatica* eggs capable of embryonating and giving rise to viable miracidia with the potential to infect intermediate hosts suggest a possible role of this species as a secondary reservoir in this Spanish region.

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Evaluation of the antimalarial properties of Mexican oregano (*Lippia berlandieri* Schauer) in a murine model of disease.

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BACKGROUND: Mexican oregano (*Lippia berlandieri* Schauer) is a potent antimicrobial and antioxidant plant endemic to most areas of the country, and it is widely produced in the northern state of Chihuahua. Oregano is used primarily in the food industry and its antibiotic properties against bacterial pathogens and some fungi have been widely reported, although few studies have evaluated its antimalarial properties. In this study we evaluate the role of the essential oil of Mexican oregano administered to a murine model of malaria.
METHODS: Pathogen free BALB/c mice from 6-8 weeks, of the same sex and caged individually, were challenged with $5x10^3$ parasitized red blood cells with lethal strain *Plasmodium yoelii yoelii* administered i.p. Mice were tail-bled daily and screened for weight loss and percentage of parasitemia. Essential oil emulsion of *L. berlandieri* Schauer was administered i.p., s.c., or orally at a dose of 100 mg or 200 mg/kg to treated mice. Control groups were treated either with 25 mg/kg cloroquine (CQ) or vehicle.
RESULTS: Mice treated with essential oil showed a reduced parasitemia compared to vehicle-control group that died, or were humanely sacrificed (weight loss >20% than mean on Day 0), on Days 6-7 post-infection. All mice from the pilot experimental group, died over the same period of time with <45% parasitemia, regardless of the route of administration of the essential oil.
CONCLUSIONS: Further research is required to complement the preliminary observations of partial reduction in parasitemia associated with treatment of essential oil of *L. berlandieri* Schauer. Additional evaluation of its purified active constituents *in vitro*, as well as in alternate murine models of the disease, would also be advantageous.

Bertiella spp. identified in human at the Tropical Medicine Foundation Dr. Heitor Vieira Dourado, Manaus, Amazonas, Brazil

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BACKGROUND: The *Bertiella* genus (Cestoda: Cyclophyllidea, Anoplocephalinae) bears various species parasiting wild animals, particularly in nonhuman primates. Two species of *Bertiella*, *B. studeri* and *B. mucronata*, have been observed in human in Asia, Africa and the Americas. In Brazil, only five cases of human Bertiellosis have been reported. The first case was observed in 1930 in the state of São Paulo. *Bertiella mucronata* is endemic in the Americas and its diagnosis is based on eggs with pyriform hooklets and proglottis. These structures are smaller compared to *B. studeri* eggs. Here, we report three individuals infected by *Bertiella* spp. These were individuals were diagnosed at the Tropical Medicine Foundation Dr. Heitor Vieira Dourado (FMT - HVD) Amazonas, Brazil.
METHODS: Stool samples were examined by wet sieving methods, Lutz and Kato/Katz for the diagnosis of proglottids and eggs of the parasite. The dimensions of the proglottides and eggs were measured.
RESULTS: Two females, aged thirty months and 35 years respectively, and a male of 43 years were attended at the FMT-HVD. The child was from the municipality of Santarém - PA and the two adults from Manaus - AM, Brazil. The three patients were with diarrhea and abdominal pain and had spontaneous elimination of the proglottides in diapers and underwear. Fifty-three eggs in the stools were examined by microscopy and showed spherical shape with a mean diameter of 44.29µm (range 36.92 to 52.11 microns), and clearly showed typical pyriform apparatus. Thirteen proglottides were analyzed and showed a mean width of 0.63mm (0.4 to 0.9) and a mean length of 1.66 mm (1.0-2.1µm). The patients were treated with a single dose of praziquantel (10mg/kg). The child and one the adult stopped eliminating the parasites after one day. The other adult continued spontaneous elimination of the parasites for nearly 20 days.
CONCLUSIONS: In the Brazilian Amazom, the number of cases continues to increase and must be an alarm to the health authorities.

Docking studies in Arginase from *Leishmania mexicana* to find potential inhibitors.

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BACKGROUND: *Leishmania spp.* is a protozoan that causes a number of diseases that can affect either appearance or cause death in the patient. Pentavalent antimonials are the most widely treatment used, however, these drugs are inadequate due to their toxicity, high costs, and the emergence of resistant strains. Therefore it is important to looking for new drugs against leishmaniasis. In this regard, various studies have shown that arginase, which catalyzes the hydrolysis of L-arginine to L-ornithine and urea, is important for the survival of both the intracellular and extracellular forms of the parasite. Here we used this enzyme as starting point to search molecules with potential leishmanicidal activity.
METHODS: Docking studies were made using the crystal structure of arginase from *Leishmania mexicana* (LmARG, PDB:4IU0) and the "Fragment" library of small molecules from Chembridge. The search of potential inhibitors was performed with MOE (www.chemcomp.com) and Glide (www.schrodinger.com) software, having the catalytic site as the binding pocket.
RESULTS: The three best matched molecules among both programs were compounds 303, 236 and 1061. An extra precision docking procedure in Glide applied to them, showed binding energies of -7.662, -7.373, and -5.101 Kcal/mol, respectively. Compound 303 made hydrogen bond interactions with Asp194 and Glu197; while 236 formed it with Ser150, His154 and Glu288. Finally, compound 1061 made hydrogen bonds with Asp14, Ser150, and Asn152. Predicted drug likeness score from these molecules was in the range to be considered as potential drugs.
CONCLUSIONS: These molecules could be potential inhibitors of LmARG and serve as a guide in the search of a new chemotherapy against leishmaniasis.

Traditional Iranian food and human Fascioliasis

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BACKGROUND: Contamination by fasciolids takes place through ingestion of metacercariae attached to vegetables. Experimental studies were performed with plant-made foods suggesting a role in human contamination in Iran. In this study we tested the viability of *F. gigantica* metacercariae in two types of Iranian foods, delar and zeitoon-parvardeh.
METHODS: The metacercariae obtained from the snail of the species *Lymnaea gedrosiana* were divided into three groups: 1) added to zeitoon-parvardeh; 2) added to delar; and 3) kept in water as control. Microscopical analyses included the weekly checking of metacercarial viability, metacercarial cysts recovered from the foods were studied under a light microscope. This method was used for metacercariae kept in zeitoon-parvardeh only for 2 weeks after food preparation and for delar until the 4th week after preparation. These metacercariae were than used to infect mice and hamsters.
RESULTS: In the foods assayed, the viability decreases with time, and a high percentage of the metacercariae was still alive 2 and 4 weeks after preparation. Infection of laboratory animals proved that metacercariae kept their infectivity.
CONCLUSIONS: The results here obtained show that the two traditional foods analyzed seem to accelerate the decreasing processes of viability and infectivity of metacercariae. Moreover, data obtained indicate that delar has a greater negative impact than zeitoon-parvardeh on both metacercarial viability and infectivity, most probably because of the high salt concentration of delar.

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Identification of potential cardiac biomarkers in chagasic cases from a rural community in the southeast of Mexico.

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BACKGROUND: Chagas disease caused by the protozoan *Trypanosoma cruzi* (*T. cruzi*), has an annual incidence of 28,000 cases in America and affects 6 to 8 million people, causing about 12,000 deaths a year. There are no efficient pharmacological and immunological methods for treatment and a significant percentage of positive cases develop heart disease. So the aim of this study was to identify changes in clinical settings Myeloperoxidase (MPO) and Nitric Oxide (NO) which could be used as potential markers for heart disease in patients infected with *T. cruzi.*
METHODS: Samples from 40 cases previously identified and confirmed as seropositive for *T. cruzi* from a rural community in Yucatan Mexico (Mayapan) were analyzed to determine the concentration of MPO and also of NO. MPO activity was measured according to the method of Bradley et al. Serum samples mixing a potassium phosphate buffer and a mixture of O- dianisidine and 0.0005% H2O2 was added. The change in absorbance was measured after the samples have been exposed to dianisidine solution and the reaction was stopped with sodium azide. For the determination of NO, the principle of the assay is the reduction of nitrate and sulfate, zinc cadmium, followed by color development with Griess reagent. Subsequently, a test of T, where the average concentrations recorded were compared with the average obtained during the clinical measurement of these values in the sera of healthy individuals was performed.
RESULTS: MPO levels, which can be measured as a marker of neutrophil activation, increased 5.3 fold in the serum of seropositive/chagasic subjects compared to that observed in the seronegative samples. With regard to NO, nitrite concentration was increased 12.24 times more in the serum of seropositive/chagasic subjects compared to the measured concentration in seronegative individuals.
CONCLUSION: These results were analyzed values increase is observed, suggest that activation of neutrophils (MPO) and macrophages (iNOS/NO) contribute to the inflammatory state in chagasic seropositive patients, however the identification of other clinical values that are altered in chagasic patients is needed should be identified that can be used as potential biomarkers to aid in the early detection of heart disease in an efficient, quick and easily.

First *in vitro* feeding of the tick *Hyalomma lusitanicum*

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BACKGROUND*:* Today, research procedures are conducting to reduce or eliminate the use of animals in experimental assays. In consequence, the design of *in vitro* methods to cultivate vector arthropods is essential for the development of new drugs with insecticide and/or acaricide activities and to perform assays to demonstrate vectorial capacity. Until now there are just a few in vitro protocols to feed ticks that avoid the use of animals but they cannot be directly applied to all the species of ticks. The aim of this study was to adapt the *in vitro* feeding assay designed by Dr. Kroeber and Guerin (to feed *Ixodes ricinus*) to *Hyalomma lusitanicum* adults, the most abundant exophilic tick in mesomediterranean environments.
METHODS: the protocol used was based on that described by Kroeber and Guerin in 2007 modified by the use of commercial sterile ovine blood, red deer hair, red deer hair extract, preconditioning periods of ticks or the order of adding males and females into the feeding units.
RESULTS: That is the first time that engorged female of *H. lusitanicum* has been obtained by in vitro feeding.

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Development of a model of oral infection with two Mexican TcI *Trypanosoma cruzi* strains.

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BACKGROUND: The acquisition of *T. cruzi* by the oral route, is currently the most important mechanism of infection with this parasite in parts of South America. In humans, oral infection with *T. cruzi* has a high mortality during the acute phase due to the development of severe myocarditis. This condition may be due to tissue tropism of the isolates of *T. cruzi* lineage belonging to TcI that have been reported on this mechanism of infection. However, the study of the infectivity of *T. cruzi* by the oral route, has been restricted to South American strains. The aim of this study was to develop an oral infection with two Mexican TcI *T. cruzi* strains.
METHODS: Female mice of the Balb/c strain were infected orally with $1x10^5$ trypomastigotes of Querétaro (Qro) or Ninoa strain. The control group was inoculated with blood of non infected mice. The presence of parasite nests and inflammatory infiltrate was assessed by IIF and H / E staining on histological sections. Curves of parasitemia and the survival rate for infected groups were determined. By indirect ELISA-IgG and secretory IgA-antibodies were evaluated in serum and stool supernatants, respectively.
RESULTS**:** In both experimental groups parasites were observed in blood, having higher parasite load the mice infected with Qro strain. Nests of amastigotes were found in the stomachs of infected mice with Qro strain. In infected mice with the Ninoa strain the nests were only observed in the heart. Inflammatory infiltrate in the stomach, heart and skeletal muscle was found in both experimental groups. The presence of IgG in sera was observed in both groups. IgA-secretory antibodies anti-*T. cruzi* were founds in stool supernatants only in the Ninoa infected group. A mortality of 33 % was observed only in infected mice with Qro strain. This value was lower than those observed when the parasite was inoculated by intraperitoneal route.
CONCLUSION: The Mexican TcI Qro and Ninoa *T. cruzi* strains are able to infect orally and establish an infection in the digestive tract and also at systemic level, inducing a humoral and cell immune response.
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Searching potential inhibitors of phosphoglycerate mutase 1 from *plasmodium falciparum* through virtual screening.

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BACKGROUND: Malaria, caused by the parasite *Plasmodium falciparum*, has a prevalence of 250 million clinical cases and a letal rate of 1 million people per year. In view of the parasite has developed resistance to drugs already established as the treatment, it is necessary to develop new drugs that can resolve this problem. In this regard, the parasite is dependent on glycolysis as the sole source of energy, enzymes from this pathway, such as phosphoglycerate mutase (PGAM), which catalyzes the conversion of 3-phosphoglycerate to 2-phosphoglycerate, are considered as an excellent target for the search of new inhibitors that can serve in the development of new drugs against malaria.
METHODS: Virtual screening was made using the crystal structure of PGAM1 from *P. falciparum* (PfPGAM1, PDB: 1XQ9), and the “Fragment” library of small molecules from Chembridge. The search of potential inhibitors was performed with MOE (www.chemcomp.com) and Glide (www.schrodinger.com) software, having the catalytic site as target.
RESULTS: From the 7677 molecules docked, compounds 1901, 3383, and 1708 were the best matched among MOE and Glide. According to an extra precision docking, the binding energy from these molecules was -3.898, -4.439, and -3.983 Kcal/mol, respectively. Hydrogen bond and cation-π interactions were formed between these molecules and some residues at the catalytic site. Additionally, a predicted drug likeness score of -1.37, -1.45 and 0.84 for 1901, 3383, and 1708 was obtained, respectively; indicating that these molecules could be considered as potential drugs.
CONCLUSIONS: The three compounds found trough virtual screening, have the potential to inhibit PGAM1 and could be used as hits to obtain new antimalarial drugs.

Anaemia in advanced chronic Fascioliasis

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BACKGROUND: Fascioliasis is considered an important human disease caused by two liver fluke species: *Fasciola hepatica* and *F. gigantica*. The last stage of the disease in humans encompasses an obstructive or chronic phase, which may develop after months to years of infection, including mild to moderate anaemia, especially in heavy infections. The association between fascioliasis-induced anaemia and related factors has been quantified in a rodent model.
METHODS: Haematological parameters were analysed in Wistar rats at 20 and 60 weeks post-infection (p.i.). Pigment stones and bile specimens were collected. Serum IgG1, IgG2a and IgE were determined in rat serum samples. Cytokine levels were correlated with haematological parameters. The screening for gastrointestinal bleeding was carried out.
RESULTS: Multivariate analysis suggested an association between anaemia and the following factors: fluke burden, eggs per gram of faeces, body area of parasite, presence of blood in faeces, IgG1 and eosinophil levels, and % of splenic weight.
CONCLUSIONS: Of all variables analysed, fluke burden is the one, which presents the highest anaemia risk, even exceeding the variable presence of blood in faeces. The development of anaemia appears to be complex and may involve multiple mechanisms. The results of the rodent model lead to the assumption that a high risk of anaemia in subjects with a heavy parasitic burden in human hyperendemic areas of fascioliasis is to be expected.

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Phenotypic characterization of *Fasciola hepatica* from altiplanic and valley South American human endemic areas

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BACKGROUND: Fascioliasis is a zoonotic parasitic disease caused by *Fasciola hepatica* and *F. gigantica*. Of both species, *F. hepatica* is the only one described in the Americas. Human fascioliasis endemic areas are mainly located in high altitude areas of Andean countries. Given the necessity to characterize *F. hepatica* populations involved, the phenotypic features of fasciolid adults infecting sheep present in human fascioliasis endemic areas were analysed in the Cajamarca Valley and Mantaro Valley (valley transmission patterns) and the northern Bolivian Altiplano (altiplanic transmission pattern). A computer image analysis system (CIAS) was applied on the basis of standardized measurements.
METHODS: The aforementioned highland populations were compared to standard lowland natural and experimental populations of European origin. Liver fluke size was studied by multivariate analyses. Two phenotypic patterns could be distinguished in *F. hepatica* adult size: the valley pattern (Cajamarca and Mantaro, Peru) and the altiplanic pattern (northern Altiplano, Bolivia).
RESULTS: Results showed that the Andean valley population and European standard populations presented a phenotypic homogeneity. The Altiplano population showed a large size range with a lower minimum size indicating that uterus gravidity is reached at a smaller size than in valley populations.
CONCLUSIONS: The results demonstrate that there is no apparent relationship between the shape of fasciolid adults with regard to altitudinal difference or geographical origin and that allometry-free shape appears as a more stable trait than size in fasciolid species.

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27-28 kDa antigenic fractions of *Fasciola hepatica* an alternative for the development of serological diagnostic methods for Human Fascioliasis

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INTRODUCTION: Human fascioliasis is an important parasitic disease caused by *F. hepatica* in Peru. The routine diagnosis of this parasitosis is the examination of the feces. Parasitological methods (sedimentation rapid, spontaneous sedimentation, tube sedimentation have a low sensitivity (30-40%) and also not detected in patients with acute fascioliasis. While serological methods (ELISA , Arch II and Western Blotting) using total metabolic antigens secreted and excreted (AgMTFh-E/S) with IgG, have high sensitivity but low specificity due to cross-reactions occur with other parasitosis as *Paragonimus spp.*, *Ascaris lumbricoides* , *Giardia lamblia*, *Blastocystis hominis* etc .
Therefore, it is important to purify the highly immunogenic 27-28 kDa antigenic fractions considered, to help us to increase the sensitivity and specificity of serological tests, avoiding cross-reactions that exist; while developing a diagnostic method that allows us to detect the onset of the disease in the acute and chronic phase.
METHODS: The adult worms of *F. hepatica* were obtained from naturally infected sheep livers, they cultivated in minimum essential medium in vitro conditions for 16 hours at 37 °C; recovering spit; These were centrifuged at 10 000 xg, where the supernatant was used as secreted-excreted, to purification by chromatography on Sephadex G-100 metabolic antigens. An electro transfer at constant voltage of 55V is then performed to observe their reactivity with immunoenzymatic patient serum fascioliasis.
RESULTS: The purified antigenic proteins were visualized on SDS-12% PAGE and analyzed in the documenter Gel (BioRad) comparing the electrophoretic mobility, presenting an approximate molecular weight of 27-28 kDa.
CONCLUSIONS: The purified fractions 27-28 kDa antigen immunosorbent presented to immunoglobulins G immunoblot for the diagnosis of patients with fascioliasis reaction.

Evaluation of the naturally acquired IgG antibodies to a chimeric *Plasmodium vivax* Reticulocyte Binding Protein-1: Lack of association with HLA-DRB1*/DQB1* in malaria exposed individuals from the Brazilian Amazon.

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BACKGROUND: The development of modular constructs that include antigenic regions targeted by protective immune responses is an attractive approach for subunit vaccine development. However, a main concern of using these vaccine platforms is how to preserve the antigenic identity of conformational B cell epitopes. In the present study we evaluated antibody responses to a chimeric protein engineered to contain a previously defined immunodominant domain of the *Plasmodium vivax* reticulocyte binding protein-1 located between amino acid positions K_{435}-I_{777}. The construct also includes three regions of the cognate protein (F_{571}-D_{587}, I_{1745}-S_{1786} and L_{2235}-E_{2263}) predicted to contain peptide sequences that bind to multiple human MHC class II alleles.
METHODS: Plasma samples from 253 naturally exposed individuals were tested against this chimeric protein named PvRMC-RBP1and a control protein that includes the native sequence $PvRBP1_{23\text{-}751}$ in comparative experiments to study the frequency of total IgG and IgG subclasses reactivity. HLA-DRB1 and HLA-DQB1 allelic groups were typed by PCR-SSO to evaluate the association between major HLA class II alleles and antibody responses.
RESULTS: We found that the chimeric PvRMC-RBP1 and the $PvRBP1_{23\text{-}751}$ were recognized respectively by IgG antibodies of 47.1% and 60% of the studied population. Moreover, the reactivity index (RI) of IgG antibodies against both proteins were comparable and associated with time of exposure ($p<0.0001$) and number of previous malaria episodes ($p<0.005$). IgG subclass profile showed a predominance of cytophilic IgG1 over other subclasses against both proteins tested.
CONCLUSIONS: Collectively these studies suggest that the chimeric PvRMC-RBP1 protein retained antigenic determinants in the $PvRBP1_{435–777}$ native sequence. Although 52.9% of the population did not present detectable titers of antibodies to PvRMC-RBP1, genetic restriction to this chimeric protein does not seem to occur, since no association was observed between the HLA-DRB1* or HLA-DQB1* alleles and the antibody responses. This experimental evidence support the maintenance of antigenic identity of conformational B cell epitopes of the protein in the chimeric form.

Phylogenetic analysis of trypanosome isolates of veterinary interest and first report of *T. equiperdum* in Venezuela

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BACKGROUND: *T. equiperdum* is the only trypanosome not transmitted by an insect vector, is the etiological agent of dourine or *mal du coit* sexually transmitted between horses. In turn, *T. equiperdum* is considered a phylogenetically related species/ subspecies of *T. brucei*. The literature has reported the presence of small mutants of *T. brucei* that have totally or part of the structure of maxicircle (kDNA) lost, as is the case of *T. equiperdum*, which lost the ability to biological transmission by tsetse fly.
METHODS: In this study we compared two Venezuelan trypanosome isolates of veterinary interest (horse, natural host) expanded experimentally in a rat model, with conducting genomic DNA extraction, PCR amplification and sequencing of four specific genes of maxicircle (Cytochrome b , cytochrome oxidase subunit 1, ATP synthase subunit 6 and NADH dehydrogenase subunit 8), was evaluated phylogenetic relatedness of the isolates from the study by phylogenetic trees (maximum likelihood and maximum parsimony) taking several sequences available in the GenBank database.
RESULTS: The two field isolates from horse possess the four specific genes maxicircle studied. Phylogenetic analysis of these genes maxicircle studied from the two isolates related with *T. brucei* and *T. equiperdum*, being of greater significance the phylogenetic relationship by sequence analysis of the cytochrome b gene, which showed close regarding *T. equiperdum* isolates from other geographic areas.
CONCLUSIONS: Although not reported dourine (*mal du coit*) so far in Venezuela, this study provides the first molecular evidence for the presence of *T. equiperdum* in Venezuela.

Molecular analysis of genetic structure and isolation distance of primary malaria vector *Anopheles darling* in Colombia.

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BACKGROUND: *Anopheles darlingi* Root 1926 is a primary vector of malaria in the Neotropic region, a specie not just highly antropophilic but very efficient in transmitting plasmodium species and is considered to be most important vector in the Amazon region. The principal main of this study was determine the genetic structure of *An. darling* populations using microsatellites (STR) as genetic markers applied in 300 anofeline females which were collected from six populations distributed in occidental and oriental regions of Colombia.
METHODS: DNA extraction was done with the cited protocol of Gonzales (2007) band using the *Genomic PrepTM Cell and tissue Isolation* commercial kits. We used the STR reported by Conn et al (2001).
RESULTS: The analysis with STR proved a high genetic diversity and significant alterations from the Hardy-Weinberg balance. The greatest count in diversity was The Mitú,-Vaupés (Na=14, Ho= 0.520). On the other hand, the least count of genetic diversity was Pueblo Nuevo-Córdoba (Na=12, Ho= 0.457). The oriental region and the Mitú populations presented the highest number of provate alleles (Ap=30; Ap=13; Ap=9.), whit variations between 0.010-0.097. The AMOVA analysis show that the whole population underwent moderated genetic differentiation (Frt=0.063, p<0.05). The same differentiation was noticed (0.06<Fst>0.06, p<0.05) with five of the six included populations in this job and a low differentiation in the Margaritas-Santander area (Frt=0.02s3, p<0.05).
CONCLUSIONS: Our results suggest a slight positive correlation which does not show a statistical significance between the geographic and genetic distance, probably suggesting that the moderated genetic differentiation found between the couples in populations not be explained for the hypothesis of separation by distance.

Fasciola hepatica and _F. gigantica_ phenotypic intermediate forms in buffaloes from Central Punjab, Pakistan

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BACKGROUND: Fascioliasis is an important food-borne parasitic disease caused by the two trematode species, *Fasciola hepatica* and *F. gigantica*. The phenotypic features of fasciolid adults and eggs infecting buffaloes from the Central Punjab area, Pakistan, have been studied to characterize fasciolid populations involved. Morphometric analyses were made with a computer image analysis system (CIAS) applied on the basis of standardized measurements.
METHODS: Since it is the first study of this kind undertaken in Pakistan, the results are compared to pure fasciolid populations: (a) *F. hepatica* from the European Mediterranean area; and (b) *F. gigantica* from Burkina Faso; i.e. geographical areas where both species do not co-exist. Only parasites obtained from bovines were used.
RESULTS: The multivariate analysis showed that the characteristics, including egg morphometrics, of fasciolids from Central Punjab, Pakistan, are between *F. hepatica* and *F. gigantica* standard populations. Similarly, the morphometric measurements of fasciolid eggs from Central Punjab are also between *F. hepatica* and *F. gigantica* standard populations.
CONCLUSIONS: These results demonstrate the existence of fasciolid intermediate forms in endemic areas in Pakistan.

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Brazilian *Morinda citrifolia* fruit (Noni) juice induces increased NO production and death of *Leishmania amazonensis* amastigotes inside BALB/c peritoneal macrophages

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BACKGROUND: The effects of brazilian *Morinda citrifolia* fruit (Noni) juice (BNJ) have been researched against several bacterial affections and cancer diseases. Our group has previously demonstrated that BNJ has activity *in vitro* against *L. amazonensis* promastigotes, however its effect in amastigotes inside macrophages needed to be investigated. Therefore, the aim of this study was to analyze the BNJ effects in peritoneal macrophages infected with *L. amazonensis*.
METHODS: Peritoneal macrophages from BALB/c mice were infected with *L. amazonensis* promastigotes and treated with BMJ (100µg/mL). After 48 hours of treatment, the amount of intracellular amastigotes were counted in 100 cells, the nitrite production was estimated in culture supernatant using Griess reagent and iNOS expression was quantified by RT-PCR. We repeated the experiment in cells pre-treated with aminoguanidine, a selective inhibitor of iNOS, and analyzed the same parameters to study the impact of NO.
RESULTS: BNJ treatment presented an IC50 equal to 63,6µg/mL against intracellular amastigotes and led to an increased nitrite production in infected and non-infected macrophages. When macrophages were pre-treated with aminoguanidine the IC50 increased to more than 120µg/mL.
CONCLUSIONS: Our results reveal that BNJ treatment can increase NO production by peritoneal macrophages and that ability is involved in the killing of *L. amazonensis* intracellular amastigotes.

Finding potential inhibitors of *Plasmodium falciparum* triosephosphate isomerase through a "*hit-consensus*" strategy.

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BACKGROUND: *Plasmodium falciparum* causes Malaria, a disease which is responsible of around 650,000 deaths per year worldwide, and more than 3 billion people live in areas at risk. Unsuccessful efforts to control and eradicate malaria are, in part, due to an increasing resistance to clinically used drugs. Therefore, there is an urgency to find new drugs for the treatment of this disease. An important metabolic characteristic is that *P. falciparum* depends on glycolysis as the unique ATP source for cellular work. Under this perspective, several groups have pointed the glycolytic enzyme triosephosphate isomerase (PfTIM) as a good target for antimalarial drug discovery.
METHODS: *Hit-consensus* was performed through virtual screening using Glide and MOE software. To this end, the crystallographic structure of PfTIM (PDB ID: 3PSW) and the ChemBrigde small molecules database were used. Because the PfTIM is only active in its dimeric form, the dimer interface was selected as the target site for virtual screening.
RESULTS: After analyzing and ordering by binding energy the output files from Glide and MOE, the top three common molecules were compounds 5403532, 5484635, and 5326476. Flexible docking studies showed a binding energy of -6.38, -3.51 and -4.24 Kcal/mol, respectively. These molecules made hydrogen bonds and cation-π interactions with residues at the dimer interface. Furthermore, a predicted drug likeness score of -0.37, -0.39, and 0.55 for 5403532, 5484635, and 5326476, respectively, supports that they could be considered as potential drugs.
CONCLUSIONS: The three compounds found by *hit-consensus* could potentially inhibit PfTIM, and serve as a starting point in the search of a new chemotherapy against Malaria.

Screening of plants from Brazilian Amazon and Cerrado against *Leishmania amazonensis*

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BACKGROUND: Since 1912, treatment against leishmaniasis has not evolved.. The usual antimony pentavalent treatment is expensive and difficult to administer. In addition, worldwide resistance to treatment has been increasingly reported. Therefore new antileishmanial drugs are urgently needed. Brazil has one of the richest flora biodiversity on the planet, which is an inexhaustible source of research into new drugs with therapeutic potential. This study presents a screening of 16 plants from the two major biomes of south America: Amazon and Brazilian Cerrado, against *Leishmania amazonensis*.
METHODS: Plants were harvested and used to produce the hydroalcoholic extract. The hydroalcoholic extracts were tested at different concentrations against *L. amazonensis* promastigotes from axenic culture to calculate the inhibitory concentration of 50% (IC(50)) of parasites.
RESULTS: Two extracts showed significant growth inhibitory activity against promastigotes. The extract from *Arrabideae chica* and *Vernonia polysphaera* inhibited the growth of promastigotes, with IC(50) values of 155.9 and 10.6 µg/mL, respectively. The others extract showed low or no activity against *L. amazonensis* promastigotes.
CONCLUSIONS: Our result supports the use of the biodiversity of the Amazon and Brazilian Cerrado fauna in the search for natural products as source of new antileishmanial drugs.

Evaluation of the effect of clodronate-liposomes injections on *Plasmodium falciparum* infection in neo-tropical primates *Saimiri sciureus*

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BACKGROUND: One of the constraints in the development of antimalarial vaccines is the limited availability of experimental models that reliably reproduce the immune responses observed in human malaria. The best experimental models are neotropical primates of the genus *Saimiri* and *Aotus*, but they have as important limitation the need of splenectomy for expression of high and consistent parasitemias. The clodronate-liposomes (CL) have been used to deplete macrophages in several experimental models and we believe that their use in *Saimiri* can allow the development of consistent parasitemias without splenectomy.
METHODS: First experiment: six animals non-splenectomized were divided in two groups receiving 1mL PBS or CL (5mg/mL) and were infected at d0 with *P. falciparum* (FUP strain). Second experiment: 14 animals non-splenectomized were used in six groups - three uninfected control group, with 2 animals each one, which received 1mL PBS, 0.5mL or 1mL CL and three groups infected at d0 and that received the same injections (PBS-group with 2 animals and CL-groups with 3 animals each one). In both experiments the injections were done intravenously twice weekly from d0. Follow-up and histological examination were performed.
RESULTS: In the first experiment, animals receiving CL had higher parasitemia than animals receiving PBS. In the second one, infected animals receiving 0.5mL CL showed parasitemia higher than the other two groups. In all infected animals, temperature was related to parasitemia. Hemoglobin and hematocrit concentration decreased reaching minimum values during or after clearance of parasite. The animals tolerated the CL injections, but signs of liver toxicity were observed on histopathology. Animals receiving CL showed decreased marking of iron in spleen.
CONCLUSIONS: Our results suggest that CL is able to promote higher parasitemias in *S. sciureus* causing infection in the same way that splenectomy. Further studies are needed and are been undertaken to confirm data.

Expression of pro-inflammatory molecules in osteoblast by *Trypanosoma cruzi* infection.

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Abstract.
Background: *Trypanosoma cruzi*, the etiological agent of Chagas disease, uses multiple strategies to ensure its establishment and persistence in the host. The parasite has the ability to infect different organs and tissues. During the acute phase, a large number of parasites are present in the bloodstream, and all types of nucleated cells in the host are potential targets for infection. Although the parasite is capable of infecting nearly any nucleated cell *in vitro*, cardiac and skeletal muscle are believed to be the main tissues involved in the development of the pathology *T. cruzi* persistence appears to be associated with its capacity to replicate inside specific host cells and evade immune system recognition, but cell tropism is still poorly understood. Here, we evaluated the effects of *T. cruzi* infection in osteoblasts (MG-63).
Methods: Osteoblast MG-63 cell was cultured in Dulbecco's Modified Eagle medium supplemented with 10% fetal calf serum, and infected with different ratios of *T. cruzi* parasites (1:5, 1:1, and 2:1 parasites/osteoblast, MHOM/MEX/1990/H1/*T. cruzi* I strain). Intracellular parasites were observed by Giemsa staining, and culture supernatants were collected at different times, to determine the production of nitric oxide by the Griess method, and of cytokines and chemokines by ELISA assays. Gene expression for adhesion molecules and metalloproteinases was determined by RT-PCR.
Results: Infection induced the production of proinflammatory cytokines (IL-1β, IL-6, TNF-α) and chemokines (CCL-3, CCL-4, CCL-5, CCL-11, IP-10), and the expression of metalloproteinase-2 and -9 and adhesion molecules ICAM1 and VCAM1).
Conclusions: This work evidences for the first time the infection with *T. cruzi* in osteoblasts suggesting that osteoblasts could play a key role in the homeostatic regulation of osteoblastogenesis and bone resorption during *T. cruzi* infection.

Monogenea (Platyhelminthes) diversity in cultured exotic freshwater fish from Morelos, México.

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BACKGROUNDS. In the past ten years, freshwater aquaculture in México has experienced a constant development; in particular, in the State of Morelos ornamental fish has become established as an industry. Most of fish cultured are exotic to Mexico and many of the species carry monogenean fauna. Information about introduction of monogenean fauna along with exotic fish is scarce, despite the fact that these parasites may cause heavy mortalities and diseases. The present study evaluates the monogenean fauna diversity of cultured fish of Morelos.
METHODS. Fish belonging to eight families were collected from different localities (farms). Fish families were Cichlidae Characidae, Cyprinidae, Ictaluridae, Loricariidae, Pangasidae, Poeciliidae and Osphronemidae. The parasites were fixed in heated 4% formalin and were processed using ammonium picrate glycerine.
RESULTS. Eigth species of *Cichlidogyrus*, one species of *Enterogyrus* and one of *Scutogyrus* were collected from *Oreochromis* spp.; one species of *Enterogyrus* from *Hemichromis* sp. and one *Gussevia* from *Pterophyllum scalare*; two species of *Silurodiscoides* were collected from *Pangasianodon hypophthalmus*; four species of *Dactylogyrus* from *Cyprinus carpio* and *Carassius auratus*; and one species of *Trianchoratus* from *Trichogaster trichopterus.*
CONCLUSION. The present study is the first evaluation of monogenean fauna diversity from exotic ornamental and food fish cultured in Morelos. With the exception of *C. sclerosus* and two species of *Dactylogyrus*, the rest of the monogenean species are reported for the first time from Morelos.

Morphological and proteomic characterization of post-blood feeding midgut epithelium and peritrophic matrix of malaria vector *Anopheles albimanus*

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BACKGROUND: The midgut of female mosquitoes is the organ that receives and digests the blood during feeding. To accomplish blood processing midgut needs to resist strong stretching, synthesize and secrete enzymes and hormones and initiate an immune response, because different infectious agents are introduced and proliferate in subsequent hours. In addition, the midgut constructs and secretes the peritrophic matrix (PM), a thick extracellular sheath, which plays several physiological roles. The aim of this work was to characterize the structure and differential proteins during bloodfeeding and early digestion in *Anopheles albimanus*, a major malaria vector in Mexico.
METHODS: Sugar- and bloodfed *An. albimanus* adult female midguts were observed by electron microscopy and protein analysis was carried out by two-dimensional electrophoresis. To identify the peptides, differential protein spots were analyzed by ESI-LC-MS/MS.
RESULTS: Before blood feeding, midgut epithelial cells contained numerous electrondense vesicles distributed in a central to apical distribution. After a bloodfed, vesicles content was secreted to the luminal side of the midgut by the apical cellular surface. At early times after blood ingest, the PM is formed near microvilli as a granulous amorphous material and after it consolidates forming a highly organized multi-layered structure. Proteomic comparative analysis of sugar and bloodfed midguts showed differentially expressed proteins, which are involved in innate immunity, cytoskeleton structure, stress response, signaling, and digestion, detoxifying and metabolism enzymes. The same analysis for peritrophic matrix showed the presence of innate immunity and signaling proteins.
CONCLUSION: Since midgut is an strategic territory during *Plasmodium*-anopheline vector interaction which happen after a boodmeal, the PM, cytoskeleton, enzymes and signaling proteins modified by feeding could participate in interactions with the parasite and in some cases to block its development, such as investigations of midgut and PM proteins may lead to new insights for control of disease transmission.

Antileishmanial assessment activity of bark extract from *Croton blachetianus* Baill

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BACKGROUND: Pentavalent antimony compounds have been used as first line drugs to treat leishmaniasis for 50 years. However, their high levels of toxicity and drug resistance acquired by parasites, make them a poor clinical agent. Therefore, the development of alternatives treatments is urgent. The aim of this study was to assess the inhibitory activity of the extract from barks of *Croton blachetianus* (known in Brazil as “marmeleiro") over the growth and survival of *Leishmania infantum* and *L. amazonensis*.
METHODS: Promastigotes in logarithmic growth phase were plated at $1x10^6$ cell/ml in a 96-wells plate and treated with *Croton blachetianus* extract (10.0 to 500 µg/mL). The cell viability was assessed after 72 hours by fluorometric assay using Resazurin and the 50% inhibitory concentration (IC_{50}) was determined. Peritoneal macrophages from Balb/c were infected with promastigotes in the stationary growth phase using a ratio 1:3 (for *L. amazonensis*) and 1:5 (for *L. infantum*) at 37 °C for 3-4h. After remotion of non-interiorized parasites, the infected cultures were incubated with 5.0, 10.0, 20.0 or 30.0 µg/mL of *Croton blachetianus* extract for 72 hours. Afterward, the cultures were stained using Panótico staining and the survival index was determined.
RESULTS: The IC_{50}/72h values were 108.90 and 42,63 µg/mL for promastigotes of *L. infantum* and *L. amazonensis*, respectively. For amastigotes, these values were 9.00 and 5.98 µg/mL. Morphological changes such as rounded forms and double flagelum were observed in promastigotes submitted to concentrations higher than 50 µg/mL.
CONCLUSIONS: The crude extract of *Croton blachetianus* efficiently inhibited the parasite growth of both species. Although the treatment has led to changes in the promastigotes morphology, the amastigotes forms shown to be more sensitive. So, we can consider the extract of *Croton blachetianus* as a promising source of compounds with leishmanicidal activity.

Intestinal protein-based vaccines against *Haemonchus contortus*

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BACKGROUND: Parasitic nematodes contain and secrete various proteases (proteolytic enzymes) which are known to have or are proposed to have many purposes including the penetration of host tissues barriers, to digest host protein for nutrients and to evade host anti-parasite immune responses. Hence, proteases and other intestinal proteins have been targeted as vaccine components.
METHODS: Proteases required for digestion, including ES proteins, are associated with vaccination-induced protective immunity in cattle and sheep against the abomasal parasite *Haemonchus contortus*. Proteins found on the intestinal surface of the latter are amongst the most efficacious vaccine antigens identified to date for any helminth parasite.
RESULTS: This overview will discuss a variety of endo- and exopeptidases required to digest haemoglobin in the blood meal, this being undertaken by an ordered and partly conserved protease cascade by analogy to hookworms and *Plasmodium spp*. In addition to cysteine proteases, other intestinal proteases include aspartic and metalloproteases, aminopetidases and dipeptidyl peptidases. Equivalent proteins and proteases have been implicated in effective vaccination against *Mecistocirrus digitatus*, a regionally important blood-feeding nematode parasites which affects cattle and buffalo in tropical regions of the world. A major barrier to vaccine production is that recombinant protease vaccines lack the efficacy of their native counterparts for a variety of reasons including incorrect protein folding and/or the lack of, or inappropriate, post-translational modifications such as glycosylation. These issues are being addressed by the use of eukaryotic expression systems such as insect cells and recent work indicates that the free-living nematode *Caenorhabditis elegans* can be also engineered to produce functional heterologous nematode parasite proteases, particularly many of the above.
CONCLUSIONS: Native protein vaccines provide an effective means for controlling *Haemonchus contortus* infections in the field and the challenge of producing equivalent, recombinant versions is being addressed, with some success, using *C. elegans.*

Cryptosporidium and the potential impact of aquatic ecosystems

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BACKGROUND: *Cryptosporidium* is widely considered a waterborne pathogen. Other routes and vehicles of transmission to humans include person- or animal-to-person spread, food and fomites. However, drinking and recreational waters account for the majority of outbreaks and a substantial proportion of cases of cryptosporidiosis, especially where filtration is poor or absent. Monitoring and detection of oocysts by microscopy does not differentiate species, or give indication of their source, for which genotyping is required. Occurrence studies have focused mainly on land dwelling mammals, with few studies of aquatic wildlife which may be hosts or vectors in source waters. We are developing a catchment pathogen model for human exposure to *Cryptosporidium,* investigating land use change scenarios. The model will be populated with *a priori* and new data regarding the abundance and human infectivity potential (species) of *Cryptosporidium* from primary (host) and secondary (vector) sources and events in catchments.
METHODS: Parameter estimates will be derived from published data. Where no suitable data are available, prospective sampling and testing has been undertaken, especially aquatic hosts and vectors, (fish guts, gills and faeces, invertebrate larvae, biofilm from river stones and aquatic mammals). DNA was extracted using freeze-thaw, proteinase k digestion and spin-columns and screened for *Cryptosporidium* by nested PCR ssu rRNA gene. Positive samples were subjected to qPCR.
RESULTS: The lack of data and a complex exposure pathway for *Cryptosporidium* collectively lead to simplifying assumptions the model. New data have been generated where *Cryptosporidium* was detected in fish samples, indicating they may be a host, or act as a transport vector for *Cryptosporidium hominis*.
CONCLUSIONS: Aquatic biota may influence oocyst detections in source waters but the main impact is likely from land mammals, including humans, and modeling the effect of land use change scenarios may help to predict human exposure to *Cryptosporidium* in aquatic ecosystems.

Anthelmintic resistance: the more we know, the more we realize how little we know

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The age of modern parasite control through the use of highly effective broad-spectrum anthelmintics began in 1961 with the introduction of thiabendazole. And thus the age of anthelmintic resistance began as well. With the introduction of each new anthelmintic class, reports of resistance soon followed. By the early 1990's the mechanism of resistance to benzimidazole (BZ) anthelmintics was elucidated, and molecular diagnostic assays for detecting BZ-resistance were developed soon thereafter. Given this accomplishment, and the exciting new technologies available, many parasitologists shared optimistic expectations that discovery of the mechanisms of resistance and the development of molecular diagnostic assays for the other major anthelmintic classes would soon follow. However, it is now quite clear that these expectations were overly optimistic; almost 30 years after the first report of ivermectin resistance in *Haemonchus contortus*, we still know quite little about the mechanisms responsible. This failure is not due to lack of effort; but rather, it appears that resistance to macrocyclic lactones (ML) drugs is quite complicated, as are the mechanisms of action (MOA). For many years we thought that the ML exerted their anti-nematodal effects by binding to glutamate-gated chloride channels, leading to pharyngeal and somatic muscular paralysis and worm death. However, we now know that these drugs have additional MOA. Abamectin has recently been shown to antagonize some subtypes of nematode nAChR, and ivermectin recently was shown to have profound inhibitory effects on chemosensory behavior. If we are only discovering these novel mechanisms now after decades of research, how many more might there be that we have not yet discovered? This is especially humbling when one considers that we don't know the function of >40% of all nematodes genes. The only reasonable conclusion is that the more we learn about anthelmintic resistance in parasitic nematodes, the more we realize how little we actually know.

Clinical update on cryptosporidiosis

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BACKGROUND: Many pathogens cause diarrhoea but one of the most notable findings of the recent Global Enteric Multicenter Study (GEMs) study is the importance of cryptosporidium as a cause of moderate to severe diarrhoea in young children in low to middle income countries in sub-Saharan Africa and south Asia (Kotloff *et al*., Lancet, 2013). *Cryptosporidium* was a significant pathogen at all sites regardless of HIV prevalence, the second most common pathogen in infants, and was associated with increased risk of death in toddlers aged 12–23 months. In addition, *Cryptosporidium* is increasingly associated with growth shortfalls, even when controlled for nutritional status. Patients with severe T-cell immune deficiency are also at increased risk from cryptosporidiosis due to development of chronic or intractable diarrhoeal disease, and complications arising from pancreato-biliary infection or, more rarely, tracheo-bronchial involvement. Even in high income countries gastrointestinal symptoms can be prolonged, persisting for up to a month, during which relapse, indicating persistent infection, occurs in over a third of cases. Long-term sequelae have been reported that require further investigation. To investigate health sequelae (including irritable bowel syndrome, IBS) occurring after resolution of acute *C. parvum* infection in adults associated with a food-borne cryptosporidiosis outbreak in England, we undertook a longitudinal study.
METHODS: A total of 54/197 (27%) patients, aged 16 years or over, diagnosed with *C. parvum* during May 2012 completed questionnaires at 6 months after diagnosis. 39/54 (retention rate 72%) completed the 12-month follow-up questionnaire.
RESULTS: Pre-existing IBS was reported by 11 people. At 6 months follow-up, 9/54 (17%) reported symptoms which fulfil the Rome III criteria for IBS, 2 of whom did not report pre-existing IBS. At 12-month follow-up 44% of those with pre-existing IBS reported worsening of IBS symptoms in the 12 months after acute cryptosporidiosis.
CONCLUSIONS: The significance of reported rates of IBS and IBS-consistent symptoms requires further investigation; a Wales-wide prospective investigation of post-acute health sequelae is currently underway.

Sexual steroids modulate oxidative stress in CBA/Ca female mice infected with *P. berghei* ANKA.

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BACKGROUND: Sexual dimorphism is a characteristic of malaria, and men are more prone to develop higher parasitaemia and mortality rates than women. The main differences between the sexes are due to sexual hormones, and the elimination of this parasite by the immune response is strongly associated with phagocytosis and oxidative stress. In this work, we tested whether sexual hormones differentially modulate oxidative stress levels in gonadectomised CBA/Ca female and male mice that had been infected with *P. berghei* ANKA.
METHODS: We decreased the level of gonadal steroids in female and male mice that had been infected with *P. berghei* ANKA by gonadectomy and analised the subsequent impact on the activities of catalase, super oxide dismutase and gluthatione peroxidase. In addition, nitric oxide (NO) levels and malonildialdehyde levels were also measured in both blood and spleen.
RESULTS: Gonadectomized female mice exhibited lower specific catalase-, superoxide dismutase- and glutathione peroxidase- activities in their blood and spleen tissues compared with gonadectomised males. Intact, sham-operated and gonadectomised female mice exhibited higher levels of nitric oxide in the blood and spleen compared with male mice. MDA levels were higher in all of the female groups.
CONCLUSIONS: Gonadectomy significantly increased the oxidative stress levels in females but not in males. These findings may explain, at least in part, the gender differences that are typically observed with regard to susceptibility to malaria and should be taken into account for the treatment and design of future effective vaccines against *Plasmodium*.
This work was supported by PAPIIT IN216914 and IN217412

In vitro infectivity of metacyclic forms of *Leishmania* (*Viannia*) *braziliensis* and *Leishmania* (*Viannia*) *peruviana* in macrophage

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BACKGROUND: Leishmaniasis is a disease that affects the skin and/or mucous; caused by protozoa of the genus *Leishmania*; and transmitted by the bite of a female mosquito of the genus *Lutzomyia*. This protozoan has a life cycle that includes two developmental forms: promastigotes and amastigotes; developed in: an invertebrate host (transmission vector) and a vertebrate host (mammals, including humans). It is in the digestive tract of the vector where occurs the conversion of some infective promastigote forms (procyclical) to form infective promastigotes (metacyclic); process called metacyclogenesis that allows the promastigote acquire the ability to infect and survive within its host cell (macrophage).
METHOD: the metacyclic promastigotes were inoculated in cell culture of macrophages; infectivity was determining the percentage of infected macrophages and the average of amastigotes per infected macrophage were evaluated as a parameter for infectivity.
RESULTS: This study showed that *L. braziliensis* and *L. peruviana* have the ability to infect the macrophage cell line dog, "In vitro"; statistically infective capacity of both species don´t have significant difference to a C.I 95%.
CONCLUSIONS: The species *L. braziliensis* and *L. peruviana* produce different clinical display; *L. braziliensis* being the most aggressive species, it cause damage to mucous level feature that *Leishmania peruviana don´t present*. However, both species are molecularly related, in this work we can see similarities in infectivity. This characteristic is inherent to each strain of *Leishmania* spp., in relation to environmental factors influencing each.

Usefully of the internal transcribed spacers (ITS) as markers for population genetic structure analysis of *Blastocystis* spp.

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BACKGROUND: *Blastocystis* spp, is a parasite with high genetic polymorphism, genetic subtypes (ST)1-4 are most common in human; however, the frequency of these STs appears to differ substantially across regions. The objective of present study was to assess the genetic variation of *Blastocystis* ST recovered in symptomatic children carriers from Michoacan, Mexico; analyzing partial sequences of the internal region of the small subunit rDNA gene (SSUrDNA) and the internal transcribed spacers (ITS).
METHODS: Serial fecal samples of 47 *Blastocystis* children carriers, attended in the Hospital Infantil de Morelia "Eva Samano de Lopez Mateos" due to presented gastroenterological symptoms, negatives for pathogen virus and enterobacteria were analyzed. PCR was performed on DNA from all stool samples to identify *Blastocystis* ST, amplifying a fragment of SSUrDNA and the ITS region (ITS-1, 5.8S, ITS-2). Amplicons were purified, sequenced and aligned with other sequences deposited in GenBank, after were analyzed for diversity and population genetic structure according to geographic areas.
RESULTS: The *Blastocystis* ST found were 24 (51%) for ST1, 11 (23%) for ST2, 9 (19%) for ST3 and 1 (2%) for ST7. The haplotype network tree and a Bayesian inference exhibited the presence of two novel variants of ST1, clustering some sequences in ST1A and ST1B. The values of nucleotide diversity (π) for ST1, ST2 and ST3 ranged from 0 (ST1, African sequences) to 0.91 (ST2, European sequences), while for the haplotype polymorphism (Θ), ranged from 0 (ST1, African sequences) to 1 (ST2, African sequences). The ratio coancestry coefficient (FST)/migration index (Nm) showed the highest differentiation between Africa and Asia with ST2 (0.282/0.63); in contrast, a high flow gene was observed between Europe and America with ST1 (0.003/84).
CONCLUSION: The internal transcribed spacers (ITS) can be used as genetic markers to assess the genetic variation of *Blastocystis* spp.

Transmission zones in the context of elimination of Onchocerciasis in Africa

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BACKGROUND: Currently, five NTDs (Onchocerciasis, Lymphatic Filariasis (LF), Schistosomiasis, Soil transmitted helminthiasis and Trachoma) are targeted for elimination or control through preventive chemotherapy. Of these, Onchocerciasis and LF transmitted by insect vectors have been earmarked for elimination. To ascertain that elimination has been achieved, entomological assessments provide a means of determining whether there is still on-going transmission in areas where epidemiological surveys indicate zero infection prevalence in human populations or whether infections could be introduced into areas freed of the disease. In this report analysis of chromosomal inversion frequencies of members of the *Simulium damnosum* complex vectors of human Onchocerciasis was used to delineate a transmission zone across areas in Cameroon, Chad and Central African Republic (CAR) in the African Programme for Onchocerciasis Control (APOC) in the context of elimination of onchocerciasis.
METHODS: Larvae of members of the *S. damnosum* s.l. were collected from rivers in border regions of Cameroon, Chad and CAR. Polytene chromosomes were prepared from the samples for cytotaxonomic examination and chromosomal inversions (fixed and floating) recorded. Fixed inversions were used to determine species composition and floating inversion frequencies analysed for population variation.
RESULTS: *Simulium squamosum*, *S. damnosum* s.s and *S. sirbanum* were identified from Cameroon and CAR while only the latter two species were found in Chad. Analysis of inversion frequencies showed similarities of *S. damnosum* s.s and *S. sirbanum* in the border areas of the three countries indicative of population movements within that zone.
CONCLUSIONS: The results imply that for onchocerciasis elimination these areas within the three countries should be considered as a single transmission zone.

New infection site of an eye fluke trematode *Philophthalmus lachrymosus* (trematoda: philophthalmidae) on the vitreous humor of capybaras *Hydrochoerus hydrochaeris* (mammallia: caviidae): accident or specialization

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BACKGROUND. The avian eye trematode *Philophthalmus lachrymosus* Braun, 1902 is for the first time referred naturally occurring on the vitreous humor of a non-human mammalian host.
METHODS AND RESULTS. In this paper it is reported the first case of philophthalmosis on wild capybaras *Hydrochoerus hydrochaeris* (Mammallia: Caviidae) collected on Brazil and infected with *P. lachrymosus*. Morphometric data of the parasite are here presented. Previously, infections with *P. lachrymosus* and other species of *Philophthalmus* have been reported only on the conjunctival sacs of man and other mammals. Several previous papers have reported the infection of mammals and humans by *P. lachrymosus* and other species of *Philophthalmus*.
CONCLUSIONS. These cases could indicate an alert to the society, indicating a more dangerous infection site, whereas the removal of the parasite from the intra-ocular cavity could provoke eyesight complications.

Natural infection with *Trypanosoma cruzi* in two mammal species: white-nosed coati and raccoons.

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BACKGROUND: The Chagas disease caused by *Trypanosoma cruzi* is one of the most important parasitic infections in Latin America. This disease is a zoonosis that occurs not only in humans but also other mammals acting as natural reservoir hosts. Our aim here was to determine the infection by *T. cruzi*, in two species of mammals in semi-wild conditions: white-nosed coati (*Nasua narica*) and raccoons (*Procyon lotor*) in the Tabasco state examined for over a period of 5 years. Our results shed light on our understanding of the role of these mammals in the maintenance of *T. cruzi* infection.
METHODS: Multiple capture-recaptures of mammals were carried out at the Zoological Park "Parque Museo de La Venta" in Villahermosa City, Tabasco. Captures (using Tomahawk traps and anesthetic dart shots) were done in 2009-2013 with two samplings per year (summer and winter). Each sampling lasted ten days, during which mammal blood samples were obtained. PCRs were performed using DNA from blood samples to identify *T. cruzi* infection and the prevalence of infection was determined for both mammal species.
RESULTS: Overall, raccoon samples showed higher relative infection values compared to white-nosed coati samples. This difference was significant in summer 2012 ($P<0.00001$), summer and winter 2013 ($P=0.03$ and $P=0.02$ respectively). Relative infection value was independent from whether samples were captures or re-captures. Prevalence changed along the study for both species: 0-33% for white-nosed coatis, and 0-93% for raccoons. For both species, higher prevalence was detected in summer 2012 and winter 2013 which were significantly different when compared to other time periods. For coatis, summer 2012 was different to the other periods ($P\leq0.02$) except for winter 2013 ($P>0.05$); and winter 2013 was different to summer 2009, 2010, 2013 and winter 2009, 2010 ($P\leq0.04$). For raccoons, summer 2012 was different to all periods ($P=0.00$); and winter was different to the others periods ($P\leq0.01$) except for summer 2013 ($P>0.05$). Because of this difference by capture period, all the other comparison (specie, sex and age) were done by specific capture period.
CONCLUSION: Our results indicate the importance of these mammal species as natural hosts of *T. cruzi*. Furthermore, these hosts may play an important role in linking sylvatic and domestic cycles of transmission in the southern part of Mexico.

Experimental treatment of cutaneous leishmaniasis with nanoformulations containing antimony

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BACKGROUND: The treatment of leishmaniasis relies primarily on the use of compounds of parenteral administration with serious toxic effects and variable clinical efficacy. The development of more effective drugs, low cost, less invasive and with fewer harmful to the host adverse reactions, has favored to carry out research of new chemotherapeutic approaches and new targets for drug development for the treatment of Leishmaniasis. This study aims to evaluate the biological activity in vivo antileishmanial of nanoformulations administered by topical and intradermal (intralesional) pathway.
METHODS: hamsters (*Mesocricetus auratus*) were used as an animal model (Animal Ethics Committee CEUA / INPA, n ° 009/2012) of the experimental tests. These were inoculated in the snout with 10^6/mL of *Leishmania amazonensis* promastigotes (IM 5584). The design for biological evaluation was determined in experiments, which contained five groups receiving treatment and one negative control group (without treatment). The formulations were applied to the wound once a day/animal for 30 days with an interval of two days per week. The total area of the lesions was measured and recorded daily. Then, each hamster was euthanized (according to the rules for animal use) and fragments of lesions were isolated and cultivated to the parasite viability determination that was performed. The parasite quantification was also carried out.
RESULTS: The nanoformulations used demonstrated reduction in the size of skin lesions compared to the negative control group, especially with Sb3, Sb2 and cream formulations 03/Sb3 at least 30 days of local treatment.
CONCLUSIONS: A reduction of the lesion size in animals treated was observed. However, there is no parasitological cure. Longer treatment and other nanoformulations are in development for full resolution of the skin lesions.

Serum levels of IFN-γ in patients infected with *Leishmania guyanensis* from state of Amazonas, Brazil.

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BACKGROUND: American Cutaneous Leishmaniasis (ACL) is an infectious disease caused by parasitic protozoa of the genus *Leishmania*, whose major circulating species in the state of Amazonas is the *Leishmania (Viannia) guyanensis*. As the etiologic agent is a mandatory intracellular parasite, the cellular immune response becomes the main mechanism involved in curing the disease, with the participation of several cytokines, among which IFN-γ is a potent activator of T_H1 lymphocytes.
METHODS: The concentration of IFN-γ in the sera of 15 patients infected and in the same number of uninfected (negative control group=*ncg*) was determined. Coinfections by HIV, HTLV, HCV or *Trypanosoma cruzi* were investigated because of the possibility of such infections distort the host immune profile.
RESULTS: The serum IFN-γ value in *ncg* was 60 pg/mL, whereas in patients with ACL the levels ranged from 64.35 pg/mL to 117.26 pg/mL, with an average of 80 pg/mL in this infected group. The difference between groups was statistically significant ($p<0.0001$). Serology for coinfections surveyed was negative for throughout the sample.
CONCLUSIONS: According to the results, *L. (V.) guyanensis* infected patients have higher serum levels of IFN-γ as compared to control individuals, demonstrating the activation of cellular immune responses in the clinical course of the disease. The variation in the levels of IFN-γ among infected individuals demonstrates the action of this cytokine and its involvement in the cure, control, and possible influence in treatment process.

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Differences between the generation times during the in vitro culture of *Blastocystis* spp., from patients with irritable bowel syndrome and asymptomatic carriers

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BACKGROUND*:* The intestinal protozoan parasite *Blastocystis* is one of the most common parasites worldwide in humans and although its ability to cause disease has been questioned, some reports have demonstrated that this microorganism is associated to the development of irritable bowel syndrome (IBS). We assessed the generation times (Tg) of *Blastocystis* in vitro cultures, from patients with IBS and asymptomatic carriers.
METHODS: Fecal samples of 50 IBS patients and 50 healthy controls, positives for *Blastocystis spp.* by coprological analysis were collected. Using templates of the Kato-Katz technique, 50 mg of feces were cultured in Barret´s and Pavlova´s mediums, both complemented with 10% horse serum. Before to culture and 48h after, parasitological burden was measured by Neubauer chamber counter and DNA was obtained to performance a quantitative PCR (qPCR) previously reported. Furthermore, 58 amplicons yielded by qPCR were sequenced to identify their genetic subtype (ST).
RESULTS: Mean Tg for isolates of IBS patients and controls in Barret´s medium were 34.7h and 27.6 h, respectively (*P*=0.024), whilst in Pavlova´s medium were 74.1h and 32.4h (*P*=0.0001). Absolute quantification by second derivate method for qPCR data corroborated the differences between the parasitological burdens in both mediums. Alignments and the phylogenetic inference showed that isolates were 22(38%), 12(21%), 24(41%) and 2(3%) for ST1, 2, 3 and 7, respectively. None association was found within ST and their Tg and clinical symptoms.
CONCLUSIONS: We identified that Tg of isolates of *Blastocystis* from IBS patients were longer than controls; thus, this biological feature could support to explain the chronicity of symptoms during IBS.

An Investigation of Giardiasis and Cryptosporidiosis in Cambodia and Malawi

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BACKGROUND: Giardiasis, caused by *Giardia duodenalis*, is one of the most common intestinal protozoal infections reported worldwide particular in children. *Cryptosporidium* spp. is a protozoan parasite and emerging pathogen that has become recognised as a significant cause of protracted diarrhoea in both immunocompetent and immunocompromised individuals worldwide. Genotyping of human isolates of both parasites from the Malawi and Cambodia can provide crucial information about transmission routes and epidemiological differences between *G. duodenalis* assemblages and *Cryptosporidium* spp. The purpose of this study was to determine the prevalence and range of *G. duodenalis* assemblages and *Cryptosporidium* spp. infecting children from Malawi and Cambodia.
METHODS: Faecal samples collected from children with diarrhoea (< 5 years of age), living in diverse geographical regions in Malawi and Cambodia. Parasite isolates were typed by using a combination of both PCR and restriction fragment length polymorphism (RFLP) and/or sequencing of ssu-rRNA, *β-giardin*, *tpi* and *gdh* genes.
RESULTS: In Cambodia, genotyping results by *tpi* gene showed that *G. duodenalis* assemblage B was the most predominant (83.78%), followed by assemblage A (10.8%), and mixed infections *G. duodenalis* assemblage A/B (5.4%), respectively. In contrast, genotyping results of *G. duodenalis* from Malawian children (*tpi* gene) showed that assemblages A/B was the highest prevalence (33.93 %), followed by assemblage B (26.79%), and assemblages A (14.29%), respectively. PCR-RFLP analysis of ssu-rRNA showed that 45.40% PCR-positive samples had *C. hominis*, 36.40% had *C. parvum* and 18.20% infected both species (*C. hominis/C.parvum*), sequence analysis of the SSU rRNA and GP60 genes confirmed the species identification.
CONCLUSIONS: Mixed infections with both *G. duodenalis* assemblage A and B (A/B) appeared to be predominant in Malawi whereas *G. duodenalis* assemblage B was the predominant in Cambodia. The predominance of *C.hominis* in this study indicated that the anthroponotic route plays a major role in *Cryptosporidium* spp. transmission in Malawi.

Preliminary report about alpha-L-Fucosidase in Blastocystis spp: a glycosidase involved in the colonization of the host?

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BACKGROUND: Alpha-L-Fucosidase, (ALF) is a glycosidase with mucolytic activity, able to release residues of fucose from the non-reducing end of mucins and other glycoproteins present in the intestinal mucus. It has been proposed that for various pathogenic micro-organisms, mostly bacteria, intestinal mucus can serve as a source of nutrients, allowing them to multiply and colonize. The aim of present study was to characterize the ALF of Blastocystis spp. and compare its expression in isolated obtained from patients with gastrointestinal disorders and asymptomatic carriers.
METHODS: Positive samples for *Blastocystis* were collected from patients with diarrhea and irritable bowel syndrome according to Roma III criteria, as well as asymptomatic carriers. The samples were cultivated in Pavlova medium modified supplemented with horse serum 10% and antibiotics-antifungal, at 35°C. DNA extraction of cultures was performed using a commercial kit. PCR protocol consisted of 35 cycles of 96°C for 30 sec, 60 sec for 30, 72°C for 30 sec and final extension step at 72°C for 7 min, the primers used were ALF forward 5'– TAT TCA ACC CGG TGA AGC TC-3' and reverse 5'– GGT CCC AGG GAG AGA GGT AG-3'. Amplicons were purified and sequenced by a commercial supplier. Blastocystis subtypes (ST) also were identified.
RESULTS: PCR performed in samples of 25 patients and 11 asymptomatic carriers showed a specific band of 250 bp., Amplicons were sequenced and after alignments, we found a high identity with ALF of *Blastocystis* spp. Interestingly 50% of samples correspond to ST2, whilst ST1 and ST3 were 20% and 30%, respectively.
CONCLUSIONS: Our results show that ALF was present in all samples studied, however it is necessary to evaluate differences its expression between symptomatic and asymptomatic carriers.

Ceragenin CSA-8 modulates the activation of splenic macrophage infected with *L. infantum.*

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BACKGROUND: Ceragenin CSA-8 are amphiphilic compound consisting of cholic acid backbone that is attached to several cationic amines, designed to mimic amphiphilic characters of natural antimicrobial peptides, and a previous susceptibility study demonstrated that CSA-8 has a anti-*Leishmania* activity. Macrophages according to classical or alternative activation exert a function of effector cell or just as host cell of the *Leishmania* parasite.
OBJECTIVE**:** To determine the immunomodulatory effect of CSA-8 on the activation of splenic macrophages in infection with *L. infantum*.
METHODOLOGY: splenic macrophages from BALB/c mice obtained on days 0, 2, 21, 53 post infection with *L. infantum*, "in vitro" were subjected to various doses (5, 14, and 20µg/mL) of the antibiotic. Activation was assessed by flow cytometric determination of NOS-2, Arginase-1 and cytokines (IL-12, IL-23, IL-10, TNF-α).
RESULTS: 2h post-treatment with 14 and 20 ug/mL of the antibiotic, the expression of NOS-2 in macrophages of day 2 (early infection) increased 45 ($p=0.02$) and 54% ($p=0.0004$), respectively, compared to untreated macrophages. On day 21 (high parasite load) the increase was 61% ($p<0.001$) with 5 ug/mL. Meanwhile decreased arginase-1 day 2 pi ($p<0.01$), and day 21 with 20 ug/mL decreased 88% ($p <0.001$). With respect to cytokines, day 21 with different doses of CSA-8, showed an 80% increase of IL-12, meanwhile the cytokines IL-10 and TNF-α showed a reduced expression of 60% and 70% respectively ($p<0.001$). The key cytokine IL-23 decreased on day 2 p.i. 65% ($p<0.001$) in CSA- 8 treated macrophages (14µg) while increasing 21 and 53 pi.
CONCLUSION**:** This suggests that this antibiotic act as an immunomodulator, which enhances macrophage activation to NO production and cytokines involved in the elimination of the parasite. Financed by FONACIT Proyecto G-2005000375, PSU09-7878-2009/ CDCH
KEYWORDS: macrophage activation, CSA-8 experimental visceral Leishmaniasis.

Plasmodium falciparum, but not _P. vivax_ infection, is related to erythrocytic apoptosis

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BACKGROUND**:** Apoptosis can occur in red blood cells (RBC) and seems to be involved in anemia related to many diseases. In malaria it is well known the participation of parasitized RBC (pRBC) in anemia; however, non-parasitized RBC (npRBC) apoptosis could amplify this malaria-associated hematologic event. In fact, increased levels of apoptosis were observed in npRBC during anemia of lethal *Plasmodium yoelii* 17XL infection, but in human malaria erythrocytic apoptosis has never been studied. The present study was performed to investigate if npRBC apoptosis also occurs in *P. vivax* and *P. falciparum* infections.
METHODS**:** Apoptosis of npRBC was evaluated in blood samples of *P. vivax* malaria patients and clinical health individuals living in Manaus, Brazil, both *ex vivo* and after incubation of RBC for 24h. Additionally, it was tested the capacity of *P. vivax* or *P. falciparum* plasma patients to induce *in vitro* apoptosis of normal RBC from a clinical health individual living in non-endemic malaria region. Apoptosis was detected by flow cytometry using annexin V staining.
RESULTS**:** Contrasting with experimental malaria that significantly increase the levels of apoptotic npRBC both *ex-vivo* and after 24h of incubation, no significant alteration on apoptotic npRBC rates was detected in *P. vivax* infected patients, when compared with non-infected control individuals. Similar results were observed when plasma of these *P. vivax* patients was incubated with normal RBC. Conversely, plasma from *P. falciparum*-infected subjects induced significant apoptosis of these cells.
CONCLUSION**:** Apoptosis of normal RBC can be induced by plasma from individuals with *P. falciparum* (but not with *P. vivax*) malaria. This finding could reflect the existence of erythrocytic apoptosis during infection that could contribute to the anemia associated to *falciparum* malaria.

The influence of *Schistosoma mansoni* on the locomotory and reproductive activities of *Biomphalaria glabrata*

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BACKGROUND: The infection and development of a parasite in their host may cause physiological, morphological and behavioral changes due to the use of energy resources of the host. The aim of present work is to evaluate the locomotory and reproductive activities of *Biomphalaria glabrata* before and after experimental infection with *Schistosoma mansoni*.
METHODS: The image analysis biomonitoring system was based on the use of a Videomex V® (Columbus Instruments) using the software Travelled Distance of Multiple Objects. Five parameters were analyzed: Distance travelled, Ambulatory time, Stereotypic time, Resting time and Average speed. Each locomotory activity was performed during 1h and 20 min. Sixty nine non-infected snails were individually recorded (control group). The same group was subsequently exposed individually to 8-10 miracidia (exposed group) and after five weeks, were likewise biomonitored by image analysis. During the eight weeks of experiments, the number of eggs masses/snail, eggs/snail and eggs/eggs masses were counted weekly in order to evaluate the reproductive capacity of the snails.
RESULTS: Out of the 69 snails infected with *S. mansoni*, 33 (47.8%) shedded cercariae and 36 (52.2%) did not demonstrate cercariae shedding until the end of the experiments. The locomotory activity of exposed and positive snails increased significantly when compared to the control group. The positive snails when compared with the exposed not shedding cercariae showed a significant increase of the Resting time and a reduction of 27% in the eggs laying (Chi-square test: $X^2 = 5.67$, $p < 0.01$).
CONCLUSIONS: The *Schistosoma mansoni* is able to affect the locomotory activity and the reproduction of their intermediate host *Biomphalaria glabrata.*

Analysis of the *Entamoeba histolytica* nuclear proteome and its response to epithelial cell interaction

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BACKGROUND: *Entamoeba histolytica* is the protozoan causal agent of human amebiasis. The adhesion, lysis and phagocytosis of this parasite results in tissue damage of host cells. Interaction of trophozoites with host cell receptors, sparks a series of signaling pathways ending in the nucleus. There are several proteins within the nucleus such as transcription factors, polymerases and others involved in the nuclear architecture. However, their participation on expression of genes involved in pathogenesis is still unknown. Therefore, the aim of this work is identify nuclear proteins with differential expression during host cell interaction.
METHODS: The nuclear proteome of *E. histolytica* was obtained after trophozoites interacted with MDCK cells. Nuclear protein extracts, with and without interaction, were resolved by two-dimensional (2D) gel electrophoresis. Differential spots were identified by MALDITOF-MS. For a selected differentially express protein we corroborate its identity and modulation by western blot assays. Finally we confirm its nuclear localization in the trophozoite by confocal microscopy.
RESULTS: *E. histolytica* modifies the expression of at least 31 nuclear proteins during its interaction with MDCK cells. Among these up-regulated proteins, we identified a 60 kDa chaperonine and confirm its nuclear localization. The increase of the 60 kDa chaperonine was validated by western blot and localized at the nucleus by inmunofluorescence.
CONCLUSIONS: *E. histolytica* differentially expresses nuclear proteins during the host cell interaction. Our data suggest a role of a 60 kDa chaperonine protein which is located in the nucleus and was up-regulated during the adhesion process between the parasite and its target cells.
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Use of the mitochondrial serine transfer RNA (UCN) of *Lutzomyia columbiana* (Diptera, Psychodidae) in phylogeographic studies

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BACKGROUND: Sand flies are small diptera of Psychodidae family of medical importance due to their role like vectors of *Leishmania sp*., Acording to the low knowledge of molecular characteristics of this specie in our region, the principal main of this study was evaluate the genetic diversity of mitochondrial gene encoding serine (*ser-tRNA*), in natural populations of *Lu. columbiana* in Nariño, southern Colombia.
METHODS: The species status was confirmed by using the taxonomic key generated by Young and Duncan (1994) and Galati (2003). The genetic analysis was performed by amplification by PCRof gene *ser-tRNA* using the primers identified by Ready et al (1997), which have as target sequence the 3' end by the 3' Cyt b'subunit 1 gene of NADH dehydrogenase (*NAD*). The same conditions for PCR reported by Perez (2008) were used.
RESULTS: The *ser-tRNA´s* gene amplification was necessary to modify the PCR conditions reported by Pérez (2008). In a final volume of 12.5 ul used 1X PCR buffer, 1.5 mM $MgCl_2$, 1.5 U of Taq polymerase (Promega GoTaq Kit), 0.2 mm dNTP's (Promega), 0.3 um of each primer and 6ul of DNA. The *ser-tRNA´s* gene consisted of 67 base pairs. The sequencing analysis show value of S:0 to number of polymorphic sites, Eta:0 to total number of mutations, h:1 to number of haplotypes, and Haplotype (gene) diversity, both variance of haplotype diversity and standard deviation of haplotype diversity values below 0.
CONCLUSIONS: The number of haplotypes found in populations of *Lu. columbiana* exhibited genetic variance possibly mainly due to geographical diferences, however is necessary increase the number of individuals analyzed to confirm the utility of the gene in phylogeographic studies.

Production and characterization of monoclonal antibodies Anti-*Toxocara canis*

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BACKGROUND: *Toxocara canis* is a geohelminth whose definitive hosts are canids, the human being produces visceral or ocular larva *migrans*. It is estimated that one billion people suffer from ocular larva *migrans* and is more common in children 3-5 years old. The diagnosis of larva *migrans* is based on the detection for antibodies, but there may be cross-reactivity with other parasitic diseases, not necessarily detect active infection and commercial kits are imported and expensive. Therefore, it is necessary to develop monoclonal antibodies that efficiently capture circulating antigen larvae of *T. canis*. The aim of this work was to produce monoclonal antibodies against excretory-secretory antigens of larvae of *T. canis* and to characterize them.
METHODS: Mice of BALB/c were inoculated intraperitoneally with live larvae of *T. canis*. They were monitored every week and when the antibody titer was 1:8000 the mice were sacrificed, the spleen was obtained and lymphocytes were fused with myeloma cells X63Ag8.653. The hybridomas producing antibodies against the excretion-secretion antigen of the larvae of *T. canis* were cloned.
RESULTS: We obtained two positive clones (1E4G4C2 and 1E4B7B12) that: a) They do not cross-react with antigens of *T. canis* adult stage, nor with 10 other species of parasites; b) They are IgG1 subclass antibodies; c) Captured 0.4 ng/ml of antigen of *T. canis* in 9 of 29 samples that were positive with a commercial kit that detects antibodies.
CONCLUSIONS: It is a monoclonal antibody that detected 0.4ng/ml from circulating antigen of *T. canis* larva and can be using for detection of active *larva migrans* in human.

Trophic interaction between parasitic mites and africanized bees on apiaries of Rio de Janeiro State – Brazil

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BACKGROUND: Although bees have major ecological and agricultural importance, nowadays, with the focus on climate change, there is huge concern about the health of these insects. Parasitic mites are a real threat to survival of these insects, emphasizing *Acarapis woodi* Rennie, 1921 and *Varroa destructor* Anderson & Trueman, 2000. This study aimed to detect and evaluate quantitatively and qualitatively the fauna of parasitic mites in beehives of Guapimirim, Barra do Piraí and Seropédica counties situated on the State of Rio de Janeiro - Brazil.
METHODS: Ten Langstroth beehives of africanized honey bees (*Apis mellifera*) were investigated during a year study (Aug/12-Sep/2013) on each county. Each bee had it body inspected under a stereomicroscope and it thorax dissected at the 1st respiratory spiracle open on the search of *Acarapis woodi* mite. Yet the contents resulted from the collection bottles were held in fine mesh sieve, where the mites were separated, counted and preserved in an Ependorf type container filled with isopropanol. Finally the mites were mounted in Hoyer´s liquid in a slide and a cover slip, and examined under light microscopy.
RESULTS: *V. destructor* were found parasitizing all beehives examined revealing a parasitism prevalence of 2,24%(G), 4,95%(BP) and 1,84%(S). Witch corroborates with literature on the low level records of its infestation on Africanized bees in Brazil (Gonçalves, 1986). Still, the presence of *A. woodi* on the tracheal branches in the insects studied wasn´t observed.
CONCLUSIONS: A symbiotic relationship between *Apis mellifera* and *Varroa destructor* occurs in the Rio de Janeiro State-

Trophic interaction between mites and bees in apiaries of Rio de Janeiro State – Brazil

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BACKGROUND: Although bees have major ecological and agricultural importance, nowadays, with the focus on climate change, there is huge concern about the health of these insects. Parasitic mites are a real threat to survival of these insects, although non-parasitic mites also benefit from the favorable conditions of the hive for its own benefit. This study aimed to characterize the fauna of mites in beehives of Guapimirim, Barra do Piraí and Seropédica counties situated on the State of Rio de Janeiro - Brazil.
METHODS: Ten Langstroth beehives of africanized honey bees (*Apis mellifera*) were investigated during a year study (Aug/12-Sep/2013) on each county. Each bee collected had it body inspected and it thorax dissected on the search of mites. The contents collected from the bottom of the beehives were examined under a stereomicroscope where the mites were separated and preserved in an Ependorf type container filled with isopropanol. Finally the mites were mounted in Hoyer´s liquid and examined under light microscopy.
RESULTS: *V. destructor* were found parasitizing all beehives examined. Althought the presence of the tracheal mite *A. woodi* wasn´t observed, 09 different Acari specimens of the parasitiformis and Acariformis order were found cohabiting the beehives. 03 mites of these were identified as on Acaridida suborder, 04 on Actinedida suborder, 01 on Gamasida suborder and 01 on Oribatida suborder. Finds similars with those of Sammataro (2000), on the common suborders of non-parasitic mites associated with bees.
CONCLUSIONS: A symbiotic relationship between *Apis mellifera*, *Varroa destructor* and non-parasitic mites occurs in the Rio de Janeiro State.

Subcellular localization of a key protein in the NAD$^+$ metabolism of *Trypanosoma cruzi*

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BACKGROUND:The current pharmacological strategies against Chagas disease are ineffective due to the progressive increase in resistance of the parasite. Additionally, these strategies present severe adverse effects and nonspecific mechanisms of action. Therefore, the study of the basal metabolism of *Trypanosoma cruzi* is fundamental for the identification of new therapeutic targets and the development of effective and safe strategies of control.
The biosynthetic pathways of the Nicotinamide Adenine Dinucleotide (NAD$^+$) converge in the enzyme Nicotinamide/Nicotinate Mononucleotide Adenylyltransferase (NMNAT, EC 2.7.7.1/18). Our research group has identified the NMNAT of *T. cruzi* (TcNMNAT), whose primary structure presents exclusive insertions that are not in the human orthologs.
In order to determine the subcellular localization of NAD$^+$ biosynthesis in *T. cruzi*, the location of the TcNMNAT enzyme was assessed in the parasite.
METHODS: Polyclonal antibodies were generated in murine models using the purified His-TcNMNAT recombinant protein as an antigen. The obtained antibodies were implemented in the standardization of Western blot and indirect immunofluorescence protocols using cell extracts and fixed epimastigotes, respectively.
RESULTS: A specific signal of ~35 kDa (expected molecular weight) was detected and a cytoplasmic distribution pattern of the TcNMNAT protein was observed.
CONCLUSIONS: The NMNAT protein is expressed in *T. cruzi* epimastigotes and is located in the cytoplasm. This localization pattern is explained because in the cytoplasm occurs the synthesis of calcium-mobilizing molecules such as NAADP, as well as NAD-dependent protein deacetylation. Both processes requiring a constant supply of NAD$^+$.

Spacial analysis of the distribution of malaria in the municipality of Baiao, at Para State, Brazil , in 2009 to 2013.

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INTRODUCTION**:** Malaria is a systemic, parasitic disease caused by protozoa of the genus Plasmodium, transmitted to humans by invertebrate vectors of the *Anopheles* genus, having as predominantly symptoms the triad: fever, chills and headache. The main species are *P. Malariae*, *P. Vivax*, *P. Falciparum* and *P. Ovale*. According to World Health Organization, in Brazil the epidemiological situation of the injury is worrisome, because the cases were reported above 300,000 patients. The Legal Amazon is considered an endemic area, concentrating 99.8 % of cases in the country. The goal of this study was to analyze the spatial distribution of malaria cases in the municipality of Baiao-PA, in the period 2009 to 2013.
METHOD**:** The study began by interdisciplinary literature review and collection of epidemiological data bases of SIVEP - Malaria, which were purified to remove inconsistencies and selection of the variables employed. Following, the georeferencing was performed in the field locations where positive cases occurred, using a Global Positioning Receiver (GPS) model GARMIN Montana 650. Subsequently, the data were aggregated to the IBGE cartographic databases and satellite image Landsat TM - 5. Finally, a map of the distribution of malaria cases by location was established, from which we could observe the spatial dispersion of cases.
RESULT**:** Among 836 cases examined in the period, 65 were reported in 2009, 194 in 2010, 326 in 2011, 208 in 2012 and 43 in 2013. The locations that had the highest number of cases were Angelim with 147 cases (17:58%), Baixinha with 85 cases (10.16%), with 62 cases Araquembaua (7.41%) and 53 cases with Chico Mendes (6.34%).
CONCLUSION**:** Since 2009, the municipality of Baiao suffered an outbreak of malaria. However, through preventive actions of municipal health department, distribution of mosquito nets and spraying, the number of cases of this disease has been drastically reduced in 2013.

Morphological and functional changes of pancreatic β cells TC-6 infected by *Toxoplasma gondii tachyzoites*

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BACKGROUND: *Toxoplasma gondii* chronically infects approximately 20-50% of the global population, and it is able to invade all kind of nucleated cells, however there is not data about the invasion and proliferation in pancreatic β cells. In this study the mean goal is to determine the morphological and functional changes of pancreatic β cells TC-6 infected by Toxoplasma.
METHODS: Invaded cells TC-6 with *Toxoplasma gondii* tachyzoites were stimulated with glucose and then the insulin secretion was determine by ELISA in order to observe the variation of the levels of insulin according to the time of proliferation of *T. gondii*. Regarding of the morphology cells, the cell culture was proceeding by Scanning Electron Microscopy and the Tanaka technique was used.
RESULTS: *Toxoplasma gondii* was able to invade and proliferate in TC-6 cells and the insulin secretion determined by ELISA was affected significantly compared with the control cells without tachyzoites. By Tanaka´s method we can see an atipycal arrangement of tachyzoites in the parasitophorus vacuole, since that was observed in a rosette and cluster form, finding that at 6 hours 80% of these vacuoles contain at least two parasite inside, while the cellular morphology of TC-6 was apparently unaffected.
CONCLUSIONS: *Toxoplasma gondii* tachyzoites are able to invade and proliferate in pancreatic β cells TC-6, leading a normal dynamic cells proliferation. In addition *Toxoplasma gondii* affects insulin secretion from pancreatic β cells which may lead to chronic conditions such as diabetes.

American Tegumentary Leishmaniasis in a endemic region in Maringá, Paraná, Brasil.

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BACKGROUND. The American Tegumentary Leishmaniasis (ATL) is considered one of the five infectious and parasitic diseases more relevant, and public health problem in 80 countries in the Americas , Europe, Asia and Africa. The present study aimed to evaluate the level of knowledge and risk factors for cutaneous leishmaniasis among elderly residents of the neighborhood Borba Gato in Maringá - Pr
METHODS AND RESULTS. This is a field research with quantitative approach - qualitative nature descriptive held in the months of August and September 2012, were included in the sample, 80 seniors, with more than 65 years, residents in the neighborhood. When evaluated on knowledge of the term zoonosis , 78.75 % reported not knowing about the American cutaneous leishmaniasis, 75 % reported knowing the disease, too, 75 % reported knowing how their transmission occurs, however, 56.25%, never received any guidance on preventive measures. About garbage attract the mosquito transmitter of LTA, 83.75 % recognized this practice as a risk factor ; 83.75 % have knowledge about the existence of a forest in the district , 85 % know that the forest provides conditions for the proliferation of the mosquito.
CONCLUSIONS. Considering the situation presented , it became evident the need for improvement of strategies for surveillance and control of leishmaniasis in the neighborhood Borba Gato in Maringá - Pr , considering that the elderly population presents some risk factors that increase susceptibility to the same contracting zoonoses parasitic.

Prevalence and genotypes of *Giardia intestinalis* from calves less than three months old from Tizayuca, Hidalgo, Mexico

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BACKGROUND: *Giardia intestinalis* (Syn: *G. duodenalis* and *G. lamblia*) is a zoonotic intestinal protozoan with worldwide distribution. In cattle produces malabsorption and failure of feed conversion. The morphologic simplicity masks a great genetic diversity. Actually, there are eight assemblage/genotypes described. Assemblages A and B are zoonotic; it has been suggested that assemblages C to H are species specific. It has also documented that *Giardia* assemblage E is highly prevalent in young ungulates, but there are reports of mixed infections involving zoonotic genotypes. In Mexico there are not data about predominant *Giardia* genotypes on calves. Therefore, the aim was to search for prevalence and genotypes predominance of *Giardia* in calves less than three months old, settled in the Dairies Basin of Tizayuca, Hidalgo, Mexico.
METHODS: Sampling was performed on 13 stables, 222 faecal samples were obtained. Faeces were processed with $ZnSO_4$ to concentrate *Giardia* cysts. These were identified microscopically. To genotyping the DNA was isolated with a QiaGen kit following instruction of providers. A glutamate dehydrogenase segment was amplified by a nested PCR and the amplicon of ≈ 432 bp was restricted with *Nla IV*.
RESULTS: The prevalence of *Giardia* was of 35.58 %. In 12/13 stables there was calves with *Giardia*. 27 samples were genotyped and the assemblage E (17) was most prevalent. With zoonotic genotype: AI (1), AII (1), B (1); mixed of genotypes: B+E (1), AI+AII+E (2), A1+AII+B+E (4).
CONCLUSIONS: Based on these data we could conclude that calves settled on this place are reservoirs and disseminators of *Giardia* from genotypes A-1, A-II, B, and E.

Parasites and climate change: framework of generalized host-parasite interaction

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BACKGROUND: Climate change alters the dynamics of infectious diseases. One hypothesis is that future environmental conditions will alter parasite transmission dynamics, increasing the severity and frequency of disease outbreaks and lead to a parasite range expansion. To know which type of host-parasite systems would be the most sensitive to this, there have been some predictive models to estimate risk and define control strategies for parasites of humans and wildlife. 'Metabolic Theory of Ecology' is proposed for predicting how these interactions change.
METHODS: An extensive bibliographic review was done, highlighting the most relevant points to evaluate which factor is the one which has the most important effects on the demographic parameters and how theories have change with recent studies.
RESULTS: The most studied and mentioned factor that can alter the interaction host-parasite is temperature and the most supported theory is "Metabolic Theory of Ecology".
CONCLUSION: Predicting the impact of climate change on dynamics of host-parasite systems is challenging due to the intrinsic complexity of multi-species interactions and the numerous ways in which the environment can influence such dynamics.

Characterization of parasites in fish poeciliids threatened and endemic of Lake Catemaco, Veracruz, México.

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BACKGROUND: There are a relatively high number of endemic species in Lake Catemaco, including fish of the family Poeciliidae. Currently, the species *Poecilia catemaconis, Poeciliopsis catemaco, Xiphophorus milleri* and *X. kallmani* are threatened, probably due to activities related to tourism, local fisheries and introduction of invasive species. However, the information about their parasitic biota is limited. Therefore, the objective of this work was to characterize the composition and infection parameters of protozoan and metazoan of three species of poeciliids.
METHODS: There were analyzed 60 *Heterandria tuxtlaensis*, 22 *P. catemaconis* and 28 *X. kallmani*.
RESULTS: We registered 26 types of parasites (5 protozoan and 21 metazoan). *Heterandria tuxtlaensis* (omnivore-insectivorous) showed the greatest species richness of helminthes (20), compared with *X. kallmani* and *P. catemaconis* (both omnivores-herbivores), with 12 species. Metacercariae of the genus *Ascocotyle* were present in all fish species analyzed, some with prevalence of 80% to 100% and mean intensities of 46±93 (*A. Phagicola nana*) or 171±166 *A. A. tenuicollis* in the hearth of *H. tuxtlaensis*. In *Poecilia catemaconis*, the ciliate *Ambiphrya* sp. was presented with prevalence of 60% and mean intensity of 91±120 and *Myxobolus* sp. with 85% prevalence and mean intensity of 46±54 xenomas.
CONCLUSIONS: It was recorded for the first time the ciliate *Ichthyophthirius multifiliis* ("Ich"), *Ambiphrya* and *Chilodonella*, as well as *Myxobolus*, *Gyrodactylus* and a dactilogyrid. The presence of "Ich" represents a risk because it has been associated with mass mortalities of native wild fish; as well as *Myxobolus* parasitizing muscle, fins, gills, intestine and sporadically the heart of the fishes, because caused mortalities relatively high during the acclimatization of *P. catemaconis* to laboratory conditions. Given the potential danger of the myxosporans and gyrodactylids, is necessary to identify taxonomically to the parasites, and to elucidate their life cycles and susceptibility among the fish of Lake Catemaco.

Taenia solium postoncospheral stage-specific proteins as a candidate to Neurocisticercosis diagnosis

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BACKGROUND: Neurocysticercosis (NCC) is a disease of the central nervous system caused by the larval stage or cysticerci of the cestode *T. solium*. Immunological methods are useful to diagnose NCC, but the sensitivity and specificity of these methods are low in patients that have only a single cyst in the brain. There remains a need for new antigens which improve immunological diagnosis methods. We evaluated *T. solium* postoncospheral (PO) stage proteins as candidate antigen for the diagnosis of NCC.
METHODS: PO stage proteins were obtained by in vitro culture of activated oncospheres to intestinal HCT-8 monolayer cells. PO stage proteins were extracted by sonication which were then compared with oncosphere and cysticerci proteins using electrophoresis and western blot (WB). The identification of PO stage diagnostic proteins was done by WB.
RESULTS: PO stage shared some proteins with oncosphere and cysticerci. Some proteins present in oncosphere were absent in PO stage while proteins present in cysticerci were present also in PO stage suggesting a change in the protein patrons during parasite development. We identified a band of 60 kDa which was PO stage-specific and is recognized by antibodies present in the serum of NCC patients. This band is not recognized by sera of patients with hidatidosis, hymenolepiasis and teniasis for *T. solium* and *T.saginata.*
CONCLUSIONS: Our results suggest that the bands of 60 kDa of *Taenia solium* found in PO stages could be used to diagnosis of NCC.

How to implement prevention, test, treat and track (P+T3) amongst populations at higher risk of malaria: lessons from western Cambodia.

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BACKGROUND. Since 2009, due to the emergence of artemisinin resistance, a series of interventions amongst populations at higher risk of malaria have been undertaken in Cambodia. Today, falciparum malaria is in danger of becoming untreatable in western Cambodia. Eliminating falciparum malaria will eliminate resistance. For this reason, Cambodia has set itself pre-elimination goal by 2015. In Cambodia (and in most malaria elimination settings), as transmission reduces, malaria increasingly becomes a disease of certain demographic groups. Amongst these are mobile, migrant and groups with limited access to services. This paper discusses strategies used in the program to Prevent, Test, Treat and Track (T3) malaria in these populations and evaluates the effectiveness in this context – Lessons learned from Cambodia may provide guidance to other elimination settings.
METHODS. Data used included that collected through a mixed methods study was conducted in six provinces of Western Cambodia in the artemisinin resistance containment zones 1&2. Additionally analysis from an informal survey among taxi drivers, farm owners, NGO staff and public health department staff; market survey, participant observations, secondary analysis of national data sets and content analysis of workshops and other grey literature through October 2011 till April 2013 have been used.
RESULTS. The strategies used in the various interventions are classified as:

a) Prevention: interactive voice response (IVR) technology, taxi scheme, LLIN-lending scheme, mass media, mobile broadcasting units, listener viewer clubs, focus group discussions, IEC and BCC materials, positive deviance.
b) Test/Treat: cross-border screening, active case detection, village malaria workers, mobile malaria workers, plantation malaria workers.
c) Track: respondent driven sampling, multiple cross-sectional surveys, movement mapping, farm and plantation mapping and MMP-information system.

Various levels of effectiveness and factors influencing that success will be described.
CONCLUSIONS. Targeting these groups is a priority in both malaria eliminating and drug resistance settings. This study has found out that some solutions exist to these problems and that village malaria workers are the backbone of all above described interventions. However, factors such as dark media zones, low-literacy, low exposure to intervention, high mobility affect the effectiveness of the strategies in some contexts and need to be accounted for in programming.

Molecular and functional characterization of TgTOR in *Toxoplasma gondii* tachyzoites.

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BACKGROUND: The protozoan *Toxoplasma gondii* is an intracellular parasite that infects humans and a broad variety of animals. In immuncompromised individuals it causes a severe disease and death. T. gondii is able to invade all the cells in the organism through dynamic mechanisms such as gliding motility, conoid extrusion and secretion from different organelles. How this lytic cycle is regulated is unknown but it can involve a role for protein kinases like mTOR.
METHODS: An in silico analysis was made in order to determine the isoforms of TOR present in *T. gondii* genome by using BLAST, domain analysis, multiple alignments and modeling. RT-PCR and Western Blot were carried out in order to know the expression of TgTOR. The effect of inhibitors of mTOR (rapamycin, Wortmanin and Torin-2) were examined in the processes of conoid extrusion, invasion and proliferation of Toxoplasma gondii in cell culture.
RESULTS: *T. gondii* has an isoform of TOR which retains the HEAT, FAT, FRB, FATC, PIKK domains conserved in TOR proteins in all species. By RT-PCR we observed a fragment corresponding to the catalytic domain of TgTOR and by using an antibody directed against the binding domain of rapamycin we observe the specific recognition of a protein in total extracts of tachyzoites. Also we noted that the presence of rapamycin, wortmanin and torin-2 inhibited the conoid extrusion, invasion and proliferation of T. gondii in cultured Hep-2 cells.
CONCLUSIONS*: T. gondii* tachyzoites expressed an isoform of TOR (TgTOR) and it apparently is essential in the pathology of this protozoan parasite.

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What risks of becoming parasitized are there in the new ecotourism, extreme tourism and adventure tourism?

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BACKGROUND: The term “ecotourism” includes any kind of tourism that has a positive contribution to the conservation of the natural environment; whereas both “adventure tourism” and “extreme tourism” refer to visiting dangerous and exciting places like caves, mountains, jungles, canyons or deserts, among others. This is, in fact, one of the main reasons people travel. Nevertheless, most people go sightseeing and enjoy these activities without even knowing about the risks of catching a disease.
METHODS: With the aim of raising awareness of all the risks of becoming parasitized related to ecotourism, an extensive bibliographic review was done.
RESULTS: There are a lot of communicable diseases with risk for travellers, such as malaria, dengue, Chagas disease or dermatological conditions, like leishmaniasis or cutaneous larva migrans, among others. However, these clearly depend on the region that is being visited.
CONCLUSIONS: It is extremely important to be aware of these diseases in order to take the appropriate preventive measures.

Trichinella spiralis may contribute to prevent fatal outcome in a cerebral malaria infection.

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BACKGROUND: Helminths strongly influence the host immune system. Particularly *T. spiralis (Ts*), direct the immune system towards an anti-inflammatory response. In this study we explored the effect of *Ts* infection in the outcome of cerebral malaria, a fatal complication associated with an exacerbated inflammatory response.
METHODS: C57BL/6 mice were separated in four groups. Group I: uninfected; Group II: infected with *PbA (5x10*^{4} *infected red blood cell (iRBC)*; Group III: infected with *Ts (100 larvae)*; Group IV: coinfected with *Ts and PbA*. Parasitemia and survival rate were assessed.
RESULTS: *PbA* infection was lethal by day 11 postinfection (p.i.) (group II) while mice infected 41 days previously with *Ts,* showed a 25% survival at day 21 p.i. (group IV). Animals from groups I and III survived along the experiment. Additionally, the course of parasitemia due to *PbA* was modified in co-infected groups *vs*. *PbA* infected animals (days 9-11 p.i.). Later in infection, an increase in parasitemia was detected ranging around 60% iRBC in coinfected mice.
CONCLUSIONS: Infection with *Ts* enhanced the resistance of mice against fatal infection with *PbA* probably by dampening the exacerbated inflammatory response induced by *PbA.*

Brain infectivity by *Toxocara canis* larvae on experimentally infected mice

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BACKGROUND: Toxocariasis is one of the most reported zoonotic infections in the world. Humans and mice are paratenic hosts, and during *Toxocara canis* infection the hatched larvae migrate systematically in the body and could reach critical organs, such as liver, lung and brain, resulting in tissue damage due to the inflammatory responses. In the present study, we investigated the liver and brain histopathology and parasitology on mice experimentally infected by *T. canis*.
METHODS: Thirty-two Balb/c female mice were orally infected with a single dose of 1,000 embryonated *T. canis* eggs each. Groups of eight animals were euthanized at 7 and 14 days post-infection (p.i.). Liver and brain were removed and fixed in 10% formalin and subsequently embedded in paraffin, then submitted to haematoxylin and eosin staining for histopathological examination. The parasitological examination was determined by larval recovered from tissues.
RESULTS: The results showed higher larval recovery in the brain in the group of 14 days p.i.. However, no difference in parasite burden was observed in the liver on the parasitological exam independently of the duration of the infection. In the brain, we observed more damaged tissue in the 14 days p.i. group, which is characterized by hemorrhagic areas and larval tunnels. Damaged tissue was observed in the liver, due to the inflammatory infiltrate with eosinophils predominance induced by the presence of larvae in the 14 days p.i. group.
CONCLUSIONS: Our results suggest that migratory behavior and infectivity of tissue larvae is more likely to affect the brain of mice infected with *T. canis*, and the tissue damage is proportional to the duration of the infection. Hence, these findings indicate that infected-mice by *T. canis* are a suitable model to neurotoxocariasis study.

Preclinical Development of Diversity-Oriented Synthesis Derived Small Molecule, ML341, with Cidal Activity Against *Trypanosoma cruzi*

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BACKGROUND: The Broad Institute and Eisai have partnered to exploit novel chemistry, Diveristy-Oriented Synthesis (DOS) collection, to discover a small molecule-based anti-infective agent, with nanomolar cidal activity against the intracellular form of *T. cruzi*.
METHOD: In order to develop effective, non-toxic chemical leads, a high throughput screen testing the Broad Institute's DOS collection was developed using recombinant Tulahuen strain of *T. cruzi* co-cultured with host cell, mouse fibroblast NIH3T3. To confirm activity and ensure compounds were effective against intracellular amastigotes, a high content imaging assay was developed detecting the nuclei of amastigotes within a host cell was used as confirmation. Compound activity was tested against three separate *T. cruzi* strains (Tulahuen ,CA-I/72, and Benznidazole resistant Y strain). Once a chemical lead series was identified, the team has performed lead optimization of the cidal small molecule, ML341.
RESULTS: A HTS was performed on 22,378 compounds derived from the DOS collection. ML341, a SnAr 8-ortho scaffold, was identified to have nanomolar potency, cidal to amastigotes, and have minimal toxicity to host cells. In the lead optimization phase of the program, we have identified a suitable analog of ML341 with sufficient bio-availability and microsomal stability for *in vivo* efficacy studies in mice.
CONCLUSIONS: We have identified a potent, cidal compound, optimized for *in vivo* mouse studies. Mouse model efficacy studies are currently being undertaken.

Targeted selective deworming in sheep with hair during rainy season, in Suchiapa, Chiapas

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BACKGROUND: Gastrointestinal parasitosis produces large losses in the national livestock sector, joined to the problematic of resistance generated at trying to control it. Because of it was evaluated the efficacy of Targeted Selective Deworming (TSD), in an explotation in Suchiapa town during rainy season understood from June to September.
METHODS: Were selected 100 animals of which was evaluated FAMACHA© degrees, corporal condition and egg's number per gram of feces eliminated, thereby was established to which animals apply anthelmintic treatment using Levamisole. The breed and age determined the effect of month. For the results analysis was used descriptive statistics, the Chi square test and variance analysis.
RESULTS: Was found a short number of animals treated in June: 7.77%, July: 10.12%. August: 1.09% and September: 13.79% with influence of the month on this variable ($P<0.05$). The breed did not has effect on the response to the treatments ($P>0.05$). The month influenced high significance on FAMACHA©, corporal condition and egg's counting per gram of feces ($P<0.01$). Breed did not influence on FAMACHA© degree, corporal condition ($P>0.05$), but it has influence in the elimination of HPG, in August ($P<0.05$), the Pelibuey breed removed highest number of egg in feces.
CONCLUSIONS: It was concluded that system reduces the number of animals to try but is considered complex the application of this to field level.

Consequences of Restricting ACT Treatment to RDT Positive Children in South west Nigeria; A Preliminary Report.

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BACKGROUND: The WHO directs that malaria diagnosis be parasite based in endemic areas thereby restricting antimalarial treatment to persons with confirmed malaria. Malaria rapid diagnostic tests (RDTs) are the most viable options for doing this in most of sub-Sahara Africa.
METHODS: In an ongoing study, 144 febrile children presenting with fever or a history of fever within 48hours in a rural study centre in Southwest Nigeria where malaria transmission is intense were enrolled and followed up for 28 days. Malaria parasite was evaluated using SD-Bioline RDT (HRP-2 based) and microscopy of Giemsa stained blood film - gold standard on days 0, 1, 2, 3, 7, 14, 21 and 28. Blood culture was done with BACTEC system. Urine, throat and ear swabs culture were also done as deemed necessary. RDT result was used in determination of ACT treatment. RDT +ve children received artesunate-amodiaquine (Sanofi Aventis) at standard dosage supervised. Other clinical conditions were treated appropriately.
RESULTS: The mean age of enrolees was 27.21months ± 14.79 (range 5-59). There were 96 male (66.7%). The prevalence of malaria parasitemia were 54.9%(79/144) and 48.6%(70/144) by RDT and microscopy respectively. Parasite density ranged from 40-125,600/µL. Sensitivity and specificity of RDT was 81.4% and 74.3% at all parasite densities and 93.4% and 93.2% at parasite density >200/µL respectively. False negative (FN) rate for RDT was 17.1%(12/70). Nine of 12 patients with FN results became +ve between days 1 and 2. Parasitemia in all enrolees cleared before day3. Parasite recurrence at days 21 and 28 were 8.85% and 12.23% respectively. ASAQ was well tolerated. Three of 136 (2.7%), 21/118 (17.8%) were positive at culture. There was concomitant bacteraemia and malaria in three patients while 8 patients had concomitant UTI and malaria.
CONCLUSIONs: Restricting ACT treatment to RDT positive febrile children led to no untoward effect.

Parasites of fishes of the Amazon Basin in Brazil: A science metric study

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BACKGROUND. This paper presents a science metric study of the parasites of fishes of the Amazon basin in Brazil, including significant literature review.
METHODS AND RESULTS.The methodology was based on researching articles taken in March 2014 in three databases: Web of Science, SciELO (Scientific Electronic Library Online) and Google Scholar. The number of articles on fish parasites in the Amazon region is growing (currently 240), an increase from 1980, with the state of Amazonas further researched. She is considered the largest watershed in the world and has great biodiversity with representatives of almost all fish taxa were Characidae and Pimelodidae the most studied families. The number of publications with fish farming is still low compared to the number of natural environments. Crustacea was the most prevalent group, followed by parasitic Myxozoa and fauna. The main issue was addressed in articles taxonomy, followed by ecology and pathology. The periodical publications was over Acta Amazon and most of the articles were in journals without impact factor. It is concluded that there is need for substantive efforts to further research in the area, since the region has great biodiversity and the same is still imperfectly known.

Development of a specific kit for the diagnosis of Chagas disease in Peru for the Enzyme-linked Immunoelectrotransfer Blot (EITB) technique.

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BACKGROUND: The diagnosis of Chagas disease continues to be a problem in Peru because diagnostic techniques currently used do not reach a specificity of 100% and usually two assays are used to diagnose the disease, so it is required a confirmatory technique. The aim of this study was to develop a specific kit for the diagnosis of Chagas disease for the Enzyme-linked Immunoelectrotransfer Blot (EITB) technique
METHODS: The standardization of the EITB was performed using excretory-secretory antigens of epimastigotes of *Trypanosoma cruzi*, serum samples of patients with Chagas' disease, other parasitic and non- infected patients to perform the EITB assay, according to the methodology described by Tsang et al. (1989), and the kit according to ISO 9001-2008 requirement Design and Development validation for qualitative methods.
RESULTS: Antigens 10, 12 , 14,15 , 19, 20 , 23, 26 , 30 , 33, 36 , 40, 42 , 46, 58 , 63, 69 , 112 91,100 KDa were detected using a pool of sera from Chagas disease patients. Antigens 10, 12, 14, 15, 19, 20, 23, 26 KDa were considered as specific because not to react with sera from patients with other parasitic infections and sera from non-parasitized individuals. The developed of the prototype or kit, contains nine components for the detection of IgG antibodies in patients with Chagas disease, a sensitivity of 94.4 %, specificity of 100%, repeatability, reproducibility and robustness 100%, a diagnostic accuracy of 96.6 % and a shelf life of 12 months.
CONCLUSION: The Kit is efficient for the diagnosis of Chagas disease in Peru and can be used as a confirmatory test for specificity of 100%.
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Ecological aspects of endoparasite fauna of corvine, *Plagioscion squamosissimus* (Heckel, 1840), in Madeira and Negro rivers, the Amazon Basin, Brazil

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BACKGROUND. Geological and geomorphological differences in the source region of water bodies determine the types of water in the Amazon rivers. For these characteristics, the Madeira River is considered white water, while the Negro blackwater. Have the parasite communities of the same host species may not behave the same way in different locations, and there may be different degrees of similarity between parasites distinct locations within a given geographical and within the same ecosystem area.

METHODS AND RESULTS. By analyzing the parasitic fauna of 61 specimens *Plagioscion squamosissimus* collected at different points of the Madeira and Negro rivers, the region in which this fish is native, there were 14.467 specimens of parasites being 10.332 specimens of the Black River (71.42%) and 4.135 of the Madeira River (28.58 %), belonging to two taxonomic groups : Nematoda (all larval) and Acanthocephala (all in adult form) . Rio Madeira negative correlation was observed between the total length of hosts and the abundance of *Neoechinorhynchus veropesoi.* The prevalence and abundance of this species were different between males and females, being the most infected males than females. Furthermore , we observed differences in diversity indices between the rivers, where the Madeira River have had significantly higher diversity and evenness than the Negro.

CONCLUSIONS. This shows that despite the great similarity found between species of rivers (81.81%), biotic and abiotic factors can alter the species diversity of parasites present in a host and consequently its dominance

IDENTIFICATION OF A CYCLOOXYGENASE-LIKE ENZYME IN *Leishmania mexicana* PROMASTIGOTES

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BACKGROUND: Infections with *Leishmania* spp. associate with overproduction of prostaglandins (PGs), arachidonic acid (AA) metabolites. Cyclooxygenases 1 and 2 (COX1/2) enzymes are responsible of AA conversion to PGG2 and PGH2 as intermediaries for the generation of PGs such as PGE_2, $PGF_{2\alpha}$, and PGD_2. PGs influence the pathogenesis of *Leishmania* favoring a Th2 type T cell response as well as inhibiting macrophage effector functions. In this work we explored the possibility that *L. mexicana* could have evolved pathogenic mechanisms involving a COX-like activity.
METHODS: COX-like activity was measured by chemiluminescence and enzymatic activity was reported as Relative Light Units (RLU) per 100 mg of protein. Purification was done by ion exchange chromatography and prostaglandin production was followed by mass spectrometry.
RESULTS: COX-like activity was detected in total extracts with an optimal pH around 7.5. Similarly to higher eukaryotes, this activity was membrane-bound: 75% of activity was associated with membrane fraction whereas 25% remained in the soluble fraction. Addition of exogenous AA induced the expression of the enzyme, finding an increase of 50% in the enzymatic activity and 5.5 fold PGE_2 production. Partial purification of COX-like activity was accomplished by ion-exchange chromatography through a DEAE-Cellulose column, showing a negative net charge in contrast to what has been reported for COX enzymes from higher eukaryotes. A 67 kDa protein was identified with a monoclonal anti-mouse COX-2 antibody. Finally, using HPLS/MS it was possible to confirm* the prostaglandin production, reinforcing the original proposal of *L. mexicana* having a COX-like activity.
CONCLUSIONS: Taken together, these results suggest that *L. mexicana* possesses a COX-like activity, which could play a major role in pathogenesis and immune evasion, and that shares some characteristics with those of COX-2 enzymes from mammals, such as optimal pH, membrane association and inducibility, but differs in others, such as the lack of sequence homology and its anionic charge, making this enzyme a possible target for drug design.

Molecular identification of *Myxobolus* sp. from *Piaractus mesopotamicus* kidney.

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BACKGROUND: Myxosporideos are common parasites of fish, which are differentiated by morphological characterization of spores. However, there is much difficulty in this identification due to morphological similarity, requiring the use of alternative techniques such as molecular biology. This study used the technique of polymerase chain reaction (PCR) to identify the mixosporideo found in the kidneys of pacu.
METHODS: kidneys from infected *P. mesopotamicus* were fixed in Bouin and immersed in paraffin for DNA extraction. The samples were removed from the paraffin by washing in xylene, absolute ethanol, and finally washing in PBS. The material was frozen in liquid nitrogen and unfrozen in a water bath at 42° C for 15 minutes each, repeating 15 times for spore lysis. Proceeded to DNA extraction by Qiamp DNA Mini Kit protocol for tissue. After it was read in nano drop spectrophotometer and PCR was performed for the target 18S rRNA gene, using the primers MX5 and MX3 for Myxobolidae family.
RESULTS: The reaction resulted in a 1600 bp fragment, confirming that the parasites belong to Myxobolidae family. The sequencing will be performed in order to confirm the species.
CONCLUSION: The technique of mixosporideo DNA extraction from kidney fixed in Bouin and immersed in paraffin was efficient for analysis by PCR.

Characterization of potential protein-protein interactions of hure1-bp and potential participation of this protein in several biological processes in *Entamoeba histolytica*.

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BACKGROUND: EhURE1-BP is an *Entamoeba histolytica* protein that belongs to the family of multifunctional Tudor and Staphylococcal Nuclease (TSN) proteins. This protein is found both in the nucleus, participating as a transcription factor that binds to the cis-activation motif URE1, as in the cytoplasm where it´s possibly involved in other biological processes.
METHODS: To analyze the localization of this protein, immunoelectron microscopy and immunofluorescence assays were performed using antibodies coupled to gold particles and Alexa 488, respectively. To identify proteins that interact with EhURE1-BP pull-down experiments were performed using the recombinant protein expressed in *E. coli* BL21 alpha. Once the protein complex is obtained, these complexes were identified by mass spectrometry.
RESULTS: Immunolocalization assays showed that this protein is found in the nucleus, where it is involved in transcription, but it is also located in the cytoplasm, possibly participating in other biological processes. Using, immunoelectron microscopy we found that EhURE1-BP is localized in small cytoplasm vesicles and by pull-down assays we identified some proteins that interact with EhURE1-BP. These protein are involved in different cellular processes, such as transcription, metabolic processes and the organization of the cytoskeleton, and others.
CONCLUSIONS: Based on these results it is suggested that EhURE1-BP is a multifunctional protein similar to proteins of the TSN family.

Evaluation of the effect of piplartine and their analogues in cultures of *L. amazonensis* promastigotes

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BACKGROUND: Leishmaniasis is a major vector-borne infectious disease and is caused by protozoa of the genus *Leishmania*. Her therapy based on administering pentavalent antimonial compounds that cause side effects and toxicity inducing the formation of resistance mechanisms. Thus it is essential to search for new compounds that may combat these parasites that minimize toxicity and adverse effects caused by conventional drugs. Studies with piplartine, an amide extracted from *Piper tuberculatum*, show your potential antiparasitic. The aim of this study is to determine the effect of piplartine and analogues (A3, A33, A7, A39 and A40) in promastigotes of *Leishmania amazonensis* in vitro targeting future applications in the treatment of leishmaniasis.
METHODS: Cultivation of *Leishmania* promastigotes (10^6/200 µL) were treated with different concentrations of piplartine, A3, A33, A7, A39 and A40 (0, 1, 2, 4 , 8, 16 , 32, 64 , 128, 256 µg / mL) and incubated at 26 ° C for 2 hours, then added 10 µL of MTT solution (5 mg / mL) to the cultures , and after 4 h incubation were added 50 µL of 10% SDS for reading spectrophotometer 570 nm.
RESULTS: Different concentrations of piplartine and analogues, A7, A39 and A40 , decreased the percentage of MTT reduction , with the highest concentration (256 µg / mL) decreased by 76.9 % to piplartine and 93 % for A7 , 79.5% to A39 and 74.2% to A40 (ANOVA followed by Student- Newman-Keuls test , $P < 0.05$). The other analogues A3 and A33 decreased the percentage of MTT reduction for higher concentrations.
CONCLUSIONS: The results indicated that both piplartine and its analogues have inhibitory effect of *L. amazonensis* promastigotes and drive studies to identify their effect on crops amastigotes and macrophages which are the target cells to establish a protozoan infection.