On the morphology of *Anisakis pegreffii*: a comparative analysis of three microscopic techniques used to build a new parasite atlas

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BACKGROUND: Human anisakidosis is a parasitic anthropozoonosis caused by larval nematodes of the family *Anisakidae*. Here, we report a detailed description of the morphology of *Anisakis pegreffii* third-stage larva performed using a conventional light and confocal microscopy, and scanning electron microscopy (SEM) that provide a basis for both phenotypic studies and genetic mutations.
METHODS: The collected larvae from fish were morphologically identified as Anisakis larvae Type I, and they were characterized by PCR-RFLP to identify the *Anisakis pegreffii* specie. Using NC5/NC2 primers, ribosomal genomic regions ITS1, 5.8 SrRNA and ITS2 of DNA were amplified and PCR products were sequenced. Fifteen larvae belonging to *Anisakis pegreffii* were fixed, sectioned, and examined with a light and confocal microscope and by SEM.
RESULTS: In our studies, have been acquired detailed ultrastructural images, which have been integrated with those derived from the dissection of the parasite, obtained with light and confocal microscopy. The structural and ultrastructural images concerning the third stage larvae of *Anisakis pegreffii* have been studied, analyzed and compared among them. The derived overall view has allowed detecting new interesting details of a well-known parasite and has been schematically showed.
CONCLUSIONS: The aim of this study is to furnish an updated atlas of *Anisakis pegreffii*. Confocal microscopy, as well as the light and electron microscopy have played a pivotal role in the accumulation of new scientific data regarding the anatomical structures of this nematode. This work is the result of one year of engagement by the Authors and the outcome is a comprehensive atlas on *Anisakis pegreffii* microscopy.

Molecular identification of abomasal bacteria in sheep naturally infected with *Haemonchus contortus*

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BACKGROUND: The widespread occurrence of anthelmintic resistant gastrointestinal nematodes in sheep production systems, especially *Haemonchus contortus*, has driven the need to develop alternative control strategies. Little is known about the abomasal microbiota in sheep and the existence of a relation to local parasitism. This study aims to molecularly identify bacteria in the abomasum of sheep, in the presence of high and low parasitism.
METHODS: Based on fecal egg count (FEC) and quantification of *H. contortus*, we classified 8 sheep naturally infected with *H. contortus* into 2 groups: high (n = 4) and low parasitism (n = 4). We collected samples of abomasal content, abomasal mucosa, and adult *H. contortus* parasites, then grouped into 6 pools: content (CH), mucosa (MH), and parasites (PH) of sheep classified with high parasitism, and content (CL), mucosa (ML), and parasites (PL) of sheep with low parasitism. The molecular identification of the bacteria was based on bacterial 16S rRNA gene amplification, 16S rDNA clone library construction and subsequent gene sequencing.
RESULTS: A similar distribution of phyla was observed between pools CH/CL and PH/PL. For pools MH/ML, there was a significant difference (p = 0.01) in the proportion of phyla observed. The bacterial phyla predominant for libraries CH/CL were, respectively: Firmicutes (82% and 62%) and Bacteroidetes (10.4% and 17.6%); for MH/ML, Firmicutes (76.9% and 56%) and Proteobacteria (10.2% and 38.4%); and for PH and PL, Proteobacteria (42.8% and 55%) and Firmicutes (31.6% and 40%).
CONCLUSIONS: We observed differences with regard to the samples analyzed: content, mucosa and parasite, suggesting that there are various bacterial communities closely associated with the various materials analyzed, even in the same environment. The identification of some specific genera observed in the parasite, which could suggest the existence of endosymbiosis, points out the possibility of developing new targets for controlling *H. contortus*.

Lyso bis-phosphatidic acid, a novel phospholipid involved in endocytic pathway of *E. histolytica.*

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BACKGROUND: In other eukaryotic cells, the multivesicular pathway is involved in many cellular events and it is regulated by many molecules, including the LPBA phospholipid which is abundantly detected in late endosomes and it is essential for the correct functioning of the endosomal/lysosomal system. Also, the acyl chains are linked at the position C2 (C2´) of the glycerol backbone of the LBPA, conferring a different structure in comparison with other glycerolphospholipids. The invasiveness of *Entamoeba histolytica* and virulence factors such as the ingestion and degradation of microorganisms and host cells, suggest an important membrane remodeling in cell surface and organelles throw vesicular traffic. However, the composition and the role of the lipids during these processes are poor understood. In this work, we detected LBPA phospholipid of *E. histolytica* and checked out its participation during the endocytosis.
METHODS: we isolated phospholipids by TLC and analyzed the position of the fatty acids in the glycerol backbone of the compounds by ^{1}H-NMR. To know the molecular species, the single TLC spot was resolved by reverse-phase HPLC-ESI/MS. Fragmentation of the parent ions were performed to confirm the identification of LBPA ions. Furthermore, by confocal and electronic microscopy, we detected the phospholipid using a specific anti-LBPA antibody during endocytic events and in basal conditions.
RESULTS: RMN spectra revealed compounds with the fatty acid chains linked at C-2, C-2´ and C-3 and C3´. With HPLC-MS analysis, we found some ions with different unsaturation; the major molecular specie was 20:4/20:4. By confocal and electronic microscopy, we showed that the phospholipid is located in vesicles, which colocalize with and phagosomes and a lysosomal marker.
CONCLUSIONS: LBPA is present in *E. histolytica* and our overall results suggest that this phospholipid could play a dynamic role in endocytic events in the same way as those described in mammalian cells.

Comparison of two commercial rapid in-clinic serological test for detection of antibodies against *Leishmania infantum* in Paraguay

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BACKGROUND Canine visceral leishmaniosis is an important vector-borne disease widely distributed in Paraguay and there is a need for sensitive and specific rapid diagnostic tests for this disease. The aim of this study was to compare the agreement between the results obtained by serological techniques enzyme-linked immunoassay (ELISA) and the rK39 immunochromatographic test for diagnosis of visceral leishmaniosis in dogs attended at the Hospital of the Faculty of Veterinary Science, National University of Asuncion.
METHODS All the 195 canine blood samples collected were included in this study and evaluated using the immunoassay test enzyme-linked (SNAP® Leishmania) (IDEXX Laboratories, Inc , Westbrook, ME, USA) and immunochromatographic test with the recombinant antigen rK39 dipstick, Kalazar Detect™ Rapid Test, (Inbios®, USA), according to the manufacturer's instructions.
RESULTS The results showed that 92/195 (47.1%) dogs were positive for *L. infantum* by enzyme-linked immunoassay test (SNAP® Leishmania) and 89/195 dogs tested were positive for rK39 (Inbios®), immunochromatographic test (45.6%). We found that the agreement between the tests was higher compared to 90% (kappa 0.9) , indicating that the evidence is consistent and concordance was very good.
CONCLUSIONS The very good agreement between the reported rapid serological tests for the diagnosis of visceral leishmaniasis suggest that both rapid in-clinic serological tests showed an adequate diagnostic accuracy and can be used for the fast detection of antibodies against *L. infantum* in dogs

Trypanosoma cruzi calreticulin inhibits tumor growth in a model of mammary adenocarcinoma

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BACKGROUND: *Trypanosoma cruzi* (*T. cruzi*) is the etiologic agent of Chagas disease. In 1931, the Soviet researchers Roskin and Exsemplarskaja discovered an antagonism between Chagas disease and the development of some types of tumors. Besides, they observed that *T. cruzi* exhibited tropism towards tumor tissue. *T. cruzi* calreticulin (TcCRT) is a pleiotropic molecule resident in the endoplasmic reticulum (ER). We have previously shown that TcCRT, is translocated from the ER to the exterior of the parasite, and also an antiangiogenic activity of TcCRT *in vitro*, *ex vivo* and *in vivo*. We propose herein that the antitumor effect reported for *T. cruzi* infection is mediated, at least in part, by TcCRT.
METHODS: *In vitro*: Using flow cytometry, endothelial cells (EAhy926) were incubated for 2, 4 and 6 hours with TcCRT bound to FITC, and either: i) whole anti-TcCRT antibodies; ii) anti-TcCRT $F(ab')_2$ immunoglobulin fragments and iii) Fucoidan. *In vivo*: Five mice were inoculated (s.c.) in the dorsal region with a mammary adenocarcinoma (TEA3-MTX) and either i) recombinant TcCRT (50 µg, s.c.); ii) Anti-TcCRT or preimmune antibodies (80 µg, s.c.), on alternate days.
RESULTS: *In vitro*: TcCRT-FITC was internalizated by the endothelial cells after 4 and 6 hours, but both Fucoidan and the anti-TcCRT immunoglobulin fragments inhibited this effect. *In vivo*: TcCRT inhibited, tumor growth, an effect that was at least partially reverted when anti-TcCRT antibodies were also inoculated (p = 0, 0313).
CONCLUSIONS: Our results are consistent with the notion that the antitumor effect reported for *T. cruzi* infection is due to the capacity of the parasite protein to access the endothelial cell, thus generating antiangiogenic activity and consequent tumor growth inhibition.
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Hot Spots of *Rhodnius prolixus* and *Triatoma dimidiata* in Santander, an endemic area of Chagas disease in Colombia

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BACKGROUND: *Rhodnius prolixus* and *Triatoma dimidiata* (Hemiptera:Reduviidae:Triatominae) are the main vectors of Chagas disease in Colombia, and therefore the priority of the programs of prevention, surveillance and control. This work aimed to record the distribution of risk and hot spots of these species in the department of Santander (Colombia) using spatial autocorrelation analysis.
METHODS: A database of triatomine of CINTROP's entomology lab registered in the period 1996-2013 was used. Records of this species at locality level of each municipality were selected and the number of dwellings where their presence has been reported was quantified. This information was linked with the centroid of the polygon corresponding to each locality. The distribution of risk and hot spots was determined and visualized by calculating the local Getis-Ord index with ArcView v.10 program.
RESULTS: For *R. prolixus* the report in 38 towns, 195 villages and 1103 dwellings was used and 50 hot spots ($z<2.58$, $p<0.01$) located in the contiguous municipalities of Cepita, Curiti, Mogotes, San Joaquin, and Molagavita and in Gambita were detected. For *T. dimidiata* the report in 27 towns, 128 villages and 1757 houses was used and 10 hot spots ($z<2.58$, $p<0.01$) located in the town of Macaravita and 42 ($z=1.65$-2.58, $p=0.1$-0.05) in the contiguous municipalities of Capitanejo, San Miguel, Enciso, Concepción, Carcasi and San Andrés were detected.
CONCLUSIONS: Hot spots and stratified risk of the localities with presence of *R. prolixus* and *T. dimidiata* were determined. The use of spatial tools can help to health services to prioritize actions for prevention, surveillance and control, maximizing the efficiency of their activities and resources.

Serological survey on *Leishmania infantum chagasi* in dogs from central region of Rio Grande do Sul, Brazil

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BACKGROUND: Visceral leishmaniasis is a zoonosis of worldwide distribution which can infect a wide variety of animal species. *Leishmania infantum chagasi* is the etiologic agent of this disease and the dogs are the main domestic reservoir and the major source of infection to humans. In Brazil, the disease is endemic in almost all regions, except the South, however, recent studies have observed the spread of the disease to areas not endemic. The present study aimed to verify the occurrence of seropositive dogs for *L. infantum chagasi* in Santa Maria (29 ° 41 'S and 53 ° 48' W), Rio Grande do Sul State, South of Brazil.
METHODS: Serum samples were collected from 210 dogs of routine clinical procedures of the Veterinary Teaching Hospital of the Federal University of Santa Maria between December 2012 and March 2013, and kept frozen at -20 ° C until analysis. For the detection of anti-*L. infantum chagasi* antibodies, the serum samples were tested by Indirect Immunofluorescence Test (IFAT ≥ 40).
RESULTS: Of the total samples, three (1.4%) were positive to the cutoff point (1.40) and 2 (0.9%) samples positive for titer 80 , totaling five (2.4%) samples positive to the test.
CONCLUSIONS: The results here obtained indicate the circulation of the parasite among dogs in the studied area and the necessity of more studies that approach ecological and epidemiological aspects to subsidize activities directed to the vigilance and control of this disease in this state.

Anti-Schistosomal activity of Silver nano-particles and Praziquantel-Silver nano-particles composite in *Schistosoma mansoni*-infected mice

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BACKGROUND: Schistosomiasis is a parasitic disease of considerable health problem globally. Schistosomiasis is effectively treated by Praziquantel (PZQ), but no vaccine or no drug available against schistosomulae with rapid re-infection rates due to low hydrosolubility and quick first pass metabolism in the liver. Silver nanoparticles (AgNPs) have novel antimicrobial and infection-fighting properties with promising anti-parasitic application. In this study the chemotherapeutic effects of AgNPs and their possible improvement of anti-schistosomsis Drug; PZQ as PZQ-AgNPs composite where tested in infected mice with Egyptian strain of *Schistosoma mansoni* (*S. mansoni*).

METHOD: Silver nanoparticles (Ag-NPs) solution of ~60nm size was prepared by chemical reduction of AgNO3. A solution of PZQ was mixed with AgNPs solution, to form the residue product; PZQ-AgNPs composite of 60-70 nm size. Age-matched Swiss albino mice, weighing 20 ± 2 gm were grouped into 6 groups; GI: un-infected, untreated (normal group), GII: un-infected, treated with AgNPs (GIIA), & PZQ-AgNPs composite (GIIP) as a single oral dose and other two groups infected with Egyptian strain of *S. mansoni* cercariae (90 ± 10 cercariae/mouse) cutaneously, infected non-treated group (GIII), infected treated 3 weeks post-infection group (GV3: G3VA and G3VP) and 6 weeks post-infection group (GVI6; GVIA6 and GVIP6). The drugs efficacies were evaluated parasitologically; measuring worm burden, tissue egg load and oogram pattern, status of egg maturation and viability, pathologically by measuring granuloma size and numbers and using electron microscope.

RESULTS: AgNPs showed a chemotherapeutic effect against juvenile and adult *S. mansoni* in infected mice compared to PZO. Also, AgNPs improved the antiparasitic activities and reduced dose of PZQ using PZQ-AgNPs composite against both juvenile and adult *S. mansoni* comparable to full dose in 3 and 6 weeks post-infection treated group in the form of reduction of worm burden, tissue egg load, hepatic granulomata size and numbers. Scanning Electron Microscopy revealed that AgNPs and PZQ-AgNPs induced tegumental damage in adult schistosomes.

CONCLUSION: The obtained results may introduce AgNPs as a novel antischistosomal compounds and their ability to modify the key features of the available drug (PZQ) with improving of its hydrosolubility and bioavailability.

New tools for Chagas disease vector control

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BACKGROUND: The Mendoza Chagas disease control program introduced the use of a vehicle-mounted automatic sprayer (AS) (Malanca) for house spraying with pyrethroid insecticides. The AS appears to be more efficient than the traditional manual compression sprayers (MS) used by vector control programs, especially in rural houses with large peridomestic structures, but the comparative performance of both treatments has not been investigated.
METHODS: To evaluate the effectiveness and efficiency of insecticide spraying operations using AS *versus* MS we conducted a randomized intervention trial in Lavalle Department (Mendoza, Argentina) between May 2013–2014. Experienced vector control personnel assessed house infestation at site level using timed manual collections with a dislodjant aerosol at 0, 1, 4 and 12 months postintervention. All houses positive for *T. infestans* at baseline were randomly allocated to AS or MS and sprayed with SC deltamethrin (Bayer) at an intended dose of 25 mg/m^2. For each house we quantified total sprayed area, insecticide applied, water required, and time to complete the house insecticide spray. Houses found infested after initial interventions were re-sprayed with the same treatment.
RESULTS: *T. infestans* was found in 41 (55%) of 76 houses in domestic (4%), peridomestic (41%) or both habitats (9%) at baseline. The interventions reduced house infestation from 100% to 16%, 0% and 11% at 1, 4 and 12 months postintervention, respectively, and did not differ significantly between treatments. Endpoint infestations were restricted to peridomestic habitats. Although the insecticide applied per m^2 and water required were similar between treatments, AS was faster than MS.
CONCLUSIONS: Both treatments had similar effectiveness and efficiency, but did not completely suppress infestations. The main advantage of AS was reduced physical effort under harsh field conditions. These encouraging results justify the execution of larger field trials in different settings before issuing a final recommendation.

Giardia intestinalis invading the duodenal epithelium of a girl with lactose malabsorption

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Giardiasis is a cosmopolitan neglected parasitic disease that produces diarrhoea in humans and animals. Actually, there are gaps in the knowledge of pathogenic and immunologic mechanisms. We demonstrated here that in *Giardia intestinalis* (Sin. *G. duodenalis, G. lamblia*) populations, there are trophozoites adapted to penetrate the duodenal epithelium. This conclusion was supported by data from human duodenal biopsies, cultures of invasive *G. intestinalis* isolates, experimental infection in suckling gerbils and the immunohistochemical detection of *Giardia* trophozoites within duodenal tissue. We found parasites in the midst of epithelial intestinal cells, under absorptive enterocytes, between the lamina propria and near the central lacteal. These results break with the widely established belief that *Giardia* trophozoites only live on the microvillus surface and do not ingress into epithelial tissue. This evidence opens new ways towards the explanation of phenomena such as: pathogenic mechanisms, immunological responses of the host, immunological evasion of the parasite and invasion mechanisms.

Immunopathology of *Trypanosoma cruzi* in mice CBA and C57BL/10 infected by intragastric and intraperitoneal routes

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BACKGROUND: Most of the experimental studies on Chagas disease use mice infected by intraperitoneal route, although oral infection is a common form of transmission in the wild. In this work we used the SC2005 isolate which was obtained from an orally infected patient and classified as TCII. This study aims to obtain data that can assist in understanding the mechanisms involved in oral infection and pathogenesis of the disease, as well as the analysis of the genetic influence of mice with different degrees of susceptibility.
METHODS: CBA and C57BL/10 mice were intraperitonealy (IP) and intragastrically (IG) infected by 10^7 trypomastigote forms of *T. cruzi* derived from cell culture. Parasitemia, mortality and global leukocytes were evaluated for 33 days after infection. After 11 and 18 days of IP infection and 26 and 33 days of IG infection, mice were histologically analyzed. Presence of parasites was evaluated by PCR and the immunological profile was evaluated through Th1/Th2/Th17 cytokine production.
RESULTS: Mice infected by IG route, independent of the mouse strain, presented lower and later parasitemia. Mortality was observed only when animals were infected by IP route, being higher in CBA (100%) than in C57BL/10 (30%) mice. CBA mice infected by IP route showed leukopenia while mice infected by IG route presented leukocytosis. Both infection routes were able to induce tissue damage, but intragastrical infection resulted in lower parasitic loads than intraperitoneal inoculation. Both routes induced a Th1/Th2/Th17 response; however, the CBA mice infected by IG route showed increased production of Th2/Th17cytokines. PCR could amplify DNA of *T. cruzi* in all analyzed organs.
CONCLUSIONS: There are significant differences in infection with *T. cruzi* strain SC2005, which are dependent on the route of infection and host genetics. Both infection routes may promote immunopathological alterations, which were less severely in animals inoculated by IG route.

Comparative analysis of differential expression between strains of *T.cruzi* sensitive and resistant beznidazole

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BACKGROUND: Therapeutic options for the treatment of Chagas disease are limited to Benznidazole and Nifurtimox. The use of proteomic techniques in trypanosomatids is particularly important because these organisms do not use transcription initiation as a regulatory step to control gene expression. The present study aimed to investigate differentially expressed proteins in benznidazole resistant and sensitive strains of *T. cruzi* obtained of acute Chagas patient.
METHODS: Epimastigote forms of two isolates of *T. cruzi* rJCRcl3 and rSylvioX/10 that show induced resistance in vitro to benznidazole with their respective counterpart's susceptible sJCRcl3 and sSylvioX/10 were subjected to two-dimensional gel electrophoresis. The detection of spots and comparison of the protein expression was carried out using the PDQuest software. The identification of the proteins was performed by MALDITOF mass spectrometry and searching database Mascot.
RESULTS: Out of 71 spots analyzed through MS, 44 were identified as 40 distinct proteins. Out of the 40 distinct proteins, 22 were present in resistant and 21 in susceptible phenotype. Among the proteins identified in resistant samples, 2 up regulated proteins were common in resistant phenotype (D isomer specific 2 hydroxyacid and acetyl ornithine deacetylasa) and 2 in susceptible phenotype (prostaglandin F2 alpha synthase and sterol 24 C methyltransferase).
CONCLUSIONS: In conclusion, the identification of various proteins associated with energy production, protein synthesis and antioxidant defense mechanism identified in this study would be consistent with the proposed model of the participation of different mechanisms acting in a coordinated response to reduce the stress generated by the drug.

Molecular detection of *Wuchereria bancrofti* DNA in human blood from filariasis endemic areas in Egypt

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BACKGROUND: Lymphatic filariasis (LF) is an important vector-borne health problem, *Wuchereria bancrofti* (*Wb*) is the major (90%) cause of LF worldwide and is focally endemic in Egypt. The aim of this study was to determine *Wb* molecular prevalence in cohort of Egyptians and to compare the diagnostic performances of the conventional methods (parasitological and immunoassays) with semi-nested PCR assay.

METHOD: This is a cross sectional study. Collected blood samples from residents in filariasis endemic areas were subjected to; parasitological concentration technique, immunoassay and molecular assay using semi-nested PCR for detection of *Wb* microfilaria, antigens and DNA.

RESULTS: *Wb* was an important pathogen among study individual with a clearly high molecular prevalence of 16.7% (n=45). ELISA detected antigen in 3.7% (n=10) of cases with 2.6% (n=7) of them were negative by PCR.

CONCLUSION: Conventional methods couldn`t be used as a consistent single detection method due to their lowered sensitivities. Considering PCR as a reference standard, ELISA assay for *Wb* detection surpassed microscopy but it showed imperfect specificity. Being had the highest diagnostic yield; PCR is a more attractive diagnostic and going to replace conventional methods for reliable detection of *Wb* and a routine procedure in filarial endemic areas.

Immunolocalization of protein phosphatases 2C of *Cryptosporidium parvum*

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Cryptosporidium parvum is an apicomplexa parasite that causes severe watery diarrhea in immunocompromised individuals. To date there is no effective treatment against infection by this parasite. Phosphatases have been considered potential therapeutic targets in many diseases due to their role in the regulation of various cellular functions. In infectious microorganisms, it has been suggested that protein phosphatases 2C are involved in virulence, infection and parasite life stages. The study of these enzymes in *C. parvum* is limited. In this work, we show the presence of several phosphatases PP2C in total extracts of *C. parvum* oocysts by Western blot using two polyclonal antibodies raised against a PP2C of *Leishmania spp.* Furthermore, indirect immunofluorescence microscopy allowed us to visualize the distribution of these proteins in the apical region of sporozoites. Additionally, using another polyclonal antibody against a 72 kDa PP2C of *C. parvum* showed localization in the nucleus and the apical region in sporozoites. Finally, an alignment with known ortholog sequences allowed us to observe the degree of conservation of these enzymes and estimate, with an unrooted tree, a possible relationship and function with PP2C of other organisms.

Wolbachia as target for filariasis control: valuable information about antibiotic resistance genes recovered from _Wolbachia_ genomes.

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BACKGROUND: *Wolbachia* obligate endosymbionts are found in the major causative agents of lymphatic filariasis and onchocerciasis. *Wolbachia* are attractive targets for control of these parasites due their essential role in their survival. So, an alternative drug for filariasis control, via *Wolbachia* are antibiotics such as tetracyclines or rifamycines proved to be effective against some filarial nematodes. However, it is well know, the emergence of antibiotic resistance among bacteria and the distinct resistance profiles that can occur within bacteria species. Currently, there are five complete genomes of *Wolbachia* endosymbiont of nematodes. This scenario allowed an *in silico* search for the presence of genes related to antibiotic resistance in *Wolbachia. In vitro* studies with obligate endosymbionts as *Wolbachia* are a hard task. So, the genomic analyses would improve the establishment of potential antibiotics to be used in the control of these infections. The aim of this study was to identify the antibiotic resistance genes in genomes of *Wolbachia* endosymbiont of filarial.
METHODS: All complete genome sequences of *Wolbachia* from nematode, were retrieved from the Genbank. Based on The Comprehensive Antibiotic Resistance Database (CARD) (http://arpcard.mcmaster.ca/), we performed BLAST searches to identify such genes in *Wolbachia* genomes. Alignments were performed by Muscle software and phylogenetic analysis by Maximum likelihood.
RESULTS: We found polymorphisms in *Wolbachia* DNA-dependent RNA polymerase (RpoBC), rifamycines target, that could reflect in the resistance phenotype. The *rpo*BC were highly conserved across these Wolbachia genomes. CARD analyses revealed the presence of putative determinants of antibiotic resistance such as mupirocin in the genome of *Wolbachia* endosymbionts from *Wuchereria bancrofti.*
CONCLUSIONS: Our results showed the presence of targets associated with antibiotic resistance in *Wolbachia* endosymbiont of some nematode. Therefore, it is a useful approach to explore *Wolbachia* genomes aiming further antibiotic use in the control and elimination of filarial nematode infections.

Trichomonas vaginalis Exosomes Deliver Cargo to Host Cells and Mediate Host Cell Colonization

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Trichomonas vaginalis is a common sexually transmitted parasite that colonizes the human urogenital tract where it remains extracellular and adheres to epithelial cells. Infections range from asymptomatic to highly inflammatory, depending on the host and the parasite strain. Symptomatic women typically present with vaginitis, whereas infection in men is usually asymptomatic but can lead to untreated, chronic inflammation of the prostate. *T. vaginalis* infection is associated with increased incidence and severity of prostate cancer. Our laboratory focuses on the role of *T. vaginalis* surface proteins and secreted vesicles in pathogenesis. We have found that *T.vaginalis* produces and secretes microvesicles with physical and biochemical properties similar to mammalian exosomes. Parasite-derived exosomes are characterized by the presence of core mammalian exosomal proteins as well as parasite-specific proteins. We have demonstrated that *T. vaginalis* exosomes fuse with and deliver their contents to host cells and modulate host cell immune responses. Exosomes from highly adherent parasite strains increase the adherence of poorly adherent parasites to vaginal and prostate epithelial cells. Additionally, exosomes from *T. vaginalis* strains that preferentially bind to prostate epithelial cells, relative to ectocercival epithelial cells, can confer this binding preference to other *T. vaginalis* strains. Studies on a *T. vaginalis* homologue of the human macrophage migration inhibitory factor (TvMIF) presence in parasite exosomes have shown that TvMIF can mimic human MIF by inducing cellular pathways linked to inflammation and increasing prostate cell proliferation and invasiveness: properties underlying the promotion and progression of prostate cancer. In summary, parasite exosomes modulate host:parasite interactions, promote colonization and may contribute to increased risk of prostate cancer in men infected by the parasite.

MOSQUITO CELLULAR RESPONSES TO STRESS

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Mosquitoes are the main vectors of pathogens transmitted by hematophagous insects, including, between others, *Plasmodium* parasite (*Anopheles* mosquitoes) and Dengue virus (*Aedes* mosquitoes), both health concerns in Mexico. Historically, actions directed against the mosquitoes have been the more successful and they include insecticide application, environmental engineering and recently have been proposed transgenic-based strategies in order to reduce the mosquito populations. As part of the evolutive intercourse between insects and control activities, insecticide resistant populations have been raised such as mosquitoes that are invading new niches and the effects of climate changes on insect populations are now visible. These scenarios indicate the importance to continue the study of the molecular mechanisms that mosquitoes use to confront the environmental insults and pathogens. In the laboratory we have studied the responses of the midgut during the blood feeding and the presence of xenobiotics and the hormone prostaglandin. We have observed that *Anopheles* and *Aedes* midguts synthesize and secrete proteins in different manners and we identify and characterize some of the responding molecules. Several of the molecules described *in vivo*, are tested in cell lines in order to facilitate the dissection of molecular mechanisms involved in the complex responses to different stimuli.

Purification of recombinant *Taenia solium* calreticulin and antibody determination post-oral immunization

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BACKGROUND: Few helminth molecules have been identified to be involved in triggering immune responses. Oral immunization with recombinant functional *Taenia solium* calreticulin (rTsCRT) and Colera toxin as adjuvant elicited high fecal specific IgA. The aim of this study was to scale-up the purication of rTsCRT and to evaluate the humoral immune response induced after oral immunization with rTsCRT in absence of adjuvant in Balb/c mice.
METHODS: rTsCRT was obtained using a bacterial expression system (*E. col*i BL-21) and purified by differential centrifugation and electroelution. Protein determination of rTsCRT concentration using different methods was compared Protein concentration was determined by Lowry and Bradford methods, as well as by 260/280nm absorbance and SDS/PAGE densitometric analysis. Mice were orally immunized four times at one week intervals and specific anti-rTsCRT antibodies in serum and supernatants from intestinal flush were identified by ELISA.
RESULTS:. The purification protocol was optimized to produce high concentrations of rTsCRT for oral immunization.The average protein concentrations obtained with the Lowry method were 67% and 30% higher than with Bradford and 260/280 absorbance respectively. The estimated concentration of rTsCRT by spectrophotometric analysis and densitometry of stained gels was comparable and represented the average of the values obtained by the two colorimetric methods. Regarding the immunizations, rTsCRT in absence of adjuvant did not induce significant amounts of anti-rTsCRT specific IgG. Low levels of specific IgA antibodies were found in intestinal flushes from rTsCRT-immunized mice compared to the control.
CONCLUSION: For rTsCRT determination, one of the most reliable methods is densitometry analys in SDS-PAGE and the best colorimetric method is Bradford because it proportion amino acid residue of basic and aromathinc aminoacids is very close to that of BSA (standard protein used for the standard curve). Our immunization protocol did not induce an adequate humoral immune response.

Community-based intervention for behavioral change in the prevention of Chagas disease in Guatemala: An Eco-Bio-Social approach

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BACKGROUND: Sustainable Chagas disease control is challenging in regions where cultural practices promote triatomine colonization of animal nests inside homes. A previous study showed that rodents were a risk factor for *Triatoma dimidiata* persistent intradomicilliary infestation and posed a threat for continued parasite transmission.
METHODS: A community-based cluster-randomized study was conducted from September 2012 to April 2014 in eighteen high infestation risk communities of Comapa, Guatemala. Communities were randomly assigned to control or intervention. Controls received community-based pyrethroid spraying according to National guidelines (December 2012 to February 2013). The intervention consisted of monthly participatory meetings on Chagas disease, risk factors, and rodent biology and control, in combination with community-based enhanced spraying and mechanical rodent control (September 2012-July 2013). Baseline and final entomological and rodent surveys were performed simultaneously with knowledge, attitudes and practices (KAP) surveys. Instar infection and human blood meals were determined to evaluate recent transmission and human contact after intervention.
RESULTS: Intervention group showed improved KAP scores regarding the disease, risk factors and surveillance compared to control. Intradomiciliary infestation decreased from 20 ± 11% to 8±7% and 19 ± 9% to 6 ± 6% in intervention and control, respectively, at least one year after spraying. *Trypanosoma cruzi* prevalence in first to third instars was 10.8% (4.3-24.7) in intervention (n=37) and 50% (34.1-65.9) in control (n=34). Human blood meals in *T. dimidiata* were detected in 7% in the intervention, and 13% in the control groups.
CONCLUSIONS*:* Even though infestation was reduced to similar levels in both intervention and control groups, the reduced human contact with infected vectors indicate a tendency towards lower transmission risk in the intervention. We propose a Chagas disease prevention strategy that integrates behavioral change to modify transmission risks with improved community-based vector and rodent control.

Major sylvatic vectors of *Trypanosoma cruzi* in the Gran Chaco region

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Of the nearly 20 species of triatomine bugs usually found in sylvatic habitats of the Gran Chaco and adjacent regions across Argentina, Bolivia and Paraguay, only a few may be relevant for public health. The most abundant sylvatic vectors are *Triatoma sordida,* the closely related cryptic species *Triatoma garciabesi* (sometimes considered a subspecies of *T. sordida*), and *Triatoma guasayana*. The range of *T. sordida* extends from the humid (eastern) Chaco to the 'cerrado' in Brazil, whereas *T. garciabesi* and *T. guasayana* occupy different habitats and predominate in the dry Chaco. Adult specimens of *T. sordida* and *T. guasayana*, but not *T. garciabesi*, frequently invade human habitations and may colonize them transiently; and bite humans and domestic animals. Neither *T. garciabesi* nor *T. guasayana* colonized human habitations during a 10-year study conducted in the dry Argentine Chaco despite the domestic occurrence of *Triatoma infestans* was nearly suppressed. Similarly, a three-year longitudinal study conducted in Pampa del Indio, in the humid Argentine Chaco, assessed site-specific house infestation with triatomine bugs before and every 4-5 months after a community-wide residual spraying with deltamethrin. The prevalence of house infestation with *T. sordida* decreased from 18.3% at baseline (mainly in peridomestic sites) to 0% up to 12 months post-spraying (MPS), and then increased gradually to return to baseline levels by 28 MPS. House invasion and recolonization of peridomestic habitats most likely originated from sylvatic bug colonies. The prevalence of bug infection with *T. cruzi* determined by kDNA-PCR before and 4 MPS (6.3-6.4%) were similar and six-fold greater than infections determined by microscopical examination of bug feces. (Peri)domestic *T. sordida* were infected with TcI, V and VI, suggesting it may be involved in (peri)domestic transmission cycles and may also act as a bridge vector of TcI, which is frequently found in local *Didelphis* opossums.

Analysis of fibrillarin as a nucleolar marker in *Trypanosoma cruzi* epimastigotes.

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BACKGROUND: Ribosome biogenesis is an essential biological process. In eukaryotic organisms, this process is initiated and almost driven to completion in the nucleolus. Our research group is interested in the study of nucleolar formation and ribosome biogenesis in the pathogenic parasite *Trypanosoma cruzi*. This organism organizes a bipartite nucleolus in proliferative developmental stages of epimastigotes and amastigotes. Interestingly, the nucleolus is not assembled in trypomastigotes (non-proliferative cellular stages). Therefore, the nucleolus can be thought of as being developmentally controlled during the life cycle of this species of trypanosomes. We have begun an analysis of the molecules involved in several steps of ribosomal biogenesis process. We study the *T. cruzi* fibrillarin, the methyl-transferase functional subunit of snoRNPs complexes that methylate the pre-rRNA along its maturation pathway. The *T. cruzi* genome encodes two potential fibrillarin genes. We present data on fibrillarin expression and nucleolar localization in epimastigotes.
METHODS: Two DNA probes were generated by PCR for each pair of annotated fibrillarin alleles. These probes were used in northern blot assays. We generate chimeras fused to EGFP of both fibrillarin genes in the endogenous expression vector pTEX. The transfected epimastigotes were analyzed by fluorescence microscopy. Polyclonal antibodies were used in western blot assays and immunofluorescence.
RESULTS: Both genes are expressed transcriptionally. The transcripts have 1.3 kb length. Ectopic expression of the two EGFP chimeras, indicate a nucleolar localization pattern. We detect the fibrillarin protein (weight: 30 kDa) that localize in the nucleolus of exponential epimastigotes. In stationary stage and in metacyclic trypomastigotes, fibrillarin appears as an extra nuclear punctuated signal.
CONCLUSIONS: Both *T. cruzi* fibrillarin genes are expressed as nuclear proteins in exponentially growing epimastigotes. The relocation of this protein in stationary epimastigotes and in metacyclic trypomastigotes may be postulated as a regulatory mechanism operating during a down regulation of ribosome biogenesis pathway.

Participation of JNK and p38 MAP kinase in the inhibition of apoptosis of dendritic cells by *Leishmania mexicana*

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BACKGROUND: Inhibition of host cell apoptosis is a strategy employed by multiple pathogens to ensure their survival in the infected cell. *Leishmania* parasites have been shown to protect macrophages, neutrophils and dendritic cells from both natural and induced apoptosis. Nevertheless, the mechanisms involved in the inhibition of apoptosis by *Leishmania* have not been established. In this study we analyzed the phosphorylation of p38 and JNK in monocyte-derived dendritic cells (moDC) infected with *L. mexicana* amastigotes and the role of these kinases in the inhibition of apoptosis of moDC by *L. mexicana* amastigotes.
METHODS: moDC were infected with *L. mexicana* amastigotes, treated with camptothecin, and apoptosis was determined by the externalization of phosphatidylserine by flow cytometry and caspase-3 activation by Western blot. Protein extracts were prepared from infected cells and the presence of phospho-p38 and phospho-JNK was determined by Western blot. Inhibitors of both kinases were used and their effect on DNA fragmentation was tested.
RESULTS: As shown in this study, the infection of moDC with *L. mexicana* amastigotes inhibited apoptosis as determined by the externalization of phosphatidylserine and the activation of caspase-3. The phosphorylation of the MAP kinases p-38 and JNK was significantly diminished in the infected cells and the inhibition of both kinases diminished DNA fragmentation, but in a major extent was the reduction of DNA fragmentation when p38 was inhibited.
CONCLUSIONS: Our results suggest that the capacity of *L. mexicana* amastigotes to diminish MAP kinases activation is probably one of the strategies employed to delay apoptosis induction in the infected moDC and may have implications for *Leishmania* pathogenesis by favoring the invasion of its host and the persistence of the parasite in the infected cells.
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Sharp global declines in shark populations: implications for marine tapeworm biodiversity

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BACKGROUND: Globally, there are widespread declines of top predators in oceanic ecosystems, particularly sharks. Despite shark species being described at unprecedented rates, shark populations are declining on average by 11% per year globally. Hence, there is a likelihood of shark species going extinct prior to their discovery. Considering that only 30% of shark species have been examined for parasites, the tapeworm biodiversity of sharks is likely to exceed the 3600 estimated unknown tapeworm species yet to be described from known sharks. The objective of this study was to assess the impact of steep global declines in shark populations on tapeworm discovery and biodiversity.
METHODS: Generalized linear and linear mixed models were used to: (1) calculate the average and 95% confidence intervals for discovery rate of tapeworms from sharks; (2) estimate the global tapeworm diversity from shark hosts; (3) identify predictors influencing the year of discovery of tapeworm species from shark hosts; and (4) identify the predictors influencing the time lag between descriptions of sharks and their tapeworms.
RESULTS: Data indicates that we are currently in the midst of an increasing rate of tapeworm discovery and that the cumulative frequency distribution curve for these parasites in sharks is far from reaching an asymptote. Furthermore, larger tapeworms tend to be discovered prior to small ones and host features are most important in explaining variation in time lag between sharks and their tapeworm parasites.
CONCLUSIONS: Unless further global biosystematics and conservation initiatives are undertaken in the near future, we are at risk of losing small host specific tapeworm taxa prior to their discovery, in large part due to the demise of their shark hosts. This potential loss of biodiversity may hinder efforts to better understand the ecological roles these tapeworms play in our marine ecosystems.

Use of the intestinal loop model in gerbils (*Meriones unguiculatus*) as a tool to analyze damage to intestinal epithelial cells by purified cathepsin B like from *Giardia duodenalis* and signaling pathways that modulate intestinal epithelial homeostasis

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BACKGROUND. Interaction of *G. duodenalis* with epithelial cells induces the release of parasite proteins which in turn may cause damage on these cells. We have recently isolated and characterized a cathepsin B like-protease from *G. duodenalis* that has proteolitic activity. When added to epithelial cell monolayers *in vivo,* cathepsin B like-protease induces degradation of intercellular junction proteins and cell apoptosis. To analyze the *in vivo* effect of this protein as well as some signaling pathways triggered by this protein in intestinal epithelial cells a model of dudodenal loop ligation in gerbils (*Meriones unguiculatus*) was performed.
METHODS. A cathepsin B like protein was purified by affinity chromatography using mAb 1G3. Then defined amounts of the protein were inoculated into the duodenal loop of gerbils while non-treated animals served as control. Interaction was carried out for different times and the intestinal loops were recovered to analyze the effect of cathepsin B like in representative sections of intestine and staining with H&E, by inmunofluorescence and Western blot. Further temsirolimus was used in this model to analyze the role of mTORC1 in intestinal epithelial cells.
RESULTS. The purified protein induces alterations of the intestinal villus, compared with the control group as shown by the presence of ulceration, cellular infiltration and hemorrhagic areas. In addition, edema at the sub-mucosal layer and a reduction in the integrity of the brush border were observed. Furthermore, western blot assays showed changes in the expression of mTOR pathway proteins which were more evident at later times pi and a significant reduction on the expression of some of these proteins {Raptor and p- mTOR (serine 2481)} was observed in animals treated with temsirolimus. As expected immunofluorescence assays showed changes in the distribution and expression of the cell junction proteins b catenin and E-cadherin, and the proliferation and protection markers phospho-histone serine 10 and muc-2, respectively.
CONCLUSIONS. These results showed for the first time that a purified cathepsin B like protein form *G. duodenalis* has a marked effect in gut homeostasis by inducing damage on intestinal epithelial cells. Project supported by SEP-Conacyt grant No.128426.

Evaluation of topical treatment of experimental cutaneous leishmaniasis with formulations containing antimonials

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BACKGROUND: Leishmaniasis is an endemic infectious disease, non-contagious, vector-borne and caused by protozoa of the genus Leishmania. Pentavalent antimonials are the first-line drugs for treatment of this disease in humans, however require parenteral administration and are always associated with several side effects such as pancreatitis, hepatitis myalgia, arthralgia and cardiac toxicity, as well as with the development of drug resistance by the parasites. With the aim of searching for the most effective, low cost and with fewer side effects to patients formulations, this study has been developed with the purpose of evaluation of effects after application of topical formulations containing antimonial in Hamters (*Mesocricetus auratus*).
METHODS: Four of four groups of hamsters were infected with 1,0 × 10^6 *Leishmania* (L.) *amazonensis* promastigotas (MHOM/BR/2009/IM5584), in the region of nose, and were submitted to different treatments of 12 days duration starting on the 40th day after inoculation. Group 1 was treated with 100 mg/animal/day cream with antimonial, group 2 was treated with a combination of 50 mg/animal/day cream with antimonial alternated with vinilin, group 3 was treated with an hydrogel containing antimonial and Group 4 (control) received any treatment. The protocol for these experiments was previously approved by the Ethics Committee of Animals, n◦.009/2012. The formulations were applied once per day in the area of the lesion. The total area of the lesions was measured/recorded daily using a calliper. After animal euthanasia, fragments of lesions were sowed in complete RPMI medium for determination of parasite viability and tissue impression smear. Statistical analyses (ANOVA) and Turkey test were made in Minitab and *P* values of less than 0.05 were considers significant.
RESULTS: In all experimental groups, viable parasites of *L. amazonensis* were still detected in NNN medium, after 12 days of treatment. All groups showed no statistical significance for the total area of lesions, when reference *p* value > 0.05. These results showed necessity of a longstanding topical treatment to detect the effective action of formulations in skin lesions.
CONCLUSIONS: Formulations for topical treatment of cutaneous leishmaniasis should be studied with other concentrations so that in the future may contribute to more effective treatment and fewer side effects.
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Effect of zinc supplementation on the control of *Eimeria* in two sheep breeds in Colombian high mountains

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BACKGROUND: Infection with *Eimeria* spp., and nematodes in sheep are prevalent worldwide, sometimes death occurs. Cellular response (Th1) control the infection, and is well recognized that Zinc deficiency decreases Th1 and Th2 responses. The aim of this study was compare the effect of Zinc enriched diet with two forms of Zinc on *Eimeria* oocysts shed by lambs.
METHODOS: A total of 21 pregnant ewes of Criolla and Hampshire breeds, were divided in three groups. Each group was supplied with 60mg/Kg of dry matter of ZnO, Chelated zinc and control, from 3 months of pregnancy until the lambs were 8 weeks old. Samples of faeces were examinated with McMaster methodology
RESULTS. With ZnO there was an increased oocysts shedding until 45 days old lambs, and then the percentage of reduction of oocysts sheeding was observed, in both Criolla and Hampshire races but it was higher in Criolla. Animals supplemented with chelate showed the same pattern, the Hampshire breed with the highest reduction percentage of oocyst. The highest number of oocyst excretion were 920000 for control group, 154000 for chelated zinc and 81900 for inorganic oxide (ZnO), the least number of oocyst after the peak of excretion was 0 for quelate in a Hampshire ewe, in Criolla race was 150 with the treatment of inorganic zinc, in contrast in the control group was 1450 in a Hampshire ewe.
CONCLUSIONS: Immune deficiency is related with nutrition. As animals supplemented with ZnO and chelate zinc had a reduction percentage of oocyst shedding compared with control, this response depends on other factors, just as genetic of the animals.

Monitoring of *Giardia* spp. and *Cryptosporidium* spp. and biodiversity of ciliated protozoa in surface water and sediment samples of the Atibaia river, Campinas, São Paulo, Brazil.

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BACKGROUND: The knowledge of ciliated and pathogenic protozoa may allow inferences about the degree of contamination of a water body as well as the possible contamination sources and thus contribute to the monitoring of the environmental quality.
METHODS: Water and sediment samples from two points of Atibaia River, Campinas, SP, were collected monthly for 19 months. Qualitative (*in vivo* observation and after silver impregnation) and quantitative analysis (*in vivo* enumeration and Quantitative Protargol Stain) were performed for ciliated protozoa. For *Giardia* spp. and *Cryptosporidium* spp. research, immunomagnetic separation (IMS) was employed. For molecular confirmation of pathogenic protozoa, aliquots positive by immunofluorescence were subjected to DNA extraction and PCR.
RESULTS: 70 morphospecies of ciliated protozoa belonging to 50 genera were identified in surface water and sediment samples over the 19 months. Species of ciliates recorded are characterized in the saprobic system, with 14 indicators of alpha-mesosaprobic environment. In surface water samples, *Giardia* spp. cysts were detected in 63.1% and *Cryptosporidium* spp., in 21.0%. Considering the sediment samples, cysts were detected in 26.3% of them, while positivity for oocysts was 13.1%. A total of 43 samples positive for *Giardia* sp. were subjected to sequencing (56.5% of total samples).
CONCLUSIONS: Since most samples were characterized as *Giardia* AII assemblage, these findings highlight the high degree of sewage contamination from human origin in the Atibaia river.

Use of the intestinal loop model in gerbils (*Meriones unguiculatus*) as a tool to identify the signaling pathways affected by TPCK treated *Giardia duodenalis* trophozoites to modulate intestinal epithelial homeostasis

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BACKGROUND. Giardiasis is an intestinal parasitic disease widely distributed in the world caused by *Giardia duodenalis*. The parasite factors that may cause damage in the intestinal epithelial cells have not been fully elucidated. Recently, we have observed that trophozoites treated with TPCK express a cysteine protease B like and induce damage in epithelial cell lines. Thus to study their effect on intestinal homeostasis an experimental model of intestinal loop ligation in gerbils was performed and molecules involved in the signaling pathways controlling proliferation, differentiation or apoptosis were analyzed.
METHODS. Gerbils were used as experimental model to perform duodenal loop ligation by surgery under anesthesia. Then loops were inoculated with 40 x 10^6 TPCK treated trophozoites, animals were sacrificed under anesthesia and duodenal loops were removed at different times post infection (30 min, 1 and 3 hrs). Representative intestinal tissue was used to obtain histological sections for H&E staining and for immunofluorescence. Likewise intestinal tissue was processed to determine by Western blot signaling pathways proteins involved in the above mentioned processes and temsirolimus treatment was used in this model to analyze the role of mTORC1 signaling pathway in the regulation of intestinal homeostasis.
RESULTS. H&E staining of histological sections showed at 1h pi parasites attached to intestinal epithelium, increase in goblet cells number, and light edema in the sub-mucosa and inflammatory infiltrate in the lamina propria. At 3h pi an increase in sub-mucosal edema, in the inflammatory infiltrate on the lamina propria and in the production of mucus on epithelial cells was detected. Moreover, western blot assays demonstrated changes in the expression of mTOR pathway proteins, including Raptor, Rictor and phospho-mTOR (serine 2481). At 30 min a decrease of these proteins was observed. At 1hr pi a slightly increased was detected while a significant increase of these proteins was observed at 3 hr pi. A significant reduction on the expression of some of these proteins, particularly, Raptor and p- mTOR (serine 2481) was observed in animals treated at the loop with temsirolimus (mTORC1 inhibitor). As expected immunofluorescence assays showed changes in the distribution and expression of the cell junction proteins b catenin and E-cadherin, and also in the proliferation marker phospho-histone serine 10 and in the mucus component muc-2.
CONCLUSIONS. TPCK treated trophozoites induce changes in the gerbil intestinal homeostasis which influence the expression of proteins of the mTOR signaling pathway a regulatory mechanism that could be important in the maintenance of intestinal epithelial cell homeostasis. Project supported by SEP-Conacyt grant No.128426.

Effect of *Leishmania mexicana* amastigotes of strains isolated from patients with localized and diffuse cutaneous leishmaniasis in the regulation of L-arginine metabolism in classically and alternatively activated macrophages

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BACKGROUND: Macrophages are decisive effector cells that either kill or host intracellular amastigotes of *Leishmania* depending on the balance of two inducible enzymes: nitric oxide synthase 2 (iNOS) and arginase 1 (Arg-1), that share L-arginine as substrate. Classically activated macrophages (CAMφ) express iNOS, which metabolizes L-arginine into nitric oxide (NO), a compound with great leishmaniacidal activity. On the other hand, alternatively activated macrophages (AAMφ) express Arg-1, which metabolizes L-arginine into L-ornithine, being the last the main source for the synthesis of polyamines that allow *Leishmania* intracellular development. In Mexico, infection with *L. mexicana* can cause two distinct clinical forms of cutaneous leishmaniasis: localized cutaneous leishmaniasis (LCL) and diffuse cutaneous leishmaniasis (DCL). In the present work, we analyzed the ability of *L. mexicana* amastigotes of strains isolated from Mexican patients with LCL (LCL-Am) and DCL (DCL-Am) in the regulation of L-arginine metabolism through iNOS and Arg-1 in CAMφ and AAMφ.
METHODS: Murine bone marrow macrophages were differentiated *in vitro* with M-CSF, classically or alternatively activated with TNF-α+IFN-γ or IL-4, respectively, and infected with LCL-Am and DCL-Am. Enzymatic activities of iNOS and Arg-1 were determined using the Griess and Archibald reactions, changes in protein synthesis of both enzymes were analyzed by Western blot, and parasite burdens were evaluated by counting the number of intracellular amastigotes released after macrophages lysis.
RESULTS: In AAMφ, infection with DCL-Am resulted in a higher activity of Arg-1 than infection with LCL-Am, whereas infection of CAMφ with both types of amastigotes led to similar iNOS activity. No changes were detected in protein synthesis for Arg-1 in AAMφ and iNOS in CAMφ after infection with both types of amastigotes. Regarding to parasite burdens, infection of AAMφ with DCL-Am resulted in a higher parasite load than infection with LCL-Am, while CAMφ were able to eliminate both types of amastigotes.
CONCLUSIONS: Our results suggest that LCL-Am and DCL-Am differentially regulate L-arginine metabolism through Arg-1 and iNOS in AAMφ and CAMφ as a survival strategy.
AKNOWLEGDMENTS: This work was supported by grant IN217913 from DGAPA, UNAM.

Antileishmanial effect of N^6-(ferrocenmetil)quinazolin-2,4,6-triamina (H2) against intracellular amastigotes of different *Leishmania* species *in vitro*

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BACKGROUND: For new drugs in the treatment of parasitic diseases the enzymes DHFR and PTR have been proposed as therapeutic targets. *In silico* we have developed conjugates with two structures with inhibitory activity of these enzymes, ferrocene and quinazoline. Based on this prediction the synthesized N^6-(ferrocenmetil)quinazolin-2,4,6-triamina (H2) compound showed anti parasitic activity for *Leishmania mexicana in vitro*. In order to extend its potential to other species, we tested their activity against *L. chagasi*, *L. V. panamensis*, and *L. V. guyanensis*.
METHODS: Conjugate H2 was synthesized and mixed with ciclodextrine (H2CD) and 15, 7.5, 3.75, 1.87 and 0.937 µg/mL were added to human macrophages - U937 ($4x10^4$/well) previously infected with promastigotes transfected with the luciferase gene at a 10:1 parasites:macrophage ratio. Infected cells and H2CD were incubated during 48 h at 34°C in RPMI with 10% HI-FCS. Parasite viability was measured by luciferase activity and expressed as relative light units (RLU). Macrophage viability was determined based on acid phosphatase activity and evaluated at 405 nm using an ELISA reader (MRX Revelation, Dynex Technologies).
RESULTS: H2CD shows a high antileishmanial activity for intracellular amastigotes at 15 µg/mL (1.42 µg H2) for *L. chagasi, L. V. panamensis* and *L. V. guyanensis*. No toxicity of H2CD at 15 µg/mL was found for the U937 macrophage line.
CONCLUSIONS: H2CD has potent effect in eliminating intracellular amastigotes of *L. chagasi*, *L. V. panamensis*, and *L. V. guyanensis* with no toxicity for the macrophage line U937. The low concentration employed of H2 suggests that it could be a promising compound meriting preclinical evaluation.

Spatial analysis of schistosomiasis mansoni cases in an peri-urban area of the city of Aracaju, State of Sergipe, Brazil

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BACKGROUND : Schistosomiasis mansoni is a serious parasitic disease, waterborne and chronic evolution, whose etiologic agent is the *Schistosoma mansoni*. This is one of the most prevalent parasitic disease in the world. In the State of Sergipe , the disease has expanded from rural to peri-urban areas , with the causal factors of this process of expansion and urbanization from this disease still unclear , thus demonstrating a potential public health problem. The present study aimed spatialize the occurrence of schistosomiasis human cases of in peri- urban area of the municipality of Aracaju/Sergipe, in the year 2011.
METHODS: This is an epidemiological and cross-sectional study. The research was conducted through census survey by parasitological Kato - Katz method and analysis of georeferenced of schistosomiasis human cases. The human cases were diagnosed and recorded residential block Spatial analysis of the distribution of infection was carried through the *GPS TrackMaker* and terraView 4.1.0 programs using intensity Kernel estimator.
RESULTS: 444 cases of schistosomiasis were identified; the prevalence of infection was 5.4 %; mild infection corresponded to 72.7 % according to parasite load (OPG); males accounted for 63.7 % of the infected subjects. The largest deletions eggs schistosomiasis occurred among adolescents and young adults aged 10-39 years. Spatial analysis of cases of schistosomiasis indicates the existence of three large clusters of infection in the neighborhood under study and visualization of areas of highest concentration of cases exposed to different degrees of ris. Clusters of higher intensity were represented by darker shades.
CONCLUSIONS: The results of the survey allow offering health services a methodological tool to facilitate the understanding of the occurrence and spatial distribution of schistosomiasis. It is necessary to a reorganization of the preventive measures for the realization of this endemic disease control in the community analysis.

Characterization of two putative TATA binding proteins in *Trichomonas vaginalis.*

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BACKGROUND: The protozoan parasite *Trichomonas vaginalis* is a common human pathogen, one of the earliest-diverging eukaryotic lineages. A classic TATA box is not found in *T. vaginalis* genes, and only the highly conserved Inr element surrounds the transcription start site, and is recognized by the Inr Binding Protein (IBP39). This element is essential for transcription and directs the selection of transcription start sites. Interestingly, BLAST analyses of the *T. vaginalis* genome have shown the presence of two putative TATA binding proteins (TvTBPs). The aim of the present work is to determine if these proteins have a similar function as a canonical TBP.
METHODS: Gene expression of the TvTBP genes was evaluated by reverse transcriptase PCR (RT-PCR). We used a yeast complementation assay to determine if TvTBP1 and TvTBP2 have a similar function as yeast TBP. The ability of TvTBPs recombinant proteins to bind DNA was tested by EMSA assays with probes corresponding to DNA promoters of the three RNA polymerases in *T. vaginalis,* and a TATA-containing yeast promoter probe. Pull down assays were carried out to test protein-protein interactions with other *T. vaginalis* putative transcriptional factors, such as TvTFIIB and TvBRF1.
RESULTS: Both TvTBPs genes are expressed in *T. vaginalis*. None of the TvTBPs were able to complement a TBP mutation in yeast, or were able to bind to the DNA tested en EMSA assays. However, TvTBP1 binds both putative transcriptional factors TvTFIIB and TvBRF1, whereas TvTBP2 binds only to TvTFIIB.
CONCLUSIONS: Our results suggest that *T. vaginalis* express two genes with different characteristics to a canonical TBP. We propose that these TvTBPs might play a role in transcription in a similar way to a canonical TBP in TATA-less promoters, without binding DNA.

Evaluation of two genes of *Onchocerca volvulus* involved in resistance to Ivermectin

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BACKGROUND: Onchocerciasis is caused by the nematode *Onchocerca volvulus*, which is transmitted to human beings by black flies of the *Simulium* genus. Onchocerciasis is the second cause of blindness over the world. In Mexico the onchocerciasis is a public health concern. In order to control and eliminate this disease, ivermectin has been given to the whole population at risk twice a year since 1991. It has been reported that due to mutations in the phosphoglycoprotein and in β-tubulin genes, *O. volvulus* has developed resistance to ivermectin. In this study we cloned and sequenced genes that encoding a phosphoglycoprotein and β-tubulin from adult worms obtained from onchocercomata nodules excised over several years.
METHODS: We amplified β-tubulin gene of 79 samples and obtained 50 sequences. We amplified 7/60 samples of the phosphoglycoprotein and six sequences were obtained. All the sequences were analyzed and aligned with the sequences reported in the GenBank.
RESULTS: It was found that the sequences of the β-tubulin from the Mexican samples were grouped together with the allele B sequences (of resistance to ivermectin) as has been reported for other parasites. The phosphoglycoprotein of the Mexican samples did not exhibit homology with the sequences reported in the GenBank for this protein, neither with the sequences of 4 phosphoglycoprotein reported for *Caenorhabditis. elegans.*
CONCLUSIONS: We can conclude that the *Onchocerca volvulus* samples studied in the present work have selected resistance to ivermectin after of almost 8 years under selective pressure. These results indicate that the elimination of the onchocerciasis in Mexico and in other endemic countries of the world will not happen in the short term have to expect a long time.

Rab21, the endosomal traffic of the β1*Eh*FNR, and their participation in amoebic pathogenesis.

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BACKGROUND: The *E. histolytica* genome encodes as much as 91 Rab family G proteins, suggesting an unusually high degree of complexity underlying vesicular trafficking regulation. Many integrins are constantly endocytosed and recycled to the plasma membrane, facilitating targeting of these adhesion receptors during cell adhesion and migration. Integrin trafficking requires the spatially regulated activity of a number of kinases in conjuction with Rab-dependent endosomal compartments. The ubiquitously expressed, small GTPase Rab21 has recently been shown to associate with the α tails of several integrins via the shared conserved membrane proximal sequence, thus regulating cell adhesion and migration via controlling the endo/exocytic trafficking of most integrin heterodimers. The aim of this project is to determine the role of Rab21 during the mobilization of the β1*Eh*FNR-(the 140 kDa integrin-like molecule)- both *in vitro* and *in vivo* conditions.
METHODS: The gene that codifies for Rab21 was cloned and the recombinant protein expressed and purified. Then, polyclonal monospecific antibodies were produced in mice and used to localize Rab 21, together with the β1*Eh*FNR by the use of a MAb (3C10), and some organelles such as lysosomes; these analyses were done by confocal microscopy, in trophozoites cultured *in vitro* as well as in trophozoites present in amoebic colitis tissue sections by immunohistofluorescence, from samples of patients with fulminant amoebic colitis (FAC). Furthermore western blot assays were performed in extracts of *E. histolytica* with different degrees of virulence.
RESULTS: The amoebic integrin-like receptor is translocated in association with Rab21. In addition the patients with FAC contain trophozoites with several patterns of expression; Rab21 is associated with β1*Eh*FNR but not with the 220 kDa lectin, (L220), an antigen not related with a mobilization process.
CONCLUSION: These data provide information into how integrin-like molecules are targeted to intracellular compartments both *in vitro* and *in vivo,* therefore establishing that Rab-regulated vesicular trafficking is important for *E. histolytica* biology and pathogenesis.

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Spatial patterns of schistosomiasis-related mortality in Brazil, 2000-2011

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BACKGROUND: Schistosomiasis is a parasitic disease determined in space and time by environmental and behavioral factors in residents of endemic areas. In this study, we analyzed the spatial patterns of schistosomiasis-related mortality in Brazil.
METHODS: We performed an ecological spatial clusters analysis, based on secondary mortality data. We included all deaths in Brazil from 2000 to 2011, in which schistosomiasis (ICD-10: B65) was mentioned on the death certificate, either as underlying or as associated cause of death. We calculated average annual mortality rates (per 100,000 inhabitants) for each municipality of residence in four-year intervals and the entire period. Local Empirical Bayesian method was used to minimize random variation in mortality rates because of the population size in the municipalities. To evaluate the existence of spatial autocorrelation, global and local Moran's I indices were used.
RESULTS: During the study period, 12,491,280 deaths were recorded. Schistosomiasis was identified in 8756 death certificates. The nationwide average crude mortality rate was 0.39 deaths/100,000 inhabitants/year. In the period, 22% (1,225/5,565) of Brazilian municipalities in 24 of the 27 states registered at least one schitosomiasis-related death. Average annual mortality rates reached a maximum of 20.95 deaths/100,000 inhabitants. Independently from the statistical cluster analysis, was identified high risk clusters of schistosomiasis-related mortality encompassing a geographic range in the east coast of the Northeast region, extending from the east areas of the Rio Grande do Norte state to south of the Bahia state and north of the Minas Gerais state.
CONCLUSIONS: In this nationwide population-based study, we identified spatial clusters of municipalities with high mortality rates, located mainly in schistosomiasis-endemic areas. The disease control programs still need to sustain increase coverage, intensify and focus measures, not only to reduce transmission, but also to prevent the occurrence of severe forms and deaths from the disease.

Enviromental and Immunogenetic Risk Factors for Ocular Toxoplasmosis

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BACKGROUND: A low percentage of individuals infected by *Toxoplasma gondii* develop ocular toxoplasmosis (OT) and the reasons underlying this susceptibility remain unclear. This study investigated environmental and immunogenetic factors as potential predictor for OT.
METHODS: Consecutive patients were recruited over a period of four year. OT was confirmed by indirect binocular ophthalmoscope analysis and *T. gondii* serology (IgG) was assessed by ELISA. Epidemiological data recorded in a questionnaire. HLA-DRB1 alleles and Le^a carbohydrate were determined by PCR-SSO and PCR-RFLP, respectively. Patients with and without OT both with positive serology were compared. The data were analyzed chi-square. Odds Ratio and confidence interval at 95% values were calculated ($p<0.05$).
RESULTS. Contact with cats and raw or undercooked meat consumption were not associated with the development of OT. Age (OT: 48·2±21·2 years vs. non-OT: 69.5±14.7 years, $p<0.0001$) and the low level of schooling/literacy (OT vs. non-OT: OR: 0·414, CI 95%: 0.2231–0.7692, $p=0.007$) were associated with OT. Le^a carbohydrate (OR: 1.937, CI 95%: 1.148 – 3.268, $p=0.015$) was associated with OT and the HLA-DRB1*01 allele was strongly associated with OT relapse (OR: 9.831; CI 95%: 2.541 - 40.06, $p=0.0006$).
CONCLUSIONS. Cats as well as raw or undercooked meat consumption are not environmental risk factor for the development of OT among infected individuals. Le^a carbohydrate and HLA-DRB1*01 allele are immunogenetic risk factors for the development and for relapse of OT, respectively.

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Natural infection of *Plasmodium* in anophelines in the State of Maranhão, Brazil*

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BACKGROUND: Malaria is an endemic disease, which generally follows the spatial distribution of Anopheles. In Brazil the anopheles fauna is quite diverse, so does the State of Maranhão due to phytogeographical position of the transition area. It has conducted studies in the reserve areas to identify the possible effect of the coexistence of vector-primate-human transmission of malaria. Entomological and natural infection studies were carried out using anophelines (Diptera: Culicidae) captured in the reserve areas to identify the possible effect of the coexistence of vector-primate-human transmission of malaria.
METHODS: The study was approved by ICMBIo (No. 34282-2). The anopheline captures were performed in the area of the Maracanã Environmental Protection and Private Reserve Aguahy, both on the island of São Luis, Maranhão State, Brazil. Daily captures were made from January, June and July 2013 used CDC-light traps, Shannon and protected human bait were used for three consecutive hours, 18:00 to 21:00 to capture anophelines in the following habitats: near the houses, in open areas (at ground level) and inside, and at the margins of the forest (canopy and ground level). Mosquitoes were caught identified according to specific keys in the Laboratory of Entomology FUNASA/São Luis and stored in isopropyl alcohol in pool 10 specimens to perform molecular tests in the Laboratory of Immunoparasitology FCAV-Unesp, campus Jaboticabal. Genomic DNA from blood-fed mosquitoes was extracted using the QIAamp DNA Mini Kit and the protocol described by the manufacturer was followed. We utilized three conventional PCR protocols to develop a molecular consensus (positive results in, at least, two protocols) and protocol for real-time PCR
RESULTS: A total of 416 specimens within six species were captured, with Shannon and protected human bait: *Anopheles aquasalis, A. evansae, A. mediopunctatus, A. goeldii, A. nuneztovari* and *A. shannoni.* Only four pools were negatives real-time PCR: *A. mediopunctatus* (1) and *A. aquasalis* (3). The *P. malariae/brasilianum* infections detected by conventional PCR.
CONCLUSION: These results suggest that a possible interaction between human and simian malaria coming from a zoonotic cycle cannot be discarded because simians that live in the areas of the Amazon Forest could play a role as a reservoir for *Plasmodium*.

Synthesis and function of base J in trypanosomatids

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BACKGROUND: β-glucosylhydroxymethyluracil (base J) is a modified DNA base found only in Euglenozoa. Base J synthesis requires two enzymes (JBP1 and JBP2) that catalyze hydroxylation of thymine, as well as a glucosyltransferase that modifies the resultant HOMeU. Knockout of *JBP2* in *Leishmania tarentolae* caused gradual loss of J, transcriptional read-through at convergent strand-switch regions (cSSRs) that contain internal J (iJ), as well as false-starts at transcription initiation sites. Bromodeoxyuridine (BrdU) treatment of *JBP2*KO parasites caused further reduction in iJ levels, and eventual death of the cells.
METHODS: We used RNA-seq and Nanostring technology to quantify mRNA levels in *L. tarentolae* cells containing different levels of J. To investigate the signals responsible for J insertion, we cloned several different sequences into plasmids, and sequenced the DNA using Single Molecule Real Time (SMRT) sequencing to reveal the exact location of J nucleotides after episomal replication in *L. tarentolae*.
RESULTS: Transcriptome analyses of *JPB2*KO and BrdU-treated cells revealed consistent and substantial (>4-fold changes) in mRNA expression of several genes located near strand-switch regions (SSRs). Plasmids containing the telomeric hexamer repeat, and two different convergent SSRs (which contain J in WT genomic DNA) accumulated J when grown in WT cells, but not *JBP2*KO; while plasmids containing an atypical cSSR (which lacks J in WT cells) accumulated no detectable J. SMRT sequencing of the J-containing plasmids revealed that most Js occurred as pairs on opposite DNA strands separated by 12 nucleotides.
Conclusions: We hypothesize that JBP2 recognizes the signal for *de novo* J insertion, while JBP1 is responsible for J maintenance (following DNA replication) by recognizing this J and inserting a new J downstream on the opposite strand. Loss of J induces de-repression of genes deleterious to cell growth, and/or down-regulation of essential genes (due to accumulation of antisense RNA at cSSRs).

Low plasma level of IgG antibodies anti-*Toxoplasma gondii* correlates positive PCR in blood donors

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BACKGROUND**:** The infection by *Toxoplasma gondii* is food and waterborne spread but blood transfusion and organ transplantation constitute important forms for its transmission. This study aimed to investigate the prevalence of *T. gondii* infection in blood donors.
METHODS: 303 voluntary blood donors from Sao Jose do Rio Preto, Sao Paulo, Brazil were enrolled. IgG anti-*T. gondii* antibodies were assessed by ELISA. ELFA was used to confirm IgM antibodies. PCR was performed by amplification of a fragment carrying 288 base pairs with the primers JW62 and JW63 followed by a re-amplification of a fragment carrying 115 base pairs with the primers B22 and B23 of the B1 gene.
RESULTS**:** From the overall, 90 were female (mean age: 33.6±11.0 years) and 222 were male (mean age: 35.7±10.5) ($p>0.05$). 159 (52.5%) blood donors presented positive serology [IgM-IgG+: 153, 96.2%; IgM+/IgG+: 6, 3.8%] and 144 (47.5%)], negative serology. Blood donors with PCR positive and IgM-IgG+ serology (n=13, 8.2%) presented low level of IgG antibodies anti-*T. gondii* (204.3±46.4 IU) in comparison with blood donors with PCR negative IgM+IgG+ (n=6, 239.8±8.6)($p=0.0002$) but not with blood donors with PCR negative IgM-IgG+ (n=140, 214.1±29.1) ($p=0.467$).
CONCLUSIONS. The low plasma level of IgG antibodies anti-*T. gondii* correlates positive PCR in blood donors and could act as facilitator for parasitemia.

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Induction of apoptosis by Ethyl-4-bromofenilcarbamate and Ethyl-4-chlorophenylcarbamate in ovary cells of *Rhipicephalus microplus.*

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BACKGROUND: The new synthetic carbamates, ethyl-4-bromophenyl-carbamate and ethyl-4-chlorophenyl-carbamate, negatively affect biological parameters and reproduction of the cattle tick *R. microplus*. Microscopically, carbamates induced picnosis, degeneration and vacuolization of the tick ovary cells. No studies have described the action mechanism of these carbamates. The aim of this study was to study apoptosis induction of the new carbamates in ovary cells of *R. microplus*.
METHODS: One-hundred fifty engorged female ticks of *R. microplus* were used. Ticks were treated by the Adult Immersion Test with 1mg/ml of ethyl-4-bromophenyl-carbamate (*n*=50), 1mg/ml of ethyl-4-chlorophenyl-carbamate (*n*=50) or were non-treated (*n*=50). Ten ticks from each treated group were dissected on days 0, 1, 3, 5 and 7 post-treatment (p.t.). Ovaries were processed for conventional histology and a TUNEL assay kit was used for determination of apoptotic bodies per mm^2.
RESULTS: The number of apoptotic bodies was higher from day 3 p.t. in ovaries of ticks treated with ethyl-4-bromophenyl-carbamate and from day 7 p.t. in ovaries of ticks treated with both carbamates than the respective control group ($p<0.05$). Also, treated ticks oocytes had degeneration of the germinal vesicle, nucleolar fragmentation, vacuolization, alterations in the cell membrane and reduction of chorion deposition.
CONCLUSIONS: The results of this study show that one of the main effects of the carbamates is apoptosis induction in ovary cells. The aforementioned can explain the inhibition of oocyte maturation and the reduction in the quantity and viability of the eggs produced by carbamates in *R. microplus*.

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Standardization of ELISA tests for the detection of antigens and antibodies anti-*Toxocara canis* and cross reactions with others nematodes, in cattle from a slaughterhouse in Puebla, Mexico

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BACKGROUND: *Toxocara canis* produces the most frequent helminthiasis in dogs worldwide. In paratenics hosts, provokes larva *migrans*. Reports in humans associate the ingestion of raw or undercooked infected meat or liver from paratenic hosts with larva *migrans* by *T. canis*. The aim of this study was to evaluate the frequency of excretory-secretory-antigens of *T. canis* larvae (ESTcl) and anti-*T. canis* antibodies (anti-TcAb) in naturally infected cattle from Mexico and detect cross reactions with other nematodes.
METHODS: Standardizations: 1) ELISA-IgG: ESTcl (0.5-2.0µg/mL), serum from an inoculated cow with Tcl/ESTcl (1:16-1:64), protein-A-HRP (1:500-1:32000). 2) ELISA-ESTcl: MoAb (64-356µg/mL); ESTcl (0.01-10µg/mL). Serum samples were collected from 127 cows at a slaughterhouse in Puebla, Mexico. Detection of IgG anti-Tc and ESTcl were performed. Additional ELISA-IgG tests were carried out using 1.0µg/mL of non-purified helminth antigens (*T. canis*, *T. cati*, *Ascaris suum*, *Trichinella spiralis*, *Taenia* spp., *Ancylostoma caninum*, *Dipylidium caninum*) and an ELISA-IgG were done using cow serum pre-absorbed with helminths extracts (1.0µg/mL).
RESULTS: 1) ELISA-IgG: ESTcl 1.0µg/mL, protein-A-HRP 1:500, serum 1:16. 2) ELISA-ESTcl: MoAb 356µg/mL. Anti-TcAb were detected in 20.47%(26/127) and ESTcl were found in 12.59%(16/127) of the serum samples. Cross-reactions were found in positives serums. Nevertheless, in pre-absorbed *T. canis* positive cow sera, were found: anti-TcAb in 32.43%(12/37) which suggests chronic infection; anti-TcAb/ESTcl in 13.57%(10/37) probably for an active infection; ESTcl in 16.21%(6/37) suggestive of acute infection and in 24.32%(9/37) weren´t found either ESTcl or anti-TcAb.
CONCLUSIONS: ELISA tests were standardized. ESTcl and anti-TcAb were detected in cattle sera. Therefore, cattle could be a source of infection for human and dogs. Cross-reactions were found; leading to the conclusion that non-purified antigens of helminths share epitopes, but still are useful for a sensitive diagnostic test and its specificity can be increased if serum is pre-absorbed with other helminth antigens.

Prevalence of bovine hydatidosis diagnosed by macroscopic lesions in municipal slaughterhouse Fresnillo, Zacatecas, Mexico, 2000 - 2007

Díaz-García, Luis-Humberto, Mata-Abrego, Leonardo, , López-Huitrado, Lilia-Patricia., Bonilla-Cid, Alfredo, Muro-Reyes, Alberto, Perea-Lugo, Adriana-Lucía., Herrera-Alonso, María-de-Lourdes y Sataray-Cabral, Ernesto.*

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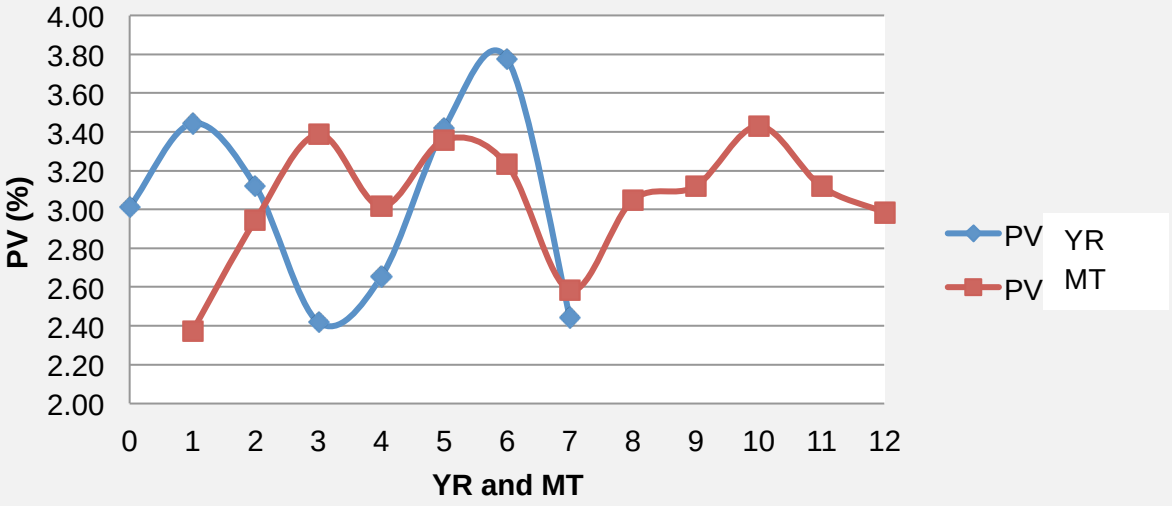
Epidemic status on bovine hydatidosis in Mexico is inconclusive and limited to occasional reporting findings in humans or frequencies reported at slaughtered animals; additionally, few cases and inadequate knowledge of the disease have been made this disease not to be considered for epidemiological surveillance programs, which further hinders the ability to clarify the current status (Rodríguez-Prado, 2011). The objective of this research was to estimate the bovine hydatidosis prevalence (PV) diagnosed during the 2000-2007 period at the municipal slaughterhouse of Fresnillo, Zacatecas, Mexico. Data used for this study were collected from the officially reported cases; which were processed in a Microsoft Excel sheet to obtain and compare PV by years (YR) and months (MT) of time. These data were analyzed with a GLM procedure of SAS using Tukey as a power test and Pearson correlation coefficient to determine differences among PV YR and PV MT and its relationship; difference (p=0.001) between 2006 (3,78 %) was observed compared with 2003, 2004 and 2007 (2,42, 2,65 and 2,44 %, respectively). The present epidemiologic information will be used to asses and actualize the status of bovine hydatidosis.

Prevalence (PV) by year (YR) and month (MT) of bovine hydatidosis in the Municipal Slaughterhouse Fresnillo, Zacatecas during the period 2000-2007



Metabolic and parasitic comparison of two sheep breeds and their crosses of livestock company zacatecas

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When it is established an animal breeding program, parasite resistance is most useful to incorporate as the effect will be to reduce parasite numbers, arising animal performance and decreasing parasite pasture load (Gray *et al*., 1987; Gasbarre *et al.,* 2001). The aim of this study was to compare serum metabolite levels on two breeds and cross breed parasite sheep on a feedlot at the town of Jerez, Zacatecas. 42 lambs (males) were used with an average age of 170 ± 10 days, which were divided by race as follows: DPR: 15 Dorper lambs, KTN: 12 Katahdin lambs and CRZ: 15 crossbred lambs. We took two samples (blood and dung) with an interval of 28 days (0 and 28) to determine glucose (GL), triglycerides (TG), Urea (UR) and Total Protein (TP) serum concentrations; nematode eggs (NM) and *Eimeria* oocysts (EM) per gram of feces (epg) were determined. Repeated measures design using time and breed effect were computed; data were analyzed using GLM and Tukey test as a power test to asses differences on main effects. CRZ data reported higher ($p < 0.05$) GL and TG than in the DPR; however, no significant differences on KTN were detected. Also, CRZ was different ($p < 0.05$) in the NM and EM obtained the lower levels, then KTN and finally DPR. It was concluded that cross of Dorper and Katahdin breeds may be a good alternative in commercial enterprises for supply having better metabolism and more parasite resistance

.Table 1. Average metabolites and parasite load (±SD) of each of the breeds evaluated in the two samples.

VARIABLE	TREATMENTS (BREED)			p Value
	DPR	KTN	CRZ	
GL	67.46±26.45^{b}	75.42±21.87^{ab}	85.28±30.56^{a}	0.0216
TG	34.56±20.89^{b}	43.56±17.97^{ab}	50.55±19.87^{a}	0.0115
UR	43.50±17.40	41.56±19.96	42.57±13.80	0.9737
TP	7.08±1.94	7.75±2.40	7.67±1.96	0.3286
NM*	3.36±0.24^{a}	3.21±0.38^{ab}	3.06±0.47^{b}	0.0154
EM*	2.84±0.61^{ab}	2.97±0.43^{a}	2.63±0.41^{b}	0.0429

DPR: Dorper; KTN: Katahdin; CRZ: Cross Breed; GL: Glucose; TG: Triglycerides; UR: Urea; TP: Total Protein; NM: Nematode; EM: *Eimeria*.
^{abc} literals with different superscripts in same line denote significant difference ($p<0.05$).
* Conversion Log10.

In vitro acaricidal effect of entomophatogenic fungi isolated from soils of cattle farms against _Rhipicephalus microplus_ (Acari: Ixodidade)

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BACKGROUND: Biological control of insect pests (causing epidemics) by microorganisms that live in habitats is one of the most important factors of balance in natural ecosystems. Within these bio-regulators microorganisms there are nematodes, protozoa, viruses, bacteria, parasites and fungi. Entomophatogenic fungi are one of the main agents used for biological control of ticks. The objective of this study was to evaluate the efficacy of 10 entomophatogenic fungi strains isolated from soils of cattle farms against larvae and engorged females of *Rhipicephalus microplus* ticks.
METHODS: Ten entomophatogenic fungi strains were isolated from 10 cattle farms distributed in five municipalities from Veracruz, Mexico. These strains were isolated by the bait method using *Galleria mellonella* larvae. Each fungi strain was identified and characterized using taxonomic keys and reproduced in media with Potato dextrose agar. Then, the acaricidal effect of fungi was evaluated against larvae and adult stage of *Rhipicephalus microplus*. Ticks were obtained from a farm in the region identified as multi-resistant to chemicals acaricides. The evaluation was conducted by dipping for one minute ticks in a conidia suspension at a dose of $1x10^8$ conidia per ml. Ticks were incubated under suitable temperature and humidity and assessed daily for 20 days to observe the acaricidal effect of fungi (measure as ticks mortality and reproductive efficiency). For each strain evaluated, the treatment effect on mortality of larvae or adults was assessed using the U Mann Whitney test. The effect of treatment on oviposition and larval hatching was evaluated by ANOVA test.
RESULTS: Three fungi strains (MaV12, MaV08, MaV04) have shown acaricidal effect on female engorged mortality compared with control group ($P<0.05$). The same strains have a good effect on oviposition ($P<0.001$). No effect was observed on egg hatching ($P>0.05$). Mav12 and MaV08 strains have acaricidal effect against larvae stage ($P<0.05$).
CONCLUSIONS: Two entomopathogenic fungus strains were identified as potential biocontrol agents against larvae and adults of *Rhipicephalus microplus* multiresistant to chemical acaricides. One strain (MaV04) has only shown acaricidal effect on adult ticks.

Risk factors associated to Chagas disease in patients treated in a tertiary school hospital, Brazil

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BACKGROUND: Chagas disease is caused by the protozoan *Trypanosoma cruzi,* which is transmitted to humans commonly in the faces of a hemipterous popularly known as barber. Here we report the three clinical forms of Chagas disease in Brazilian patients and the related risk factors.

METHODS: Epidemiological data from 302 patients attending a tertiary school hospital in NW region of São Paulo state, Brazil, were compared according to the clinical forms of Chagas disease: cardiac, megaesophagus, megacolon. Twenty eight potential risk factors were compared among these groups using the Chi-square test and the exact Fisher's test ($p \leq 0,05$).

RESULTS: In the cardiac form 54% were female and 46% male (mean age 63.59 ± 10.63 years); in the megaesophagus form 46% were female and 53% male (mean age 64.79 ± 12.12 years); in the megacolon form 35% were female and 65% male (mean age 64.77 ± 10.42 years). Statistical analysis of the risk factors showed significant differences in relation to gender only for comparisons involving the cardiac and megacolon forms ($p = 0.0253$); blood transfusion ($p = 0.0242$); lived in ($p = 0.0409$) and/or still live in rural areas ($p = 0.0449$); in the house of clay or wattle and daub ($p = 0.0386$).

CONCLUSIONS: Four risk factors were predominant among the analyzed groups being megacolon in males, in patients who underwent blood transfusion, who have lived or still live in rural residence in houses of clay or wattle and daub.

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Variable expression of gp82 and gp90 proteins in *T. cruzi* isolates involved in outbreaks of acute Chagas in Santander, Colombia.

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BACKGROUND: In recent years, in Santander (Colombia) there have been presented acute Chagas disease outbreaks. Epidemiological studies indicate that these outbreaks could be caused by oral transmission. Some studies have shown invasive ability of metacyclic trypomastigotes (TM) in the gastric mucosa cells associated with the expression of surface proteins such as gp82 and gp90. Therefore, the objective of this study was to evaluate the expression of surface molecules associated with the ability of invasion by oral route in TM isolated from patients involved in outbreaks of acute Chagas disease.
METHODS: 12 clones of *T. cruzi* isolates obtained from patients involved in outbreaks of acute Chagas presented in the department of Santander were used. Metacyclic trypomastigotes were obtained by incubation of epimastigotes in TAU3AAG medium. The expression of surface proteins gp82 and gp90 was determined by Western blot assays using the monoclonal antibodies 3F6 and 1G7 respectively.
RESULTS AND DISCUSSION: In the present study, it was found that all of the clones analyzed expressed both gp82 as gp90, however, variable expression of both glycoproteins were observed between them. Oral transmission is an important factor for the emergence of disease outbreaks of acute Chagas which is characterized by high mortality and is associated with eating food contaminated with *T. cruzi*. This study demonstrates the presence of surface glycoproteins in isolates from outbreaks of acute Chagas that have been identified as essential to produce orally invasion.

Analysis of a VDAC-like protein of *Rhipicephalus microplus* midgut cells in response to infection by *Babesia bigemina*

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BACKGROUND: Babesiosis is an infectious disease caused by parasites of the genus *Babesia*, which are transmitted by ticks to their animal and human hosts. *Babesia bigemina* is one of the causative agents of bovine babesiosis. This disease has a very important economic impact in the livestock industry, due to significant losses in the production of milk and meat. Nowadays the development of new alternatives on the control of the disease is based on the study of the tick-*Babesia* interface as part of the efforts to determine the interactions and molecular mechanisms that occur so that the infection can take place. The parasites of the genus *Babesia* infect several organs into the vector, in which the following organs are found: the Malpighi tubules, salivary glandules, ovaries and gut. Interestingly, the tick midgut is the first organ that the parasite has to go through to get transmitted and ensure its survival. We previously, through proteomic tools, identified and reported the VDAC-like protein of the gut of the tick that interacts specifically with sexual stages of *Babesia bigemina*. The objective of this study was to identify the sequence of the *Rmvdac* gene and to determine the changes in the expression of both the gene and the VDAC protein in the process of infection by *Babesia*.
RESULTS: This is the first report of the study of *R. microplus* VDAC protein in response to the infection by *Babesia*. RESULTS: In this research we got the partial sequence of the *Rmvdac* gene that was reported in the GenBank with the access number GU994210.1. Our results show that the level of expression of the gene increases as well as the one of the protein in the gut of the infected ticks over the non-infected ones; especially at 24 hours post-repletion. These results suggest that the VDAC protein can be important in the process of invasion by *Babesia* to the gut of the vector; although it is important to carry out future *in vivo* studies into the tick to determine this.
CONCLUSIONS: The identification of the proteins expressed in the *Rhipicephalus microplus* midgut cells in response to the invasion by *Babesia* provides information about the molecular mechanisms and will allow the identification of potential targets to development of anti-tick vaccines, or transmission blocking.

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Seroprevalence of *Neospora caninum* in dual purpose cattle

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BACKGROUND: Neosporosis is a parasitic disease that causes large economic sector worldwide livestock losses, its agent *Neospora caninum* is a protozoan that has become important to be involved as one of the main pathogens causing abortion in cattle. The aim of this study was to determine the seroprevalence of *Neospora caninum* in dual purpose cattle, from Culiacán and Navolato municipalities.
METHODS: Serun samples from 226 cattle and females regardless of history of abortion were collected from the jugular vein or tail vein needle with vacutainer glass vacuum tubes without anticoagulant. The samples were transported in a cooler to the Laboratory of Parasitology, Faculty of Veterinary Medicine of the Autonomous University of Sinaloa for analysis. Subsequently the blood was centrifuged at 3000 RPM for 15 minutes to extract at least 1 mL of serum, which was stored same eppendorf tubes and frozen at -20 ° C until analysis by indirect ELISA.
RESULTS: The overall seroprevalence of anti-*Neospora caninum* antibodies was 20.79 %. In the analysis results by municipality was observed 22.72 and 19.56 % respectively in Culiacán and Navolato, found no statistically significant difference ($P \geq 0.23$).
CONCLUSIONS: This study reveals the presence of serological Neospora in these regions; therefore studies have to continue to know more about its epidemiology.

The prevalence of *Demodex folliculorum* and *Demodex brevis* in Antofagasta, Chile

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INTRODUCTION**:** In human skin is possible to find a variety of microorganisms. Among them must be include the microscopic mites of the genus *Demodex*. There are two species that affect humans: *Demodex folliculorum* and *Demodex brevis*. These mites, can be found in hair follicles and sebaceous glands of the skin, especially on the face. In this study, the presence of *Demodex sp.* and their relationship with age, gender, work activity and allergic diseases was investigated.
MATERIAL AND METHODS: A total of 400 subjects were examined by using the standardized surface skin biopsy. The study group was composed of people whose age ranges were between 18 and 83 years.
RESULTS: From the total of individuals examined, 24.0% were infected with *Demodex sp*. A higher prevalence was observed in males (62.5 %), whereas women displayed a prevalence of 37.5 %. Similarly, a higher percentage of *Demodex sp.* infection was observed in patients of age groups from 48 to 87 years (34.6 %). Regarding the species found, the highest percentage corresponded to *D. folliculorum* (91.7 %) while *D. brevis* was found in 8.3% of cases. No relation was observed beetween presence of the mite and allergic disease.
CONCLUSIONS: Human infestation by *Demodex spp*., was frecuent in Antofagasta, where the parasite was more frecuently found in males. In the same way, *D. folliculorum* was the most prevalent specie.

Immunization with enolase of *Trypanosoma cruzi* confers protective immunity against acute phase of Chagas disease in mice.

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BACKGROUND: Chagas disease is a major public health burden in Latin America and a potentially serious emerging threat to a number of countries throughout the world. Currently, there is no vaccine. For this reason, the aim of this study was to determine the immune response generated by the immunization with the *T. cruzi* enolase during the Chagas disease in mice.
METHODS: The enolase gen was obtained from cDNA by RT-PCR, the PCR product was cloned into pCR™2.1-TOPO® Vector and afterwards was sub-cloned to obtain pRSETB::TcENO. The recombinant protein (rTcENO) was obtained by nickel affinity chromatography. BALB/c female mice were immunized with rTcENO. A second group received only PBS. The isotype of antibodies was determinated by ELISA. Mice were infected with 8 X 10^4 blood trypomastigotes; then were bled every three days and the parasitemia was determinate. The survival was monitored daily. Cytokines were analyzed by flow cytometry. Finally, hearts were isolated aseptically and fixed in paraformaldehyde. Fixed hearts were embedded in paraffin, sectioned (5μm), stained with hematoxylin & eosin, and examined by light microscopy.
RESULTS: Mice vaccinated with rTcENO were able to generate specific antibodies (IgG1, IgG2a and IgG2b) typical for Th1/Th2 immune response. Furthermore, the group vaccinated with rTcENO showed 75 percent of survival with respect to control group. The parasitemia burden was reduced in 69.8% in vaccinated mice with rTcENO. Meanwhile, the cytokines generated by immunization with rTcENO and after parasite challenge were IFN-gamma and IL-2, showed that a type Th1 immune response was polarized. Furthermore, hearts from vaccinated mice showed minimal inflammatory response compared with control mice (non- immunized and infected) that demonstrated abundant inflammatory cells and amastigotes nests.
CONCLUSION: The findings of this study indicate that immunization with rTcENO induces protection against Chagas disease. Therefore, rTcENO may be an excellent candidate for further vaccine development.

Community mobilization an essential component of a sustainable control program for *Taenia solium* taeniosis/cysticercosis

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BACKGROUND: WHO-endorsed recommendations for use by *Taenia solium* taeniosis/ cysticercosis control program managers in endemic countries include core “rapid impact” interventions aimed at treating human taeniasis and mass treatment and vaccination of pigs, as well as longer term “supporting measures” such as community education, improving pork hygiene and better pig management. Compliance of the affected communities is essential for this package of relatively simple interventions to be effective and sustainable. Models of mobilized communities are useful for planning large scale control activities for combating *T. solium* infections.
METHODS: Focus group discussions and individual interviews were conducted in a *T. solium*-free pig raising community in cisticercosis-endemic Tanzania to determine the likely reasons for the absence of the parasite. Additionally, key components of community-led initiatives for ending open human defecation in South Asia were examined to determine the most effective ways of igniting behavior change to achieve the desired health outcomes.
RESULTS: *T. solium*-free status of the Tanzanian village was maintained through strict implementation of community regulations on sanitation and pig keeping based on standard punishments and community awareness. Community-led total sanitation efforts have been successful in mobilizing whole communities to make a collective decision and take actions to stop the practice of open defecation as a result of participatory rural appraisal to help all community members recognize the links between open defecation and ill health, directing incentives to the community and rewarding outcomes, and developing local markets by enabling local suppliers to respond to demand.
CONCLUSIONS: Innovative ways of mobilizing communities will be helpful in ensuring the effectiveness and sustainability of control efforts for combating the burden of *T. solium* taeniosis and cysticercosis.

Factors associated with complicated malaria in Colombia

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BACKGROUND: Colombia is an area of low transmission for malaria but with conditions that favor its transmission, principally in the Pacific coast where a lot of cases of complicated malaria have been reported.
METHODS: A retrospective case-control study in a 1:2 proportion was conducted, in Tumaco, Cali and Buenaventura. The sample size was 60 cases and 120 controls, patients with positive thick blood smear between the period 2009 -2013 were included, controls were patients without severity criteria.
RESULTS: 88.3% (159) of patients originated from Tumaco, 5.0% (9) from Buenaventura, and 6.67% (12) from Cali. 50.0% (30) of the cases were female. In the controls 46.6% (56) were female; regarding the distribution of parasite specie, 43 cases corresponded to *P. falciparum*, 16 to *P. vivax*, and 1 was mixed. With respect to the controls, 85 were *P. falciparum*, 32 were *P. vivax* and 3 were mixed malaria. In the bivariate analysis identified risk factors were chills OR 3,44 (IC 95%: 1,14; 10,42), coluria OR 3,49 (IC 95%: 1,02; 11,95), jaundice OR 4,00 (IC 95%: 1,72; 9,24), thrombocytopenia less than 100.000 platelets/mm3 OR 10,77 (IC 95%: 3,73; 31,10), and transfer from an institution of low medical complexity OR 3,79 (IC 95%: 1,75; 8,21). In the multivariate analysis continued as risk factors chills OR 7,24 (IC 95%: 1,00;52,45), jaundice OR 4,86 (IC 95%: 1,75;13,40) and thrombocytopenia OR 5,95 (IC 95%: 1,84; 19,22).
CONCLUSIONS: Even though jaundice was identified as a risk factor we did not establish any associations with laboratory studies due to lack of information in the medical records, which was the principal limitation of the study due to its retrospective nature.

Activities against *Trypanosoma cruzi* of aromatic glycosyl disulfide derivatives

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BACKGROUND: *Trypanosoma cruzi* is the etiologic agent of Chagas's disease, an endemic parasitosis in Latin America with 7 million people infected. Currently, there are no adequate chemoprophylactic drugs to eliminate the parasite from the blood of serologically positive donors in order to prevent transfusion-associated Chagas's disease. In the same way, only two drugs, nifurtimox and benznidazole, are available for the treatment of chagasic patients. These drugs are effective for acute infections, but their use for chronic patients remains controversial.
METHODS: Microplate serial diluitions of resarzurin, aromatic oligovalent glycosyl disulfides, and some diglycosyl disulfides were tested against three different *T. cruzi* strains. Cytotoxicity was evaluated against HeLa, Vero and peritoneal macrophage cell cultures.
RESULTS: Di-(β-D-galactopyranosyl-dithiomethylene) benzenes 2b and 4b proved to be the most active derivatives against all three strains of cell culture-derived trypomastigotes with IC50 values ranging from 4 to 11 µM at 37 °C. The inhibitory activities were maintained, although somewhat lowered, at a temperature of 4 °C as well. Three further derivatives displayed similar activities against at least one of the three strains. Low cytotoxicities of the active compounds, tested on confluent HeLa, Vero and peritoneal macrophage cell cultures, resulted in significantly higher selectivity indices than that of the reference drug benznidazole. Remarkably, several of the tested compounds strongly inhibited the parasite release from *T. cruzi* infected HeLa cell suggesting an effect against intracellular *T. cruzi* amastigotes.
CONCLUSION: Oligovalent aromatic glycosyl disulfides displayed trypanocidal activities against trypomastigotes of *T. cruzi* from different strains. This observation, suggest the potential for these compounds to be used to prevent *T. cruzi* infection in blood banks. Furthermore, it was remarkable that several members of the tested panel, like 1b, 3, 4b, 5, 6 and 11, were cell-permeable and were effective against amastigotes, the intracellular stage of the parasite.
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Prevalence of *Cryptosporidium* spp. and clinical status in calves less than three months old from Tizayuca Hidalgo, México

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BACKGROUND: *Cryptosporidium* is a zoonotic and cosmopolitan parasite. In cattle, this protozoan produces watery diarrhoea, weight loss, decrease milk production and low feed conversion; causing economic losses to farmers. In Mexico there are few data about prevalence of this parasite in calves. The aim of this study was to determine the prevalence of *Cryptosporidium* and clinical status in calves less than three months old from the Dairy Basin of the Tizayuca, Hidalgo.
METHODS: The sample size was determined with a confidence interval of 95%, error margin of 5% and expected frequency of 10%. Tizayuca, Hidalgo is located in the centre of Mexico. 12 dairies were sampled, to get to faecal samples of 212 calves to examine. *Cryptosporidium* oocysts identification was performed using the Kinyoun stain. Clinical signs were observed at the time of sampling. The following parameters were analysed: age, diarrhoea and nutritional status, with the purpose of correlate the infection. For the nutritional status were classified into three categories. We extracted DNA from faecal samples and amplified by conventional polymerase chain reaction (PCR) the 18S small subunit (SSU) rRNA gene using forwarding primer CryF: 5´-AACCTGGTTGATCCTGCCAGTAGTC-3 and reverse primer CryR: 5´-TGATCCTTCTGCAGGTTCACCTACG-3.
RESULTS: Cryptosporidiosis prevalence by kinyou staining was 30.18% (64/212) and by PCR was 31.8% (13/51) analysed by now. All dairies had calves infected with *Cryptosporidium*. One calf of 3 days old had *Cryptosporidium* oocysts, suggesting that it is possible to be infected since the first day of life.
CONCLUSIONS: There was not significant difference between the infection and the age, diarrhoea and nutritional state of the calves. The *Cryptosporidium* genus was confirmed by PCR. The environment of the dairy basin of the Tizayuca is favourable for *Cryptosporidium* transmission.

Curcumin inhibits cell motility and alters microtubules organization in *Giardia lamblia*.

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BACKGROUND: Even thought, different drugs have been extensively used for the treatment of giardiasis, there are no definitive treatment protocols. Phytotherapeutic agents have great potential in the treatment of giardiasis. Curcumin, the main polyphenol constituent of turmeric has demonstrated an irreversible cytotoxic effect in *Giardia lamblia* trophozoites, but, the mechanisms involved have not been fully characterized. In this study we have used immunofluorescence labeling methods and confocal microscopy technique to identify if curcumin affects the microtubules organization.
METHODS: *Giardia lamblia* trophozoites were cultured at 37ºC in TYI-S-33 pH 7.0, supplemented with 0.5 mg/ml bovine bile and 10% bovine serum. Parasites were treated for 12, 24, 48 and 72h with different concentrations of curcumin and compared these with parasites grown in the presence of DMSO (the curcumin vehicle). The effect of curcumin on trophozoite attachment to glass was determined in a Neubauer chamber under light microscopy. Parasites were processed by confocal microscopy to identify morphological alterations and tubulin distribution.
RESULTS: Curcumin inhibited the cell attachment and produced morphological damages on trophozoites. The microtubules dynamics was also affected by curcumin; microtubules appeared with reduced fluorescence in comparison with control.
CONCLUSIONS: Our results suggest that the antigiardial effect of curcumin is at least partially due to its capacity to affect the polymerization of microtubules.

Prediction of conserved, B-cell epitopes in the AMA-1 protein of *Babesia bovis to be evaluated as a vaccine candidate*

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BACKGROUND: Bovine babesiosis caused by *Babesia bovis* is a tick-borne disease that imposes important constraints on livestock health and economical development in tropical and subtropical regions throughout the world, including Mexico. Effective control can be achieved by vaccination with live attenuated parasites. However these vaccines have a number of drawbacks, therefore new vaccines are needed. In recent years a number of parasite proteins with immunogenic potential have been discovered, one of these proteins is the apical membrane antigen 1 (AMA-1). To date, there is little information about the presence of conserved epitopes in AMA-1 among different parasite isolates. Bioinformatics is a powerful tool for the identification of conserved, surface expose, B-cell epitopes in vaccine candidate antigens like AMA-1.
METHODS: Ticks infected with *Babesia bovis* were placed in a bovine for five days. Seven days later, infected blood was collected and processed for DNA extraction. Through polymerase chain reaction and genetic sequencing the predicted sequence of the protein AMA-1 (isolate Tecomán, Colima State, Mexico) was obtained and it was included in a multiple alignment analysis using whole sequences reported in the GenBank. Furthermore an analysis of secondary structures, presence of signal peptide and trans-membrane regions was carried out using the Protscale, TMpred, THMM and SignalIP algorithms. In this manner we made an analysis of B-cell epitope prediction on the extracellular region of the protein using several algorithms (ABCPred, BCPred, Antigenic and IEDB). These peptides were chemically synthesized, placed in a nitrocellulose membrane, and then incubated with sera of bovines naturally infected with *B. bovis* in western blot assays.
RESULTS: Through bioinformatics analysis we predicted a set of five peptides on the extracellular region of AMA-1, containing B-cell epitopes, which are exposed and conserved among several isolates. The sera of bovines naturally infected with *Babesia bovis* identified the peptides, as determined by western blot analysis.
CONCLUSIONS: To confirm biologically that AMA-1 has conserved epitopes the synthetic peptides that were designed were evaluated in an immunoassay with sera from naturally infected cattle. The presence of antibodies recognizing conserved, surface-exposed, B-cell epitopes in AMA-1 supports its further evaluation as vaccine candidate for *B. bovis*.

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Protein phosphatase PP2C activity in *Leishmania mexicana* promastigotes

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BACKGROUND**:** Recent studies suggest that the protein phosphatase PP2C of different pathogens such as *Plasmodium falciparum* and *Toxoplasma gondii* plays a role in the pathogenesis of these parasites.PP2C dephosphorylates a number of intracellular substrates such as cyclin-dependent kinase, mitogen-activated kinase (MAPK) and BAD (pro-apoptotic protein). Sanguinarine is an alkaloid obtained from the bloodroot plant *Sanguinaria canadensis*. It inhibits the enzymatic activity of PP2C, induces apoptosis and inhibits tumor formation. Therefore, in this work we analized the protein phosphatase PP2C activity in *Leishmania mexicana* promastigotes and its effect in the infection of macrophages in presence of sanguinarine.
METHODS**:** *L. mexicana* promastigotes were incubated with different concentrations of sanguinarine for five days. Every day the parasites were counted in a Neubauer chamber. After three days of culture, parasites were lysed by sonication and total extract was used to analyze the phosphatase activity. Also in this time we identified the protein phosphatase PP2C by western blot. Promastigotes were incubated with 80 nM of sanguinarine by three days were used to infect murine macrophages.
RESULTS: Sanguinarine inhibited in a dose-dependent manner the growth of parasites and phosphatase activity in extracts of promastigotes of *Leishmania mexicana.* Western blot assays showed that there wasn´t a differential expression of PP2C protein in *Leishmania* promastigotes. Parasites incubated with 80 nM of sanguinarine didn´t infect the macrophages.
CONCLUSIONS: Our results suggest the inhibition of *L. mexicana* PP2C with sanguinarine decreases the phosphatase activity in the parasites and can play an important role in the signaling pathways of the parasite.
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Antibody-covered *Toxoplasma gondii* tachyzoites enter more efficiently to endothelial cells

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BACKGROUND. *Toxoplasma gondii* is able to invade human endothelial cells, which express FcRn; this receptor mediates endocytosis and transports all IgG subclasses (and immune complexes) across the placenta. It has been observed that antibodies may enhance cytomegalovirus entrance to the chorionic villi, which probably facilitates fetal infection. The aim of this study was to determine if antibody-covered *T. gondii* tachyzoites enter more than non "opsonized" parasites to human endothelial cells and to compare this phenomenon between parasite strains and between cells subtypes.
METHODS. The expression of FcRn was determined in human microvasculature endothelial cells (HMEC-1) and in human umbilical vein endothelial cells (HUVECs) by qRT-PCR (mRNA), flow cytometry and confocal microscopy (protein). On the other hand, tachyzoites of the RH and ME49 strains, harvested from 72 h Vero cell cultures, were stained with CFSE, incubated with IgG1 positive or negative human sera and incubated for 2 h at 10:1 parasite/cell ratio with either HMEC-1 or HUVECs. The number of infected endothelial cells was measured by flow cytometry and checked by confocal microscopy.
RESULTS. Both types of human endothelial cells express the FcRn mRNA and protein. The proportion of infected cells was higher for HMEC-1 than for HUVECs, with both parasite strains. Parasites covered with specific antibodies infected more HMEC-1 cells than those incubated with negative serum; this was also observed for the RH strain in HUVECs. Positive serum does not have effect with ME49 strain in HUVECs. Infected cells were observed by confocal microscopy and all parasites were intracellular.
CONCLUSIONS. As previously reported, we found that HMEC-1 cells are more susceptible to *T. gondii* invasion than HUVECs. Also, specific IgG1 antibodies favor parasite entry to both endothelial cells, probably by means of the FcRn.

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Quantification of flavonoids, antimicrobial activitie, antioxidant activitie and giardicidal activityof the extract of *Peniocereus Maculatus*

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BACKGROUND: The species *Peniocereus* belong to the family of cacti located in arid zones in Mexico and United States. Some of this cacti has been used for their medicinal properties in the treatment of diabetics, bronchitis, bacterial infection and also as anti parasitic. During this investigation we perform in vitro essays to corroborate their biological activity.
METHODS: The extract of *P. maculatus* was prepared using dichloromethane and methanol (1:1) after a week we filtrated the extract .The antimicrobial assay were perform with the macrodilution in tube technic (NCCLS) using the following standards: *E. coli*, *C. albicans* and *S. aureus*. The antioxidant assay and the quantification of flavonoids were using the methodology previously describe using the micro plate method. The extract was tested against *G.lamblia* trophozoite.
RESULTS: The extract of p. maculatus showed antimicrobial activitie against E. coli and *C. albicans* (MIC 0.25 mg/mL). The maxim antioxidant activitie was 38 % (1mg/mL) and the giardicidal activity showed MIC 21.149µg/mL.
CONCLUSIONS: The results showed interesting activities against pathogenic microorganisms an important reason to continue their studies as with other protozoos like *T. vaginalis* and hematic parasites.

Acanthamoeba keratitis review

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BACKGROUND: Nowadays, *Acanthamoeba* keratitis, associated with the use of contact lens is an increasingly problem. It is known that *Acanthamoeba* is the most common amoebae that is founded in soil contaminated lakes, streams, and other water environments. Also it can tolerate widely conditions.
METHODS: We made an extensive research of the most recent information about *Acanthamoeba* keratitis from articles.
RESULTS: *Acanthamoeba* has two stages: trophozoite and cyst. Cysts are an ineffective stage. The majority of human infections have been associated with the T4 genotype. This microorganism can host many bacterias, allowing them to evade host defenses, to resist antibiotics actions, also to increase its virulence. Keratitis is usually associated with eye trauma that occurred before contact with contaminated soil, dust, or water. The Invasion by *Acanthamoeba* produces corneal ulceration and severe ocular pain. For the diagnosis corneal scrapping should be collected and be examined using a saline wet preparation and iodine-stained smears. This disease may respond to topical miconazole, chlorhexidine gluconate or propamidine isethionate, in some cases it may require repeated corneal transplantation or, sometimes, enucleation of the eye. Also topical application of steroids.
In Mexico City the highest number of amoebae was isolated from cisterns and roof tanks. Most *Acanthamoeba* isolates were non-pathogenic, however, their presence is a potential hazard for the development of the disease. In Mexico have been reported about 5 cases of amoebic keratitis, 80% associated with use of contact lense. But the report of this disease it is not obligated.
CONCLUSIONS: Based in our research we can conclude that there is no record neither an appropriate diagnosis of this disease.
Prevention and control of the infection is difficult because of the widely distribution of *Acanthamoeba.* Being this important because this corneal infection can lead to blindness or vision impairment.

Changes of toxoplasmosis in Mexico: related to weather?

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BACKGROUND: Incidence of *Toxoplasma gondii* infection largely depends on climatic conditions, since the oocyst is the main transmissible stage and its viability largely depends on weather. The climatic change occurred worldwide may have had an effect on *T. gondii* incidence. In Mexico, changes in temperature and pluvial precipitation had been heterogeneous along the country, thus *T. gondii* transmission may have been differentially affected.
METHODS: We made use of two National Health Surveys, one performed in 2000 and one in 2006; in both cases, perfectly stored serum banks were available; also, demographic information could be gathered. We tested 3599 and 2916 samples from the 2000 and 2006 banks for IgG specific antibodies by ELISA and confirmed by western blot. Crude, epidemiologically weighted and diagnosis-performance-adjusted prevalence values were calculated. Seroprevalence was compared between surveys and among regions (north, center and coast). Also, correlations between changes in temperature or humidity and those in prevalence were determined.
RESULTS: A global increase from 40.0% to 43.1% (weighted-adjusted prevalence) was observed between 2000 and 2006. Coastal states and children presented the largest increases between surveys, while the center of the country showed a decrease. A higher prevalence of *T. gondii* infection was observed in both surveys when compared to that performed in 1987, while a geographical re-distribution was found from 2000 to 2006, with a positive correlation between changes in temperature and in *T. gondii* prevalence in 21 states.
CONCLUSIONS: Our results suggest that temperature changes, but not humidity, were partially responsible for *T. gondii* frequency increase. Demographic aspects could not be ruled out and should be studied.

Alternative treatment using herbal extracts with antiprotozoal and immunomodulatory activity in a mice model of Chagas disease

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BACKGROUND: Chagas disease is a National importance disease in México. The most exposed population remains uninformed about many of the preventive measures and even less about the existent treatment. The treatment is regulated by the NOM-032-SSA2-2002, for the epidemiological surveillance, control and prevention of vector transmitted diseases, section 9.5. However, in Yucatan, the people with infection during 2012 to date have not yet received treatment. The medications to treat this disease are still not an effective cure for the chronical stage of the disease. Since there is an urgent need for an alternative treatment, we decided to determine *in vivo* activity of the extracts of two traditionally used Mayan medicinal plants to treat cutaneous leishmaniasis: *Allium sativum* and *Tridax procumbens* since leishmania and trypanosoma belong to the same family and the hexanic extract of *T.procumbens* had an IC_{50} < 2.90 against *T.cruzi*.
METHODS: four groups of six CD1 mice infected with 1000 trypomastigote were treated either with vehicle, extract of *T.procumbens*, *A.sativum* watery extract or the MIX, to a dosage of 100mg/Kg/day. Treatment was given 25 days post-infection. The evolution of the disease was measured by parasitemia, weight, survival rate, and heart hystology.
RESULTS: Mice treated with MIX, showed a lower load of parasites during the acute phase, after this stage, the parasites lowered in all groups. The control showed 33% survival rate, the groups treated either with *T.procumbens* or *A.sativum* had 66% of survival and the group treated with the MIX had 100% survival to day 62. The survivors were euthanized and heart histology showed that the group treated with MIX had less inflammatory infiltrate and lower amastigote nests in comparison with control.
CONCLUSIONS: The treatment with MIX, seems a promising lead for development of an alternative treatment against *Trypanosoma cruzi;* however, it is necessary to find the therapeutic dose.

Immunobiology of human congenital toxoplasmosis

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BACKGROUND: Due to the important role of the immune response for Toxoplasma gondii tachyzoite replication control, it is not surprising that fetal damage is inversely related to time of gestation at infection, i.e. it is less serious if it occurs close to delivery, when the fetal immune system is more mature. What is intriguing is that vertical transmission is highest at the end of pregnancy.
IMMUNE RESPONSE, VERTICAL TRANSMISSION AND PATHOLOGY: Some paradoxical results of several groups indicate that the response which protects the mother against pathological consequences (IFN-γ, IgG1, etc.) may be actually favoring vertical transmission or fetal pathology. We studied antibody (ab) IgG subclasses in mother/newborn pairs at birth, and found an interesting result: most mothers who were positive for IgG1 antibodies delivered ill offspring, while most negative for this subclass gave birth to apparently healthy babies. A possible explanation is that adherence of tachyzoites to the villous surface could be enhanced by IgG abs through the FcRn, concentrating the parasites and thus favoring invasion. Interestingly, FcRn expression increase on the syncytiotrophoblast parallels that of *T. gondii* vertical transmission along gestation. To test this hypothesis, we recently performed *in vitro* invasion experiments and found that IgG1-covered tachyzoites entered more endothelial cells than uncovered ones. More recently, we observed a mixed ("Th1/Th2") response profile in mothers with obstetric problems and in congenitally infected newborns with chorioretinitis and hepato-splenomegaly, while the same profile but accompanied by regulatory cytokines was observed in women without obstetric problems. Newborns who produce IgG3 or IgG4 specific abs tend to develop more clinical problems than those producing IgG1. Unexpectedly, IgG2 showed no relevance in clinical terms.
ROLE OF PARASITE TYPE IN TRANSMISSION AND PATHOLOGY: Regarding relevance of parasite virulence, we performed a systematic review of the literature and found that: a) virulent parasites are more commonly transmitted to the embryos during the first third of gestation than "avirulent"; b) type II strains ("non-virulent") are the most frequent (this is not new); and c) less "virulent" strains cause less damage than "virulent" ones (type I or atypic) acquired at the same gestation time. Thus, it seems that the biology parasite is also important in terms of transmission and pathogenesis.

Intravenous inoculation of *Plasmodium falciparum* sporozoites for controlled human malaria infection and development of highly efficacious malaria vaccines

Benjamin Mordmüller, Meral Esen, B. Kim Lee Sim, Stephen L. Hoffman, Peter G. Kremsner

Plasmodium falciparum (Pf) malaria has a large negative impact on health and development, especially in Sub-Saharan Africa. Therefore development of improved treatments and novel preventive interventions is a top-priority on the research agenda for infectious diseases. As for most other diseases, translation of basic into clinical research is extremely difficult. Controlled human malaria infection (CHMI) can accelerate development of antimalarial interventions and is a very robust surrogate for efficacy in the field. Hence, CHMI allows early, well-founded decisions on further clinical development and thereby can be used to save and potentially re-direct resources. So far, CHMI has only been available to a small community with access to an insectary suitable for production of infected mosquitoes that can be used in clinical trials. In addition, standardization of mosquito-bite-mediated CHMI is difficult. To overcome these problems, we have recently established a protocol for CHMI using intravenous injection of cryopreserved, pharmaceutical-grade Pf sporozoites (PfSPZ Challenge, Sanaria). This allows highly standardized and reproducible clinical trials and enables centers around the globe to perform single and multicenter CHMI studies. Beyond CHMI, PfSPZ are currently used to develop new immunization strategies and discover immunological mechanisms of protection.

Evaluating the residual effect of insecticides used for indoor residual spraying and long-lasting insecticide nets based malaria control programs in western Kenya.

BACKGROUND: Indoor residual spraying and long-lasting insecticidal nets have been extensively used for malaria prevention and control in Kenya. We evaluated the persistence of lambda-cyhalothrin and deltamethrin on the sprayed mud walls, and the bioefficacy of long-lasting insecticide-treated nets.
METHODS: Wall bioassays were performed monthly on artificial walls and filter papers sprayed with lambda-cyhalothrin and deltamethrin using *Anopheles gambiae* mosquitoes collected from different sites from western Kenya. Net cone bioassays were performed on nets collected from the fields using wild *Anopheles gambiae* collected from two sites. Kisumu susceptible strain was used as control in both cases. Chemical analysis of the netting material was done using gas chromatography.
RESULT: Mosquitoes from all study sites showed substantial resistance to both deltamethrin and lambda-cyhalothrin with the mortality rates ranging between 80% - 85% in four out of the six study sites and 69%- 74% in two sites. Sprayed artificial walls showed lower mortality rates compared to sprayed filter papers. Lambda-cyhalothrin had high mortality rates on the wild mosquitoes compared to deltamethrin. Wild mosquitoes collected from the field had significantly lower mortality rates to LLINs (60%-75%) compared to the control strain (100%). Net chemical content analysis indicated that there was no difference in the net chemical content in the nets between 6months and 2years old, the nets collected from the field had lower chemical content (ranging from 0.06% wt - 0.13% wt), than the positive control nets (0.14% wt).
CONCLUSION: ICON and deltamethrin sprayed persist on the wall surfaces for five and three months respectively. *Anopheles gambiae* mosquito populations from western Kenya are highly resistant to insecticides used for IRS and LLINs. Therefore, future malaria vector control programs should develop strategies to manage vector resistance.

Immune mechanisms in ehrlichial diseases

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Ehrlichia are tick-borne small obligately intracellular bacterial pathogens of humans and wild, domestic, and companion animals. Characteristically residing in modified phagosomes of monocytes or neutrophils, ehrlichiae cause severe acute illnesses and chronic persistent infections. Lacking lipopolysaccharide and peptidoglycan, ehrlichiae possess a large family of ~ 28 kDa proteins, a limited set of which is expressed in a particular host species rather than being expressed sequentially as a mechanism of immune evasion, tandem repeat containing proteins (TRPs), ankyrin (an eukaryotic protein-binding motif) repeat containing proteins (e.g., Ank 200) and heat shock proteins. Some of these have been shown to stimulate at least partial immune protection.
Ehrlichia manipulate the host to favor ehrlichial survival by inhibiting apoptosis, regulation of the cell cycle and differentiation, signal transduction, expression of proinflammatory cytokines, and membrane trafficking proteins, including inhibition of phagolysomal fusion, and degradation of $p22^{phox}$ to avoid oxidative killing. Ehrlichiae have genes for many type IV and type I secretion system components. TRP120 and Ank200 of *E. chaffeensis* are secreted, are transported into the host cell nucleus, and bind to DNA encoding genes that may explain the altered host defenses. Secreted ehrlichial TRPs also bind host cytoplasmic proteins that may modify the cellular response to ehrlichiae.
Both humoral and cellular immunity play roles as anti-ehrlichial host defenses. IFN-ɣ, TNF-α, MHC class I, $CD4^+$, and $CD8^+$ T cells, and antibody all contribute significantly to immunity. The immune system also contributes to the pathogenesis of severe acute illness associated with extremely high levels of TNF-α and IL-10, a high frequency of TNF-α-producing $CD8^+$ T cells, decreased *Ehrlichia*-specific $CD4^+$ T cells, including those producing IFN-ɣ, and low IL-l2. NKT cells are instrumental in the production of immunopathologic responses. There is evidence that IL-10 and PD-1 play roles in modifying host immunity leading to persistent ehrlichial infection.

Strategies for understanding and reducing the *P. vivax* hypnozoite reservoir in Papua New Guinean children

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BACKGROUND: *P. vivax* can cause relapse infections month or even years after a primary infection is cleared. However, it is poorly understood how much such relapse contribute to burden of infections in endemic population. Also since hypnozoite carriers can not be detected with current diagnostic test, it is not clear how one does best intervene to reduce the hypnozoite reservoir.
METHODS: We conducted two cohort studies, where children 1-5 and 5-10yrs were randomized the receive either blood stage plus liver stage or blood stage only treatment irrespective of the presence of blood-stage parasites or febrile symptoms. Children ere followed actively for infection for 9 months. Parasite were detected by real-time PCR and all *P. vivax* infection genotyped. Mathematical models were used to explore different option to reducing the hypnozoite reservoir in the population.
RESULTS. Removing hypnozoites in children 5-10yrs reduced the incidence of asexual *P. vivax* infections and *P. vivax* gametocytes by 80% and that of clinical illness by 70%. Due to a less effective primaquine treatment, somewhat smaller effects were observed in children 1-5yrs. Modelling shows that mass-screen & treat (MST) is unlikely to be an effective intervention to reduce *P. vivax* transmission. However, mass-drug administration (MDA) is potentially highly effective but only if it includes an anti-hypnozoite treatment.
CONCLUSIONS: Our results suggest that the majority of *P. vivax* infections are due to relapses. This reservoir of infection contributes substantially to the burden or *P. vivax* clinical episodes and to transmission. Efficient control and eventual elimination of P. vivax will be difficult without targeting the hypnozoite reservoir.

ENDOTHELIAL ACTIVATION INDUCED BY *P. falciparum*: Effect of cytoadherence on endothelial inflammatory response.

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BACKGROUND: The pathogenesis of malaria by *P. falciparum* is characterized by two mechanisms: inflammatory response and sequestration of infected erythrocytes (IEs) in the microvasculature, by binding to CD36 and ICAM-1 in endothelial cells. Sequestration leads to blockage of blood flow, endothelial activation and cell damage.
AIM**:** In an *in vitro* model of cytoadherence, we explored the response of microvascular dermal endothelial cells (HDMEC) to stimulation with IEs. Specifically, we evaluate the cytoadherence of four lab lines of *P. falciparum* (FCB1, FCB2, FCR3 and 3D7) and isolates by light microscopy and, as well their effect on the expression of ICAM-1, inflammatory cytokines secretion and cell viability.
RESULTS: 1. Number of bound IEs to HDMEC was different between lab lines, FCB1 showed greater adherence, followed by FCR3, FCB2 and 3D7. We also found differences in the adherence of IEs from malarial patients. 2. We found a significant increase (with $p < 0.05$) in the ICAM-1 expression in HDMEC after interaction with IEs from lab lines compared to controls (nIEs), but less than induced by TNF. Increase expression of ICAM-1 was greater with FCB1 and FCR3 compared to 3D7 and FCB-2. We also found an increase of different inflammatory cytokines secretion on supernatant culture (IL-6, IL-8 and MCP-1). 3.Colombian isolates (high or low adhesion) induced increased levels of mICAM-1, sICAM-1, IL-6, IL-8 and MCP-1. No relationship between the level of cytoadherence and inflammatory responses of cells was found.
CONCLUSIONS: The parasites studied show different binding capacity to HDMEC, but the degree of endothelial activation and cytoadherence are independent. We observed that IEs with *P. falciparum* induced pro-inflammatory and pro-adhesive endothelial activation, which could contribute to disease pathogenesis through increased adherence of IEs (via ICAM-1) and possibly the recruitment of leukocytes and release of inflammatory molecules.

Plasmodium berghei induces immune priming in _Anopheles albimanus_

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Priming in invertebrates is defined as the acquired capacity to better combat pathogens after a previous exposure to sub-lethal doses of the same organism, and it is proposed to be functionally analogous to immune memory in vertebrates. Previous studies of priming in *Anopheles gambiae* mosquitoes concluded that the enhanced response to a second challenge with *Plasmodium berghei* was dependent to the activation of hemocytes by midgut residing bacteria, which probably accessed the haemolymph through lesions produced by parasites during midgut invasion. We argue that the immune response to bacteria overlaps to that to Plasmodia, making it difficult to differentiate the specific immune and priming responses induced by the parasite. Here we documented in relatively midgut bacteria depleted *An. albimanus*, that priming was induced independently of midgut bacteria and of the parasite invasion of the midgut. Priming protection against a homologous challenge with *P. berghei* lasted as long as 21 days. The expression of antimicrobial peptides related to anti-*Plasmodium* responses (attacin, cecropin and gambicin) showed a biphasic pattern rather than sustained response. We provide the first demonstration in mosquitoes that immune priming depicts the whole marks of adaptive immune responses: long lasting, specific and biphasic.

Perspectives and mechanisms of sand fly saliva-based immunoprophylaxis in leishmaniasis.

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Leishmania parasites are transmitted by female sand flies that co- inject parasites and different products from the vector, including saliva, in the host's skin. Saliva of sand flies and of other blood feeding arthropods contains potent pharmacological components to facilitate the blood meal. Salivary proteins also play an important role during pathogen transmission as co-inoculation of sand fly saliva with the parasite exacerbates parasite infectivity. Pre exposure to *Lutzomyia longipalpis* saliva is protective against visceralizing leishmania. Several mechanisms have been implicated in this phenomenon including antibody- and/or cell-mediated mechanisms. We will present data on protection conferred by preexposure to sand fly saliva against *Leishmania donovani* infection and the perspectives of using sand fly saliva or its recombinant products as an immunoprophylatic approach against visceral leishmaniasis.

Benzimidazoles detection in sheep and cattle liver and milk samples by LC-MS/MS

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BACKGROUND: The benzimidazoles are broad-spectrum drugs used in the veterinary field having anti helminthic and antiprotozoal action against intestinal and tissue parasites. They inhibit the tubulin assembly in the cytoplasmic microtubules, alter the absorption of glucose with decreased ATP, and inhibit fumarate reductase specification of worms. In this study, liver samples of cattle and sheep in Sicily were tested by confirmatory method for the detection of benzimidazoles and its metabolites (albendazole, sulfoxid-albendazole, sulfon-albendazole, flubendazole, oxibendazole, febendazole, febantel, thiabendazole), it was analyzing also milk samples by LC-MS/MS.
METHODS: 93 samples were extracted using QuEChERS. The molecules separation was performed by UHPLC with chromatographic column Hypersil GOLD 50 mm, 2.1 mm ID, 1.9 mm. Eluents phases were 0.1% formic acid in water (phase A) and 0.1% formic acid in acetonitrile (phase B). The detector used was a triple quadrupole mass spectrometer, ESI Source.
RESULTS: The samples of cattle and sheep including 40 liver samples and 53 samples of milk have been tested. All samples were negative for benzimidazoles. The validation data has been evaluated in accordance with the Commission Decision 2002/657/EC.
CONCLUSIONS: Since benzimidazoles are used in both prophylactic and therapeutic way against parasites, they can cause chemo-resistance, aversion, vomiting, loss of appetite, increased thirst and variation volume of urine output. The use of veterinary drugs can cause the presence of residues in food of animal origin that, without adequate interruption periods, can become a hazard to consumers.

Slim Initiative for Vaccine Development against Tropical Diseases: Recent advacnes on the development of a therapeutic vaccine against Chagas disease

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The Sabin Vaccine Institute Product Development Partnership (PDP) was founded to develop and test recombinant protein vaccines targeting neglected tropical diseases, a group of chronic, debilitating, and poverty-promoting infections. In partnership with the Carlos Slim Health Institute, we recently launched Slim Initiative for Vaccine Development against Tropical Diseases. For the Chagas Vaccine Initiative, the Sabin Vaccine Institute PDP has a partnership in place and in-depth cooperation with leading R&D institutions and vaccine manufacturers in Mexico including the Autonomous University of Yucatan (UADY), the Center for Research and Advanced Studies of the National Polytechnic Institute (CINVESTAV), and Mexico's leading public sector vaccine manufacturer known as Birmex (Laboratorios de Biològicos y Reactivos de Mèxico). Additionally, we have a continuing partnership with Dr. Bruce Lee at The Johns Hopkins University (JHU) for his expertise in the economic modeling of NTD vaccines. A major goal of this initiative is the development of a bivalent therapeutic vaccine for the treatment of chronic Chagas disease. The vaccine is proposed to be comprised of two *T. cruzi* recombinant proteins formulated on alum. The vaccine development program will also incorporate the evaluation of a second immunostimulant. One of the antigens is the unique *T. cruzi* 24 kDa antigen (Tc24) and the other is the unique *T. cruzi* surface transialidase (TSA-1). Proof of concept for the protective effect of each of these antigens and their combination (as DNA vaccines) was based on immunizations conducted previously in *T. cruzi*-infected laboratory animals, together with identifiable mechanisms of protective immunities. The proposed vaccine could be administered either to individuals with chronic Chagas disease or those with indeterminate status (determined by antibody seropositivity using a licensed diagnostic kit) who may go on to develop cardiomyopathy, or in patients with early-stage evidence of clinical Chagas disease. Advances in the development of these vaccine targets will be presented including the early stage process development, analytical and biochemical characterization and propopsed pre-clinical development plans.

Selection and characterization of inmunodominant mimotopes of *Haemonchus contortus* larvae 3 from phage display libraries

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BACKGROUND: Indiscriminate use of anthelmintics for *Haemonchus contortus* control has caused the generation of resistant strains. So that the use of alternative methods for controlling this parasite is required. Applying immunodominant parasite antigens as a vaccine has been shown to significantly reduce the parasite load of infected animals, therefore, the research has been directed to the search for antigens with immunoprotective capacity. In this study, phage display system was used to identify and characterize inmunodominant mimotopes in the third larval stage (L3) of *H. contortus*.
METHODS: Antibodies against L3 of *H. contortus* were raised in SPF rabbits. The L3 antigen was obtained from the feces of infected sheep and was characterized by ELISA and Western blot., Combinatorial libraries of 7 amino acids (Ph.D 7) and 12 amino acids (Ph.D. 12) were screened with purified IgG by three selection rounds. 20 clones from each library were isolated and sequenced, their reactivity was assessed by ELISA.
RESULTS: 8 different mimotopes were identified from the 20 clones selected of the 3rd selection round from Ph.D 7. 13 different mimotopes were identified of the 20 clones of 12 Ph.D. It was observed that the most abundant mimotopes of the 20 selected clones from each combinatorial library were the most reactive in ELISA.
CONCLUSION: Different immunodominant mimotopes were identified from L3 antigen. The characterization results suggest that the expressed peptide sequence from each mimotope could be potential vaccines candidates against the parasite.

Morphometric identification of trematodes eggs parasites of sheep and cattle

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BACKGROUND: The infections caused by trematodes cause economic losses in ruminant production systems. These damages are mainly attributed to *Fasciola hepatica*, however, the presence and capacity of *Paramphistomum sp.* has been underestimated. It is necessary to establish procedures to identify the eggs of these two genera in order to stablish specific control strategies. The objective was to determine morphometric characteristics of *Fasciola hepatica* and *Paramphistomum sp.* collected from domestic ruminants.
METHODS: Adult parasites of *Fasciola hepatica* and *Paramphistomum sp.* from sheep and cattle were collected. Eggs were obtained by maceration technique of adults in saline and formalin solution. One hundred eggs of each were measured by length and width with microscopy. Average, minimum and maximum values and differences between means were determined.
RESULTS: *Fasciola hepatica* eggs had an average of 133.47 (116.55-154.83) µ long and 87.45(64.56-102.27) µ wide. *Paramphistomum sp.* eggs, collected from sheep were 116.51 (101.75-134.76) µ long and 65.03 (54.53-74.75) µ wide.
The measurements of the eggs collected from bovine were 111.35 (95.34 - 124.32) µ long and 64.10 (54.26-86.99) µ wide. Measurements were compared by Tukey test and statistical significant diferences were detected (P>0.05).
CONCLUSIONS: *Fasciola hepatica* eggs and *Paramphistomum sp.* collected from sheep and cattle in the Sierra region, Tabasco, have different measures. The micrometry can help in identifying eggs *Paramphistomum sp.* and *Fasciola hepatica*.

PfEMP1 trafficking in *P. falciparum*

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BACKGROUND: The particular virulence of the most deadly of the malaria parasites, *Plasmodium falciparum*, is associated with binding of infected red blood cells (RBCs) to sites in the brain and the placenta. Adhesion is mediated by a Velcro-like protein, called *P. falciparum* erythrocyte membrane protein-1 (PfEMP1), presented at the RBC surface. Despite its importance, the mode of trafficking of PfEMP1 and its insertion into the RBC membrane is poorly studied.
METHODS: We have used 3D-SIM, electron tomography and molecular methods to probe the organisation of the membrane network that the parasite establishes in its host cell to traffic PfEMP1 to the RBC surface via. We have examined exomembrane sculpting proteins, such as the ring-exported protein-1 (REX1), and followed the trafficking pathway for the major virulence proteins, the knob-associated histidine-rich protein (KAHRP) and *P. falciparum* erythrocyte-binding protein-1 (PfEMP1).
RESULTS and CONCLUSIONS: We have ultrastructurally characterised a range of parasite-derived structures, including the Golgi-like Maurer's cleft, ~500 nm tubular tethers and ~80 nm electron-dense vesicles. This provides a model of the exomembrane system for trafficking of virulence proteins. We show that Maurer's cleft cisternae are formed early after invasion and proteins are delivered to these (initially mobile) structures in a temporally staggered and spatially segregated manner. The tether-like structures are generated as early as 4 h post-invasion and become attached to Maurer's clefts. The tether/ Maurer's cleft complex docks onto the RBC membrane at ~20 h post-invasion via a process that is not affected by cytochalasin D treatment. We have defined the REX1 domains responsible for Maurer's cleft sculpting. We have examined the trafficking of a GFP chimera of PfEMP1 expressed in transfected parasites and show that it is trafficked via a specialised compartment at the parasite surface, before transfer to the surface via Maurer's clefts and electron-dense vesicles.

Host cell remodelling in *P. falciparum*: Sex and Drugs

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BACKGROUND and METHODS: New microscopy techniques are providing amazing views of the cellular landscape. We have used 3D Structured Illumination Microscopy (SIM) and 3D-Electron Tomography to explore the sub-cellular topography of the malaria parasite, *Plasmodium falciparum*.
RESULTS and CONCLUSIONS: We have explored changes in the cellular structure of the parasite as it undergoes a remarkable transformation to the gametocyte stage in preparation for transmission to a mosquito and sexual reproduction (*1*). We have shown that the inner membrane complex (IMC) of the *P. falciparum* gametocyte has a similar structure and function to the IMC of motile stages. We have identified a previously unrecognized actin-based cytoskeleton that is assembled in maturing *P. falciparum* gametocytes. We correlate shape changes with deformability changes and hypothesize that the circulating gametocyte adopts a banana shape to enable it to pass through the sinusoidal slits in the spleen, thereby avoiding host surveillance mechanisms.
We have probed the parasite's digestive processes and show that digestion is initiated in ring stage parasites. We show that haemoglobin uptake and digestion plays a critical role in activating the antimalarial drug, artemisinin (*2, 3*). We have investigated stage-dependent differences in artemisinin activity and have characterised differences in the responses of artemisinin-resistant parasites from the field. This gives important insights into the nature of artemisinin action and resistance.

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Cryptosporidiosis: Molecular Epidemiology, Immunity and Malnutrition.

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We aim to describe the natural history of *Cryptosporidium spp.* infection and identify correlates of mucosal and humoral immunity (serum and stool antibodies) in slum-dwelling Bangladeshi children. Children were enrolled at birth and followed for two years, with active surveillance for diarrheal illness. Diarrheal and surveillance stool samples were tested for *Cryptosporidium spp*. using real time qPCR. Antigen detection was used to test for serum anti-cryptosporidium IgG and fecal anti-cryptosporidium IgA. Anthropometric measurements were taken every 3 months. We followed 392 children from birth to age two. By age two, almost 80% of children in the cohort had been infected with *Cryptosporidium spp*. Asymptomatic infection was more common than diarrheal infection (72% vs 25%). Higher parasite burden, as measured by quantitative real time PCR, was associated with diarrhea rather than asymptomatic infection (T-test, $p < 0.0001$). Using multivariate regression analysis, we found that children with asymptomatic *Cryptosporidium spp.* infection during the first two years of life were significantly more likely to have growth stunting at age two, when compared to children who were never infected ($p = 0.0345$). Positive anti-Cryptosporidium serum IgG at 12 months of age was associated with lower risk of *Cryptosporidium spp*. infection during the second year of life (log-rank test, $p = 0.0326$), however no protective effect was seen with positive anti-Cryptosporidium IgA in stool. History of *Cryptosporidium spp*. infection in year one did not predict risk of infection in year two. We also found that over 90% of samples tested were of *C. hominis* subtype, which is consistent with previous reports. In summary, the burden of *Cryptosporidium spp.* infection in Bangladeshi children is largely subclinical, however is associated with significant growth faltering.

Effects of blood on vector physiology – they are what we feed them

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The blood feeding behavior of disease-transmitting arthropods creates a unique intersection between vertebrate and invertebrate physiology. Human blood contains a myriad of nutrients, growth factors, cytokines/chemokines, pathogens, and pathogen-associated molecules that can interact with arthropod vectors. Many of these blood-derived factors persist during blood digestion to signal in the arthropod vector. This review summarizes the conserved signal transduction pathways that are activated by these vertebrate host-derived factors, and describes their downstream effects on lifespan, reproduction, and innate immune responses of the most common arthropod vectors of human disease agents. Expanding our understanding of these complex interactions can guide novel approaches for the control of a variety of vector-borne diseases.

Plasmodium vivax: subtelomeric variant proteins, spleen adherence and development of a functional microengineered model of the human splenon-on-a-chip

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The lack of a continuous *in vitro* culture system for blood stages of *Plasmodium vivax* has severely limited our understanding of the molecular basis of pathology in this species. Noticeably, recent data have challenged the dogma that P. vivax-infected reticulocytes do not cytoadherence in the deep vasculature of internal organs. Here, we show that a transgenic line of *P. falciparum* expressing a VIR protein but not another transgenic line expressing a Pv-FAM-D protein, mediated adherence to human spleen fibroblasts. Spleen-adherence specificity was shown as neither transgenic line bound to human lung fibroblasts. To extrapolate these results to natural infections, adhesion experiments using *P. vivax*-infected reticulocytes obtained from human patients were performed on human spleen fibroblasts. Results demonstrated adherence, albeit variable, among different isolates. These data reinforce the fact that cytoadherence is not exclusive of *P. falciparum* and challenges the dogma that the spleen is the evolutionary driven force for cytoadhesion of infected red blood cells in other organs for avoidance of spleen- clearance. To further advance these studies, we have developed a functional microengineered model of the human splenon-on-a-chip. Results on its implementation for advancing spleen studies in malaria will be discussed.

"Reticulocyte-derived exosomes: an intercellular communicator in *Plasmodium vivax* malaria?"

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Exosomes are 30-100-nm membrane vesicles of endocytic origin. Recently, we have described the isolation and characterization of exosomes from reticulocytes, *rex*, of BALB/c mice infected with the *P. yoelii* 17X strain (*rexPy*). Remarkably, subcutaneous immunization of mice with purified *rexPy*+CpG induced protection and subsequent long-lasting sterile protection in 83% of the animals lethally challenged. We have now determined the molecular composition of these vesicles and found the presence of parasite proteins and yet uncharacterized small RNAs. Moreover, we found that subcutaneous administration of exosomes determines their initial fate to draining lymph nodes. Nevertheless, the spleen plays an essential role since protection does not occur in splenectomised mice. In addition, we are determining the cellular response to *rexPy* in terms of their capture, ability to stimulate immune cells and capacity to promote the appearance of memory cells in the spleen. In order to translate these results to humans, experiments of the capturing of human exosomes from vivax infections (*rexPv*) by human splenocytes and their stimulation were performed. Last, we have also determined the molecular composition of exosomes obtained from plasma of patients actively infected with *Plasmodium vivax*. Results will discuss the potential use of *rex* as a novel vaccine and platform against *Plasmodium vivax* malaria.

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Molecular techniques for differential diagnosis of *Taenia asiatica*

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Taenia solium, *T. saginata* and *T. asiatica* are human infecting taeniid tapeworms that cause taeniasis in human and cause cysticercosis in intermediate host animals (cows and pigs). Human *Taenia* tapeworms exhibit morphological similarities, particularly in the eggs and proglottids of *T. sagianta* and *T. asiatica*, which make frequent confusion in the endemic areas where the sympatric distributions of these tapeworms are seen. Consequently, it is necessary to be able to distinguish accurately among these tapeworm species; molecular diagnostic methods have been developed for rapid and accurate detection of these parasite species. Molecular approaches to the differential diagnosis of *T. asiatica* has been tried to date including the use of sequence specific DNA probes, PCR coupled to restriction fragment length polymorphism, Base Excision Sequence Scanning Thymine-base analysis (BESS T-base), multiplex PCR, Loop-Mediated Isothermal Amplification (LAMP), nucleotide sequencing based on 10% formalin-fixed paraffin-embebded specimens, DNA genotype, and immunoblot patterns. The improvement of genomic DNA extraction methods from various tissues and copro-DNA sources is prerequisite for applying molecular genetic techniques to relevant study fields. These studies have greatly contributed to the understanding of genetic diversity including genetic identification, genotypic relationships of hybrids and phylogenetic relationships, and epidemiological status of *T. asiatica*.

Versatility of eosinophils in nematode infection

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BACKGROUND: The eosinophil is a defining cellular feature of the immune response to parasitic worm infection. Research into the role of eosinophils in host defense has produced findings that appear contradictory. Eosinophil-mediated toxicity for nematode larvae was documented many years ago, while recent work has revealed that eosinophils promote survival and/or growth of larvae and worms. We have found two distinct and opposite influences of eosinophils in primary versus secondary infection of mice with the nematode, *Trichinella spiralis*.
METHODS: This study employed targeted gene knock-out and transgenic mice, together with adoptive transfer protocols, to assess eosinophil-mediated effects on immune function, parasite burden, host vitality, and larval growth. The worm completes its life cycle in a single host, first colonizing the intestine and then releasing larvae that infect skeletal muscle.
RESULTS: In neither primary nor secondary infection did eosinophils influence the survival or reproduction of intestinal worms. In contrast, during primary infection, eosinophils preserved muscle stage larvae by releasing IL-10 and promoting immunity mediated by IL-10-secreting myeloid dendritic cells and IL-10-secreting CD4+ cells. During secondary infection, eosinophils contributed to clearance of larvae in an antibody dependent manner, most likely affecting larvae as they migrated to muscle.
CONCLUSIONS: The eosinophil is a pivotal mediator of distinct immune mechanisms that protect larvae during primary infection and clear them during secondary infection.
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Blastocystis and Irritable Bowel Syndrome

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BACKGROUND: Irritable Bowel Syndrome (IBS) is a common functional bowel disorder that is characterised by a number of non-specific symptoms that are underscored by the relapsing and chronic nature of the syndrome.
The gut microbiota and specific components of the gut microbiota have been implicated in the aetiology of IBS. This includes *Blastocystis* which is single celled microbial eukaryote, found in a range of non-mammalian and mammalian hosts including humans. Although a number of past studies have reported a higher prevalence of *Blastocystis* in individuals with IBS, other studies have reported no significant association. The strongest evidence supporting a pathogenic role for *Blastocystis* in intestinal disease and IBS stems from cell-line assays and rodent models which show that *Blastocystis* can induce an inflammatory response.
METHODS and RESULTS: A recent study that used a molecular epidemiological approach to assay the prevalence of *Blastocystis* in healthy adults (n =105) living in an industrialised country (where IBS is a significant burden in the population) has shown that: 1) a high proportion (56%) of the healthy adult population are colonised by *Blastocystis*, and 2) are colonised over the long-term (10 years) without any symptoms (Scanlan *et al*., submitted). Moreover, recent unpublished data indicates that there is no significance difference in *Blastocystis* prevalence between IBS v control (14% versus 20% respectively, *P* =0.09, Danish study).
CONCLUSIONS: Even though *Blastocystis* spp. are highly prevalent in healthy individuals and less prevalent in IBS, it is possible that there may be a link between specific genotypes of *Blastocystis* and host symptoms, a host x parasite interaction, and/or a relationship between parasite load and host symptoms. Currently, the role of *Blastocystis* in IBS remains speculative.

Identification of Activated C Kinase 1 (RACK1), during stress response in C6/36 HT mosquito cells.

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BACKGROUND: Mosquitoes of Aedes genus are vectors of several parasites and viruses causing diseases of public health importance, and the analysis of the signal transduction in response to stress could give knowledge useful to understand interaction with pathogens and immune system activation. Dexamethasone, a synthetic glucocorticoid, has been used to induce stress in several systems and it has been observed that it produces pleiotropic effects affecting multiple signaling pathways. AIM**.** Identify proteins participating in signaling pathways and their interactions during induced stress.
METHODS: We treated mosquito *Aedes albopictus* cell line C6/36 HT either dexamethasone or starved for 2 h, then the proteins were analyzed by 2D electrophoresis and MS. Specific proteins expression and modification was validated by bioinformatics, western blot and qPCR, confocal microscopy and interactions with others proteins was initially investigated by immunoprecipitation (IPP).
RESULTS: We identified proteins that modified their presence in stress conditions including the protein Receptor for Activated C Kinase 1 (RACK1). This protein, which is a scaffold protein member of the tryptophan-aspartate (WD) repeat family, folds in a seven-bladed β-propeller structure that permits the association of proteins to form functionally active complexes.
CONCLUSION: RACK1 has a significant role in shuttling proteins around the cell, anchoring proteins at particular locations and regulating signal transduction that coordinate stress response, immunity and cell migration, among others. RACK1 expression was validated and their location observed demonstrating their reactivity during stress. Finally, we studied by immunoprecipitation its interactions with various proteins.

Interactions of *Trypanosoma cruzi* and Triatomines

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BACKGROUND: The interactions between *Trypanosoma cruzi* and triatomines include a regulation of the development of the flagellates by intestinal compounds and microbiota of triatomines and the effects of the trypanosomatid on the vector.
METHODS: The development of the different stages of *T. cruzi* in different regions of the intestinal tract of *Triatoma infestans* and the effect of starvation and blood ingestion is compared. Effects of the trypanosomatid are also considered.
RESULTS: In the stomach of the vector, the ingested blood trypomastigotes immediately are aggregated and develop to spheromastigotes and epimastigotes. Some strains are not agglutinated but lysed and are unable to establish. Blood ingestion and starvation directly affect the composition of the populations. Engorgement induces diuresis, and peptides in the urine induce metacyclogenesis. During starvation more spheromastigotes develop. After feeding of such long-term starved bugs, giant cells appear. Feeding also affects the symbionts, mainly developing in the cardia and stomach, and the strong multiplication as mycel form correlates with a strong increase of antibacterial activity. The *T. cruzi* can be classified as subpathogenic, i.e. in regularly fed bugs developmental times of larvae and mortality rates are not affected. However, starvation resistance and the concentrations of free amino acids and proteins in the rectum are reduced. The intestinal immune system strongly reacts to *T. cruzi* in the ingested blood. The expression of genes encoding lysozymes, defensins and nitric oxide synthase are up-regulated, but also of the protease cathepsin D. In old infections, *T. cruzi* suppresses the intestinal immunity as indicated by the development of otherwise not occurring bacteria and fungi.
CONCLUSIONS: The complex interaction of *T. cruzi* and triatomines depends on the respective trypanosomatid-vector combination, includes interactions with the symbionts and other bacteria and results in a subpathogenicity of the flagellate for the vector.

First L braziliensis meglumine gel animal efficacy data

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BACKGROUND: Meglumine antimoniate (MA) (Glucantime®) is a pentavalent antimonial, which remains one of the recommended 1st line treatments for cutaneous Leishmaniasis[1]. Pentavalent antimonials are only available as parenteral formulations, most frequently administered intramuscularly or intralesionally, to the discomfort of the patients, many of whom are children. The availability of a topical formulation of MA could obviate the need for parenteral treatment in many instances, and thus improve the comfort and acceptability of treatment for patients. In 2011, the Access to Medicines department of Sanofi initiated a topical MA development programme with formulation and pre-clinical development being carried out in Latin America, in collaboration with Sanofi R&D in France. Previous studies in the BALB/C mouse infected ear model, have demonstrated the efficacy of the gel, in *L amazonensis* infection.
METHODS: The study was performed on Golden hamsters (*Mesocricetus auratus*) infected dorsum model. The hamsters were infected with *Leishmania braziliensis* (strain M/HOMCO/UA) transfected with green fluorescence protein (GFP). 8 weeks post infection, treatment was commenced for 30 days in 5 groups of hamsters. 2 MA gel formulations, one of which was paraben free, and their placebos, were applied daily (20 mg per application), and a 5th group received MA 200 µg twice weekly intralesionally (IL group). Lesion size was measured every 2 weeks by calipers (mm2). The animals were followed for 60 days post treatment, before being sacrificed, for measurement of parasite loads animals by real time PCR and limiting dilution assay (LDA).
RESULTS: The cure rate at 60 days post end of treatment was 50 % in Paraben free MA gel fgroup, 37.5% in the Paraben containing MA gel group, and 37.5 % in the IL meglumine group. The paraben containing MA gel group was not significantly superior to the placebo gels. Lower parasite loads were seen in the paraben free MA gel and IL groups. Tolerability of all the gels, and the IL meglumine was good.
CONCLUSIONS: The two MA gels tested in this study showed differing efficacy even though they contained similar quantities of active substance. Increased duration and frequency of treatment are potential avenues of exploration to improve the efficacy of MA gel therapy.

Molecular Pathogenesis of Amebiasis,

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Entamoeba histolytica is an obligate parasite of the large intestine and a common infection in infants in low-income countries. *E. histolytica* infection in most children does not cause symptoms, but in minority can invade the intestine causing severe or even lethal infection. The origins, evolutionary selection, and regulation of amoebic virulence are complex. Amebae deplete the host mucosal barrier, adhere to the colonic lumen, trogocytose and kill epithelial cells, and invade through the colonic epithelium. Parasite damage results in colitis and, in some cases, extraintestinal dissemination. Both host and parasite genotypes influence the course of infection, as do the regulatory responses they govern at the host-pathogen interface. Here we highlight research that illuminates novel links between host, parasite, and environmental factors in the regulation of *E. histolytica* virulence.

Trogocytosis-blocking Vaccine for Amebiasis.

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Entamoeba histolytica is an enteric protozoan that is the cause of amebic colitis, diarrhea and liver abscess. The parasite adheres to the intestinal epithelium and initiates trogocytosis via a parasite surface Gal/GalNAc adherence lectin. There is no vaccine for amebiasis, but studies in children have identified an adherence-inhibitory mucosal IgA anti-Gal/GalNAc adherence lectin response with protection from reinfection. Our efforts to develop a vaccine against this pathogen have focused on an internal 578 amino acid fragment, designated LecA, located within the cysteine-rich region of the heavy chain subunit because: (i) it is a major target of adherence-blocking antibodies of seropositive individuals, (ii) vaccination with his-tagged LecA provides protection in animal models and (iii) its sequence is conserved in every isolate tested. We developed a purification process for preparing highly purified non-tagged LecA using a codon-optimized gene expressed in *Escherichia coli*. Immunization with LecA in an liposome adjuvant formulation with GLA and 3M052 provided protection from an intracecal challenge with *E. histolytica* trophozoites.

Towards a Multi-Antigen Multi-Stage Malaria Vaccine

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A highly effective malaria vaccine is a major goal of global health research and will likely require a multi-stage product. Oxford researchers are developing the concept of a highly effective multi-stage *P. falciparum* vaccine to the point of proof-of-concept phase II testing in Europe, prior to trials in malaria-endemic areas.
Remarkable recent advances in vaccine design for all four stages of the *P. falciparum* parasite's life-cycle allow testing of a multi-stage multi-component vaccine for the first time, with strong chances of success. These advances are i) the availability of a new vectored prime-boost vaccination regime based on the chimpanzee adenovirus technology that has been found to induce exceptionally potent $CD8^+$ T cell responses and high titre antibodies against multiple malaria antigens; ii) the development of an improved version of the leading partially protective RTS,S sporozoite vaccine candidate, termed R21, that lacks the excess of HBsAg in RTS,S; iii) the identification, using a vector technology screen, of the blood-stage antigen RH5 as the first antigen to induce potent strain-transcending neutralisation of blood-stage parasites in *in vitro* growth inhibition assays; and iv) the demonstration that antibodies against a mosquito-stage antigens that induce 100% transmission blocking against field isolates of *P. falciparum* in Africa are inducible by a new nanoparticle vaccine candidate.
We are aiming to undertake phase I / II clinical trials to assess the pre-erythrocytic, blood-stage and mosquito-stage components individually, and then together, using state-of-the art immunomonitoring, key functional assays of vaccine-induced immunogenicity, and sporozoite and blood-stage parasite challenges to measure efficacy prior to field testing. A viral vectored prime-boost regime has recently shown high efficacy against malaria infection in East Africa and the first combination trial of RTS,S/AS01 with these vectors is underway.
The prospects for achieving high efficacy with such combination approaches now appear very good.

Differential protein expression in stomach of *Meccus pallidipennis* infected with *Trypanosoma cruzi*

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BACKGROUND: Chagas disease is a public health problem in Latin America. Triatomines, a family of Reduviidae are strict hematophagous and are potential vectors for the Chagas disease parasite *Trypanosoma cruzi.* Triatomine bugs digestive system is divided in anterior midgut (stomach), posterior midgut and the rectum.The stomach of triatomines is essential for *T. cruzi* biological cycle. In this organ blood trypomastigote differentiates into epimastigote. In the stomach, parasites are exposed to components such as digestive enzymes, defensins and agglutinins. Differential protein expression was analyzed in the stomach of *Meccus pallidipennis* infected with *T. cruzi*.
METHODS: *M. pallidipennis* females were used to obtain the stomach at 24 h and 3, 5, 7 days after feeding. The experimental group was fed with blood of BALB/c infected with *T. cruzi* (Ninoa strain). Protein extracts from *M. pallidipennis* stomachs was analyzed by SDS-PAGE.
RESULTS: It was evident that under *T. cruzi* infection a group of proteins of approximately 123.7, 145.1, 162.6, and 178.1 kDa are not expressed relative to the control group.
CONCLUSIONS: Electrophoresis profiles showed that at day 3 and 5 post-infection there are obvious changes in the expression profile of proteins in the stomach of *M. pallidipennis*.

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, helminthiasis and HIV infections are major public health diseases in sub-Saharan region of Africa particularly among pregnant women with a staggering statistics of iron deficiency also reported among them. This study aimed at evaluating the prevalence and association of anaemia and iron status with these infections.
METHODS: Three hundred and twenty-three pregnant women were recruited from a secondary healthcare facility. Blood samples were obtained for haematocrit determination, malaria microscopy while serum samples were used for ferritin and iron level estimation using ELISA technique and Atomic Absorption Spectrophotometry respectively. Kato-Katz method was used for quantification of helminth ova in stool samples collected. Data was analysed using SPSS version 16.0.
RESULTS: 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/helminthiasis, malaria/HIV and helminthiasis/HIV co-infections respectively. Preliminary results showed that 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 (p=0.000), malaria (p=0.000) and malaria/HIV (p=0.000) infections relative to those negative for the three infections. Coinfection of malaria and HIV caused a significantly lower haematocrit value compared to helminth only (p=0.022). Also, women positive for malaria only or coinfected with helminth had higher ferritin levels compared to those with no infections. No observable difference was detected in serum iron levels among the groups.
CONCLUSIONS: Despite a lot of interventions in Nigeria, the burden of malaria either singly or in coinfection with helminth or HIV is still relatively high thus aggravating the anaemic tendencies in pregnancy. The differences in iron status based on infection are still further investigated.

Eco-Epidemiological aspects of rickettsiosis in Brazil

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In the last thirteen years the number of *Rickettsia* genus organisms described in Brazil increased from just one (*Rickettsia rickettsii*) to seven (*R. rickettsii*, *R. bellii*, *R. parkeri*, *R. amblyommii*, *R. felis*, *R. rhipicephali* and R. *monteiroi*). Others (new?) species are being reported without confirmation yet. Before that, the reports of human clinical cases were very sporadic and rare, with, in the case of Minas Gerais state, a silence period of around 40 years without any register. Actually, many groups in Brazil are investigating wild and domestic animals (potential reservoirs and amplified hosts), tick and fleas vectors, ecological and epidemiological conditions, risk factors and host-parasites interactions, among other questions. On the species reported,it has been observed different levels of pathogenesis and distinct ecological conditions involving several vertebrates (wild and domestic) and invertebrates (ticks and fleas). Anthropogenic actions are very marked and associated to the risk and/or exposure, being it the major epidemiological condition observed in urban or periurban areas in Brazil. *Amblyomma aureolatum*, *A. brasiliensis*, *A. cajennense*, *A. dubitatum*, *A. incisum*, *A. longirostre*, *A. nodosum*, *A. ovale*, *A. parkeri*, *A. triste*, *Haemaphysalis juxtakochi* and *Rhipicephalus sanguineus* are being described parasiting/involved in sylvatic and/or domestic cycles with birds, capybaras, small rodents, marsupials, dogs and/or horses, among others. Some of these *Rickettsia* species are, until now, considered as apathogenic or without known pathogenicity, being reported in isolated cases parasiting tick vectors or detected by serological response circulating among vertebrates. Despite that, disregarding the pathogenicity or level of pathogenicity of *Rickettsia* species, from 2001 when just five states in the Southeast region of the country reported human cases of rickettsiosis, nowadays we have notifications in all geographical regions. In Brazil this event is of obligatory notification, with an integrated web of public reference laboratories giving the support to (new) focus.

Malaria-Cutaneous Leishmaniasis coinfection in mice: influence on disease outcomes

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BACKGROUND: Malaria and Cutaneous Leishmaniasis are coendemic in several tropical regions of the world. Although there are no reports of *Plasmodium* and dermotropic *Leishmania* coinfection in humans, this possibility cannot be ruled out. Therefore, it is extremely important to study the possible changes in clinical progression and the balance between immune response in coinfections.
METHODS: Firstly, BALB/C mice were infected intradermaly in the ears with *L. braziliensis (Lb)* or *L. amazonensis (La)* ($1x10^5$ and $1x10^4$ promastigote, respectively). Nonlethal *Plasmodium yoelli* 17XNL (Py) infection (intraperitoneal inoculum: $1x10^6$ infected erythrocytes) was performed 3 days later. *Py* infections were monitored through blood samples stained with Giemsa. *Leishmania* lesion sizes were monitored weekly with a digital caliper and parasite loads were determined using a quantitative limiting-dilution assay.
RESULTS: The development of lesions in coinfected mice was delayed in comparison to those infected with *Lb* or *La* alone. It was also observed a decrease in the number of ulcerated lesions and a transient reduction in the load of *Leishmania* spp. parasites in earrings during patent malaria parasitaemia in coinfected groups. *Py* infection was similar in malaria and coinfected mice, except for the mean peak parasitaemia that was higher in malaria than in coinfected groups. In coinfection, we observed that, depending on the species of *Leishmania*, the course of malaria disease was altered in a different way. It seems that coinfection with *Lb* causes beneficial effect while coinfection with *La* increases gravity leading some animals to death.
CONCLUSIONS: Our data suggest that coinfection with *Py* and *Lb* or *La* may mutually affect pathogenesis and outcome of malaria and cutaneous leishmaniasis. Concurrent infections most likely modulate the host immune responses to each single parasite. Ongoing studies in our laboratory will further investigate the impact of coinfection on immune responses.

Development and analytical validation of a new kit prototype for molecular diagnosis of congenital Chagas disease

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BACKGROUND: Every year around 15000 babies are born infected with *Trypanosoma cruzi* remaining high percentage of them undiagnosed until chronic disease symptoms appears. Therefore, early diagnosis is a priority in management of vertical transmission. Accordingly, we developed and validated a kit prototype based on Real-Time PCR intended for molecular diagnosis of congenital Chagas disease.
METHODS: Methodology included DNA extraction from 1 mL of blood treated with a DNA stabilizer using magnetic beads and glass fiber columns and a multiplex Real-Time PCR based on TaqMan technology, aiming to amplify *T. cruzi* DNA and an internal amplification control simultaneously. Primers and probes sequences were designed against conservative regions within satellite repeats of all parasite DTUs.
RESULTS: The prototype was selected based on the combination of primers, probes, master mixes and UDGs that reached the best analytical sensitivity (0.2 parasite equivalents/mL). Specificity was 100% in seronegative panels and DNA from *T. rangeli* and *Leishmania* sp. Finally, the prototype was validated following CCLS guidelines and field validation for early diagnosis of congenital Chagas disease is currently undergone in mother-newborns from health centers of endemic argentinean regions.
CONCLUSIONS: The performing parameters of this prototype encourage its application to early assessment of *T. cruzi* infection in other scenarios such as oral transmission, organs transplantation from seropositive donors and monitoring patients under trypanocidal treatment.

Cryptic genetic exchange in *Trypanosoma cruzi* and *Giardia duodenalis*

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BACKGROUND: Giardiasis and Chagas disease represent serious human public health problems due to their ubiquitous geographical distribution and remarkable capacity to cause disease in people. Genetic exchange has increasingly been demonstrated as an important biologic trait in the evolution and acquisition of virulence potential and the biological success of protozoan parasites like *Toxoplasma* and *Plasmodium*. The aim of this work is to develop and apply Multilocus Sequence Typing schemes to conduct population genetic analyses in *Giardia* and *Trypanosoma cruzi* parasites with an holistic approach to understanding the phylogenomics of these parasites.
METHODS: We applied a total of 30 MLST markers on 150 *T. cruzi* isolates to understand the population genetics of *T. cruzi* based on previous reports. We identified and developed Ten genetic loci, two for each of the 5 chromosomes, to assess intra- and inter-specific population genetic diversity among 32 A and B assemblage isolates.
RESULTS: After analyzing MLST markers on *T. cruzi* isolates, putative events of recombination and an intriguing lack of linkage disequilibrium were detected. When analyzed using the 10 MLST genetic markers for *Giardia*, 24 distinct multi-locus genotypes were resolved. At any given locus, examples of both inter- and intra-specific recombination, and supported a high level of genetic exchange. The data is consistent with the majority of isolates existing as intra-assemblage admixtures of the three (A) or two (B) allelic types. Importantly, 4 isolates (12%) that were inter-assemblage recombinants based on the inheritance of alleles across the 10 loci were identified.
CONCLUSIONS: These findings demonstrate the high frequency of recombination at nuclear level across natural populations of *T. cruzi*. In the case of *Giardia*, the data support a model whereby genetic exchange is frequent among natural *Giardia* isolates.

Chances for a Biological Control of *Trypanosoma cruzi* within the Vector or of the Vectors?

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BACKGROUND: The use of insecticides for the control of triatomines is very successful in domiciliary populations, e.g. *Triatoma infestans* in the Southern Cone Countries and *Rhodnius prolixus* in Central America. Problems are the toxicity to humans (inhabitants and sprayers) and animals, the short period of action time by the insecticide, inactivation on adobe-walls, the development of insecticide resistances and the invasion of houses by sylvatic/peridomestic populations/species after successful campaigns. Therefore, an integral approach is required, i.e. a strong reduction by insecticides and then the use of biological control approaches.
METHODS: Approaches to control *T. cruzi* within the vector focus on transformed symbionts. A biological control by predators, parasitoids, the insect-pathogenic trypanosomatid, *Blastocrithidia triatomae*, fungi and viruses is reviewed.
RESULTS: Transformed symbionts of *R. prolixus* and *T. infestans*, which produce a part of an antibody against components of the trypanosomatid or cecropin A, an antibacterial compound of the innate immune system of a Lepidoptera, kill *T. cruzi* within the vector. Also the bacterium *Serratia marcescens* kills *T. cruzi* in the vector, but some strains are human-pathogenic. Vertebrate predators are not effective, because they are not night-active or not present in the microhabitat. Using invertebrate predators and parasitoids, the large scale rearing is cost intensive. *B. triatomae* affects immune responses, digestion, excretion, ecdysis, developmental times and mortality rates of nymphs and reproduction of adults. Investigations of viruses and fungi are in progress.
CONCLUSIONS: Most promising is the approach using transformed symbionts, but it includes the controversially discussed introduction of non-bacterial genes via bacteria into the environment. A biological control of the vector via viruses, fungi and protozoan parasites requires more basic research.

Synthetic alpha-galactosyl-containing neoglycoproteins of *Trypanosoma cruzi* as biomarkers for the follow-up of Chagas disease chemotherapy

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BACKGROUND: Chagas disease (CD) is a debilitating, frequently fatal neglected tropical infection caused by the protozoan parasite, *Trypanosoma cruzi.* Millions of individuals are chronically infected in Latin America, representing a substantial social and economic burden. Owing to extensive global movements, CD now affects thousands of people in nonendemic countries. Although considered partially effective, chemotherapy of chronic patients seems to be more successful and beneficial than previously assumed, based on recent clinical and immunological studies. A major outstanding issue, however, has been the scarcity of dependable and sensible molecular tools or biomarkers to assess the effectiveness of chemotherapy in both acute and chronic patients. Protective lytic antibodies have been used as markers of active *T. cruzi* infection. These antibodies have decreased titers following chemotherapy, at the same time that cross-reactive antibodies to the noninfective epimastigote forms are still detectable in high titers. The majority of lytic antibodies in Chagasic patients are produced against the highly immunogenic, immunodominant, and conserved α-galactopyranosyl (α-Gal) epitopes, abundantly found on glycoproteins of the plasma membrane of infective trypomastigote stages. Very high levels of lytic, protective anti-α-Gal Abs are observed in both acute and chronic phases of CD.
METHODS: A synthetic glycoarray containing non-reducing α-galactopyranosyl moieties related to mucin O-glycans of *Trypanosoma cruzi* was evaluated by chemiluminescent enzyme-linked immunosorbent assay with sera from patients with chronic CD and healthy individuals.
RESULTS: Our data clearly revealed the immunodominance of the glycotope Galα(1,3)Galβ, which was strongly recognized by sera from Chagasic individuals but weakly by sera from healthy individuals.
CONCLUSIONS: Fully synthetic, structurally defined Galα(1,3)Galβ-containing NGPs could be eventually useful for the reliable follow-up of CD chemotherapy.

Dipeptidylcarboxypeptidase of *Leishmania donovani*: A novel drug target

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Leishmaniasis imposes a substantial burden of mortality and morbidity affecting 12 million people globally and continues to be a neglected tropical disease. Control of the disease is mainly based on chemotherapy, which relies on a handful of drugs with serious limitations such as high cost, toxicity and lack of efficacy in endemic regions. Therefore, development of new, effective and affordable anti-leishmanial drugs is a global health priority. Over the last decade, target based drug discovery is being employed rather than the random screening of compounds. Leishmanial Dipeptidylcarboxypeptidase (DCP), an angiotensin converting enzyme (ACE) related metallopeptidase has been recently identified and characterized. Significant differences between Leishmanial dipeptidylcarboxypeptidase (LdDCP) and mammalian enzyme have been observed. Further, a correlation between inhibition of LdDCP enzyme activity and parasite multiplication strongly suggest the potential of leishmanial DCP as a novel drug target for antileishmanial drug discovery. Four compounds, belonging to two chemical classes, were identified as the selective inhibitor of LdDCP. These compounds also exhibited both *in vitro* and *in vivo* antileishmanial activity. The data suggests that these compounds provide leads to be optimized into candidates to treat these protozoan infections.

Plasmodium senses the environment. Is it a matter of survival?

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BACKGROUND: Malaria is a burden that affects roughly 500 million people each year. The complexity of parasite biology and its multiple forms represents a challenge to our understanding of its development. From the work of several labs, it is now accepted that *Plasmodium* senses the environment and exploits signaling pathways to modulate cellular functions.
METHODS: we have used a synthetic, codon-optimized form of *Plasmodium* genes involved in signaling to express malarial protein in the HEK293 or PC12 mammalian cell lines and primary hepatocytes, in order to test whether malarial proteins can couple with mammalian signaling machinery. Endogenous gene expression was also knocked down using RNAi methodology to isolate the effect of the expression of the heterologous gene. By performing time-lapse confocal microscopy, we observed agonist-induced calcium signals. We also performed experiments using a permeable caged IP_3, upon 2-photon encaging, to test more directly the role of IP_3 at host cells as well as to investigate its ability to elicit calcium in *P. falciparum*-infected RBC.
RESULTS: In the mammalian cells we managed to express PfSR10 or PfRack with a FLAG tag in HEK293T and HEK293cells, respectively. Expression in these cells shows that PfSR10 partially colocalizes with the plasma membrane while PfRack at the cytosol. Western blot using anti-FLAG antibodies detected PfPAT in HEK293T at the predicted molecular weight of ~80 kDa. The ~80 kDa band was clearly detected in immunoprecipitated fraction of HEK293T cells, both with anti-FLAG and anti- PfSR10 antibodies. The phosphorylation profile of HEK293T cells was altered upon co-transfection of PfSR10 as assessed by anti-phosphoserine and anti-phosphotyrosine antibodies.
CONCLUSIONS: Our data demonstrate that PfRack is able to interfere with the host's signaling machinery, specifically with IP3 receptors. Our data suggest that PfSR10 potentially exerts a function in *Plasmodium falciparum* intraerythrocytic cycle.

A cAMP function on the modulation of *Plasmodium falciparum* transcription factor, PfNF-YB.

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BACKGROUND: Calcium is thought to play a role in the malaria parasite cell cycle through the activation of kinases and proteases. Interplay among calcium and cAMP is a widespread circuit for signalling and is the molecular base for melatonin action in *P. falciparum*. Pf NF-YB belongs to CBF family of transcription factors that are involved in the regulation of cell cycle of many eukaryotic genes. The aim of this work was to investigate the role of calcium and cAMP in the control Pf NF-YB expression and function in *Plasmodium falciparum*.
METHODS In order to determine the protein and mRNA expression levels of intra-erythrocytic PfNF-YB, western-blot and qRT-PCR were performed. To localize the PfNF-YB protein distribution in the parasite, confocal microscopy and subcellular fractionation were carried out.. The effect of second messengers on PfNF-YB gene and protein expression during intra-erythrocytic development of the malaria parasites was followed by qRT-PCR and western blot analysis.
RESULTS: PfNF-YB is expressed throughout the intra-erythrocytic stages but is greatly detectable at the schizont stage at both the mRNA and protein levels.. PfNF-YB transcription and protein levels are down regulated in ring stage in presence of cAMP analog as well, and conversely, is up-regulated in throphozoite stage. To determine if PfNF-YB is able to binding into CCAAT motif in promoter region was performed chromatin immunoprecipitation and further ChIP-on-chip assay.. The chromatin immunoprecipitation indicated that CBF protein can bind to distal promoter regions of glycogen synthase kinase 3 (PFC0525c), transcription factor with AP2 domain (PFI1665w), and transcriptional regulatory protein sir2b (PF14_0489) genes.
CONCLUSIONS: Our data show that the role of second messengers signaling on modulation of the human malarial PfNF-YB transcription factor is distinct during the stages of parasite development. The high throughput analysis has been carried out by ChiP-on-chip assay showing 160 genes are NF-YB targets.