Aedes aegypti mosquito at university: should we be worried?

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BACKGROUND: *Aedes aegypti*, the meaning vector of Dengue virus, has been infecting thousands people annually. Especially in Brazil, many dengue fever cases are reported every year. The National Program of Dengue (PNCD) in Brazil, nowadays, is using insecticide *diflubenzuron* to control vectors populations. However, this program has a reduced efficiency, and mosquitoes are reported in all states. Thus, this study aimed at registering and monitoring the presence of *A. aegypti* at Faculdade Frassinetti do Recife, one of the most important colleges placed in Recife, Pernambuco State, Brazil, as well as investigating the influence of temperature and rainfall on its temporal distribution and egg densities.
METHODS: The study was performed from May/2013 to October/2013. Ovitraps with 30% of hay infusion and a wood paddle as an oviposition site were used for surveillance in each of the 50 points distributed throughout the college . Twelve epidemiological weeks were analyzed. After egg counting, Egg Density Index (EDI), Positive Ovitraps Index (POI), and Mean Number of Eggs (MNE) were used for data analysis.
RESULTS: The presence of *A. aegypti's* eggs, identified through POI, was registered throughout the study period in all epidemiological weeks, showed variation between 27,9% to 53,3%. The EDI registered high densities with variation among 63 to 143 eggs/epidemiological week. The NMO showed important values that were among 20 to 77 eggs/ovitrap. No significant relationships were observed between rainfall and temperature with entomological indexes values.
CONCLUSION: Once that we observed the constant presence of *A. aegypti* at FAFIRE, it reinforces the need for effective actions to control this insect at universities, especially at FAFIRE, once this college serves over 1,500 people daily. In addition, our data pointed the inefficiency of the PNCD in Brazil, and reinforce the importance of the implementation of new vector control strategies.

Tracking *Toxoplasma gondii* in freshwater ecosystems: interaction of the parasite with the exotic mustelid American mink (*Neovison vison*) in Spain.

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BACKGROUND: *Toxoplasma gondii* is a zoonotic protozoan that causes serious illness in humans and infects animals worldwide. Felids are the definitive hosts, excreting oocysts in faeces to the environment. Several authors have suggested the important role of water-borne transmission of the parasite. The objective of the present study was to analyze the seroprevalence of *T. gondii* in American minks (*Neovison vison*), a widely distributed invasive species living in freshwater ecosystems in Spain.
METHODS: Serum samples were collected from 526 American minks from Northern Spain from 2011 to 2014. Antibodies to *T. gondii* were assayed by the modified agglutination test (MAT titres ≥1:25).
RESULTS: Antibodies were found in 409 (77.76%) American minks. No significant differences were found among geographical locations (Catalonia (72.00%), La-Rioja (85.71%) and Castilla-León (77.82%)). CONCLUSIONS: This study shows high and widespread natural exposure of American minks to *T. gondii* in freshwater habitats in Spain.
CONCLUSIONS: Water-borne transmission of oocysts may be an important mode of transmission for American minks, which could be a sentinel species for *T. gondii* contamination in fresh water aquatic habitats.

Regulation of gene expression in *Entamoeba histolytica*

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BACKGROUND: *Entamoeba histolytica* trophozoites display different virulence degree under diverse environmental conditions, suggesting that changes in gene expression may be involved in this behavior. In addition, the life cycle of this parasite involving the reversible conversion of cysts to trophozoites is expected to be due to differential expression of genes. However, the molecular mechanisms involved in gene expression in this microorganism are poorly comprehended. In this study we analyzed the transcription regulation in *E. histolytica* by a transcription factor (URE1-BP). We also investigate the presence of protein arginine methyltransferases (PRMTs) that could have a role in the epigenetic mechanisms of gene regulation in this parasite.
METHODS: We obtained the URE1-BP recombinant protein and antibodies against it. By electrophoretic mobility shift assay and super-gel shift analysis we evaluated the ability of the recombinant and native protein to bind to the URE1 motif. By confocal microscopy we analyzed the localization of URE1-BP in trophozoites. Pulldown assays were used to obtain proteins that interact with URE1-BP. Genes encoding putative EhPRMTs were identified by BLAST and the activity of one recombinant EhPRMT was determined by the incorporation of the [^{3}H]methyl group to histones.
RESULTS: Electrophoretic mobility shift assay and super-gel shift analysis confirmed that URE1-BP binds to the URE1 motif. URE1-BP was located in nucleus and cytoplasm and its mobilization is involved in the transcription regulation of the EhrabB gene. Pulldown assays revealed that URE1-BP is a multifunctional protein that associates with proteins involved in several cellular pathways. We identified four putative EhPRMTs related to PRMT1 and PRMT5. One EhPRMT recombinant protein showed methyltransferase activity on histones.
CONCLUSIONS: Our results suggest that URE1-BP is a multifunctional protein involved in gene transcription and in other cellular pathways. In addition; *E. histolytica* contains PRMTs that could be involved in the epigenetic mechanisms that control gene expression.

Conventional microscopy and molecular tests for diagnosis of Giardiasis: a comparative analysis

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BACKGROUND: Despite all advances in the development of molecular tests, the conventional microscopy is still considered the reference test for diagnosing of giardiasis, a disease caused by *Giardia* spp, a protozoan commonly infecting humans and animals. However, some limiting aspects such as low parasite load, mixed infections, and others related to the quality of the samples, should be considered as factors hampering the diagnosis by of this methodology. New researches have been conducted in order to develop and to standardize new parasitological techniques with greater sensitivity. In this context, Polymerase Chain Reaction (PCR) has been widely applied in many research centers, since it is a method based on amplification of genetic material of parasite, also becoming possible subtyping different assemblages of *Giardia* spp. This study aimed to evaluate the concordance of results obtained by PCR and conventional microscopy for giardiasis diagnosis in faecal samples.
METHODS: 145 human faecal samples were collected and further analyzed by conventional microscopy after the zinc sulfate centrifugal flotation technique of Faust; and also by detection using PCR technique. The obtained results were compared and expressed in percentage of positivity.
RESULTS: By using of conventional microscopy, 60 samples showed positivity for *Giardia duodenalis,* while PCR technique was able to detect positivity in only 35 samples. In this study, PCR efficiency was 60% lower compared to microscopy.
CONCLUSIONS: It was possible to conclude that molecular tests continue to show high false negative rate compared to conventional microscopy. One hypothesis to explain these results may be the possible small amount of target DNA combined with low efficiency in the DNA extraction procedures, also the presence of PCR inhibitors. This result emphases the use of microscopy as the preferential method for laboratory diagnosing of giardiasis.Financial support: CNPq (Processo 476396/2011-5)
Keywords: Microscopy, PCR, Giardiasis.

Experiences in genetic resistance to gastrointestinal nematodes in hair sheep in tropical conditions of Mexico

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BACKGROUND: The selection of lambs genetically resistant to gastrointestinal nematodes (GIN) has being proposed. The identification and selective breeding of animals with the highest genetic resistance against GIN is a promising alternative. Such method has the advantage of providing a long-term solution that can be easily disseminated through the use of resistant rams.
METHODS: Faecal samples are taken to determinate faecal egg counts (FEC) by the McMaster technique. Blood samples are collected and the packed cell volume (PCV). The average daily weigh gain (ADWG) was recorded. Nematodes are recovered at necropsy. According to the average level of egg faecal elimination (EPG), lambs are categorized either as resistant or susceptible to GIN, considering two standard errors.
RESULTS: A high increase in the FEC occurred 35 days after the first infection. The FEC remained high until day 56 and decreased with the time. In the reinfection high EPG was recorded 35 and 42 days post-infection (PI). PCV decreased until day 35 PI (26.0 ± 4.2) in the first challenge. The FEC and PCV changed according to the type of lamb in both infections ($P<0.01$). The lambs identified as resistant showed the lowest FEC and the highest PCV. The adult nematode counts were highly variable among lambs and lamb type. The number of nematodes was a decisive factor to determine the acquired resistance.
CONCLUSION: The lowest EPG values indicated the highest acquired resistance during the re-infection, which was observed when the lambs received a previous infection; although, this depends on the natural resistance in the host.

Optimizing the use of *Duddingtonia flagrans* chlamydospores for controlling sheep parasitic nematodes

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BACKGROUND: The use of *Duddingtonia flagrans* is an alternative method of control of gastrointestinal nematodes (GIN) of sheep. This method is based on the intake of chlamydospores (spores of resistance) by the animals. Once spores are spelled with the faeces, fungi develop traps where GIN larvae are caught and killed. This process depends on factors such as spores dosage and periods of spore intake. Knowing the amount of spores into faeces after been consumed by the animals is important to establish the correct dose to be offered to the animals. The use of McMaster technique allows to visualize and to count faecal spores. After oral intake of spores only 10% survive after passing through the gastrointestinal tract (GIT) of the animals. The rest of spores are digested into the GIT. Establishing the best spore oral dose to get the best proportion of chlamydospores excreted with respect to the number of eggs per gram of faeces is the challenge. This methodology helps to achieve a more efficient use of fungal spores. The optimums fungal spore doses will depend on the degree of parasitism or adjusting the dose considering the number of faecal eggs.
CONCLUSIONS: The fungus *D. flagrans* is a useful tool of GIN control. Quantifying fungal spores in faeces is very complicated. However, the use of the McMaster´s chamber technique can be used to estimate the number of spores expelled by animals into faeces. This information can help to adjust fungal doses to get the best results in reducing the GIN nematode larvae in faeces without wasting chlamydospores.

SERO-EPIDEMIOLOGICAL SURVEY OF TOXOPLASMOSIS IN CATTLE, SHEEP AND GOATS IN PROVINCE OF M'SILA (ALGERIA)

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BACKGROUND: Described for the first time in Tunisia, toxoplasmosis is a cosmopolitan parasitic zoonosis caused by a protozoan. It occurs in most warm-blooded animals and causes abortions in pregnant females, a high rate of mortality among infants and young. By its medical importance, health and economic toxoplasmosis is not only a hindrance to the intensification of our meat production but also a real danger to man and his environment.

METHODS: Prevalence of toxoplasmosis in *Toxoplasma gondii* in 1500 cattles, 7000 sheeps and 3500 goats was carried out using serological methods (MAT and ELISA) in the province of M'sila situated in the East of Algeria.

RESULTS: In the sample studied, the overall serological prevalence was 24.83 % for sheep, 14.17% in cattle and 7.2% in goats. Region M'cif was the most affected area with a rate of 61%. Seroprevalence found in females was 29.7 % for sheep, cattle 14.7% and 7.1 % in goats. Among males, the prevalence found was 20.3 % for sheep, 13% in cattle and 7% in goats.

CONCLUSIONS: In conclusion , animal toxoplasmosis is strongly present in the province of M'sila mainly M'cif area known for its large herds of sheep, cattle and goats for human consumption; highlighting the importance of the implementation of preventive measures in order to reduce zoonotic infection by T. gondii.

Forecasting the impact of alternative multi-parasite control strategies on the geographical distribution of soil-transmitted helminths in The Philippines.

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BACKGROUND: Soil-transmitted helminth (STH) infections with *A. lumbricoides, T. trichiura* and hookworm are endemic in all 80 provinces of the Philippines. In order to increase the efficient allocation of parasitic disease control resources in the country, we aimed to predict for the first time the spatial distribution in the prevalence of *A. lumbricoides*, *T. trichiura* and hookworm across the Philippines; quantify the association between the physical environment and spatial variation of STH infection and develop predictive risk maps for each infection; and forecast the impact of alternative multi-parasite control strategies.
METHODS: Data on STH infection from 35,573 individuals across the country were geolocated at the barangay level and included in the analysis. Bayesian geostatistical models of STH prevalence were developed, including age and sex of individuals and environmental variables (rainfall, land surface temperature and distance to inland water bodies) as predictors, and diagnostic uncertainty was incorporated. Predicted STH maps were integrated with age-structured metapopulation models of STH transmission to assess the impact of alternative interventions on disease prevalence. Forecasted STH prevalence following simulated control was interpolated using Bayesian geostatistical models.
RESULTS: The role of environmental variables was different between regions of the Philippines. This analysis revealed that while *A. lumbricoides* and *T. trichiura* infections were widespread and highly endemic, hookworm infections were more circumscribed to smaller foci in the Visayas and Mindanao. Metapopulation models of STH control demonstrated a substantial improvement in efficacy with a community-wide drug distribution strategy versus targeting school aged children only.
CONCLUSIONS: Significant spatial variation in STH infection prevalence was demonstrated both within and between provinces of the Philippines. This suggests that a spatially targeted approach to STH interventions, including mass drug administration across whole communities, is warranted.

A bioinformatics-based approach towards mining of highly diverse and essential protease sequences from pathogenic helminth and protozoan parasites for predicting their subcellular localization

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BACKGROUND: Helminthic and protozoal diseases are major public health problems worldwide, affecting millions of people annually. Recent advances in genomics and transcriptomics of several parasites have revealed that proteases play a key role in their survival in the host. This study aimed at mining the highly diverse and essential proteases from these parasites and predicting their subcellular localization, potential B-cell epitopes and 3D structures.
METHODS: Protease sequences of 39 helminth and 19 protozoan genera represented in human and rodent hosts were downloaded from MEROPS-9.8 database, formatted into 'fasta' format and preprocessed by removing sequences having short length and lowly complex regions. Highly diverse and essential proteases were extracted from host's proteases and database of essential genes (DEG) through protein-protein BLAST. PROlocalizer server, BCPREDS, ABCpred and BepiPred tools were used to predict the secretory proteins and potential B-cell epitopes. ModWeb server was implemented to build their 3D structure based on homology modeling.
RESULTS: 3670 protease sequences from 27 helminth and 1936 from 13 protozoan genera were extracted. Of these, 1061 were considered essential and highly diverse- representing 505 Cysteine, 244 Metallo, 135 Aspartic, 73 Serine, 93 Mix and 9 unassigned clans, with 13%, 63%, 67%, 65%, 58%, and 100% proteases of respective clans as predicted secretory proteins; of the latter, 241 sequences were predicted as common B-cell epitopes. Among all 1061 sequences, only 458 3D structures were predicted as reliable by GA431 and zDOPE scores.
CONCLUSIONS: The freely downloadable parasite proteases data and their 3D structures provide a rich resource for future research and analysis.

Putative role of lactobacilli against *Giardia lamblia* infection

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BACKGROUND: *Giardia lamblia* is a protozoan responsible for giardiasis, the most common intestinal parasitic disease in the world. It has been established that some probiotics play a role in protection against this parasite. For instance, *Lactobacillus johnsonii* LA1 (LjLA1) prevents the establishment of *Giardia* in the gerbil. In this study, we tried to figure out the molecular mechanisms involved in this inhibitory effect.
METHODS: The aim of this project is first to establish the putative role of lactobacilli metabolism on *Giardia* growth by hemocytometer and flow cytometry. Secondly, to perform the inactivation and over-expression of candidate genes of LjLA1, putatively mediating a protective role against *Giardia* growth *in vitro*. Finally, we explored the capacity of other lactobacilli (around 30 strains were tested), to also control *Giardia* growth in cultures.
RESULTS: We have cloned several candidate of LjLA1 as His-tag fusions in a heterologous system, and purified them in order to study their biochemical properties. We have characterized their substrate specificities and are currently producing specific antibodies to further study their expression in LjLA1. In parallel, the screening of other lactobacilli strains has allowed us to highlight multiple *L. johnsonii* strains displaying comparable inhibitory effects on *Giardia*.
CONCLUSIONS: The output of this project is to propose new therapeutic strategies against *Giardia*, based on the use of probiotics in order to counteract the emergence of drug resistant *Giardia* strains.

Determination of the anti-malarial activity of *Krameria cystisoides.*

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BACKGROUND: Malaria is an important cause of death and illness in children and adults in tropical countries. Mortality, currently estimated at over a million people per year, has rise in recent years, probably due to increasing resistance to antimalarial medicines. This has led researchers to look for other alternatives, one of which is investigation of plants. In this study we worked with an extract isolated for *Krameria cystisoides*. This compound exhibited good anti-inflammatory activity reducing significantly edema, and demonstrating inhibitory effects against some important infectious organisms.
METHODS: Balb/c mice were used for the experiment. Twenty fourth mice were divided into six groups were inoculated intaperitoneal (i.p.) with 2 x 10^7 parasite of *Plasmodium berghei* at the commencement of the experiment. The 1st group received (i.p.) distilled water as negative control, the 2nd group was the positive control. The 3th group received 5mg chloroquine/kg body weight. While the groups 4-6 received 120, 240 and 480 extract/kg body weight i.p. respectively. Blood slides were from the caudal vein of each mouse, fixed with methanol, stained with Giemsa stain and percentage parasitaemia was evaluated.
RESULTS: The average percentage suppression of parasitaemia was 27.41, 32.50 and 36.17% at dose of 120, 240 and 480mg/kg per day, respectively. Chloroquine at 5mg/kg per day produced 100% chemo-suppression.
CONCLUSIONS: The 480mg/kg extract of *Krameria cystisoides* was found to produce some level of suppression of parasitaemia. The suppression of parasitaemia by chloroquine at 5mg/kg per day cleared all the parasites. Considering good result produced in high concentration could be contemplated.

Eimerian intestinal polyps in lambs – histological and electron- microscopic study

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BACKGROUND: In lambs, infections with *Eimeria faurei* and *E. bakuensis* (*E. ovina*) can cause intestinal polyps. This can be histologically confirmed with the finding of different stages of coccidian-like organisms developing in the epithelial cells of the polyp mucosa, producing a number of papilliferous growths with hyperplastic villi.
METHODS: Our research was carried out on 8 Transylvania Merinos lambs, 40 to 50 days old, 5 of which (group A) were suppressed with IMUPRIN (Remedica Ltd) then inoculated with 150,000 oocysts of *Eimeria* spp., to characterize the effects of this drug on the parasitological profile. The other (n=3) lambs (group B) served as controls (unsuppressed, uninfected).
RESULTS: In group A, lambs which were slaughtered at 4, 6, 9, 11 and 15 days after the infection, showed gross and microscopical lesions of eimeriosis. One of the lambs in the group had a 3 mm diameter polyp on the jejuna mucosa, caused by *E. bakuensis*. Histologically, the polyp was built up of deer-horn-like tubular cell proliferation with different morphological forms of some eimerial species. Electron microscopy showed that the schizont was located in a parasitophorous vacuole, containing several merozoites and granules of amylopectin. Sporozoidal forms were present in the lymphocytes' cytoplasm from the mesenteric lymph nodes.

Analysis of the diagnostic request for cysticercosis in Mexico (1988 to 2013)

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BACKGROUND: Neurocysticercosis (NCC) has significance in the public health administration due to the number of cases that are attending in the third level hospitals (HTN). High economic spend is designated to cabinet and neuroimaging studies, as well as drug and surgical treatments; such fees impact the patient socio-economic style of life since the parasite affect the quality of life and, occasionally lethal prognosis is observed. Diagnosis of NCC is made considering clinical, radiologic, epidemiologic and immunologic criteria. The ELISA and the enzyme-linked immunoelectrotransfer blot assay (EITB), which is considered in the global literature the gold standard for serology. Since the National Institute of Diagnostic and Epidemiological Reference is the leader of the Laboratory Network (LESP) in México, the aim of this work was to determine the seroprevalence in the cysticercosis diagnosis request made from 1988 to 2013

METHODS: Serum and cerebrospinal fluid samples from patients with compatible signs and symptoms of cysticercosis were analyzed to determine the presence of antibodies to *Taenia solium* cysticerci antigens. Samples were submitted by the LESP and the HTN, those submitted between 1988 and 1997 were analyzed by ELISA and, in order to improving the quality of the diagnosis, it has been used the EITB ("home made type") since 1997 until the date,

RESULTS: Immunodiagnosis by ELISA was performed in 4,637 samples (prevalence of 17.1%). A total of 7,798 samples were analyzed by EITB and the prevalence of antibody detection since 1988 until today is 13.6 %.

CONCLUSIONS: Data show that the frequency of positive is higher with ELISA, however it is associated to false positive and negative results. Demand of the diagnosis is decreased in the recent years. Further studies are needed to determine the tangible cysticercosis seroprevalence in Mexico.

Induction of cyclooxygenase-2 (COX-2) and prostaglandin E_2 (PGE_2) via MAPK in macrophages infected with *Leishmania mexicana*.

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BACKGROUND: The regulation in the expression of the *Cox-2* gene and prostaglandin synthesis involves one or more of three major MAPK in infectious processes caused by *Leishmania* parasites. It is suggested that different MAPK activation may influence the survival of the parasite. COX-2 protein catalyzes the conversion of arachidonic acid into prostaglandins, which are involved in inflammatory processes. The expression of COX-2 and the increase in the production of prostaglandin E_2 can be important in modulating parasite infection but the signaling mechanisms that mediate secretion of PGE_2 during *Leishmania* sp infection have been established only for some species. Therefore the aim of this study was to define the MAPKs involvement during *Leishmania mexicana* infection in J774 macrophages.
METHODS: In *L. mexicana* infected macrophages, we analyzed by western blot the activation of ERK 1/2, JNK, and p38. Moreover by using selective inhibitors PD98095, SB203580, and SP600125 we determined the involvement of ERK1/2, p38, and JNK respectively, in the expression of COX -2 protein and PGE_2 synthesis during infection.
RESULTS: We determined the phosphorylation pattern of the three MAPKs in infection kinetics. p38 phosphorylation level returned to baseline over a short period of time, while ERK 1/2 and JNK remain activated until the end of the trial. However, ERK 1/2 and p38 are the major signaling pathways in the induction of COX-2 expression and PGE_2 synthesis in macrophages infected with *L. mexicana*.
CONCLUSIONS: As in other parasites of the genus *Leishmania*, involvement of MAPKs in the induction of COX-2 and PGE_2 synthesis is relevant; particularly for *L. mexicana* infected macrophages, activation of ERK 1/2 and p38 MAPKs is responsible of COX-2 expression and PGE_2 production.

IL-17 Immunolocalization in *Leishmania mexicana* Infected BALB/c Mouse Ears.

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BACKGROUND: Leishmaniasis is an infectious disease caused by *Leishmania*, an intracellular parasite infecting macrophages and other phagocytic cells. It may present three clinical forms: cutaneous leishmaniasis (localized and diffuse), mucocutaneous (MCL) and visceral. Severity depends on the clinical form and the infective *Leishmania* species. IL-17 has been associated with progresion and tissue destruction in murine and human MCL. However, the distribution of IL-17 has not been analyzed in lesions of *Leishmania mexicana* infected mice.
METHODS: We used 15 BALB/c mice divided into 3 groups (basal, inoculated with saline solution and infected with $1x10^5$ *Leishmania mexicana* promastigotes). We analyzed IL-17 distribution in ear tissues on days 0, 1, 7, 14 y 28 post-infection by immunohistochemistry.
RESULTS: At day 1, infected and mice inoculated with saline solution showed similar expression of IL-17 in epithelium, connective tissue and muscle, which was higher than basal controls. Beginning on day 7 post-infection, only infected mice showed a continuos enhancement of IL-17, whereas controls diminished the cytokine expession. At day 28 of infection, samples showed the presence of parasites together with a chronic inflamatory infiltrate associated with elevated levels of IL-17 expression, whereas control mice showed expressions similar to basal tissues.
CONCLUSIONS: IL-17 is distributed in different tissues: epithelium, connective tissue and muscle at very early times both in experimental and control samples, showing high levels of production of this cytokine by a variety of cells, including cells of the innate immune response. After day 7 of infection, when the adaptive immune response has already been activated, the expression of IL-17 augmented together with the inflammatory infiltrate, which continued increasing as the infection became chronic at which point IL-17 also became closely associated to the parasite, suggesting a possible relation between IL-17 production and disease progression in a susceptible mouse model.
PAPIIT IN215212

GP63 IS RESPONSIBLE OF A COX-LIKE ACTIVITY IN *L. MEXICANA* PROMASTIGOTES

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BACKGROUND: During tissue invasion by *Leishmania* parasites, cytokines and other compounds that are released activate PLA_2 to free araquidonic acid, the substrate for Ciclooxygenase (COX) enzymes to produce, among other eicosanoids, PGE_2 that affects immune cells function. Recent work in our laboratory has shown that *L. mexicana* possesses a COX-like activity, suggesting that this enzymatic activity could be involved in the evasion of the immune response. Therefore, the aim of this work was to identify the protein responsible of COX-like activity.
METHODS: Total extract from *L. mexicana* promastigotes was prepared and soluble and membrane fractions were separated. From the soluble fraction, a protein with COX-like activity was enriched by ammonium sulfate fractionation. COX activity was measured using a commercial kit (Enzo Life Sciences Cat. No. 80-0275). The protein with COX activity was immunoprecipitated using a monoclonal antibody (D12) produced in our laboratory; the Ag-Ab complex was analyzed by SDS-PAGE and the protein was sequenced by MALDI-TOF. Glycoprotein (gp) 63 was purified by affinity chromatography with concanavalina A (ConA) from promastigotes of *L. mexicana*.
RESULTS: From the soluble fraction, a protein with COX-like activity was enriched by 40% ammonium sulfate fractionation; this protein was recognized by a polyclonal anti-mouse COX-2 antibody. Moreover, the protein with COX activity was immunoprecipitated using a monoclonal antibody (D12) produced in our laboratory; the Ag-Ab complex was analyzed by SDS-PAGE and the protein, identified by MALDI-TOF, was gp63. We further confirmed that gp63 was responsible of the COX-like activity by purification of gp63 by concanavalina A (ConA) affinity chromatography demonstrating the presence of COX-like activity in the gp63 eluates.
CONCLUSIONS: gp63, the surface protease of promastigores is responsible for the COX-like activity recognized by an anti-mouse COX-2 antibody. This enzymatic activity may participate in modulating the host immune response.

PRODUCTION OF A MONOCLONAL ANTIBODY AGAINST A *L. mexicana* PROTEIN WITH COX-LIKE ACTIVITY.

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BACKGROUND: Recent work in our laboratory has shown that *L. mexicana* presents a protein with cyclooxigenase (COX)-like activity. COX is responsible of PGE_2 and other prostanoids synthesis. PGE_2 is of particular importance because it regulates the immune system to inhibit Th1 cytokines (IL-1, TNF-α, and IL-12) and promotes production of Th2 cytokines (IL-10, IL-4, and IL-5), in this way evading the immune response and establishing into the host. Therefore, the aim of this work was to produce an antibody against the protein with COX activity.
METHODS: *L. mexicana* promastigotes in log phase were lyzed by sonication; the total extract was centrifuged to obtain soluble and pellet fractions. The soluble fraction was subjected to fractionation with ammonium sulphate at 10, 20, 30 and 40% of saturation (SAS). The 40% fraction, containing COX activity, was used to immunize BALB/c mice, 5 times, with weekly intervals. After that, the mouse with the highest antibody titer was sacrificed and the spleen was recovered to prepare the cellular suspension that was used to produce monoclonal antibodies against a possible COX-like protein.
RESULTS: A selected antibody (D12), recognized a protein of 65 kDa in promastigotes and amastigotes of *L. Mexicana*; this antibody also recognized a similar protein in other pathogens such as *T. cruzi*, *E. histolytica* and *G.duodenalis*,
CONCLUSIONS: We have produced a monoclonal antibody directed against a 65 kDa protein with COX-like activity; this antibody crossreact with other protozoan parasites. Further work is needed to confirm that the proteins recognized in other pathogens also present the enzymatic activity.

Impact of the zoonotic trematode *Philophthalmus lucipetus* on the invasive freshwater snail *Melanoides tuberculata* in three ponds of a wetland of Peru having different degrees of human disturbance

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BACKGROUND: *Melanoides tuberculata* (OF Müller, 1774) (Thiaridae) is a prosobranch gastropod with a cosmopolitan distribution. It is a dioecious, parthenogenetic and ovoviviparous species that, due to its high biotic potential, has great ecological importance as an invader and its impact on the diversity of native snails. The aim of this investigation was to determine the impact of the zoonotic trematode *Philophthalmus lucipetus* (Rudolphi, 1819) on *M. tuberculata* in three wetland ponds of Peru having varying degrees of human disturbance.
METHODS: 2537 snails were collected from three ponds of the coastal wetland Pantanos de Villa, Lima, Peru from 2009 to 2011. Snails were measured (mm) for shell length. Cercariae were obtained by subjecting snails to light, and rediae and cercariae dissected from the snail intermediate host and determined to be *P. lucipetus*. Names (sample size and level of disturbance) of the three ponds were: Mayor (n = 1019) (low human disturbance), Marvilla (n = 360) (middle human disturbance), and Delicias (n = 1158) (high human disturbance).
RESULTS: Prevalences of rediae and cercariae of *P. lucipetus* were significantly higher in the pond with low human disturbance, Mayor (38.17%, 31.01%), than those of middle disturbance, Marvilla (26.11%, 14.44%) and high disturbance, Delicias (12.35%, 8.20 %). Thus, a decline in the prevalence of rediae and cercariae of *P. lucipetus* was associated with greater human disturbance. The average shell length of *M. tuberculata* in Marvilla was higher (21.62 mm) than in Delicias (19.21 mm) and Mayor (19.87 mm). Size differences between infected (21.97 mm) and uninfected (19.11 mm) snails were found. Snails with offspring were larger. There was a negative relationship between the presence of rediae and cercariae in snails and the presence of offspring. The average depth and temperature of the three ponds did not significantly influence the rates of cercarial release from *P. lucipetus*.
CONCLUSIONS: Prevalences of rediae and cercariae of *P. lucipetus* in *M. tuberculata* were higher in ponds with lower levels of human disturbance. The presence of rediae and cercariae in snails showed a negative association with the presence of snail offspring suggesting parasitic castration of snails.

Ocular cysticercosis

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BACKGROUND: Cysticercosis is the most common parasitic infection of the CNS worldwide. The location of Cyst in the CNS and the eye (considering the retina as an extension of the CNS) are considered as neurocysticercosis. Although the cyst within the eye is considered NCC, immune response and treatment is widely different.
METHODS: This article reviews current knowledge on neurocysticercosis highlighting the importance of ocular cysticercosis detection and treatment.
RESULTS: The cysticercus may lead to blindness in 3-5 years. The immune response is dependent of the cysticercus staging. Cysts should be removed before systemic treatment is undertaken to prevent damage from the dying process of the parasite. Reviews of treated cases suggest that early removal of the organism is associated with preservation of visual function. Intraocular NCC represents the 0.58 % of all the cases. Intraocular lesions caused by cysticercosis most commonly occur in the vitreous or subretinal space.
CONCLUSIONS: Cysticercosis remains endemic in Mexico. Removal of the cyst is mandatory to remove the source of the toxins causing inflammation and early removal has been advocated. An ophtalmic examination should always precede antiparasitic treatment to rule out ocular cyst. Antiparasitic drugs may cause irreparable damage when used to treat ocular cysts while antiparasitic drugs helps in brain NCC treatment.

Cytokine Profiles Associated with Human Schistosomiasis Mansoni in New Halfa City- Sudan

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METHODS: The study was conducted among 770 population living in an area where schistosomiasis mansoni is endemic, with age range between 4-85 years old and average age of 23 ± 19 years, 475 of them were males (61.7%) and 295 were females (38.3%). One hundred twelve out of 770 (14.5%) faecal specimens were found to be positive for *S. mansoni* when examined by direct wet mount and Kato-Katz technique. For detection of human Th1/Th2/Th9/Th17/Th22 cytokines, 305 serum samples were investigated between the three groups (110 with a current infection with *S. mansoni*, 60 that had had a previous infection and 135 uninfected individuals). Thirteen cytokines (IL-2, IFN-γ, TNF-α, IL-4, IL-5, IL-13, IL-17A, IL-22, IL-6, IL-10, IL-9, IL-1β and IL-12p70) were analyzed in individuals sera using Flow cytometry technique. All these cytokines compared in two groups (infected and uninfected) by using Mann-Whitney test and in three groups (infected, infected before and uninfected) by using Kruskal-Wallis test. All above cytokines were compared with factors such as age, sex and race, which were associated with cytokines levels. Also, were compared with treatment and intensity of *S. mansoni* using Kato-Katz technique.
RESULTS: All above cytokines, which were detected in the study, showed significant differences when were compared with gender, age, intensity of *S. mansoni* using Kato-Katz technique and treatment.
CONCLUSIONS: This study indicated that *S. mansoni*-infected individuals have more IL-5 and IL-13 than those with a previous infection, less IL-17, IL-22 and IL-6 than uninfected individuals, more IL-10 and IL-9 than uninfected and more IL-1β and IL-12 than uninfected individuals.

Chaperone and co-chaperone involvement in erythrocyte remodelling by malaria parasites

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Malaria parasites modify their host cell, the mature human erythrocyte. We are interested in the molecules mediating these processes, and have recently described a family of parasite-encoded heat shock proteins (PfHsp40s) that are targeted to the host cell, and implicated in host cell modification. Hsp40s generally function as co-chaperones of members of the Hsp70 family, and until now it was thought that human Hsp70 acts as the PfHsp40 interaction partner within the host cell. Here we revise this hypothesis, and identify and characterize an exported parasite-encoded Hsp70, referred to as PfHsp70-x. PfHsp70-x is exported to the host erythrocyte where it forms a complex with PfHsp40s in structures known as J-dots, and is closely associated with PfEMP1. Interestingly, Hsp70-x is encoded only by parasite species that export the major virulence factor EMP1, implying a possible role for Hsp70-x in EMP1 presentation at the surface of the infected erythrocyte. Our data strongly support the presence of parasite-encoded chaperone/co-chaperone complexes within the host erythrocyte, which are involved in protein traffic through the host cell. The host-pathogen interaction within the infected erythrocyte is more complex than previously thought, and is driven not only by parasite co-chaperones, but also by the parasite-encoded chaperone Hsp70-x itself.

Helminth parasites of two South American amphibian of the genus *Rhinella* (Anura: Bufonidae) from Peru

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BACKGROUND: With 315 recorded amphibian species, Peru is in fourth place worldwide in relation to species richness after three Neotropical countries like Brazil, Colombia and Ecuador. Species of the genus *Rhinella* is widely distributed in South America.
METHODS: 120 specimens of amphibians of the genus *Rhinella* were purchased from markets in Lima, Peru. The acquired specimens were identified as *Rhinella limensis* (n = 40), endemic to Peru and used in animal experiments, and *Rhinella spinulosa* (n = 80), mostly Andean and used for educational purposes, in food and medicine. Both species according to the IUCN Red List are considered as Least Concern. Platyhelminths and acanthocephalans were collected, fixed and preserved in 70% ethyl alcohol, stained with acetic carmine and mounted in Semichon Canada balsam or Entellan. Nematodes were fixed in hot 70% alcohol and cleared in a mixture of alcohol-phenol.
RESULTS: A total of 2,173 parasites were collected and seven helminthes were *identified:* Digenea: *Gorgoderina chilensis*; Cestoda: *Cylindrotaenia americana*; Nematoda: *Aplectana hylambatis*, *Rhabdias sphaerocephala*, *Hedruris moniesi*, and *Physaloptera huascari*; Acantocephala: *Anuracanthorhynchus lutzi*. All hosts showed infection with at least one parasite species. The helminth species with higher prevalence (P) and mean abundance (MA) was the nematode *R. sphareocephala* (P = 95%, MA = 7.37) followed by *A. hylambatis* (P = 22.5%, AM = 6.93). Nematodes dominated in number of species (n = 4) with a total of 1855 specimens (85.3%) compared to the other taxa (14.7%).
CONCLUSIONS: Seven helminthes parasites were identified and a total prevalence of helminth parasites was 100% in two South American species of anuran amphibians, *R. limensis* and *R. spinulosa*.

The impact of immunity to sand fly salivary proteins on leishmaniasis: from basic science to translational research.

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Every time a sand fly attempts to get a blood meal it injects saliva into the skin of the host. It is remarkable that only few nanograms of injected salivary proteins are sufficient to disarm the host hemostatic system, including vasoconstriction, platelet aggregation and the blood coagulation cascade. Importantly, this small amount of salivary proteins is sufficient to induce a systemic humoral and cellular immune response. This systemic immune response has been shown to protect rodents against leishmaniasis. Using a multidisciplinary approach based on transcriptomics, biochemical and immunological assays we aimed to understand the basis of this protective effect and to identify the protective salivary protein. We observed a protective effect in rodents, dogs and non-human primates and this protection correlates to the development of a T_H1-biased delayed-type hypersensitivity response (DTH) to the salivary molecule/s and to the development of an accelerated robust *Leishmania*-specific immune response with minimized pathology. Importantly, immunity to a T_H1-DTH-inducing salivary protein protects against both cutaneous and visceral leishmaniases suggesting, as expected, that it exert its influence early after an infected bite while the parasites are in the skin and at their most vulnerable. During this presentation I will show evidence of the protection in the various models, allude to the mechanism of protection and discuss the realistic prospect for a sand fly salivary protein as a component for a *Leishmania* vaccine.

Molecular profiling of adaptive immunity to *Plasmodium* infection in humans using systems immunology

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BACKGROUND: Despite intense research for many decades, the mechanisms of protective immunity against malaria are poorly understood and there is no immune correlate of protection. Recent technological developments provide an opportunity to characterize, at the molecular level, how plasmodia modulate the host immune response to infection. To study immunomodulatory events occurring at the earliest stages of blood stage infection, we are applying cutting-edge systems immunology approaches to a unique resource of experimental *Plasmodium falciparum* blood stage infection in malaria-naïve humans to define molecular profiles associated with T cell and B cell responses to *P. falciparum*.
RESULTS: We have identified a set of microRNAs expressed in the peripheral blood in early *P. falciparum* infection that are inversely correlated with blood-stage parasitemia, and another that is positively correlated with a *P. falciparum* specific antibody response. Bioinformatic analyses associate these microRNAs with TCR and BCR signaling, as well as TP53 mediated apoptosis. In addition, we have investigated the polyfunctionality of *Plasmodium*-specific $CD4^+$ and $CD8^+$ T cells, and showed a variation over the time course of infection. We further identified a unique gene expression signature displayed by IFN-γ, IL-2 and TNF triple positive $CD4^+$ T cells compared to IFN-γ single positive $CD4^+$ T cells.
CONCLUSIONS: These data provide unique insights into the host immuno-molecular response to early blood stage *Plasmodium* infection that may have important relevance to the evolution of immunity to malaria. As well as providing novel insight into the parasite-host interaction, these data may also inform design of more efficacious vaccines or therapeutics.

Humoral Response in patients infected with *Leishmania (Viannia) guyanensis*

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BACKGROUND: The pathogenesis of American Cutaneous Leishmaniasis (ACL) is influenced by inherent factors: the host, such as genetic and immunological features and response to the parasite, as the virulence of the infecting species. The aim of this study was to evaluate serum titers of immunoglobulin isotypes (IgG, IgM and IgE) in patients with Cutaneous Leishmaniasis (CL) before and after clinical cure.
METHODS: We performed serological tests for the detection of immunoglobulins in 17 patients with CL and five individuals without infection (control group). The samples were analyzed before and after treatment with Glucantime®. The Enzyme-Linked Immunosorbent Assay (ELISA) was used for the detection of antibody titers in the serum of patients. The isolated species was characterized as *Leishmania guyanensis*, by electrophoresis of isoenzymes.
RESULTS: Based on the values of the cut-off were considered positive for IgM titers ≥ 1/20 and IgG titers ≥ 1/40. In the control group the reactivity of all immunoglobulins analyzed was 1/10. In the comparative analysis of higher titers of immunoglobulins in infected patients, there was significant difference ($p < 0.0001$) in the reactivity between IgM and IgG after treatment. The IgG titer was higher in the samples after treatment when compared with the titer of IgM in the same group. No significant difference in the reactivity of IgM and IgG was observed in the samples before the beginning of treatment. The analysis of IgE presented tested negative for the titrations, either before starting treatment and after-treatment, possibly by concentrations of this immunoglobulin being less than the tested titers (<1/10).
CONCLUSIONS: Titers of IgM and IgG were detectable in the serum of patients infected with *L. guyanensis*, with IgG titers high than 1/160. The results suggest that the parasite antigens are also stimulating Th2 response in patients infected with this species of *Leishmania*.
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Novel culture medium for *Balamuthia mandrillaris*

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BACKGROUND: Until now, for axenic cultivation of *Balamuthia mandrillaris,* the BM-3 culture medium is the only one that has been recommended. This culture medium is excellent for the growth of *Balamuthia mandrillaris*, but it has some disadvantages as it requires many components, and its preparation is laborious. Therefore, we developed a novel culture medium for *B. mandrillaris* axenic cultivation. METHODS: Using variations in components of BM-3 culture medium and Cerva's medium (Bactocasitone 2% supplemented with fetal bovine serum and antibiotics), as basal culture medium, different media were incubated at 37 °C with and without CO_2 atmosphere. *B. mandrillaris* reference strain CDC: V039, and nine *B. mandrillaris* environmental isolates from water and soil were used during trials.
RESULTS: After testing eleven combinations of BM-3 components and Cerva's medium, the basal medium complemented with Hank's balanced salts solution was the only one that supported the confluent growth of *B. mandrillaris* axenic culture. Faster confluency was obtained using CO_2 atmosphere. Cell shape and motility of trophozoites were normal. The new medium developed is as useful as BM-3 medium for axenization after cell tissue culture. We are continuing long-term cultivation using new medium over two years.
CONCLUSIONS: Our results suggest that nutrimental necessity of *B. mandrillaris* depends on an adequate salts solution; protein extracts and other nutrients obtained from the fetal bovine serum. When using axenic cultures, the development of a cheaper and easy-to-prepare medium for *B. mandrillaris*, opens the possibility of increasing its study.

Ecological approaches to schistosomiasis. A research proposal to the cercariae-rotifer relationship

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BACKGROUND: Schistosimiasis is a well-known infection that has significant importance to health and economics. In a recent Egyptian review, the relationship between cercariae, snails and rotifers was described. And there are also several references with an ecological approach in that review. On the other hand, another research has found positive chemotaxis to freezing-killed females.
METHODS: Starting from the Egyptian review, I looked for those references dealing with the snail-cercariae-rotifer relationship, or with another ecological approach. Then I gathered the data to make a comparison between the different lines of research and over the time.
RESULTS: An icreasing number of articles was found, that show an ecological approach to the interactions between snails, cercariae and rotifers. Finally I show a research proposal based on the fact that rotifers decrease the parasite burden in the snail and that freezing-killed females seems to attract other rotifers.
CONCLUSIONS: This is the proposal: it's possible to attract a higher number of rotifers to the snails by designing an experiment using these freeze-killed females, and therefore the number of cercariae may decrease, which may possibly reduce the risk of infection.

Matrix Metalloproteinase (MMP) -2 and -9 serum activity in *Trichinella spiralis* and *Trichinella pseudospiralis* mouse infection

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BACKGROUND: *Trichinella spiralis* and *Trichinella pseudospiralis* are intracellular parasites of the skeletal muscle cells where they induce different degrees of myositis in the mouse. Matrix metalloproteinases (MMPs), in particular MMP-9 (92 kDa gelatinase) are implicated in various aspects of inflammation. The aim of this study was to evaluate serum level changes of gelatinase A (MMP-2) and B (MMP-9) in mice experimentally infected with *T. spiralis* or *T. pseudospiralis*, to elucidate their involvement during the inflammatory response to these parasites. The level of TIMP-1, an endogenous *in vivo* regulator of MMP-9 and other metalloproteinases, pro-inflammatory cytokines (TNFα, IL-1β) and neutrophil gelatinase-associated lipocalin (NGAL), was also evaluated.
METHODS: We used gelatin zymography on SDS polyacrilamide gels to assess the serum level of gelatinases and *in situ* zymography on muscle histological sections to show the gelatinase-positive cells. TIMP-1 levels were quantified by ELISA, IL-1β, TNFα, and NGAL using a Luminex® screening assay.
RESULTS: In both *T. spiralis* and *T. pseudospiralis* infected mice, a significant ($p<0.05$) percentage increase of total MMP-9 was observed at 6 and 14 days p.i., compared to day 0 value. However, the MMP-9 level was lower in *T. pseudospiralis* than in *T. spiralis* infected mice. For both species, the increase of MMP-2 shifted onward when compared to MMP-9. Significant differences were also observed in MMP-2 activity between the two experimental groups. TIMP-1 levels in sera of both *Trichinella* species infected mice were significantly ($p< 0.05$) higher at 21 day p.i than in controls. *In situ* zymography showed a higher number of gelatinase positive cells in *T. spiralis* than in *T. pseudospiralis* infected muscles. Hematoxylin and eosin staining confirmed the inflammatory nature of the gelatinase positive cells.
CONCLUSIONS: MMP-9 and MMP-2 may be considered as markers of the inflammatory response for both *T. spiralis* and *T. pseudospiralis* infections.

Functional analysis of drug targets and resistance mechanisms

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BACKGROUND: Extensive use of anthelmintics has led to emergence of resistance in nematode populations worldwide. Identification of targets revealed, with the exception of the benzimidazoles (BZ), neuronal ion channels as targets of all nematocidal drug classes. In addition to drug target associated resistance mechanisms also unspecific mechanisms have been investigated for most of the currently used anthelmintics. As unspecific resistance mechanisms predominantly P-glycoproteins (Pgps) and metabolic pathways e.g. cytochrome P450 monooxygenases (Cyps) have been considered.
METHODS: Currently identification of new drug targets is mainly performed using mutagenesis screening in *Caenorhabditis elegans*. Functional analysis includes expression systems such as transgenic *C. elegans*, *Xenopus* oocytes, yeasts and mammalian cells. While the *C. elegans* system is mostly limited to rescue of respective phenotypes, the *Xenopus* system allows detailed electro-physiological investigation of recombinant channels. Exploring the unspecific mechanisms, *in vitro* analysis using inhibitors of xenobiotic transporters and metabolic pathways as well as candidate gene approaches in *C. elegans* have been described. Transport associated studies were recently conducted using an ABC-transporter deficient yeast strain transformed with nematode Pgp-expression constructs. For assessment of BZ-resistance pyrosequencing assays were designed for multiple nematode species.
RESULTS: In principle, target sites have been identified for all currently used drug classes e.g. the SLO-1 channel for emodepside and acetylcholine receptor subtypes for nicotinergic drugs. Pgps were implicated in resistance in several nematode species using specific inhibitors, expression studies or Pgp-deficient *C. elegans*. Similar to BZ-resistance associated single nucleotide polymorphisms (SNPs) in the β-tubulin, SNPs have been identified in Pgp-genes. Recently a yeast-based assay demonstrated interaction between recombinant nematode Pgp and anthelmintics.
CONCLUSIONS: Recombinant expression followed by thorough physiological analysis has significantly improved our understanding of parasite drug interaction and the development of resistance. They will also allow testing of optimised drugs with improved target binding or decreased affinity for Pgps or Cyps.

Toxoplasma retromer complex is essential for protein maturation, trafficking, biogenesis of secretory organelles and host invasion

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BACKROUND: The secretion of rhoptries, micronemes and dense granules is required for motility and host cell invasion of apicomplexan parasites. We recently identified and characterized a Golgi-endosomal-like type I transmembrane receptor called *Tg*SORTLR for *Toxoplasma gondii* Sortilin-Like Receptor, which is essential for the biogenesis of rhoptries and micronemes. METHODS: We demonstrated that the C-terminal cytoplasmic region of *Tg*SORTLR specifically binds to the *T. gondii* proteins Vps26 and Vps35, components of the retromer complex. Furthermore, we constructed the first retromer-trafficking interactomes in *T. gondii* using knock in parasite strains expressing HA tagged versions of *Tg*Vps35 and *Tg*Vps26 proteins followed by co-immunoprecipitation and proteomic analysis.
RESULTS: We now report the conditional knock out of *Tg*Vps35 that demonstrates the key roles of the retromer in the biogenesis of rhoptries and micronemes. In addition, the localization of dense granule proteins is also altered in the *Tg*VPS35 mutant, suggesting that the retromer complex is critical for protein transport to dense granules. We identified numerous known proteins involved in vesicular trafficking, but also several unknown parasite-specific proteins, suggesting that retromer-associated proteins that have not been described in mammalian cells and yeast may exist in *T. gondii*. CONCLUSION: In summary, we will describe how an endosomal-like system plays a key role in the biogenesis of secretory organelles that are essential for host invasion and infection by *T. gondii*.

Variation in apoptosis mechanisms employed by *Plasmodium berghei*: the roles of inducers, dose dependence and parasite stages

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BACKGROUND: *Plasmodium berghei* ookinetes exhibit an apoptotic phenotype when developing within the mosquito midgut or when cultured *in vitro*. Markers of apoptosis increase when they are exposed to nitric oxide or reactive oxygen species; but cell membranes were rapidly compromised by higher concentrations. Chloroquine has been used to induce apoptosis in erythrocytic stages of *Plasmodium falciparum*. The effect of chloroquine and staurosporine on ookinetes was studied. Finally, the suggestion that apoptosis may have evolved as a strategy employed by ookinetes to increase the fitness of surviving parasites was explored by determining whether increasing the ecological triggers, parasite density and nutrient depletion induced apoptosis.
METHODS: Ookinetes were grown in culture then either exposed to hydrogen peroxide, chloroquine or
staurosporine, or incubated at different densities and in different media. The proportion of ookinetes displaying positive markers for apoptosis in treated samples was compared with controls and results were analyzed using analysis of variance followed by a Turkey's test, or a Kruskal-Wallis test.
RESULTS: Hydrogen peroxide below 50 µM triggered apoptosis but cell membranes were rapidly compromised by higher concentrations, and the mode of death could not be defined. Both chloroquine and staurosporine cause a significant increase in ookinetes with condensed chromatin, caspase-like activity and, in the case of chloroquine, phosphatidylserine translocation and DNA fragmentation. However, mitochondrial membrane potential remained intact. No relationship between ookinete density and apoptosis was detected but nutrient depletion significantly increased the proportion of ookinetes with chromatin condensation.
CONCLUSIONS: It is proposed that both a mitochondrial and an amitochondrial apoptotic pathway may be involved, dependent upon the trigger, and that pathways may differ between erythrocytic stages and ookinetes, or between rodent and human malaria parasites. No relationship between ookinete density and apoptosis was detected but nutrient depletion significantly increased the proportion of ookinetes with chromatin condensation in four hours.

Ultrastructure study on vitelline cells of experimentally recovered migrating *Fasciola gigantica*

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BACKGROUND: Many ultrastructural studies were done on the different structures of *Fasciola* spp. like tegument, caecal epithelium and sprmatogenesis. Few ultrastructure studies were done on the female reproductive cells such as vitelline cells of adult *Fasciola* recovered from naturally infected hosts. In the present study, the ultrastructure of vitelline cells of experimentally recovered migrating *Fasciola gigantica* is demonstrated.
METHODS: Adult worms of *Fasciola gigantica* were collected and their eggs were incubated. After incubation, eggs hatched into miracidia. Laboratory bred snails *Lymnaea natalensis* were exposed to miracidial infection. After few weeks post infection, cercariae were shed and encysted into metacercariae, which were used to infect mice. Recovered *F. gigantica* worms were prepared for transmission electron microscopy.
RESULTS: The ultrastructure of migrating *Fasciola gigantica* shows that vitelline cells are grouped into follicles. Vitelline cell has a nucleus with obvious chromatin. At early stages of vitelline cell development, the nucleo-cytoplasmic ratio is high. The cytoplasm of the developing vitelline cells has mitochondria and rough endoplasmic reticulum. The shell protein globules surround the nucleus during development. Mature vitelline cells contain peripheral shell protein globules underneath the plasma membrane.
CONCLUSIONS: This study is the first to show the fine structure of vitelline cells of migrating *F. gigantica* recovered from experimentally infected mice.

Effects of helminth colonization on the structure and function of the host gut microbiome

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BACKGROUND: Helminths colonize more than one quarter of the world's human population. Furthermore, helminth infections pose a serious challenge to food animal production. Helminths ensure their survival by regulating host immunity through mechanisms that dampen intestinal inflammation. Our previous studies demonstrated that the abundance of approximately 13% of the genera and 26% of the metabolic pathways identified in the host gut microbiome are significantly altered by helminth infection in animal models. In humans, the gut microbiome of helminth-colonized individuals tends to have greater species richness and increased microbial diversity.
METHODS: In this study, we characterized the impacts of helminth infections on the structure and function of the host gut microbiome using deep next-generation sequencing and bioinformatics tools in an animal model. Fourteen parasite naive young goats were exposed to 5,000 drug-resistant L3 larvae of *Haemonchus contortus,* arguably the most important helminth parasite for small ruminants, for 42 days. Six age-matched animals served as uninfected controls.
RESULTS: Compared to the naive control animals, the infection resulted in a reduced bodyweight gain. Moreover, infection with *Haemonchus contortus* had a significant impact on the gut microbial habitat as evidenced by a significant increase in the abosamal pH values (P <0.05). Deep sequencing of hyper-variable V1 to V3 regions of the 16S rRNA genes (the mean number of sequence reads = 226,179, N=20) of the abomasal caprine microbiome unraveled a profound change in the microbial composition induced by the helminth infection.
CONCLUSIONS: The potential role of the gut microbiome in modulating host-parasite interactions was identified. Understanding three-way interactions between the host, its microbiome, and the parasite will provide novel insights into physiological consequences of helminth infection, which should facilitate development of novel strategies for helminth control in animals and humans.

Serological detection by ELISA of *Neospora caninum* in cattle herd in Tamaulipas

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BACKGROUND: Neosporosis is a serious disease which results in reproductive failure in cattle, mainly abortion, resulting in livestock losses; its etiological agent, *Neospora caninum* is a widely distributed parasite in domestic animals, especially dogs and cattle, but it is also found in wild animals. Since the parasite description in 1984, a number of worldwide epidemiological studies have been carried out to understand this disease. In Mexico, Tamaulipas state has an important activity in calf production. A previous study found that 16 % of dairy cattle of the north part of Tamaulipas was seropositive for *N. caninum*. The aim of the current study was to detect the presence of *Neospora caninum* antibodies in cattle and to contribute to the epidemiology of this disease.
METHODS: According to the cattle population census in municipalities' chosen from south, central west and east of Tamaulipas, the sample size was set up in 184 organisms. Blood samples from cattle, aged from 5 to 136 months, from 14 properties of six municipalities were processed for the detection of antibodies against the parasite through an enzyme-linked immunosorbent assay (ELISA), using a commercial kit (IDEXX®, Westbrook, USA). The essay was conducted according to the manufacturer's instructions and was performed at Facultad de Medicina Veterinaria y Zootecnia.
RESULTS: There were seropositive animals to *Neospora caninum* in 100% of the municipalities; with a total of 22 positive animals, and a general seroprevalence of 12%. Results showed the immunology evidence of antibodies against *N. caninum* with similar results of seroprevalence of antibodies in the neighbor states of Texas and Nuevo León, with 12.9 % and 10% respectively.
CONCLUSIONS: This is the first report of the presence of antibodies against *N. canimum* in the municipalities sampled. Future studies should be addressed to find the relationship among the pathogen and cattle reproductive problems.

Identification of microorganisms in partially fed female horn flies, *Haematobia irritans*

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BACKGROUND: The horn fly *Haematobia irritans*, is one of the most important ectoparasites of cattle, with high economical and health impact. Infestations with horn fly reduce the weight and milk production. Added to this the horn fly has been implied as vector of several pathogens. The aim of the study was to identify the prevalence of microorganisms using processing EST´s and the real time RT-PCR in partially fed female horn flies.
METHODS: Total RNA from the abdominal tissues of 1,500 partially fed adult female horn flies was isolated. A cDNA library was constructed and analyzed as previously reported (Torres *et al.,* 2011). The cDNA Annotation System software (CAS), was used for automated sequence cleanup, assembly, and blasting against multiple sequence databases. Nucleotide sequences were aligned using the program AlignX. Real-time RT-PCR was used to test the pathogen prevalence in horn fly by analyzing infectious agent mRNA levels in individual adult female flies.
RESULTS: Seven unigenes with 24 EST´s showed homology with infectious agents such as: Nora virus (3 unigenes; 8 EST´s), Wolbachia endosimbyonte (3 unigenes; 3 EST´s) and Mycobacterium bovis (1 unigene; 13 EST´s).
CONCLUSIONS: Reported herein increase the repertoire of microorganisms that can cause persistent infections or can be mechanically transmitted by horn flies and supports future studies on the role of the horn fly on the epidemiology of these pathogens in Mexico.

Cloning, expression and *in silico* characterization of actin depolymerizing factor from the Apicomplexan *Neospora caninum* (NcADF)

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BACKGROUND: Actin-binding proteins (ABP) are proteins with domains capable of interacting with actin and they are responsible for the assembly and disassembly of actin filaments. In Apicomplexan parasites 98% of actin is maintained in monomeric form, despite the necessity of filamentous actin in the glideosome for locomotion and invasion processes, suggesting that actin turnover is tightly regulated. Thus, in this study we aimed at cloning, expressing and *in silico* characterizing an ABP (Actin Depolymerizing Factor – ADF) from the Apicomplexan parasite *Neospora caninum,* a parasite associated with abortion in cattle, leading to important economic losses.
METHODS: The ToxoDB protein sequence of NcADF was aligned with their orthologues (ClustalW method and structural alignment by ESPript 3.0) and analyzed for conserved domains in Pfam. The ORFs were amplified, firstly cloned in pGEM t-easy, then in pET28(a)+ and pET32(a)+, and expressed in BL21 *E. coli* cells. The recombinant proteins were loaded on a Ni-resin column and the purification analyzed on SDS-PAGE.
RESULTS: NcADF had 89% identity with *Toxoplasma gondii* ADF (TgADF) and the actin-binding domain ADF-H was present in the sequence. Moreover, the three dimensional sequences of NcADF and TgADF were very similar. The recombinant proteins were expressed in a soluble form in pET28(a)+ and pET32(a)+ for 18 hours in 18°C, showing ~18 and ~30 kDa in SDS-PAGE, respectively.
CONCLUSIONS: The *in silico* analyses represented the beginning of NcADF molecular characterization. Furthermore, the expression of recombinant NcADF will enable the obtainment of specific polyclonal antibodies (in progress) and the accomplishment of the soluble recombinant protein is significant for future actin binding tests.

Inhibition of the expression of COX-2 by BAY-11 in macrophages infected with *Leishmania mexicana*.

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BACKGROUND: The effectiveness of *Leishmania* parasites to infect and remain viable in the host is primarily due to its ability to modulate the host's immune response. When the parasite infects the cell, it induces the expression of cyclooxygenase-2 (COX -2) enzyme, responsible of prostaglandin E_2 (PGE_2) production, a mediator crucial in modulating the immune response. In this study we analyzed the effect of BAY-11, a NF-κB inhibitor, on the expression of COX-2 in *Leishmania mexicana* infected macrophages.
METHODS: From macrophages pretreated or not with BAY-11 and further infected with *L. mexicana*, we analyzed by Western blot, the expression of COX-2 and the phosphorylation level of IκBα, and by electrophoretic shift (EMSA), the translocation of NF-κB to the nucleus.
RESULTS: NF-κB inactivation in macrophages treated with BAY-11 prevents NF-κB translocation to nucleus and their interaction with c*ox-2* gene promoter, which completely inhibited the expression of COX-2 enzyme produced by *L. mexicana* infection, however, parasitic burden is similar in the treated and untreated macrophages.
CONCLUSIONS: Results strongly suggest that NF-κB plays a role in the induction of COX-2 in macrophages infected with *L. mexicana*. In our experimental model, it appears that NF-κB is the main transcriptional factor involved in the induction of *Cox-2* gene.

An integrated multi-omics approach for studying helminth infections

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BACKGROUND: Using an integrated multi-omics approach, including nematode genomics, transcriptomics, functional genomics, proteomics, interactomics etc, we undertake innovative bioinformatic analysis and obtain genomics-based discovery of information essential for developing of novel diagnostics, vaccines and anthelmintics. More specifically, combining a variety of bioinformatics and cheminformatics approaches, along with laboratory screening of nematodes spanning the phylum Nematoda, we identify and characterize targets with a broad-control potential.
METHODS: With an average of 15 years and $800 million dollars to bring a new drug to market, drug repurposing for new diseases can lower the high cost of bringing a drug to market. Therefore our approach has been developed for genomics based target and compound prioritization that allowed us evaluating existing drugs that could serve as a shortcut to develop affordable new treatments for worm infections.
RESULTS: Here I present our progress on exploring potential targets that are conserved across the phylum Nematoda and on selecting drug-like compounds that might be active against these targets and parasitic worms. Subsequent laboratory studies showed that these predictions were correct in a high percentage of cases. Genome-wide transcriptional responses to these drug-like compounds, transcriptional regulation, coexpression networks, docking, and database mining begin revealing their mode of action.
CONCLUSIONS: The prioritized drug targets and drug-like compounds have potential to expedite the discovery of new anthelmintic drugs with broad-spectrum efficacy.

Pan-Nematoda comparative genomics with emphasis on Trichocephalida species

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BACKGROUND: We have recently facilitated evolutionary studies of the phylum Nematoda by sampling a basal representative, the adenophorean nematode Trichinella spiralis (nematode of vertebrates that can cause infections in humans primarily by eating undercooked pork). The *T. spiralis* genome provided an entry towards a deep understanding of the phylum Nematoda at the molecular level since *T. spiralis* is a species that represent a position on the evolutionary timeline marked by important changes in animal anatomy, physiology, development or behavior. While the ever-evolving sequencing technologies have provided opportunity to time- and cost-efficiently generate sequence data for many organisms *T. spiralis* is still the only published sequenced representative of this taxonomic group.
METHODS: Using Next-Generation Sequencing technologies we generated sequence data from multiple insert size whole genome shotgun libraries, assembled and annotated the genome of two other species from the order Trichocephalida, *T. nativa* and *Trichuris suis*. Furthermore, using RNAseq approach we built an expression profile atlas for *T. suis* by sampling several developmental stages. Pan-nematoda genome comparison was undertaken using all available genomes from the phylum Nematoda.
RESULTS: The pan-phylum characteristics identified advance evolutionary studies and provide new insights about the molecular characteristics of Trichocephalida, and the phylum Nematoda in general.
CONCLUSIONS: Our observations provide a much better understanding of one of the understudied taxonomic groups (Trichocephalida) and also suggest that more representatives per taxonomic group should be sequenced to better understand the diversity within Nematoda.

The murine model and his importance in the study of *Naegleria fowleri* in the last 40 years, a bibliographic review

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BACKGROUND: *Naegleria fowleri* it´s free-living amoeba commonly found in warm freshwater environments, such as hot springs, lakes, lagoons, spas, pools, is a pathogen worldwide distributed causative of primary amoebic meningoencephalitis (PAM), disease that nearly 95% of infections is fatal to the host in approximately seven days, the researches conducted worldwide, primarily those under the murine model, as these resemble a human model and allow modify to observe and discover new specific interactions with the pathogen.
METHODS: The study consisted in performing specific searches in PubMed database modifying criteria such as: date of publication, and relevance, separating them into 3 groups, the first comprehended the years of 1960 - 1980, the second from 1980 - 2000 and the third from 2000 - 2014 and finally comparing the information found among these groups and plotting them.
RESULTS: In this study was found a total number of 184 papers published since 1975 – 2014 related with the murine model in *N. fowleri*, in the first group from 1960 to 1980 the number of papers published was 33, in the second group of 1980 to 2000 were published 93 papers, and finally in the third group from 2000 - 2014 were published 58.
CONCLUSIONS: This results suggests over time it has changed the interest in the research topic with *N. fowleri* (noting the number of publications between the second and third groups) and the specificity of the topics of the papers since the development and innovation of new molecular techniques.

Genetic characterization of *Echinococcus* spp. causing echinococcosis in the Nothern Hemisphere

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BACKGROUND: Both alveolar and cystic echinococcoses (AE and CE) are widely distributed in the Northern Hemisphere. *Echinococcus multilocularis* and *Echinococcus granulosus* sensu lato (s.l.) are the causative agents of AE and CE, respectively. In the last two decades, molecular phylogenetic analyses have revealed that *E. granulosus* s.l. consists of 5 valid species, and *Echinococcus shiquicus* was newly described. Thus, reevaluation of the causative agents of echinococcoses is now needed. In the present study, we review the recent results of molecular epidemiology of *Echinococcus* spp. mainly in the Northern hemisphere, with the emphasis on *E. multilocularis*, *E. granulosus* sensu stricto (s.s.) and *Echinococcus canadensis.*
METHODS: Based on the phylogeographic analyses, 4 mitochondrial genotypes (Asian, Mongolian, European and North American) of *E. multilocularis* have been confirmed. These genotypes generally have unique geographic patterns with some exceptions. All genotypes are distributed in Russia. To date, only one human case of North American genotype has been confirmed, suggesting this genotype has a low infectivity to humans. Further molecular epidemiological survey is needed to examine the pathogenicity of each genotype.
RESULTS: Contrary to *E. multilocularis*, *E. granulosus* s.s has a cosmopolitan mitochondrial haplotype which so far has been confirmed in Eurasia, Africa, and South America. Haplotype network analyses and population genetics indicates that *E. granulosus* s.s has been rapidly dispersed worldwide through diffusion of stock rising.
CONCLUSIONS: Recent phylogenetic analyses demonstrated that *E. canadensis* consists of 3 genetically close related genotypes, camel/pig strain (G6/7) and cervid strains (G8 and G10). In the Eastern Siberia of Russia, all of the genotypes are distributed. Human CE cases of all genotypes have been confirmed, and G6/7 cases are common in Mongolia. Molecular identification of the causative agent of CE cases is now essential to clarify the infectivity and pathogenicity of *E. granulosus* s.l., especially *E. canadensis* genotypes.

Antileishmanial *in vitro* activity of *Endlicheria bracteolata* essential oil on *Leishmania amazonensis*

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BACKGROUND: Due to the numerous side effects and difficulties with the main drugs, such as the pentavalent antimonials used to treat Leishmaniasis, there is a growing interest in the search for new antileishmanial agents with fewer side effects. Thus, we investigated the antileishmanial *in vitro* activity of *Endlicheria bracteolata* against *Leishmania amazonensis* promastigotes and amastigotes.
METHODS: The antileishmanial activity was evaluated using macrophages infected with *L. amazonensis* promastigotes which were incubated with different concentrations of the essential oil of *E. bracteolata*. The cytotoxicity assay was determined by Neutral Red using J774.G8 macrophages. The evaluated transmission electron microscopy was used to determine any possible changes caused by this essential oil in promastigotes and intracellular amastigotes forms of *L. amazonensis*.
RESULTS: The essential oil inhibited 50% growth of promastigote forms with 7.93µg/ml, while the 50% inhibition of the intracellular amastigotes forms was 3.61µg/ml. The value of 50% cytotoxic concentration was 15.14µg/ml showing that *E. bracteolata* essential oil is less toxic to macrophages than to the parasite. Promastigotes and intracellular amastigotes forms of *L. amazonensis* were treated with the essential oil of *E. bracteolata*. Ultrastructural studies of treated promastigotes and amastigotes showed several alterations, such as loss of cytoplasmic organelles, including the nucleus, and the presence of lipid inclusions.
CONCLUSION: This showed that *E. bracteolata* has promising antileishmanial properties, as it can act against the promastigotes forms and is able to penetrate the cell, being also active against the amastigotes forms. These results open new prospects for research that can contribute to the development of products based on plant compounds to treat cutaneous leishmaniasis.

Novel therapeutic approaches against Free Living Amoebae infections

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BACKGROUND: Free-Living Amoebae (FLA) belonging to *Acanthamoeba* and *Sappinia* genera as well as *Balamuthia mandrillaris* and *Naegleria fowleri* species are aerobic, mitochondriate, eukaryotic protists that occur worldwide and can potentially cause infections in humans and other animals. Our research team is mainly focus on *Acanthamoeba* genus and *Balamuthia mandrillaris*. Currently there are not effective therapeutic approaches against these parasitic amoebae mainly due to the existence of a highly resistant cyst stage in their life cycle and lack of knowledge of the amoebae biology in the case of *B. mandrillaris.*
METHODS: Our laboratory is developing novel therapies based on the use of siRNAs in order to validate different cellular targets in these amoebae and to search for a chemical substitute or further develop a RNAi-based technology.
RESULTS AND CONCLUSIONS: The obtained results so far in our laboratory are being presented in this communication. In the case of *Acanthamoeba*, we have recently established a novel therapy based on statins which was elucidated using siRNAs approaches.

Potentially Pathogenic *Acanthamoeba* strains from Soil Samples in Gran Canaria, Canary Islands, Spain

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BACKGROUND: Free Living Amoebae of *Acanthamoeba* genus includes non-pathogenic and pathogenic strains that are currently classified in 18 different genotypes, T1-T18. We have evaluated the presence of *Acanthamoeba* strains in soil samples of Gran Canaria Island, Canary Islands, Spain.
METHODS: There were collected between 2012 and 2013 and inoculated onto non-nutrient agar (NNA) plates and were checked for the presence of *Acanthamoeba.* We carried on the identification of *Acanthamoeba* strains using Page´s morphologic key and characterized at the genotype level by sequencing the DF3 region located in the 18S rDNA gene of *Acanthamoeba* as previously described.
RESULTS AND CONCLUSIONS: Those results revealed the presence of T2, T5 and T4 genotypes within the studied samples. To the best of our knowledge, this is the first report demonstrating the presence of potentially pathogenic *Acanthamoeba* strains in Gran Canaria Island and the first study of *Acanthamoeba* at the genotype level in the Canary Islands.

Development and glycoprotein composition of the perimicrovillar membrane in *Triatoma (Meccus) pallidipennis* (Hemiptera: Reduviidae)

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BACKGROUND: Hemipterans and thysanopterans (Paneoptera: Condylognatha) differ from other insects by having an intestinal perimicrovillar membrane (PMM) which extends from the base of the microvilli to the intestinal lumen. The development and composition of the PMM in hematophagous Reduviidae depend on factors related to diet. The PMM may also allow the human parasite *Trypanosoma cruzi*, the etiological agent of human Chagas Disease, to establish and develop in this insect vector. We studied the PMM development in the Mexican vector of Chagas Disease, *Triatoma (Meccus) pallidipennis*.
METHODS: We describe ultrastructure changes in the midgut epithelial cells of insects in response to starvation, and at different times (10, 15 and 20 days) after bloodfeeding.
RESULTS: In starved insects, the midguts showed epithelial cells closely connected to each other but apparently free of PMM with some regions being periodic acid-Schiff (PAS-Schiff) positive. In contrast, the PMM was evident and fully developed in the midgut region of insects 15 days after feeding. After this time, the PMM completely covered the microvilli and reached the midgut lumen. At 15 days following feeding the labeled PAS-Schiff increased in the epithelial apex, suggesting an increase in carbohydrates. Lectins as histochemical reagents show the presence of a variety of glycoconjugates including mannose, glucose, galactosamine, N-acetyl-galactosamine. Also present were N-acetyl-glucosamine and sialic acid which contribute to the successful establishment and replication or *T. cruzi* in its insect vectors. By means of scanning electron microscopy (SEM) and transmission electron microscopy (TEM), the formation and structure of the PMM is confirmed at 15 days post feeding.
CONCLUSIONS: Our results showed that the formation of the PMM differs from others Triatomine species suggesting that the biochemical composition of the vectors' PMM may be important for the parasite development. One future step to clarify this is to carry out a detailed mapping of the proteins and carbohydrates of the PMM.

In vitro effects of triterpenic acids from Tunisian olive leaf extracts against promastigote stage *Leishmania* spp

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BACKGROUND: Protozoan diseases, such as leishmaniasis, are a cause of considerable morbidity throughout the world, affecting millions every year.
METHODS: In this study, two triterpenic acids (maslinic and oleanolic acids) previously isolated from Tunisian olive leaf extracts were tested in vitro against the promastigotes stage of *Leishmania* (L.) *infantum* and *Leishmania* (L.) *amazonensis*. The mechanism of action of these drugs was investigated by detecting phosphatidylserine (PS) exposure, plasma membrane permeability, mitochondrial membrane potential and ATP level production in the treated parasites.
RESULTS: The highest activity was shown by Maslinic acid with an IC_{50} of 9.32 ± 1.654 and 12.460 ± 1.25µg/ml against *L. infantum* and *L. amazonensis*, respectively. Both products produced a time-dependent plasma membrane permeabilization and surface exposure of PS in promastigotes. Both molecules reduced the mitochondrial membrane potential and decreased the ATP levels.
CONCLUSIONS: The triterpenic acids tested in this study could be a potential therapeutic alternative against leishmaniasis. Nevertheless; further studies are needed to confirm it.

Assessment of the ELISA RIDASCREEN® diagnostic performance for human toxocariasis

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BACKGROUND: New serological and molecular biology-based methods have increased the diagnostic capability and specificity in human toxocariasis, including the possibility to classify the human cases in acute or chronic infections. Commercial techniques are also available, but comparative data of their diagnostic performance are limited reported in literature.
METHODS: In this report we described the experience using the IgG antibodies avidity ELISA, the conventional ELISA, an immunoblot technique (multiple antigen binding assay, MABA using antinges of *Toxocara canis and Ascaris lumbricoides*) and a commercial kit (ELISA RIDASCREEN®) in 15 case samples and 9 control samples, including also a coproparasitological assessment looking for intestinal infection with *A. lumbricoides*. The avidity index (AI) was defined as the mean optical density (OD) of urea-treated wells/mean OD urea-untreated wells x100. Values below 30% were ranked as IgG with low avidity, between 30-50% as moderate and >50% as high avidity. In all cases, the AI was worked out with values of OD higher than 0.100.
RESULTS: Concordance between ELISA RIDASCREEN® and Avidity-ELISA and conventional ELISA was 86.6% (κ=0.727; p=0.003, both cases), however with MABA 53.3% (κ=0.001; p=0.999). Nine samples were positive for *A. lumbricoides*, among these concordance between ELISA RIDASCREEN® and Avidity-ELISA and conventional ELISA was 67.8% (κ=0.400; p=0.134, both cases), with MABA 56.7% (κ=0.270; p=0.236). Control samples with ELISA titers ≥1:64 dils were positive for *T. canis* and negative for *A. lumbricoides* at MABA (κ=1.00; p<0.001).
CONCLUSIONS: Immunodiagnostics of toxocariasis have been improved over the last decades, however in the near future other techniques (based on recombinant antigens), will be widely available. Meanwhile, combination of immune methods, including IgG avidity, clearly helps diagnosis of infection, particularly in acute phase. Nowadays, commercial test are not widely available and present performance limitations, as we seen herein, when compared with other well-established techniques.

Determination of *in vitro* encystation stages duration in *Giardia duodenalis* using a cyst wall marker and optical microscopy

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BACKGROUND: *Giardia duodenalis* respond to intestinal factors such as bile salts, alkaline pH and/or cholesterol deprivation inducing the formation of environmentally resistant cysts. This encystation process is reproducible *in vitro* and includes three main stages: induction of differential gene expression, synthesis and intracellular trafficking of cyst wall components and cyst wall assembly. Cyst wall proteins (CWP1-3) are considered useful markers for *Giardia* encystation. The intracellular trafficking of these proteins is carried out by encystation-specific vesicles (ESVs). Cell wall assembly is completed when cysts are resistance to hypotonic shock. In this work, a kinetic analysis of the duration of *Giardia* encystation stages was determined using CWP1-specific primers and monoclonal antibodies against CWP-1. Also microscopy observations of both ESVs and water-resistant cysts were performed.
METHODS: WB strain trophozoites were induced to *in vitro* encystation and cell samples were analyzed at different times post-encystation induction. CWP1 mRNA levels were determined by RT-PCR using ubiquitin as constitutive control. As marker of encystation monoclonal antibodies (5-3C) against CWP1 were used to detect encysting and encysted cells by indirect immunofluorescence. ESV-harboring cells and cysts were quantified by differential interference contrast (Nomarski) optical system.
RESULTS: RT-PCR assays showed that steady-state levels of CWP1 transcripts were observed at 20-min after encystation induction. The time-response curves allowed determining a generation time of about 2.5 hrs for ESV and 4.4 hrs for CWP1-positive cells. On the other hand, water-resistant cysts showed a generation time of approximately 5.2 hrs. Based on the fact that an early exposure of CWP1 occurs during cyst wall assembly, the duration of this process takes around 50-min.
CONCLUSIONS: These studies suggest that the inductive and cyst wall assembly stages of *in vitro* encystation in *Giardia* occur within a short time, while protein synthesis and vesicle-mediated trafficking take longer times to be carried out.

Using real-time PCR to detect *Angiostrongylus cantonensis* in host animals and infected humans

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Angiostrongylus cantonensis is the most common infectious cause of eosinophilic meningitis in humans. It can also infect animals, for example primates and birds. The geographical distribution of this disease has changed dramatically in the last few decades, fueled by the introduction of invasive species to new environments. The Hawaii islands regularly report human cases and the parasite is commonly found there in its natural hosts (mollusks and rodents). Recent studies using real-time PCR on rats and mollusks from Hawaii found infection rates of 24-100% depending on the species. In contrast, the mainland US has mostly been considered free of this parasite, with the exceptions of the New Orleans area and Miami. Using real-time PCR, the infection rate and larval burden were shown to be much lower in these locations compared to in Hawaii. This may explain why very few cases of locally acquired human infections have been reported from mainland US. However, the low level of documented human cases may also be a result from diagnostic challenges; the clinical findings are non-specific and reliable laboratory diagnostic methods are mostly unavailable. A real-time PCR assay was recently evaluated for the detection of *A. cantonensis* DNA in human cerebrospinal fluid (CSF) specimens. CSF specimens from 33 patients with eosinophilic meningitis were included: *A. cantonensis* DNA was detected in 22 patients. Immunodiagnosis and/or supplemental PCR testing supported the real-time PCR-findings for 28 patients. Based on these observations, real-time PCR can be useful for the laboratory detection of *A. cantonensis* in host animals as well as a confirmatory diagnostic method for human infections.

Molecular basis for the deformability and localization of *Plasmodium falciparum* mature gametocytes

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BACKGROUND: The transmission of *Plasmodium falciparum* from human to mosquito occurs via the sexual form of parasite called gametocytes. Gametocytes develop and mature in human red blood cells (RBCs). Interestingly, immature gametocyte-infected RBCs (iRBCs) are localized in the bone marrow and only mature gametocyte-iRBCs are found in the circulation. Immature gametocytes are rigid and get sequestered in the bone marrow tissue, while mature gametocytes are deformable and easily pass into the circulation. Rigidity of the immature gametocytes has been correlated to the presence of the STEVOR protein on the iRBC surface. Conversely, the increased deformability of mature gametocytes is associated with the absence of STEVOR from the iRBC surface.
METHODS: We have used western blotting and protease inhibitors to identify the molecular pathway involved in the loss of STEVOR from the iRBC surface.
RESULTS: In this report, we have demonstrated that during the transition of immature to mature gametocytes, STEVOR gets cleaved from the iRBC surface. The cleaved STEVOR protein can be detected in the culture supernantant by western blotting. This cleavage is likely mediated by serine proteases.
CONCLUSIONS: These findings shed light on the possible role of serine proteases in gametocyte maturation and localization.

Both endo-siRNAs and tRNA derived small RNAs are involved in the differentiation of primitive eukaryote *Giardia lamblia*

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BACKGROUND：Small RNAs (sRNAs), including microRNAs and endo-siRNAs, were found to regulate most important biological processes in eukaryotes, such as cell division and differentiation. Although sRNAs were extensively studied in various eukaryotes, the role of sRNAs in the early emergence of eukaryotes is unclear.
METHODS： sRNA transcriptomes of four different stages in differentiation of *Giardia lamblia* were deep-sequenced and analysed by both bioinformatics and experiments.
RESULTS**:** A large number of endo-siRNAs in this fascinating parasitic protozoan were identified, and we found that they were produced from alive telometric retrotransposons and three genomic regions i.e. eSGR regions. eSGR-derived endo-siRNAs were proved to target mRNAs *in trans*. Gradual upregulation of endo-siRNAs in the differentiation of *Giardia* suggested that they might be involved in the regulation of this process. This was demonstrated by the impairment of differentiation ability of *Giardia* when GlDicer, the essential for the biogenesis of endo-siRNAs was knocked down. Endo-siRNAs are not the only sRNA regulator in *Giardia* differentiation, because a great number of tRNAs derived sRNAs showed more dramatic expression changes than endo-siRNAs in this process.
CONCLUSIONS: In this work, we totally identified five novel kinds of tRNAs derived sRNAs, and found that the biogenesis in four of them might be correlated with that of sitRNA which was discovered in our previous studies. Our studies reveal an unexpected complex panorama of sRNA in *G. lamblia,* and shed light on the origin and functional evolution of eukaryotic sRNAs. (This work was supported by Grant (2011CB811300) from the National Basic Research program (“973” program) to L.-H.Q. and by Grants from the National Natural Science Foundation of China (#31272305) and Sun Yat-Sen University (12lgjc11) to Z.-R.L.)

Multiple phenoloxidases are responsible for parasite melanization, egg chorion melanization and cuticle formation in the mosquitoes

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BACKGROUND : Phenoloxidase (monophenol, L Dopa:oxidase; EC 1.14.18.1; PO) has long been suggested to be involved in various biochemical processes for the survival of insects, including parasite melanization, egg tanning, wound healing and cuticle sclerotization, etc. In mosquitoes, PO is synthesized as inactive enzyme call prophenoloxidase (pro-PO) and multiple putative pro-POs have been identified in the genomes of several mosquito species. We also identified eight distinct pro-POs from mosquito *Armigeres subalbatus*, designated As-pro-PO I to VIII. In this study we used gene silencing analysis to elucidate the function of individual PO in *Ar. subalbatus*.
METHODS: We first used RT-PCR to analysis the PO expression profiles during parasite melanization, egg tanning or developmental stages in mosquitoes to identify the potential POs involved in these reactions. Then gene silencing analysis was used to elucidate the function of individual POs in mosquitoes.
RESULTS: We found the expressions of both As-pro-PO I and V were significantly increased in microfilariae (mf)-inoculated mosquitoes. The expressions of As-pro-PO V and VII were significantly increased in mosquitoes after blood feeding and significantly reduced after oviposition. The expressions of As-pro-PO III, IV, VI and VIII were fluctuated in pupal stage. Mf melanization rates were significantly reduced in both As-pro-PO I and V-knockdowned mosquitoes. Both egg chorion melanization rate and egg hatching rate were significantly reduced in As-pro-PO V and VII-knockdowned mosquitoes. Knock down of As-pro-PO III, IV, VI and VIII resulted in significant reduce in the number of lamellae in endocuticle deposited in the cuticle pupae and adults.
CONCLUSIONS: Our results suggested that As-pro-PO I and V were responsible for filarial parasite melanization, and As-pro-PO V and VII participated in egg chorion melanization. In addition, we also found As-pro-PO III, IV, VI and VIII involved in cuticle formation in pupae and adults, a novel function for POs.

FEASIBILITY OF TH1 STIMULATORY PROTEINS AS POTENTIAL POLY VACCINE AGAINST VISCERAL LEISHMANIASIS

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BACKGROUND: Generation of a Th1 type immune response plays an important role in mediating protection against *Leishmania*. The fact that healing and recovery protects individuals from re-infection, is suggestive for the possibility of a vaccine against VL. With this in view, our earlier studies have shown that a fraction of soluble *L. donovani* proteins ranging from 89.9-97.1 kDa induces strong Th1 type response in both cured - human subjects as well as hamsters with significant prophylactic efficacy in hamster against *L. donovani* challenge. Further proteomic analysis of this sub-fraction led to the identification of potential 18 Th1 stimulatory proteins, of which, 15 were developed as recombinant ones and were further subjected to re-assessment of their immunogenicity. In this study we have further carried out immunological characterization of these recombinant proteins and identified potent T-cell epitopes using bioinformatic tools for developing synthetic polyvalent vaccine against VL
METHODS: The cellular responses (Lymphoproliferative and cytokine) of the recombinant proteins and synthetic peptides were checked in cured *Leishmania* patients as well as cured hamsters. Bioinformatic analysis of these proteins using online epitope mapping tools-(IEDB/SYFPEITHI) was carried out based on their MHC binding affinity with different HLA-alleles frequently present in VL endemic Indian population.
RESULTS: Six out of 15 recombinant proteins - elongation factor-2 (EL-2), p45, aldolase, enolase, triosephosphate isomerase (TPI) and protein disulfide isomerase (PDI) were found to be highly immunogenic. Moreover, among these only p45, enolase, TPI and PDI have shown more than 85% prophylactic efficacy in hamsters against *L.donovani* challenge. Several antigenic peptides of of six potent Th1 stimulatory proteins have been identified and synthesized and re-evaluated for their immunogenicity.
CONCLUSIONS: The results will enable us to design polyvalent synthetic vaccine that will be a step further in the process of vaccine development.

The contribution of academia to new drugs for neglected tropical diseases

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BACKGROUND: Neglected tropical diseases (NTDs), a group of about 20 diseases caused by viruses, bacteria, protozoa and helminths, represent a major health burden to a significant part of the world's population. These diseases kill each year millions of people and are responsible for vast morbidity and disability. The existing diagnostic tools and drugs are inadequate for many of these diseases, especially drugs which lack efficacy and safety or which require long and complicated application. New drugs are urgently needed to effectively launch the fight against NTDs. Product-development-partnerships i.e. the Foundation for Innovative New Diagnostics (FIND), the Medicines for Malaria Venture (MMV) or the Drugs for Neglected Diseases initiative (DNDi) are contributing to close the R&D gap and bring new products to the patients.
RESULTS: Academic groups play a major role in drug discovery and hit to lead generation. Our research Unit *Parasite Chemotherapy* is screening and evaluating new compounds for protozoan parasites using in vitro assays and animal models. Our activity/efficacy data helped our partners to bring several drug candidates to clinical trials such as the synthetic peroxide OZ439 or the spiroindolone KAE609 for malaria and aromatic diamidines or fexinidazole for human African trypanosomiasis.
CONCLUSIONS: Several clinical candidates in the drug pipelines for protozoan diseases have realistic chances to become new treatments during the next 3 years e.g. fexinidazole or the oxaborole SCYX-7158 for African sleeping sickness. NTDs are ear-marked for world-wide elimination by WHO and the international community. According to the roadmap five NTDs including African sleeping sickness are targeted for elimination by the year 2020. New effective, safe and oral drugs will be key elements in these elimination efforts.

A new approach to detecting *Schistosoma mansoni* (Platyhelminthes: Trematoda) - in *Biomphalaria* (Gastropoda: Planorbidae) host pools

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BACKGROUND In Brazil, *Biomphalaria glabrata*, *B.tenagophila* and *B.straminea* are naturally found infected by *Schistosoma mansoni*. The diagnosis is routinely performed through detection of *S.mansoni* cercariae by means of artificial light exposure or by squeezing snails between glass slides. But these traditional diagnosis methods are ruled out when sporocysts are undergoing early stages or when field collected snails are infected with different trematode species or when they arrive dead in the laboratory. Some molecular techniques proved to be efficient to detecting this parasite. More recently, the Loop-Mediated Isothermal Amplification (LAMP) was used for detection of *S.haematobium* and *S.mansoni* DNA in infected snails. In this study, the LAMP use is described to detect the presence of *S.mansoni* in: (1) differents Brazilian intermediate hosts, (2) *B.glabrata* snails during the prepatent period, (3) snails pools; and (4) the distinguishing *S.mansoni* among other trematode.
METHODS: The DNAs of parasites and snails were submitted to LAMP. The samples were divided into four groups. i) Intermediate hosts group: DNA of *B.glabrata*, *B.tenagophila* and *B.straminea* negative and shedding *S.mansoni* cercariae; ii) Prepatent period group: DNA of *B.glabrata* prepatent period (11 days after exposition); iii) DNA pool group: 1) 100 *B.straminea* negative and one shedding *S.mansoni* cercariae; 2) 100 *B. glabrata* negative and one shedding *S.mansoni* cercariae; 3) 300 *B.glabrata* negative and one shedding *S. mansoni* cercariae; iv) Other cercaria group: *Cercaria macrogranulosa*, *Cercaria caratinguensis* and *Cercaria ocellifera*.
RESULTS: The LAMP technique detected *S.mansoni* DNA in infected snails, being a rapid (average 3 hours) and sensitive technique. This methodology saves time and costs, since it avoids the use of thermocyclers and electrophoresis instrumentations.
CONCLUSIONS: We presented the possibility of detecting *S.mansoni* in the molluscs pools which facilitates its detection in endemic areas or in low prevalence regions.
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Molecular identification of *Anisakis* spp. (Nematoda: Anisakidae) in stranded cetaceans from the Mediterranean Sea, NE Atlantic Ocean and SE-SW Pacific waters, with insights into host-parasite co-phylogenetic aspects

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BACKGROUND**:** Members of the genus *Anisakis* occur worldwide in a range of cetaceans, and genetic/molecular markers have been used to study their systematics, taxonomy, ecology and phylogeny. Such markers allowed detectiing new species and helped clarify the co-evolutionary processes between *Anisakis* spp. and their definitive hosts.
METHODS**:** Different species of stranded cetaceans from worldwide seas and oceans were surveyed and several adult specimens of *Anisakis* spp. were collected. *Anisakis* species were identified by multilocus allozyme electrophoresis and sequenced at two mitochondrial genes (mtDNA *cox2, rrnS*). The genetic structure of *Anisakis* spp. specimens was compared with *Anisakis* spp. already genetically characterized.
RESULTS**:** Adult *Anisakis* analysed from *Physeter macrocephalus* from Greek coasts and from Scottish waters resulted to belong to *A. physeteris*, while specimens from *Lagenorhynchus albirostris* stranded in the same NE Atlantic area belonged to *A. simplex* (s. s.). Worms in *Ziphius cavirostris* from Greece occurred as a mixed infection (*A. physeteris* plus *A. pegreffii)*, while *Z. cavirostris* specimens from Ionian Sea and Chile coast were found infected by *A. ziphidarum*. The dolphins *Stenella coeruleoalba* and *Tursiops truncatus* stranded along the Italian coasts were infected by *A. pegreffii*; *Globicephala melas* from Chile and New Zealand coasts contained a mixed infection of *A. pegreffii* and *A. berlandi*.
CONCLUSIONS**:** Results from this study enhance knowledge about the geographical distribution and hosts of *Anisakis* spp., improving information on their systematics and ecology. Host-parasite associations observed further support co-evolutionary scenarios that have been proposed for these worms with respect to the phylogeny of their hosts.

Molecular detection of tick and tsetse-borne bovine pathogens in Nigeria

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BACKGROUND: Tick and Tsetse borne diseases are very important especially in the tropics. In Nigeria these diseases are responsible for loss in production. Control measures include chemotherapy and control of the vector tick and tsetse fly.
METHODS: A total of 129 blood specimens were collected from apparently healthy cattle from north eastern and central Nigeria. All the specimens were initially screened for the presence of *Babesia*/*Theileria* and *Erhlichia* / *Anaplasma* genomic DNA using PCR and Reverse line blot assays. The same specimens were further screened for trypanosome species using a single polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay, used to characterize all important trypanosome species.
RESULTS: The RLB results had all 129(100%) of the cattle to be positive for one or more parasites. Some of the parasites reported are; *Anaplasma marginale* 66(51.16 %), *Babesia bovis* 19 (14.73 %), *Theileria mutans* 98 (75.97 %), *Theileria velifera (68.22 %), Theileria sp. sable* 41(31.78 %), *Erhlichia sp. omatjienne* 14(10.85 %), *Babesia bigemina* 3 (2.33) and *Theileria annulata* 6 (4.65%). The PCR-RFLP had 23.26 % of the cattle to be positive for trypanosomes. The species reported are *Trypanosoma theileri* 27 (90 %), *T. brucei* 2 (7 %) and *T. vivax* 1(3 %).
CONCLUSIONS: The results show that the bovine population harbour varied haemoparasites, some of which are reported for the first time in Nigeria.

Elevation influence on Phlebotominae (Diptera: Psychodidae) distribution and American cutaneous leishmaniasis occurrence in an endemic area of southeastern Brazil

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BACKGROUND: Sandflies are the vectors of American Cutaneous Leishmaniasis (ACL), a parasitic disease that present a high incidence in the municipality of Cariacica, state of Espírito Santo, southeastern Brazil. In the endemic locality of Roda D'Água, cases of ACL occur up to 500 m above sea level. In the present study, we evaluated the local sandfly fauna aiming to incriminate the main vectors.
METHODS: We captured sandflies by means of Shannon traps in three elevation levels (up to 250 m, between 250 and 500 m, and above 500 m), each divided in two environments (forest and peridomicile). The occurrence of the species were also evaluated by seasons.
RESULTS: We collected 13,233 sandflies, distributed in 26 species, four of them highly anthropophilic. *Nyssomyia intermedia* (61.1%) was the predominant species, followed by *Pintomyia fischeri* (18.2%) and *Migoneimyia migonei* (8.7%). *Pintomyia monticola* represented 1.7% of all evaluated species. Elevation affected the distribution of sandflies in a way that *Mg. migonei* was more abundant up to 250 m, *Ny. intermedia* and *Pi. fisheri*, between 250 and 500 m and *Pi. monticola* above 500 m. *Ny. intermedia* and *Mg. migonei* were more abundant in peridomicile and *Pi. monticola* in forest. *Pi. fisheri* showed no preference for determined environment, but was the only one affected by season, most commonly found in dry periods and during the winter.
CONCLUSIONS: *Ny. intermedia* was the species predominant and seems to be the main ACL vector, and *Mg. migonei* probably act as secondary transmitter. *Pi. fisheri* does not appear to be involved in local human transmission, despite having been already incriminated in other regions. *Pi. monticola* was not considered an important transmitter, being more abundant at highest elevation in forest environment.

Validation of *T.cruzi in vitro* drug discovery cascade – identification of a novel small-molecule series with *in vivo* efficacy.

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BACKGROUND: Chagas disease is a major health problem in Latin America. The only available treatments, the nitrodrugs Nifurtimox and Benznidazole, have significant drawbacks in terms of toxicity and variable efficacy. While small-molecule drug discovery efforts for Chagas disease have recently increased, the pipeline remains remarkably empty due to high levels of attrition (unwanted MOA, lack of *in vivo* efficacy, failure in clinical trials …).
METHOD: We have developed a drug discovery screening cascade with the specific aim of improving the chance of success in the clinic based on our experience with other kinetoplastid parasites. This cascade consists of an image-based, relatively high-throughput single point and potency assay to identify hits, followed by a series of secondary assays to build confidence in the hit series. These include a cidality assessment, a CYP51 inhibition assay and testing against a panel of clinical isolates.
RESULTS: We have to date screened over 175,000 compounds in the primary assay and identified several series of interest with *in vivo* efficacy. One series has now progressed into lead-optimisation and we will discuss the results of this series in our *in vitro* screening cascade.
CONCLUSION: We have designed a robust screening cascade for *T. cruzi* to develop novel lead-optimisation candidates. The cascade has the throughput necessary to screen large compound collections and confides high confidence in the resulting compound series by means of a panel of secondary assays.

Periparturient parasitism impacts on methane production

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BACKGROUND: Since methane release by ruminants is directly related to feed intake, factors that influence feed intake, such as disease challenge, are likely to affect methane release. However, direct measurements of disease challenge on methane release are needed to account for the impact of disease *per se*.
METHODS: Twin-rearing ewes were *ad libitum* fed pelleted lucerne from day_{-32} to day_{36} (day_0 is parturition), and infected or not with 10,000 *Teladorsagia circumcincta* L_3 every Mon-Wed-Fri (n=16). A third group of 16 ewes were fed at 80% of uninfected ewes' feed intake during lactation. Feed intake was measured twice weekly. Ewe and litter bodyweight were assessed weekly. Staggered lambing allowed for four rounds of housing in one of six methane chambers for six days from day_{30} (two ewes per chamber). Methane release over the last 24 h was used for this analysis.
RESULTS: Parasitism reduced feed intake and litter weight gain by 9 and 7%, respectively and increased maternal bodyweight loss ($P<0.05$). Whilst parasitism reduced daily methane production by 10% ($P<0.05$), methane yield was similar, averaging 10.6 g per kg intake. However, additional feed intake needed for delayed weaning at similar lamb weight and compensation of additional maternal bodyweight loss suggests that periparturient parasitism increases methane output per lamb weaned by 14%. Extrapolation of our findings to lambs using published pen and field studies would predict that methane costs of lamb parasitism could be considerably greater.
CONCLUSIONS: Periparturient parasitism increases methane production arising from accounting for production losses, suggesting that ewe worm control can improve productivity and reduce environmental footprint of sheep production systems.
Funding was granted by the Scottish Government.

Geographical characterization of cutaneous, mucosal and visceral leishmaniasis in the municipalities of the state of Bolivar, Colombia

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BACKGROUND: There are few published studies that characterize the epidemiologic behavior of leishmaniasis using epidemiologic maps. This is why, this research group chose to map the incidence of cutaneous (CL), mucosal (ML) and visceral leishmaniasis (VL) in Bolívar, Colombia, from 2007 to 2011.
METHODS: A study was conducted to characterize geographically (estimated as cases/100.000pop) the annual incidence of leishmaniasis (CL, ML and VL), in 46 municipalities and the district, of the state of Bolivar, Colombia, between 2007 and 2011. The data sources were the surveillance system SIVIGILA and the national statistics institute DANE. Rates were processed with the program as Kosmo ® 3.1 as geographic information system (GIS), which allowed the creation of 15 epidemiological maps of the state of Bolivar 18 municipalities, by municipality, years and clinical forms of the disease.
RESULTS: In 2007-2011, there were 1435 cases of CL reported, 20 cases of ML and 42 cases of VL, this represents a cumulative rate of 73.21 cases/100,000pop., 1.02 cases/100,000pop. and 2.14 cases/100,000pop., respectively. During the study period, the highest rates of both CL and ML were in Santa Rosa del Sur (318.44 and 16.91, respectively, both in 2007), and for VL in El Carmen de Bolivar (27.50 in 2007), both, rural municipalities of this state.
CONCLUSIONS: Cutaneous leishmaniasis focused on southern municipalities (Tiquisio Norosi, Arenal, Santa Rosa del Sur and San Pablo) and northern municipalities (El Carmen de Bolivar, San Jacinto and Mahates) of the state of Bolivar. While the VL was more prevalent in two municipalities of the north (El Carmen de Bolívar and Cartagena de Indias). Maps developed in conjunction with the assessment of environmental, socio-economic and welfare variables would more accurately assess the behavior of leishmaniasis and help in prioritizing different interventions.

Amphibian Parasite Inventory in Hortobágy National Park (Hungary)

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BACKGROUND: Parasitological perspectives in biodiversity inventories provide powerful insights into the history, structure, and maintenance of the biosphere.
METHODS: We collected 101 individuals of the *Pelophylax esculentus* complex (Ranidae) in 2012 and 2013 from sites associated with flowing water, a fish pond system and a wetland marsh system.
RESULTS: We found the following species: Digeneans: *Haematoloechus variegatus*, *Opisthioglyphe ranae*, *Diplodiscus subclavatus*, *Pleurogenes claviger*, *Pleurogenoides medians* Nematodes: *Oswaldocruzia filiformis*, *Rhabdias esculentarum*. Acanthocephala: *Acanthocephalus ranae*. *R. esculantarum* is a new species for the Hungarian fauna and *P. ridibundus* represents a new host record for *R. esculentarum*, while *D. subclavatus, P. claviger* and *P. medians* is also a new species for the helminthofauna of the Hortobágy National Park. The overall prevalence was 76.2 % [95%CI:66.89-83.78] in the examined water frog population. The overall mean intensity was 2.44 [95%CI:2.17-2.71] helminth species per host.
CONCLUSIONS: Our current findings showed significant discrepancy from the results of baseline inventories carried out 30 years ago in HNP, but the reasons are not clear. We suspect that the helminth species previously reported that we did not encounter are restricted to a host we have not yet collected.

EVALUATION OF RECOMLINE® AVIDITY AS CONFIRMATORY TEST IN CONGENITAL TOXOPLASMOSIS

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BACKGROUND: There exists a need to improve the early identification of children with congenital toxoplasmosis by evaluating alternative confirmatory assays that could increase sensitivity or reduce the need for multiple criteria for diagnosis in newborns.
METHODS: We used the 5972 Recom-Line *Toxoplasma* IgG [Avidit.] from Mikrogen Diagnostics (Germany). We evaluated the avidity of IgG specific antibodies in 10 serum samples taken during the first three months of life in children with confirmed congenital toxoplasmosis and 10 children who became anti-*Toxoplasma* IgG negative in the absence of treatment (confirmed absence of congenital infection). Avidity was estimated by comparing bands intensity for P30, MAG1 and rSAG1 proteins with and without urea treatment. Sensitivity and specificity and confidence intervals at 95% (CI95%) were calculated.
RESULTS: Low avidity for P30 protein obtained sensitivity of 66% (IC95% 30.3-100) and specificity of 100% (IC95% 93.7-100). Low avidity for MAG1 protein obtained sensitivity of 57% and specificity of 71%. Low avidity for GRA1 protein had sensitivity of 66% and specificity of 75%. Low avidity rSAG1 had sensitivity of 85% and specificity of 42.8%. Three children without specific IgM or IgA at birth were identified by the P30 low avidity criteria.
CONCLUSIONS: Low avidity criteria for P30 protein in Western blot Recom-Line kit obtained the best sensitivity and specificity and identified children who were negative for first line diagnostic criteria.

Protein Palmitoylation in Trypanosomes: Roles in Protein Trafficking and Parasite Virulence

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BACKGROUND: Trypanosomes are single-celled eukaryotic parasites that cause neglected diseases of poverty such as African sleeping sickness and Chagas disease. Targeting of some proteins to the ciliary (flagellar) membrane of trypanosomes requires dual N-terminal acylation with myristate and palmitate and a stretch of positively charged amino acids nearby. Ciliary membrane targeting is also dependent on the unique lipid chemistry of the trypanosome ciliary membrane, a discrete cell surface membrane domain that is highly enriched in sterols and sphingolipids. The calflagins are myristoyl/palmitoyl calcium sensors that associate with lipid rafts in the flagellar membrane of the African trypanosome *T. brucei*. Prevention of palmitoylation (C3A mutant) leads to mistargeting of calflagin to the cell body membrane.
METHODS: To identify the specific palmitoyl acyltransferase (PAT) that palmitoylates calflagin, we performed an RNAi screen using cell lines in which each of the 12 TbPATs can be inhibited. This screen revealed that a single enzyme, TbPAT7, is necessary for calflagin palmitoylation and flagellar membrane targeting. Inhibition of TbPAT7 caused calflagin the same mislocalization to the cell body membrane as that observed in the C3A mutant.
RESULTS: We determined the 124-member palmitoyl proteome of *T. brucei* using acyl biotin exchange and tandem mass spectrometry. This palmitoyl proteome includes all of the known palmitoyl proteins in T. brucei as well as several proteins whose homologues are palmitoylated in other organisms. The TbPAT7-specific palmitoyl proteome was determined in TbPAT7 RNAi cells, which identified a subset of palmitoyl proteins, including the calflagins. We next investigated the trafficking of calflagin to the flagellar membrane and, in particular, the site of myristoyl calflagin palmitoylation. TbPAT7 is located in the ciliary (flagellar) pocket, a specialized membrane domain from which the flagellum emerges and through which all endocytosis and exocytosis occurs. This finding has allowed us to develop a model in which some proteins destined for the flagellar membrane are first myristoylated in the ER, traffic to the flagellar pocket in membrane vesicles where TbPAT7 palmitoylates them, and then enter the flagellar membrane compartment where they find their proper location.
Finally, treatment of parasites with 2-bromopalmitate, an inhibitor of protein palmitoylation, caused potent growth inhibition, yet there was no effect on growth by the separate, selective inhibition of each of the 12 individual *T. brucei* palmitoyl acyltransferases.
CONCLUSIONS: This suggested either that *T. brucei* has evolved functional redundancy for the palmitoylation of essential palmitoyl proteins or that palmitoylation of some proteins is catalyzed by a noncanonical transferase. Infection of mice with parasites in which individual TbPATs could be inducibly inhibited by addition of doxycycline to the drinking water revealed that some PATs but not others are virulence factors in infection.

Trypanosoma cruzi - *Triatoma pallidipennis* interactions

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BACKGROUND: The innate immune system of vectors is constituted by several molecular pathtways, between them, the synthesis of antimicrobial peptides (AMPs) display a broad activity against microorganisms as virus, bacteria and protozoa. These are peptides of less than 100 amino acids that are divided into five main groups: cecropins, insect defensins, glycine rich/proline rich peptides and lysozymes. They disrupt the integrity of the membrane by depolarization and pore formation. In triatomines, important Chagas diseases vectors, there are few studies on AMPs and nothing in the endemic triatomines of México. The aim of the present work was to describe the presence of AMPs in *Triatoma pallidipennis* during the infection course with *Trypanosoma cruzi*.

METHODS: fifth instars of *T. pallidipennis* were reared in the laboratory and feed with blood from infected Balb/c mice. At different time points, middle and posterior gut, yellow body and haemolymph were collected and analyzed for AMPs. Expression of defensins (1, 3 and 4) and lysozymes (1 and 2) were quantified. Their sequences were compared with different insect groups.

RESULTS: Differential expression of AMPs was observed in different vectors organs independently of the presence of *T. cruzi.* Especially, differential expression of peptides was observed when middle and posterior intestine were compared. Additionally, a lysozyme from the haemolymph was characterized as lysozyme type C by its activity and inhibition characteristics. These peptides share high homology (93-88%) with AMPs from *T. brasiliensis*, *T. infestans* and *Rhodnius prolixus*.

CONCLUSIONS: A diversity of expression of AMPs in different organs is a characteristic of this innate mechanism in *T. pallidipennis*. The importance of long co-evolution between the parasite and vector is shown for the apparent lack of increased expression of some AMPs in the presence of *T. cruzi.*

ACKNOWLEDGEMENTS: This work was supported by Grant IN206512 from DGAPA-UNAM and grant from Fundación Miguel Alemán to Dr. Bertha Espinoza.

Finding of *Blastocystis* sp. in *Crassostrea virginica* oysters

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BACKGROUND: *Blastocystis hominis* is the most common parasite protozoo in the digestive tract of man. This parasite has also been isolated from primates, pigs, cattle, amphibians, birds, rodents and insects. Nevertheless, there are no studies to explore whether infected raw animal consumption with *Blastocystis sp.* coud be a transmission mechanism. We studied the presence of *Blastocystis* sp. in *Crassostrea virginica* oyster obtained from shopping centers in Mexico City.
METHODS: Five hundred fifty *C. virginica* oysters were examined from June to October 2013. Oysters shells were brushed and rinsed with sterile distilled water, after this procedure, intestine snail was dissected and placed into sterile Petri boxes. Two smears of intestinal content were performed; a direct smear examination stained with Lugol's iodine and with Gomori trichrome. The smears were observed with two magnifications 100x and 400x in a Carl Zeiss microscope. Positive samples were cultured in modified Boeck and Drbohlav medium and PCR was performed with *B. hominis* specific primers (BH1: 5´ GCT TAT CTG GTT GAT CCT GCC AGT 3´ y BH2: 5´ TGA TCC TTC CGC AGG TTC ACC TAC A 3´).
RESULTS: *Blastocystis* cysts were observed in 392 out of 550 (71.3 %) oysters. It was identified a 1770pb amplicon, which is related to *Blastocystis sp*.
CONCLUSIONS: The high prevalence of *Blastocystis sp.* detected in *C. virginica* oysters suggest a possible mechanism of transmission of this microorganism to man from marine molluscs.

Comparative study of two *Trypanosoma cruzi* isolates: Queretaro and Purisima.

Gutierrez-Quiroz,Manuel[1]; Fernández-Presas,Ana María[1]; Fajardo-Tovar, Antonio[1]; Ruíz-González,Leticia[1]; Barbabosa-Martínez,Ignacio[3];Solano-Galvéz,Sandra[1];Rodríguez-Jiménez,JoséAgustín[1];Carbajal Esquivel,Fernando[1]; Molinari-Soriano, José Luis[2]

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BACKGROUND: *Trypanosoma cruzi* was originally isolated 27 years ago from feces of a *Triatoma Barberi* specimen from the State of Queretaro, Mexico. This *T.cruzi* isolate has a gradual virulence decrease detected in the susceptible murine model. Therefore, it was important to isolate a recent strain, for analysing their biological behavior and compared with the one isoloted in the State of Queretaro.
METHODS: A T. *cruzi* isolate was recovered from *Triatoma barberi* feces in Queretaro State (Purisima de la Cueva). Triatoma feces with metacyclic trypomastigotes were inoculated intraperitoneally to 2 Female mice. After 15 days of infection mice were anesthetized to obtain total blood. Blood was pooled an aliquoted; one lot was inoculated on N.N.N medium and in RPMI to analyse epimastigotes growth curve. Another lot was inoculated intraperitoneally to mice with the bloodstream forms($1x10^6$). Histophatology from the different organs was performed. The same procedure was done with the Queretaro *T.cruzi* isolate. *T.cruzi* isolates was compared for significance using analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test.
RESULTS: No statistically significant differences was found in the epimastigotes growth curves from both isolates, neither in parasitemia curves $p>0.05$.Histopathological analysis of dissected organs (heart,skeletal muscle and esophagus) showed statistical differences between the presence of amastigotes nests in mice cardiomyocytes of the Queretaro isolate *versus* Purisima $p\angle 0.01$, more amastigotes nests were found in mice skeletal muscle of the Purisma isolate when compared with Queretero $p\angle 0.001$. No differences were seen the mice esophagus from both isolates $p>0.01$.
CONCLUSIONS: The two isolates of *T. cruzi* were obtained from the same place in the state of Queretaro in The Purisima de la Cueva locality and from a *Triatoma barberi* specimen, but no significant differences in the biological behavior of both isolates were found, nevertheless, the Queretaro isolate were obtained in 1987 and La Purisima 20 later (2007). It would be interesting to perform molecular studies to determine significant differences, for example in the kDNA.

Virus-like particles in *Trypanosoma cruzi* epimastigotes.

Fernández –Presas, Ana Ma [1], Robert –Guerrero, Lilia ;[1]., Tato –Zaldívar,Patricia; Jiménez-Rodríguez, José A[1]; Solano-Galvez,Sandra[1], Molinari –Soriano,José Luis[2]

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BACKGROUND: Virus like particles (VLP) have been found in the cells of animals throughout the animal kindgdom, and the presence of virus in parasite protozoa has also been described. We have found VLP within the cytoplasm in an isolate of *Trypansoma* cruzi epimastigotes. The present study describes the ultrastructure of the epimastigotes of *Trypanosoma cruzi* and the virus like particles in *T.cruzi* epimastigotes.
METHODS: *Trypanosoma cruzi* was isolated from an specimen of *Triatoma barberi* feces obtained from the State of Queretaro, Mexico. Triatoma feces with metacyclic trypomastigotes were inoculated intraperitoneally to Female 5 CD-1 mice (4 weeks old, 20±2g).After 15 days of infection,mice were anesthetized to recover total mice blood. The blood from mice was inoculated to N.N.N medium and in RPMI-1640. Parasites (5 x 10^6) obtained from log growth phase was harvested and washed three times in fresh medium at 4°C, were processed for electron microscopy rinsed. Thin sections were cut with a diamond knife at 50-70 nm on a Leika ultramicrotome and stained sequentially with uranyl acetate and lead citrate. The specimenes were examined and photographed with a JEOL JEM-1200 EXII transmission electron microscope.
RESULTS: Some VLPs were observed in the parasite cytoplasm,,close to the Golgi apparatus or the flagellum. The virus population is comprised of different virus-like particles with varying sizes (48-60 nm) and shape (cylindrical and spherical particles) The VLPs were organized in paracrystalline clusters. Epimastigotes exhibit a plasma membrane, microtubules, Golgi cisternae, mitochondria, kinetoplast, nucleus and flagellum well conserved. Parasites has a nucleus with condensed heterochromatin around the nucleolus and close to the nuclear envelope, Lipid bodies, dense vesicles and Golgi apparatus were seen in the cytoplasm, but endoplasmic reticulum was rarely observed.
CONCLUSIONS: Further studies are necessary to identify these virus-like particles within the parasite in order to explain if they have any significance in *T.cruzi* pathogenesis

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Fernández-Presas, Ana M

a[1]; Robert Guerrero, Lilia[1]; Tato-Zaldívar, Patricia[1]

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Jiménez-Rodríguez, José A[1];

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[1] Departamento de Microbi

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