Gene regulation at post-translational level in *Giardia duodenalis*: the paradigmatic case of the g14-3-3 protein

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BACKGROUND: The 14-3-3s are a family of dimeric evolutionary conserved pSer/pThr binding proteins that, interacting with a plethora of client proteins, play key roles in multiple biological processes. *Giardia duodenalis* encodes for a single 14-3-3 isoform (g14-3-3) that, unique in the 14-3-3 family, is constitutively phosphorylated and polyglycylated on its C-terminus. Alteration of g14-3-3 phosphorylation and polyglycylation status affects the parasite encystation. To further investigate the role of these PTMs, the crystal structure of the g14-3-3 was solved.
METHODS: A recombinant GST-g14-3-3 was expressed in *E. coli* and used to produce diffraction-quality crystals. Various g14-3-3 mutants were produced and studied by native PAGE, chemical cross-linking and transmission electron microscopy (TEM). Molecular dynamic simulation was used to analysed the protein behavior with or without phosphorylation.
RESULTS: The crystal structure of the g14-3-3 in the apo form was solved. Oligomers of g14-3-3 were observed due to domain swapping events at the protein C-terminus. The formation of protein filaments was supported by TEM. Mutational analysis proved that polyglycylation is necessary to prevent oligomerization. In silico phosphorylation and molecular dynamics simulations supported a structural role for the phosphorylation of Thr214 in promoting target binding.
CONCLUSIONS: Our findings highlight unique structural features of g14-3-3 opening novel perspectives on the evolutionary history of this protein family and envisaging the possibility to develop anti-giardial drugs targeting g14-3-3.

The Lipophosphoglycan of *Leishmania amazonensis*: intraspecific variation and interaction with macrophages.

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BACKGROUND: Intraspecies variations in the lipophosphoglycan (LPG) from New World species of *Leishmania* have been assessed only in *Leishmania infantum.* This glycoconjugate is highly polymorphic among species with variations in sugars that branch off the conserved Gal(β1,4)Man(α1)-PO_4 backbone of repeat units. The structure and the degree of intraspecies polymorphism in LPG of *Leishmania amazonensis*, the causative agent of diffuse cutaneous leishmaniases, are not known. In this study, intraspecific variation in the repeat units of LPG was evaluated in two strains of *L. amazonensis* (PH8 and JOSEFA) from Brazil.
METHODS: LPGs from both strains were extracted and purified using phenil-sepharose. The LPGs were depolymerized using mild acid hydrolysis and the repeat units were dephosphorylated using alkaline phosphatase. The neutral repeat units were subjected to fluorophore-assisted carbohydrate electrophoresis (FACE) and the profiles analyzed after enzymatic treatment with β-glucosidase. LPGs from both strains were incubated with murine peritoneal macrophages from Balb/c, C57BL/6 and respective knock-outs for TLR2 (-/-) and TLR4 (-/-). NF-kB translocation was assessed using Chinese Hamster Ovary cells (CHO) using CD25 as the reporter gene. Translocation was detected using flow cytometry.
RESULTS: One strain (PH8) was originally isolated from the sand fly and the other (Josefa) was isolated from a human case. Both strains exhibited structural polymorphism, with the former possessing 2-4 β-glucose side chains and the latter being poly-glucosylated. The significance of these modifications was investigated during in vitro interaction with murine macrophages and CHO cells. In macrophages, only the LPG from PH8 strain was able to activate TLR2, resulting in higher nitric oxide and cytokine production (TNF-α, IL-1β and IL-6). However, the opposite was observed with live parasites from Josefa strains. Interestingly, in CHO cells, both LPGs were not able to translocate NF-kB.
CONCLUSION: Those data demonstrated the LPGs of *L. amazonensis* from both strains are very pro-inflammatory without triggering NF-Kb translocation.

Toxoplasma gondii: Inflammation and Immunity in the Mucosal Response to Infection

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The intracellular protozoan *Toxoplasma gondii* elicits strong Th1 responses that are necessary to protect the host and ensure survival. In the intestine following oral inoculation, chemokine-mediated recruitment of Th1 cells and inflammatory monocytes ensures appropriate activation of the latter to control infection. While usually assuming a protective role, mucosal CD4+ T cells can also cause proinflammatory lesions in the intestine. Thus, it is critical that the appropriate balance between inflammation and protective immunity is achieved to enable host and parasite survival.

Effect of combined therapy with Albendazole and Sutrim in experimental cryptosporidiosis.

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BACKGROUND: Many chemotherapeutic agents have been tested against cryptosporidiosis.This work is a trial to elucidate the effect of a combination of a broad spectrum antihelmintic: Albendazole, and sutrim on experimental *C.paruim* infection.
METHODS: Male albino mice were obtained from Schistosome Biological Supply Centre (SBSC), Theodor Bilharz Research Institute (TBRI). Viable *Cryptosporidium* oocysts were obtained from stool samples of infected cows. A group of 40 albino mice were infected orally with 10,000 *Cryptosporidium* oocysts per mouse, mice were divided into infected control (Grp.1), Infected treated with albendozole orally (0.4 mg/ mice) daily for five consequence days two weeks postinfection (Grp 2). Infected treated with Sutrim orally (1.2 mg/ mouse) daily for five consequence days two weeks postinfection (Grp 3). Infected treated with half the doses of both drugs orally (0.2 mg Albendazole / mouse + 0.06 mg) daily for five consequence days two weeks postinfection(Grp.4). *Cryptosporidium* oocysts counts in feces and intestinal contents as well as histopathological changes in intestines of were evaluated.
RESULTS: A significant difference in the number of cysts/gm stool was recorded between control and infected treated Grp.4. The percent reduction in the number of cysts/gm stool was 28.6%, 54.2% and 55.3% in infected treated groups Gp.2, Gp.3 and Gp.4 respectively. A highly significant reduction was found in the mean number of *Cryptosporidium* oocysts/ gm stool five days post- treatment. The reduction in oocysts excreted in the stools the groups II, III, VI were 49.9%, 74.3%, 75.7%, respectively compared to infected control group. Again, the reduction in *Cryptosporidium* stages in intestinal villi was 54.7%, 81.1%, 80.4%, respectively compared to infected control group. Histopathological study confirmed parasitological study.
CONCLUSIONS: Albendozole and Sutrim are efficient anti-cryptospoidial drugs. Sutrim gave high efficacy as well as their combination and may be considered as new therapeutic approaches.

Overwhelming strongyloidiasis – from bed to bench –

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BACKGROUND: Overwhelming strongyloidiasis is often seen in the immuno-compromised hosts. To elucidate the underlying mechanisms of the opportunistic infection of strongyloidiasis, clinical features of overwhelming cases were analyzed by literal survey. In addition, possible cellular effector mechanisms for expulsion of *Strongyloides* from rodent hosts were reviewed.
METHODS: Overwhelming strongyloidiasis cases were gathered by literal survey in Japan during 2000 and 2013 to analyze their clinical features. Effector/regulator mechanisms in the mucosal defense against *Strongyloides* worms were elucidated using rodent models.
RESULTS: We collected a total of 80 overwhelming strongyloidiasis cases in Japan during 2000 and 2013. Residential history were available in 65 cases; 10 cases were the immigrants, 4 engaged in military activity in Southeast Asia during the World War II ("war strongyloidiasis") and 55 born and grown in the Nansei Island, Japan, where ATL/HTLV-1 infection is endemic. Except for the immigrants, the age of the patients was over 50 years old. Among 80 cases, 36 were HTLV-1 positive and 25 have a history of being treated with steroids. In contrast to our results and of others in Japan, association of overwhelming strongyloidiasis with HIV/AIDS remains unclear and controversial.
In the animal model studies, the role of mast cells in the mucosal immunity against Strongyloides worms was highlighted. Expulsion of *Strongyloides* worms was retarded in mast cell deficient *W/W^v* mice. Similarly, gene-knock out of mast cell growth/differentiation factors such as IL3, IL18 and PI3K caused prolonged infection. Interestingly, Mongolian gerbils, of which mast cells are quite different from those of mice and rats in regard to phenotype and subset, are unable to expel *Strongyloides* worms.
CONCLUSIONS: From clinical features of patients, *Strongyloides* worms can differentiate the immune-compromised status caused by HTLV-1 and HIV infection. Mast cells may be an important key to bridge strongyloidiasis research findings between bed and bench.

Efficacy of newly introduced synthetic compounds for treatment of experimental intestinal giardiasis.

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This study was conducted to evaluate the anti-protozoal effect of the two new compounds, (4-Hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-2-oxoacetic acid (M1) and Ethyl 2-(4-Hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-2-oxoacetate (M2**)** on *Giardia lamblia* infection and to study the pathological impact of these drugs on the duodenal mucosa of infected hamsters. Fourteen hamsters were used and divided into four groups. Group (1): *G. lamblia* infected-untreated hamsters served as control. Group(2): infected with *G. lamblia* and treated with metronidazole. Group (3): *G. lamblia* infected and orally-treated with M1 compound (100 mg\kg/3 successive days). Group (4): *G. lamblia* infected and orally-treated with M2 compound (100 mg\kg/3successive days). RESULTS: Treatment of Giardiasis 2 weeks post-infection orally administered with compounds M1 and M2 gave a highly statistical significant reduction ($p< 0.001$) in the number of cysts\gm stool (76.38% &82.71%,respectively). Number of vegetative forms (trophozoite) in the small intestine of sacrificed hamsters was reduced significantly ($p< 0.001$) (71.99% after M1 treatment , & 80.12% after M2 treatment) when compared to infected-untreated control group. Pathological examination of the upper third of the duodenum revealed complete villous shortening and atrophy, polymorphonuclear and eosinophilic cells with diffuse loss of brush border microvillus surface area on group of hamster treated with compound M2. Compound M2, had a promising effect on the *Giardia lambila* infection and was superior than treated with Metronidazole. Key words: Infected hamsters ,*Giardia Lamblia* cysts, Intestinal giardiasis, Newly synthetic compound, Histopathological Studies .

Epidemiology of Intestinal Parasites Versus Knowledge, Attitudes and Practices of Residents of a Low-Income Community of the *Complexo de Manguinhos,* Rio de Janeiro, Brazil.

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BACKGROUND: The reach of intestinal parasites both globally and regionally is indisputable: more than two billion people worldwide, roughly a third of the global population, are estimated to be parasitized (WHO 2005a) with 42% of the population of Latin America infected (Hotez et al. 2008). In Brazil intestinal parasitic infections (IPIs) caused by protozoa, specifically amebiasis and giardiasis, affect 15.5 million school-aged children, with an estimated prevalence of 30%, yet studies are limited and often do not include community knowledge, attitudes and practices (KAP) vital for developing preventive measures. The purpose of this study is to assess the risk factors, prevalence and KAP of IPIs in an urban low-income community (Parque Oswaldo Cruz/Amorim) of the Complexo de Manguinhos, Rio de Janeiro, Brazil.

METHODS: A questionnaire to evaluate the socioeconomic profile of the eligible, participating households and a KAP survey were utilized and answered by an adult household representative. Fresh fecal samples of the household residents were collected and analyzed using the Modified Lutz method. Individuals with positive samples were referred to the participating local public health clinic for medically supervised treatment. Data were entered into an ACCESS (Microsoft Office 2007 for Windows) database created specifically for this study and exported to Statistical Package for the Social Sciences (SPSS Version 21) for statistical analysis.

RESULTS: Of the 1862 residents in the study, 60.20% (n=1121) returned fecal samples, of which 19.9% were found positive for protozoa or helminthes.

CONCLUSION: Further research is needed to measure the effect of the use of this data in educational interventions in order to direct prevention efforts.

Knowledge, Attitudes and Practices of Health Professionals of the Family Health Strategy Regarding Intestinal Parasites

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BACKGROUND: In an attempt to relieve the burden of intestinal parasitic infections (IPIs), prevention strategies based on popular education have been elaborated utilizing the knowledge, attitudes and practices (KAP) of community members to better develop interventions and understand high-risk groups. However, the KAP of health workers concerning IPIs or the population they interact with has not been addressed. In Brazil, all citizens are entitled to receive free public health services from the *Sistema Único de Saúde* (SUS) making these professionals an essential source of health education, surveillance and promotion, especially in low-income areas.
METHODS: This study aimed to identify the knowledge, attitudes and practices of health professionals of the Family Health Strategy (FHS) responsible for the public primary health care services of residents of the *Complexo de Manguinhos* (CM), an agglomeration of low-income communities in Rio de Janeiro, RJ, Brazil, through the application of KAP surveys divided into 4 categories: individual characteristics; intestinal parasitic infections and comorbidities; clinical approach to IPIs; and social approach to IPIs. Survey answers were categorized as *correct, partially correct* and *incorrect* and analyzed utilizing EpiInfo Version 3.5.1.
RESULTS: The professionals generally presented correct information on intestinal parasitic infections and comorbidities, but answers diverged when addressing the clinical and social approaches utilized.
CONCLUSIONS: This study suggests the need for homogenization of the approach to these neglected diseases, which includes consideration of both biomedical and social factors of IPI vulnerability.
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Frequency of emerging parasites in stool samples from HIV and Oncological patients by coprological and molecular analysis.

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BACKGROUND: Several protozoa species have been associated with diarrhea in immunocompromised patients including *Cryptosporidium* spp., *Microsporidium* spp., *Isospora belli*, *Giardia intestinalis*, and *Cyclospora* spp. The magnitude of the public health threat for these emerging parasites is undervalued. Other countries have reported the frequency of opportunistic parasites, but the results have been variable, possibly due to insensitive methods used to detect and identify the infective species. The aim of this study was to determine the frequency of emerging parasites in two groups of immunosuppressed patients, including individuals infected with the human immunodeficiency virus (HIV) or having acute lymphoblastic leukemia (ALL), with or without diarrhea.
METHODS: Stool samples were collected from 96 HIV and 77 ALL patients. Screening under a light microscope to find cysts, oocysts and/or spores of opportunistic parasites was carried out by the Faust method and Ziehl Neelsen staining. DNA was extracted from all fecal samples and then, polymerase chain reaction (PCR) was performed to each parasite.
RESULTS: 22.9% of HIV fecal samples were positive for emerging parasites, including *Cryptosporidium* spp. (7.3%), *Microsporidium* spp. (5.2%), *Isospora belli* (1.0%), *Giardia intestinalis* (2.6%), and *Cyclospora* spp. (7.3%). In ALL fecal samples, 32.5% were positive for emerging parasites, including *Cryptosporidium* spp. (9.1%), *Microsporidium* spp. (19.5%), *Isospora belli* (1.3%), and *Giardia intestinalis* (2.6%).
CONCLUSIONS: The presence, frequency, and distribution of opportunistic or emerging parasites in patients with a deficient immune system is very important, as it impacts not only the quality of life of these patients (if manifested as chronic diarrhea) but may also have effects on the disease course, therapeutic efficacy and tolerability, and mortality. Improved diagnostic methods for these parasites will feature higher specificity and sensitivity to facilitate more accurate and early diagnosis so that the appropriate therapeutic intervention may be initiated in a timely manner.

Effect of new ethyl carbamates against *Rhipicephalus microplus* in vivo

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BACKGROUND: For many years, the most common strategy for *R. microplus* control has been the use of chemical acaricides although the high selection pressure extended by the use of current commercial acaricides has led to acaricide resistance. Carbamates are currently used as antiparasitics and insecticides. The aim of this study is to evaluate the effect of new carbamates on *R. microplus* larvae, nymphs and adults on infested cattle.
METHODS: A triple-resistant tick strain of *R. microplus* was used. Approximately 1000 larvae were collocated into hand-made cloth chambers on the back of the bulls. The treatment with the new ethyl carbamates at egg hatching inhibitory concentration (obtained *in vitro)* was applied in the different parasitic development stages (larvae, nymph or adult). After 21 days the engorged females were collected and collocated in Petri dishes for incubation until the egg hatching. The total engorged females by chamber, the percentage of females laying eggs and egg hatching inhibition percentage with *Etil 4-clorofenil carbamate* were determined.
RESULTS: The number of engorged females by chamber decreased ($p<0.001$) with the treatment in the three stages larvae, nymph and adult, compared with their control (8.37±4.313 and 226.5±15.5; 104.9±32.81 and 402.5±15.50; 148.9±18.92 and 353.5±120.5, respectively). The percentage of females laying eggs was not affected; but compared to untreated females, eggs produced by treated females had a dark, dry, opaque appearance and were less adhered. Finally, the egg hatching inhibition percentage increased ($p<0.001$) with the treatment in the stages of nymph and adult compared with their control (19.48± 4.041 and -1.738e-008± 6.319, 43.94± 3.465 and 7.749e-005± 2.294 respectively).
CONCLUSIONS: The study with Etil 4-clorofenil carbamate showed its good acaricide potencial, nevertheless, it is important to continue with future studies to consider it as a new pharmaceutical option for tick control.

This study was supported by PAPIIT/UNAM

Isolation and *in vitro* cultivation of *Taenia Crassiceps* germ cells

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BACKGROUND: Neurocysticercosis caused by the larval stage of the flatworm *Taenia solium* is the most frequent parasite disease of the human brain. Considerable advances on the understanding of cysticercosis have been achieved using the murine model of cysticercosis based on a close species: *T. crassiceps*. Recent reports have described methods for the transient and stable transfection of different parasite organisms, however, in the case of cestodes, only transient transfection has been developed for *E. multilocularis*. Our final goal is to develop a stable transfection of germ cells and we are working on two directions: 1. Isolation of *T. crassiceps* germ cells, and 2. Developing plasmid constructs with transposon machinery for integration into the *T. crassiceps* genome. Here we describe the identification, isolation and *in vitro* cultivation of germ cells from *T. crassiceps* larvae.
METHODS: Immunolocalization studies on tissue sections of *T. crassiceps* cysticerci were carried out using α-VASA polyclonal antibodies and a fluorescent CY3 secondary antibody. Isolation of germ cells was achieved through physical disruption of the larval tissue followed by tryptic digestion. For cultivation, we used a McCoy medium supplemented with 10% whole vesicular fluid.
RESULTS: Immunolocalization assays showed the presence of abundant positive cells among subtegumentary cytons. Western blotting confirmed the presence of VASA-like proteins in crude extracts of *T. crassiceps*. Blast searches in the *T. solium* genome database indicated that the most probable target for the anti-VASA antibodies was a PL10-like DEAD box RNA helicase. Isolated germ cells support *in vitro* cultivation for at least 1 month.
CONCLUSIONS: We have succeeded identifying, isolating and *in vitro* cultivating germ cells from *T. crassiceps* cysts with a remarkable proliferative capacity. Current efforts are directed to the transfection of these germ cells using plasmid constructs with transposon machinery.

Leishmania exosomes are enriched in short RNA sequences

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BACKGROUND: We hypothesized that exosomes released by leishmania may contain parasite RNAs, and that these molecules may play a role in pathogenesis.
METHODS AND RESULTS: Indeed, our recent findings show that exosomes released by *Leishmania donovani* and *Leishmania braziliensis* contain RNA sequences. Interestingly, in both species the size range of exosomal RNA was considerably narrower than that of total cellular RNA, with the majority of sequences being shorter than 200 nt. To characterize leishmania exosomal RNA in more detail, RNA extracts were processed for paired end sequencing with Illumina MiSeq. When aligning the *L. donovani* and *L. braziliensis* exosome libraries with their respective reference genomes, the vast majority of reads mapped to known non-coding RNA, such as transfer RNA (tRNA) and ribosomal RNA. Only a small number of reads mapped to annotated protein coding sequences and these were present in a very low abundance. We found evidence for the presence of tRNA-derived fragments, a phenomenon recently described in other Trypanosomatids. In addition, a number of sequences mapped to regions of the genome that currently lack annotation, suggesting that leishmania exosomes may contain novel, previously uncharacterized transcripts. Northern blotting with probes for tRNA-derived fragments and novel transcripts revealed that several of these seemed to be enriched in exosomes versus total leishmania RNA.
CONCLUSIONS: Our data suggest that RNA in leishmania exosomes is subject to selective packaging rather than random cytosolic sampling. Current experiments are focused on understanding the function of these RNA sequences in leishmania biology, particularly pathogen-host interactions.

Parasites of two forage fish in the Biological Reserve of the Foz do Rio Aguapeí in the Southeast of Brazil

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BACKGROUND: Fish parasites had been recognized as an important component of biodiversity influencing ecosystem functioning. Although many studies about fish parasites have been realized in last years in Brazil, there is still many freshwater fish species unexploited, especially in relation to forage species. For this purpose, we conducted a study to investigate the parasitic fauna of two forage species, *Satanoperca pappaterra* and *Laetacara araguaiae*.
METHODS: The fishes were collected in ponds in the Biological Reserve of the Foz do Rio Aguapeí on the dry (August, 2013) and rainy (February, 2013 and January, 2014) seasons. Once collected, fish were weighed (g), measured (cm) and dissected in the field to obtain the parasites.
RESULTS: Four helminth parasite species were recovered in *S. pappaterra*: metacercariae of *Austrodiplostomum* sp. free in eyes (prevalence [P] = 27, mean abundance [MA] = 1.4 ± 0.7), *Clinostomum* sp. in muscle and fins (P = 54, MA= 4.1 ± 1.85), *Sciadicleithrum satanopercae* in gills (P = 36, MA = 4.8 ± 2.4), and larvae of a not identified anisakid in mesentery (P = 27, MA = 0.4 ± 0.2). *Laetacara araguaiae* was parasitized by three helminth parasite species: metacercariae of *Clinostomum cf. heluans* in fins (P = 2.9, MA = 0.03 ± 0.02), a not identified digenean in intestine (P = 1.5), and *Paracymothoa* sp. in mouth (P = 7.3, MA = 0.07 ± 0.03). We tested whether differences between seasons and ponds had an effect on parasite communities of these cichlids, but no significant statistical association was found. In contrast, we observed a positive correlation between the length of *S. pappaterra* and abundance of *S. satanopercae* ($r = 0.673$; $p = 0.017$) and *Clinostomum* sp. ($r = 0.0.862$; $p < 0.05$).
CONCLUSIONS: A potential explanation for the association between length and abundance of these parasites is that the increase of the surface area of the body influenced the increment in the abundance with the advance of the host's age. Our result suggests that these forage species had an important role in the metacercariae transmission in ponds and lotic river in the Brazilian freshwater ecosystems.

Trichinella spiralis infection induced the expression of thymosin β4 to stimulate angiogenesis in normoxic condition.

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BACKGROUND: *Trichinella spiralis* (*T. spiralis*) has been reported to up-regulate the expression of the angiogenic molecule vascular endothelial cell growth factor (VEGF) during nurse cell formation.
METHODS: In order to analyze the induction of angiogenesis by *T. spiralis*, the expression patterns of angiogenesis-related proteins were investigated by immunohistochemical analysis. VEGF expression was induced in the infected muscles at an early stage of infection (10 days after infection) and diminished after 3 weeks.
RESULTS: Thymosin β4 (Tβ4), a major factor which induces VEGF, showed increased expression in muscle fibers 10 days after infection, and the expression remained high in the nurse cells for 6 weeks, when the formation of the nurse cell complex was completed. The hypoxia inducible factor (HIF)-1α showed a diffuse expression pattern around the infected muscle fibers and was strongly expressed in inflammatory cells but was not related to the hypoxic condition caused by nurse cell formation. Localization of the hypoxic regions by the hypoxia marker, pimonidazole showed *T. spiralis* infection does not induce a hypoxic condition in nurse cells. We also found Tβ4 and β-actin were co-localized in the *T. spiralis*-infected nurse cells from 10 days to six weeks. The expression patterns of other actin-binding proteins, including Tβ10, subunits of the Arp2/3 complex, subunits of Capping protein, profilin, and cofilin, were also analyzed at the mRNA level. Tβ10 expression was also increased during nurse cell formation. Expressions of the Arp2/3 complex was increased at 21 days after infection and Capping proteins was increased during nurse cell formation but shows different expression patterns, depending on the subunit. Profilin and cofilin were specifically increased in the muscle fibers from 14 days after infection.
CONCLUSIONS: These results suggest that the expression of VEGF and Tβ4 induce angiogenesis and the Tβ4 was co-localized with β-actin to regulate nurse cell formation and maintenance.

Urbanization increases *Aedes albopictus* larval habitats and accelerates mosquito development and survivorship

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BACKGROUND: *Aedes albopictus* is considered one of the most invasive and aggressive vectors that had caused many outbreaks of dengue fever, chikungunya, and yellow fever in many countries globally. Vector ecology and disease epidemiology are strongly affected by environmental changes. Urbanization is a worldwide trend and is one of the most ecologically destructive phenomena. The purpose of this study is to determine how the environmental changes due to urbanization affects the ecology of *Aedes albopictus*.
METHODS: Aquatic habitats and *Aedes albopictus* larval population surveys were conducted from May to November 2013 in three areas which represented rural, suburban and urban settings in Guangzhou, China. *Ae*.*albopictus* adults were collected monthly using BG-Sentinel trap. *Ae*.*albopictus* larvae and adults life-table experiments were conducted with 20-replicates in each of the three study areas.
RESULTS: Urban area had the highest and rural area had the lowest number of aquatic habitats and proportion of *Ae*. *albopictus* larval positive habitat. Densities in larval stages varied among the areas, but urban area had almost two-fold higher densities in pupae and three-fold higher in adult populations compared to suburban and rural areas. Larvae developed faster and adult emergence rate was higher in urban area than in suburban and rural areas. The survival time of adult mosquito was also longer in urban than in suburban and rural areas. Study regions, surface area, water depth, water clearance, surface type, and canopy coverage were important factors associated with the presence of *Ae*. *albopictus* larvae.
CONCLUSIONS: Our results indicated that urbanization can substantially increase the density, larval development rate, and adult survival time of *Ae*. *albopictus*, which in turn can potentially increase the vectorial capacity and therefore the disease transmissibility. Further studies on mosquito infectiousness of dengue virus should be compared among different environmental settings.

Dengue virus replication is positively regulated by miR-281: an abundant midgut-specific miRNA of vector mosquito *Aedes albopictus*

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Abstract
Emerging evidence indicates that host miRNAs involve in viral replication. Here, we report the role of an abundant midgut-specific mosquito *Aedes albopictus* miRNA in the host-virus system. We found that miR-281 specifically expressed in female midgut where dengue virus invaded firstly. After dengue virus serotype-2 (DENV-2) infection, this miRNA showed an increase at day 4. Functional intervention analyses in vitro with the specifically designed miR-281 mimics and corresponding antagomiRs demonstrated that miR-281 positively regulated DENV-2 viral replication. Further the depletion of miR-281 in female mosquitoes by injection of its specific antagomiRs led to significant reduction in DENV-2 abundance. We confirmed the interaction between miR-281 and its predicted target, DENV-2 genomic 5'untranslated region (UTR), by co-transfecting of miR-281 mimic and an enhanced green fluorescent protein-based reporter system. These findings identify that miR-281, an abundant midgut-specific miRNA, facilitates DENV-2 replication through targeting the viral 5'UTR sequence.

Trypanosoma cruzi antigens as biomarkers of pathology in Chagas disease and therapeutic efficacy

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BACKGROUND: Chagas' disease (ChD) affects 10 million people and currently has spread to non-endemic areas. To date, the unique accepted criterion of therapeutic efficacy in ChD is the absence or reduction of anti-*T. cruzi* antibody titers measured by conventional assays. These tests are not useful for a short- and medium-term post-treatment monitoring.
METHODS: The recognition of K11-PFR2-H70 and 3973 *T. cruzi* antigens by 162 sera from ChD patients at different stages of the sickness and 50 sera from non-infected subjects has been analyzed by ELISA. IDO activity has been quantified in PBMCs from 168 chronic patients and 13 healthy donors through the measure of kynurenine using a spectrophotometric assay.
RESULTS: Sera from ChD patients recognized K11-PFR2-H70 and 3973 molecules with high sensitivity and specificity. Shortly after benznidazole treatment, a statistically significant decrease in reactivity against these molecules was observed. The antibody levels against 3973 epitope detected in symptomatic ChD patients are higher than those detected in sera from asymptomatic patients. We also show that the IDO activity of symptomatic chronic ChD patients was higher than that detected in asymptomatic patients. Remarkably, the benznidazole treatment induced a long-lasting decrease in IDO activity from symptomatic patients.
CONCLUSIONS: Our results suggest that K11-H70-PFR2 and 3973 biomarkers are a useful tool for monitoring the treatment effectiveness of ChD and could discriminate between different degrees of illness severity. Furthermore, it is evidenced that a pro-tolerogenic state would be associated with the severity of Chagas disease and that benznidazole treatment is a valuable tool for breaking the parasite-driven immune tolerance in the symptomatic chronic phase.

HDV-ribozyme function is associated to the Pr77-hallmark of trypanosomatids mobile elements: a conserved active sequence in fossil elements?

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BACKGROUND: Trypanosomatid genomes are highly colonised by repeats of long and short interspersed nucleotide elements (LINEs and SINEs, respectively) and their degenerated versions (DIREs and SIDERs, respectively) which have accumulated a large amount of mutations being unable to mobilise by themselves. All the mentioned elements bear a 77 nt-long conserved sequence at their 5'-ends, known as the Pr77-hallmark. The high degree of conservation of this sequence in a variety of mobile elements suggests an important function for this sequence.
METHODS: The putative HDV-like ribozyme structure of the Pr77 signature of different restrotransposons from trypanosomatids was manually explored. Several constructs were generated to evaluate in vitro and in vivo Pr77-signature functionality. Co-transcriptional cleavage activity and cleavage point determination were measured by resolving the transcription reactions of each ribozyme in denaturing polyacrylamide gels and by primer extension.
RESULTS: The Pr77-hallmark is a well-conserved sequence of the L1*Tc*/*ingi* clade retrotransposons in trypanosomatids. Many of these sequences adopt a folding compatible with an HDV ribozyme and exhibit in vitro and in vivo HDV ribozyme function. The upstream and downstream regions flanking HDV-like ribozymes are capable of influencing ribozyme catalytic activity. Some copies of the Pr77-hallmark are located in 3´UTR regions of genes that have a low transcription rate.
CONCLUSIONS: Pr77-hallmark promoter and ribozyme activities may play important roles in trypanosomatid genetic regulation. In *Leishmania spp*, the pervasive presence of these HDV-like ribozyme-containing mobile elements in certain 3'-untranslated regions of protein-coding genes has been linked to mRNA downregulation.

Parasitic Infections of Medical Importance in Thailand

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BACKGROUND: Thailand locates in the tropical zone of SE Asia where infections of many species of medically important parasites are endemic.
METHODS: Parasitic infections of Thailand were reviewed from the first control implementation to the present situation.
RESULTS: Like any countries in the region, in the fiftieth Thai people suffer high parasitic burden in particular soil-transmitted helminthiases (STH) and opisthorchiasis. Ministry of Public Health launched nationwide control program continuously and consistently from 1980 to 2000 using school based control for STH and a mobile team of stool diagnosis and treatment in the endemic area for opisthorchiasis, and able to bring down the infection rate and intensity to a level which no longer considered a public health importance. After 2000, the control program was decentralized and only actively implementing in the areas where it requires. Recent nationwide stool examination survey revealed slightly high rates of opisthorchiasis and hookworm infection, and it affirms the need of effective prevention and control measures that could be sustainably employed throughout the country.
CONCLUSIONS: School health based control activity sould be one of the important measures to keep the parasitic infections at a low or an eliminating level.

Protective intranasal vaccination against *Aspiculuris tetraptera*. Study of the immune response.

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The continual appearance of resistance to drugs used to control helminths in livestock, plus the social awareness concerning the accumulation of drugs in meat, requires a new methods of parasite control, such as vaccination. Recently, several members of our research group have published the levels of protection achieved after intranasal immunization with a recombinant peptide corresponding to the catalytic region of serin threonin phosphatase PP2A of *Angiostrongylus costaricensis*. In these works, high levels of protection were measured both at the immunological as well as the parasitological level. In the present communication, using the murine species of nematode *Aspicularis tetraptera* as a parasite model, we present the study, after the intranasal immunization (0.2 mg/ Kg) and two doses spaced 15 days apart, for the protection levels and the immune-response levels of the immunized challenged animals, the infected animals, and the immunized animals compared with the non-vaccinated, non-infected control animals. The results indicate a protection level of 81.5%. Also, it was found that recombinant PP2A, free of endotoxins, is capable of significantly activating *in vitro* the nuclear receptor NFK b, inducing, after the intranasal inoculation, a response in the mucosa and serum of specific IgA, IgM, and IgG. The confocal microscopic study of the intestinal wall of the immunized and infected animals showed a notable increase in CCL2, CCL17, and CXCL15, both on the edge of the vellosities as well as in the intestinal crypts. The levels of interleukin expression in the spleen of the vaccinated animals infected by RTqPCR, showed a rise in interleukins IFNg, IL2, TNFa IL10, and TGFb in spleen, while in the mesentery ganglion a lower levels of Th1/Th2/Th17 interleukins were found in vaccinated and infected animals compared to infected control.

Intraclonal expression of mucin-associated surface proteins (MASPs), proteins specific to *Trypanosoma cruzi*

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BACKGROUND: MASPs (*mucin-associated surface proteins*) of *T. cruzi* comprise a family of 1377 genes and 433 pseudogenes. They have highly conserved *N*- and *C*-terminal regions, sites for *N*- and *O*- glycosylation, and a hypervariable region. MASPs constitute N-glycosylated proteins. The mechanisms that have allowed the expansion of the MASP family are unknown, although they may involve the defence system of the host. The stimuli that induce expression of the different MASPs also remain unknown. The expression of the members of the MASP family among different strains and stages of *T. cruzi* differ, maintaining constant the differential expression of MASP genes among strains.
METHODS AND RESULTS: By confocal microscopy with antibodies against constant regions, we describe how these proteins are expressed during cell invasion. We observed a differential expression in the different stages of the parasite, with higher MASP-expression levels in trypomastigotes. The cloning of the strain Pan4 (TcI a,e) and the study of the differential expression of MASP genes by Nested RTPCR and by capillary electrophoresis show a different and constant expression pattern between clones, with a differential recognition on the part of sera of individuals with different Chagas pathologies. The conserved C-terminal region of the MASP proteins constitute antigens that can be recognized by the IgM as well as by the IgGs, showing a greater avidity for the IgMs than for the IgGs in acute infections in mouse.The mapping of the conserved C-terminal in 7 peptides activates TLR in different ways, with the peptides C1 and C5 being the ones that trigger the greatest activation of the NFKβ receptor. These peptides are recognized in a different way by the Chagas serum of different origin or pathology.
CONCLUSION: The presence of antigens of the constant N-terminal region in particulate antigens of the parasite present in the biological fluids of Chagas patients opens the possibility of new diagnostic systems.

Biological functionality and immunogenicity of proteins prohibitin 1 and 2 in *Leishmania major*

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BACKGROUND: The genus *Leishmania* includes a broad group of species, causing around 14 million cases leishmaniasis worldwide. Despite the sequencing of its genome, many problems remain unresolved. Treatment is still particularly complicated and lacks immune prevention. Thus, it is necessary to study the proteomics of the parasite and the disease from a new perspective. In this sense, the search for new proteins and their biological functionality, as well as their use as therapeutic or immunological targets, has led us to study proteins known as prohibitins.
METHODS AND RESULTS: These proteins Prohibitin 1 (Phb1) and 2 (Phb2), which have been described in eukaryotic cells, are small proteins involved in numerous functions in the mitochondria and nucleus, including the most striking feature, its role in apoptosis.
The purification, sequencing, and cloning of LeishPHB1 and LeishPHB2 from *Leishmania major*, by our group, have shown 52% homology between the two proteins and retain the common domain PHB characteristic of the Prohibitin family.
The expression of these prohibitins varies from the stage of the parasite and the species, being higher in those with strong virulence. Immunolocalization studies show that prohibitin 1 is located in the vicinity of the mitochondria and flagellar pocket, while prohibitin 2 is located mainly in the mitochondria. Both Prohibitins show domains with binding capacity for iron ions. The iron in the culture medium increases the expression of both proteins. It has been observed that, in situations of high oxidative stress, their location may vary. In addition having an antioxidant role, they prevent the formation of ROS.
The immune response induced by the recombinant protein linked to a fatty acid vinyl sulfone gives a Th1/Th17 type reaction, capable of somewhat protecting from the challenge of infective metacyclic forms of *L. major*, reaching the highest inhibition levels in the pathogenesis and higher splenocyte cell-stimulation index.

Influence of growth conditions on exosome excretion by *Trypanosoma cruzi*

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BACKGROUND: Exovesicles act in cell communication and are being used as markers in many diseases, e.g. cancer. The presence of exovesicles has been demonstrated in protozoan parasites of the Kinetoplastidae family such as *Leishmania* sp., *Trypanosoma brucei,* and recently *T. cruzi*. In this work, we show the detection of exovesicles secreted by *T. cruzi* into the culture medium and how this secretion differs depending on the conditions under which the parasites are grown.
METHODS: The exosomes were purified by differential ultracentrifugation after the growth of $5x10^6$ parasites/ml of epimastigotes and metacyclic trypomastigote forms of *T. cruzi* in RPMI medium with 10% of SBF (free exovesicles) and modified Grace's medium respectively. By TEM after negative stain and by dynamic light scattering the size was determined. The different culture conditions were: temperature (18º, 28º, 37º, and 45ºC), nutritional stress (50 mg/l of 2-desoxy-glucose), and adding cytoskeleton inhibitor (10 µg/ml of cytochalasin B) and after different time of culture with and without synchronization of life cycle. The measurements were made by scintillation β spectrometer after labelling the parasite culture with 0.5 µg/ml with radioactive [3H]leucine (specific activity 35-70 Ci/mmol). RESULTS: The TEM and dynamic light scattering studies show exovesicle sizes of parasites to be between 30 and 100 nm. The optimal conditions of secretion of exovesicles were 12 h at 28 ºC in the epimastigote culture and at 37 ºC for metacyclic trypomastigote forms. The decrease of exosome production in nutritional stress was 83.64% from the non-treated cultures and the inhibition with the cytoskeleton inhibitors was a 91.51%. In the S phase of the parasite cell cycle, a decrease in labelled (3H leucine) exosomes was found.

Tick complement system and its role in the immune response to microbial infections

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BACKGROUND: Ticks are blood-feeding ectoparasites that transmit the widest variety of pathogens to their vertebrate hosts. The hard tick *Ixodes ricinus* is the most serious vector of Tick-Borne Encephalitis and Lyme disease in Europe. The transmission of pathogens depends on their ability to evade the tick defense mechanisms that are based on the pathogen recognition and humoral immune reactions that are associated with phagocytosis by tick hemocytes. Among the immune molecules present in the *I. ricinus* hemolymph, we have described fibrinogen-related proteins (FREPs), complement-like molecules (TEPs), and putative convertase-like factors.
METHODS: We have carried out functional analyses, based on RNA interference, to evaluate interactions between the tick hemocytes and the pathogens by using an *in vitro* phagocytic assay.
RESULTS: Here, we show the expression profiles of all tick TEPs, FREPs, and convertase-like factors in the response to immune-challenge by different microbes. The *in vitro* phagocytic assay shows specific roles of TEPs, FREPs, and convertase-like factors in phagocytosis.
CONCLUSIONS: Our results shed light on function of components of the primordial complement system in ticks. Also, this study contributes to the general knowledge on evolution of this key innate immunity mechanism and offers new concepts for efficient blocking of tick-borne pathogens transmission.

Supported by the Grant Agency of the Czech Republic (grants No. P506/10/2136 to P.K. and postdoctoral grant 13-27630P to O.H, by the project Postdok_ BIOGLOBE (CZ. 1.07/2.3.00/30.0032) co-financed by the European Social Fund and state budget of the Czech Republic.

Vaccination against *Teladorsagia circumcincta* with a recombinant antigen cocktail – a relationship between efficacy and age

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BACKGROUND: *Teladorsagia circumcincta* is the major cause of parasitic gastroenteritis in small ruminants in temperate regions. As sheep can acquire a protective immune response against *T. circumcincta* in natural and experimental circumstances, vaccination is a possible alternative for control. We have developed a strategy to identify putative host protective antigens by studying local antibody responses directed at proteins specific to post-infective larvae. Antigens were also selected on the basis of their potential immunomodulatory role at the host/parasite interface.
METHODS: Recombinant versions of five immunogenic molecules were combined with recombinant versions of three proteins that have potential immunoregulatory activities and were administered to sheep as a single vaccine formulation. The animals were subsequently subjected to a trickle challenge infection regime with *T. circumcincta* infective larvae. The trial was performed three times in lambs of 7, 6 and 4.5 months-old.
RESULTS: In both trials where lambs of ≥6 months old were used, vaccinated sheep had significantly lower mean faecal egg counts (FEC) over the period of the experiment, with an overall mean FEC reduction of 72% (Trial 1, 7 month-old lambs) and 58% (Trial 2, 6 month-old lambs). During the peak egg shedding periods vaccinated sheep shed 92% and 73% fewer eggs than control sheep in Trials 1 and 2 respectively. At post mortem, vaccinated sheep had 75% (Trial 1) and 57% (Trial 2) lower nematode burdens in the abomasum than those in the control group. When lambs of 4.5 months old were used, the effects on FEC and worm burden were substantially diminished.
CONCLUSIONS: Successful, repeatable, vaccination against *T. circumcincta* with a recombinant sub-unit vaccine has been demonstrated but, as with naturally-acquired immunity, the strength of protection may be age-related.

Frequency of parasitosis in children with DM1 and their siblings, and their relationship to FoxP3 and HLA-DR.

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BACKGROUND: Diabetes mellitus type 1 (DM1) is a major chronic childhood diseases implies a significant reduction of hope and quality of life. One factor associated with the prevention and development of DM1 is the possible presence of parasites such as inducing the expression of FoxP3, which would act as a protective factor by reducing the expression of proinflammatory cytokines thus protecting the pancreatic B cell and develops DM1.
METHODS**:** A case-control study was done in which we assessed the association between parasitosis, FoxP3 and DM1 in children who attended the clinic for care of children with diabetes Children's Hospital of Mexico, as well as their siblings. Questioning was conducted in patients with DM1, and their siblings, and three coproparasitoscopic (CPS) analyses by using the Faust technique of morphological identification: of *E. histolytica, A. lumbricoides* and *Toxocara sp.* Also, IgG antibodies were determined by ELISA against aforementioned parasites. HLA-DR was analyzed as a factor associated with DM1 and FoxP3 expression as a protective factor in the expression of proinflammatory cytokines that damage the islet B cells of the pancreas.
RESULTS: We analyzed 38 samples of feces and sera, 17 belong to patients with DM1 and 21 belong to their siblings. Parasites were identified by CPS and *Endolimax nana* and *Entamoeba coli*. Fiftyfive point three percent of DM1 children´s siblings showed these parasites. A significant difference was found between antibodies vs *Ascaris lumbricoides* in siblings of children DM1 (71.4 %), with respect to children with (DM1) patients (52.9 %).
CONCLUSIONS: There was a significant relationship between the presence of *Ascaris lumbricoides* and the absence of the manifestation of DM1 in the siblings of sick children allows us to infer their possible involvement in this autoimmune disease.

Diversity and host specificity of *Lepidapedon* spp. (Digenea: Lepidapedidae) in the Mediterranean deep-sea

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BACKGROUND: *Lepidapedon* Stafford, 1904 (Lepidapedidae) is the most spacious and dominant group of digeneans in deep-sea teleosts. It is believed that species distributions are restricted to colder waters, the majority of the records being from the north Atlantic and northeastern Pacific. However, no deep-sea investigations into fish parasites have been carried out in other warmer zones.
METHODS: Five deep-sea fish species were sampled using bottom trawling at depths 600–700 m (*Phycis blennoides* and *Trachyrinchus scabrus*) and 1,000–2,000 m (*Coelorinchus labiatus*, *Lepidion lepidion* and *Mora moro*) in the Mediterranean between the Balearic Islands and the Catalonian coast of Spain. Specimens of *Lepidapedon* were characterised both morphologically and molecularly. A large number of isolates was sequenced for the mitochondrial *nad1* gene and *lsr*DNA gene. Both loci individually and concatenated data were used to test the morphological identification and to reconstruct the phylogenetic relationships of *Lepidapedon* spp.
RESULTS: Two species were identified based on the integrated data analysis: *L. desclersae* Bray & Gibson, 1995 and *L. guevarai* Lopez-Roman & Maillard, 1973. Both species are characterised molecularly from their type-hosts for the first time; both exhibited high haplotype diversity and low host specificity.
CONCLUSIONS: This first sampling of deep-sea fish parasites in the Mediterranean suggests low diversity and host specificity of *Lepidapedon* in warmer zones.
The study was supported by the MICINN (Spain) project ANTROMARE (CTM2009-12214-C02-02) and the CSF (Czech Republic) project ECIP (P505/12/G112).

Molecular and morphological characterisation of larval and adult isolates of *Diplostomum* (Digenea: Diplostomidae) from Spain

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BACKGROUND: Recent molecular studies have revealed high species diversity of *Diplostomum* in central and northern Europe. However, no data exist for infections in the intermediate and definitive hosts in southern Europe; this study aims to fill this gap in our knowledge.
METHODS: Totals of 20 fish species and six fish-eating bird species were sampled opportunistically in three regions (Catalonia, Extremadura and Aragon) in Spain. All isolates of *Diplostomum* spp. were characterised morphologically and molecularly. Partial sequences for the *cox1* gene and complete sequences for ITS1-5.8S-ITS2 gene cluster were used for molecular identification.
RESULTS: Integrated morphological and molecular analyses demonstrated the presence of three species among the larval and adult isolates: *D. spathaceum* (in fish and birds) *D. pseudospathaceum* (in birds) and *D. spathaceum*/*parviventosum* referred to as Clade Q (*sensu* Georgieva et al., 2013) (in fish). We detected ten haplotypes among the isolates of *D. spathaceum* with only one haplotype shared with adult isolates from central and northern Europe. No specific geographic pattern of the distribution of the novel haplotypes was found.
CONCLUSIONS: This first molecular confirmation of the diversity of *Diplostomum* spp. in southern Europe indicates much lower species richness compared with the northern regions of Europe.
The study was funded by the Czech Science Foundation (ECIP P505/12/G112).

Transforming Growth Factor β: A Host Factor Specifically Recognized by TGFβ-like Receptors in *Taenia crassiceps* and *Taenia solium* Cysticerci

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BACKGROUND: Taeniids exhibit an unusual degree of adaptive plasticity that facilitates their establishment, growth, and reproduction. Several cytokines are locally secreted in the sites where parasites are lodged; one of them is the Transforming Growth Factor β (TGFβ). This study was designed to explore the role of TGFβ on *Taenia solium* and *Taenia crassiceps* cysticerci in humans and mice, respectively, as well as its relevance for the outcome of the host-parasite relationship.
METHODS: Type-I and II TGFβ-like receptors were searched in the *T. solium* genome database, and were immunolocalized in *T. solium* and *T. crassiceps* cysticerci slides using rabbit anti-TGFβRI and anti-TGFβRII polyclonal antibodies.
The function of these parasite receptors was evaluated *in vitro,* in cultivated cysticerci exposed to different TGFβ concentrations, and *in vivo,* in neurocysticercosis patients resistant to cysticidal treatment.
RESULTS: Homologous TGFβ receptors (*Ts*Type I and *Ts*Type II), as well as several members of the TGFβ signal transduction pathway downstream were found in *T. solium* genome. The expression of both TGFβ receptors was confirmed by immunohistochemistry, being located on the external tegument of *T. solium* and *T. crassiceps* cysticerci. Evidence was found of TGFβ operating as a factor promoting growth, reproduction, and survival in both *T. solium* and *T. crassiceps* cysticerci. Moreover, before drug treatment, increased TGFβ levels were found in cerebrospinal fluid and in the supernatants of peripheral mononuclear cells from patients suffering subarachnoid neurocysticercosis at the base of their brain, when specifically stimulated with antigens from *T. solium*; these patients were non-responder to cysticidal treatment, pointing thus to the relevance of this growth factor for parasite survival in its human host.
CONCLUSIONS: Altogether, these results suggest a possible role of TGFβ produced by the murine or human host in parasite growth and resistance to treatment, pointing to its relevance in the host-parasite relationship.

Cryptosporidium parvum and _Cyclospora_ spp. in school children of three public elementary schools of northwest Mexico.

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BACKGROUND: The intestinal *coccidium* has cosmopolitan distribution but its prevalence is higher in extreme poverty and shortage of basic services such as drinking water, and sewage in underdeveloped countries. In recent decades, coccidiosis has been associated to low nutritional status of the general population and to act as an opportunistic infection in populations with high immune depression and social marginalization. Currently, information on the prevalence of coccidiosis at local and national levels is limited. Therefore, the purpose of this study was to determine the prevalence of *Cryptosporidium parvum* and *Cyclospora* spp. In school children of three public primary schools in the city of Hermosillo, Sonora, Mexico.
METHODS: Of the 1,680 officially registered school children, 201 fecal samples were analyzed and 176 socioeconomic surveys were applied. Samples were processed and analyzed by the methods of Faust, technical spontaneous sedimentation tube (TSET), technical sporulation-linked immunosorbent assay (ELISA) and modified Ziehl-Neelsen.
RESULTS: Prevalence of 36.8% and 23.8% were estimated for *Cryptosporidium parvum* and *Cyclospora* spp. respectively. *Cryptosporidium parvum* was statistically associated ($P < 0.05$) with crowding conditions ($P = 0.020$) and lack of health services ($P = 0.001$). *Cyclospora* spp was associated with lack of sewage ($P = 0.040$).
CONCLUSIONS: An unexpected prevalence of coccidiosis was found in our study population. Local authorities should be aware that strategies on health and hygiene and basic services had to be introduced as priority in the affected population, like that our study area.

Cryptosporidium parvum in drinking water system of the city of Cananea in northwest Mexico.

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BACKGROUND: Water is able to transmit various types of pathogens associated with intestinal infections. In 2009, Mexico reported a rate of intestinal infections of 46.75 per 1,000 inhabitants while Sonora reported a rate of 50.67 and its municipality of Cananea reported a rate of 77.9. Some studies have revealed a high prevalence of *Cryptosporidium parvum* (*C. parvum)* in drinking water supplies. This intestinal protozoan is characterized by its association with outbreaks of gastrointestinal infection transmitted through water worldwide. Probably *C. parvum* is a factor contributor to the high rate of intestinal infections in the town of Cananea. The purpose of this study was to determine the prevalence of *C. parvum* in drinking water supply of the town of Cananea.
METHODS**:** Water samples were collected of both 14 wells that supply the drinking water system of the town and 105 households statistically estimated and randomly selected of a total of 10,667 with active service water in the distribution system with active. Each sample was analyzed using the technique of concentration and dry sediment was obtained after centrifugation and vacuum process for analysis and diagnosis of *C. parvum* using ELISA. RESULTS**:** The prevalence of *C. parvum* was 14.2% (n = 2) in samples of untreated water (wells) and 9.5% (n = 10) in treated water of the households.
CONCLUSIONS: Population of the town of Cananea is a high risk of cryptosporidiosis transmitted by its drinking water system and actions should be taken by the proper authorities.

The capacity of *Taenia solium´s* enolase to bind and activate human plasminogen

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BACKGROUND: Enolase is a key glycolytic enzyme present in the cytoplasm of prokaryotic and eukaryotic cells that contain the plasminogen domain. Recently, enolase is considered a multifunctional protein as its identification on the surface of several pathogenic organism suggests that acts as a plasminogen receptor, concentrating proteolytic plasmin activity on the cell surface. Binding and activation of human plasminogen (Plg) have been associated with the invasive potential and establishment of certain parasites. The aim of this work is the characterization and immunolocalization of *Taenia solium* enolase and the evaluation of its capacity to bind and activate human plasminogen.
METHODS: *T. solium* enolase was identified and characterized using the database genome of *T. solium*. Immunohistochemical localization of enolase on tissue sections of *T. solium* cysticerci and worm were carried out using mouse α-Enolase polyclonal antibody. The confirmation of the protein was carried out by immunoblot analysis. Ligand blotting assays identified Plg-binding spots (enolase) in *T. solium* cysticerci protein extracts. The binding was inhibited by εACA, to indicate the specificity of this interaction.
RESULTS: The sequence identified in the genome database has the characteristic domains of the enolase protein. Immunolocalization assays showed the presence of enolase in the cysticerci and worm surface. Enolase is high expressed on *T. solium* cysticerci. The 47kDa protein was observed in immunoblot, corresponding to enolase molecular weight. The ligand blotting assays identified several major Plg-binding spots in different proteins extracts. Through LS-MS/MS it was confirmed that *T. solium* enolase is one of the proteins that bind human plasminogen.
CONCLUSIONS: *Taenia solium´s* enolase is a plasminogen binding protein that could be participate in the host-parasite relationship and contribute in the parasite establishment.

Educational impact of web-based identification keys in the learning process of veterinary parasitology

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BACKGROUND: Animal parasitic infections of medical, economic and public health importance are common in many places in the world. Proper identification of parasites is very important in order to apply preventive and control measures aimed to reduce either economic losses or an impact in public health. A method to identify parasites consists of using dichotomous identification keys. Nevertheless, they can be difficult to use as they require previous training or expertise in parasitology taxonomy and morphometrics. Besides, their availability is limited for large numbers of students. Web-base keys are a helpful alternative and have been widely used in some botanical and zoological areas. Nonetheless, there are few studies regarding their benefits and acceptability in veterinary parasitology. This study aimed to compare the performance of first-year veterinary medicine students that studied using online interactive keys recently developed to identify veterinary parasites.
METHODS: Students were required to undertake an examination of 20 unlabeled parasite specimens that infect domestic animals. The same examination scores were compared with those of a previous scholar cycle, when the online keys were not yet developed. This work also sought to assess students' perceptions of the web-based identification key through a survey.
RESULTS: Analysis of results demonstrated that students who used the web-based key performed significantly better than those who used traditional paper-based keys to study. Similarly, the online-based keys showed a high level of acceptance as assessed with the survey.
CONCLUSIONS: It is therefore concluded that web-based identification keys are a valuable educational tool to identify parasites of veterinary importance.

Prevalence of gastrointestinal parasites in adults and young sheep backyard in Oaxaca, Mexico

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BACKGROUND: Gastrointestinal diseases caused by parasites are one important cause of economic losses in sheep. The aim of this study was to estimate the helminthes and protozoan infection in young lamb and adults.
METHODS: Fecal samples from seventy 1-year-old sheep and one hundred nine 2.5-year-old sheep in Oaxaca, Mexico were examined using coprological techniques to detect and count coccidia oocysts and eggs of gastrointestinal nematodes and cestodes. Eimeria-positive samples were cultured to identify species. Fecal cultures were carried out to identify strongylid larvae. Spearman correlation coefficient (rs) was used to evaluate the relationship
RESULTS**:** Forty-five percent of young sheep were positive to *Eimeria* spp., and the most prevalent species was *E. pallida* (50%). The infection rates of *Strongyloides papillosus*, *Haemonchus contortus*, *Trichostrongylus axei*, *Cooperia* spp., *Chabertia ovina* and *Oesophagostomun* spp. were 23.60%, 25.84%, 13.48%, 15.73%, 11.24% and 10.11% respectively. The animals were infected with *Trichuris ovis* (8.57%) y *Moniezia* spp. (34.29%). Twenty-three adult sheep were positive to *Eimeria* spp., and the most prevalent species was *E. ovinoidalis* (41.62%). The infection rates of *Haemonchus contortus*, *Trichostrongylus axei*, *Cooperia* spp., *Chabertia ovina* and *Oesophagostomun* were 30.19%, 11.32%, 16.98, 22.64% and 18.87% respectively. The animals were infected with *Trichuris ovis* (3%) y *Moniezia* spp. (29%). A negative and significant ($p \leq 0.05$) correlation was found between parasite burden and age.
CONCLUSIONS: The present study demonstrated that gastrointestinal parasitic infections occur frequently in young and adult sheep around Oaxaca, Mexico, especially coccidian infections. Parasite burden and age are negatively correlated and this could be perhaps due to a stronger immunity in older animals.

Geographic distribution and ecology of *Triatoma dimidiate* (Latreille, 1811) in Colombia

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BACKGROUND: *Triatoma dimidiata* is the second most important vector of Chagas' disease in Colombia. It has a great capacity to adapt to different environments. Control of *T. dimidiata* must take into account its enormous variation and the diversity of its habitat.
METHODS: We divide the country in seven biogeographical regions. In a representative sample of houses we proceeded to search for triatomines and calculated the entomological indexes. Indoor and outdoor niches were searched and infestation by triatomine bugs in palm trees and caves was done by live baited traps.
RESULTS: This species was found in 8 departments and in seven biogeographic zones. Intradomestic infestation was determined in 8.6% of the houses and peridomestic infestation in 6.3%. All of the houses with *T. dimidiata* infestation were in the biogeographic zones of east Andes, east slope of the Sierra Nevada of Santa Marta and upper Magdalena. The predominant life zones in these areas were humid premontane forest and dry tropical forest. No domestic and peridomestic infestation by *T. dimidiata* was found in the biogeographical zones of Uraba and Caribbean plains. In these regions the predominant life zones are humid tropical forest and *T. dimidiata* was only collected in palm trees.
CONCLUSIONS: In Colombia *T. dimidiata* is a species that occupies a wide variety of ecosystems, from transformed ecosystems in the Andean biome with shrub and xerofitic vegetation to very dense forests in the humid tropical forests in the SNSM. Two populations of this species are present in the country, the population of the east region presents a complex distributional pattern including sylvatic, peridomestic and domiciliated ecotopes and occupying a great variety of life zones and is of epidemiological importance.

13th INTERNATIONAL CONGRESS OF PARASITOLOGY
AUGUST 10th - 15th, 2014
ICOPA XIII MÉXICO

Long-term parasitological study in raccoons (*Procyon lotor*) and coatis (*Nasua narica*) from Mexico

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BACKGROUND: Raccoons (*Procyon lotor*) and coatis (*Nasua narica*) are carnivores, members of Procyonidae family, which are distributed across North and Central America. Raccoon has become an invasive animal in Europe and Asia suggesting a public health risk because their role as reservoir host of infectious aetiological agents as the nematode *Baylisascaris procyonis*, the major concern of *larva migrans* syndrome in USA during the last years. Data about the reservoir host role of coatis are scarce. Both procyonids have been well adapted to urban-touristic tropical zones in Mexico; thus, the aim of this work was to perform a long-term parasitological study in raccoons and coatis from Parque Museo La Venta, Tabasco, Mx.
METHODS: Animals were trapped in six different events, two per year since 2009 to 2011. After bleeding, animals were released, a total of 231 serum samples and 296 fecal swabs, were obtained. Antibodies to different helminthes taxa were determined by ELISA using antigens of *Ascaris suum*, *Toxocara canis*, *Fasciola hepatica* and *Taenia crassiceps*. Reactivity of ELISA positive samples were corroborated using Western blotting. Stool samples were analyzed via direct fecal smear method.
RESULTS: Antibodies were only detected to *Ascaris*, the prevalence was 19.0% (n=171) in coatis and 8.4% (n=60) in raccoons. The fecal survey registered four species of nematodes, one digenea, three cestodes and two protozoans. Prevalence of fecal parasites was 29.0% (n=228) for coatis and 39.7% (n=68) for raccoons. Previous reports indicate that some secreted-excreted antigens of *Toxascaris* and *Baylisascaris* show similar molecular weight to *A. suum*. Ascaride eggs were identified in one raccoon and one coati sample, but dissimilar morphologies were seen. The first resembles *B. procyonis* literature morphology but coati egg differs in the granulated shell because is less rugose.
CONCLUSIONS: Further studies are necessary to characterize the parasite-fauna of Mexican procyonids.

Host-specificity of *Blastocystis* subtypes: Comparison of humans with domestic pigs and wild rodents in a small community in Sumba Island, Indonesia

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Blastocystis is known to be one of the most common intestinal protozoan parasites in human fecal samples. Since the genetic diversity among Blastocystis isolates from humans and animals are extensive, *Blastocystis* isolates from humans and other mammalian and avian hosts are currently classified into 9 subtypes based on the small subunit rRNA gene (SSU rDNA) phylogeny. Based on the subtype or sequence analyses in a variety of human populations and animals, all 9 subtypes are mixed with human and animal *Blastocystis*. Although, transmission of *Blastocystis* infection between human-to-human or animal-to-human is not conclusively demonstrated, several molecular studies indicated those closely related domestic or wild animals to humans as being a source of human *Blastocystis* infection. Considering the fact that only 10 cysts of *Blastocystis* derived from the feces of infected rats showed 10 to 100% infectivity to laboratory rats, the transmissions between humans and animals might be occurred commonly under a poor hygiene condition.

In this study, we had examined *Blastocystis* subtypes of the humans, domestic pigs and wild rodents in a small community in Sumba Island, Indonesia for evaluation of the transmission of the same *Blastocystis* subtypes between humans and animals. As expected from the close condition of humans and animals; resident people are living in a traditional open house with several domestic animals bred under their house, and the low sanitary condition; their water for daily use depending on low water from river or well and no restroom in most houses, high-level infections of intestinal parasites, such as *Ascaris lumbricoides*, *Trichuris trichiura*, *Giardia intestinalis*, *Entamoeba* spp., and *Blastocystis* spp. were detected microscopically. However, the result of subtype analyses of *Blastocystis* isolates from the resident people and animals revealed clear differences of genotype distribution depending on host species. The resident people were infected with *Blastocystis* subtypes 1-3, while domestic pigs were infected with subtypes 5 and 7, and wild rodents were infected with subtype 4. Although human and

animal fecal contamination could be highly taking place in the communities, the host specificity of *Blastocystis* seems to restrict the transmissions among human, domestic pigs and wild rodents.

Bon appétit: severe toxoplasmosis and consumption of bizarre meat

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Although recent improvements in detecting a specific antibody to a 11-kDa sporozoite protein in serum samples showed that oocyst-induced infections in humans are largely underestimated, *Toxoplasma gondii* cysts in undercooked meat remains an important source of infection. Most cyst-induced infections worldwide are linked to the consumption of farm animals raised for human consumption (such as poultry, sheep, pork, and beef) and most otherwise healthy people infected with *T. gondii* after birth are asymptomatic or develop a mild disease in some cases. However, *T. gondii* is a highly successful parasite and is supposed to infect all warm-blooded animals, i.e. all birds and mammals. Consequently, *T. gondii* infection in humans is theoretically possible with all kinds of meat including wild game from remote areas or domestic animals that are not raised primarily for human consumption. However, little is known about the clinical consequences of a primo-infection with *T. gondii* strains from these unusual meats.
Here we review the cases of life-threatening toxoplasmosis described in otherwise healthy patients from French Guiana and linked to the consumption of undercooked wild game hunted in the Amazonian rain forest. Taking horsemeat as an example, we also focus on cultural differences allowing some people to eat meat from animals raised as companion animals, not as food producing-animals. Horsemeat is regarded as a taboo in many English-speaking countries whereas this kind of meat may be appreciated in other countries such as France or Italy. In France, there is a higher risk of severe toxoplasmosis with horsemeat because it is frequently eaten raw and is mostly imported from Canada or South America where highly virulent atypical *T. gondii* strains are more common than in Europe.

A new species of *Kudoa* (Myxozoa) from the somatic muscle of Pacific bluefin tuna *Thunnus orientalis* possibly associated with food poisoning in humans

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BACKGROUND: Since *Kudoa septempunctata* in olive flounder *Paralichthys olivaceus* was demonstrated to cause food poisoning in humans, other *Kudoa* species are suspected to have pathogenic potential. Recently, another *Kudoa* was detected from flesh of Pacific bluefin tuna (PBT) *Thunnus orientalis* associated with food poisoning. Spores of the *Kudoa* were similar to those of *K. neothunni* which is a causative myxosporean of post-harvest myoliquefaction in yellowfin tuna (YFT) *T. albacares*.
METHODS: Commercially available PBT and YFT samples were collected to examine the spore morphology (light microscopy (LM) and low-vacuum scanning electron microscopy (LV-SEM)) and the ribosomal RNA gene sequences (18S and 28S) of the parasites. From specimens of sliced raw PBT (sashimi) associated with food poisoning cases occurred in Tokyo from 2011 to 2012, *Kudoa* infections were investigated by real-time PCR and LM.
RESULTS: Morphological observations by LM and LV-SEM revealed that there were two morphotypes; pointed- and round-type spores, which were significantly differentiated by the ratio of suture width to spore width. Furthermore, the two morphotypes were genetically distinguishable by the 28S rDNA sequence. Heavy infections with the round-type *Kudoa* were found in PBT samples from food poisoning cases.
CONCLUSIONS: The morphological and molecular evidence validates that the two *Kudoa* morphotypes are separate species; the pointed-type is referred to as *K. neothunni*, whereas the round-type is new to the science. The pathogenicity of the new *Kudoa* to humans remains to be studied.

When markers tell the story of *T. gondii* infection

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Since 2002, the French parasitologist network for *Toxoplasma* isolate collection has collected more than 1,000 *Toxoplasma gondii* strains or DNA samples in patients and animals. This collection is maintained at the *Toxoplasma* Biological Resource Center (BRC Toxoplasma, France, http://www.toxocrb.com). The geographic range of samples is worldwide but most of them were collected in Europe, especially in patients with congenital toxoplasmosis. To a lesser extent, this collection also contains samples of animal origin from South America and sub-Saharan Africa or clinical isolates from severe toxoplasmosis in immunocompromised and otherwise healthy patients. All strains and DNA samples were genotyped with 15 microsatellite markers in a single PCR multiplex assay. Here, we review how these markers improved our knowledge on the epidemiology and pathophysiology of clinical toxoplasmosis. These highly polymorphic markers are useful tools for differentiating closely related strains such as those belonging to the same clonal lineage. These markers are therefore optimal to know whether two isolates are genetically identical or different and have been successfully used to unambiguously reveal a common source of infection in outbreaks, a mixed infection with two different strains or a laboratory contamination. Moreover, some allelic combinations with microsatellite markers exhibit strong geographic patterns and it is possible to cluster strains from certain geographical areas with the genotyping data. Consequently, these markers permitted to find out that some unexplained severe clinical cases diagnosed in France were in fact due to imported strains from South America and sub-Saharan Africa.

Alterations in carbohydrates amounts of *Rhipicephalus (Boophilus) microplus* ticks in response to Fluazuron

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BACKGROUND: The ectoparasite *Rhipicephalus (Boophilus) microplus* has high negative implications in world´s livestock economy. During blood feeding, it ingests, among other nutrients, carbohydrates to support its metabolism. Glucose is a precursor for a variety of intermediates metabolites, working in biosynthesis of chitin, for example. The mechanisms involved in chitin metabolism of *R. (B) microplus* and the regulation by insect growth regulators, have not been fully characterized. In this study, we have made biochemistry analysis to characterize the changes in glucose amounts and lactate dehydrogenase (LDH) activity (E.C 1.1.1.27) in *R. (B) microplus* hemolymph and glycogen concentration of females's fatty body in response to Fluazuron exposure.
METHODS: Two bovines experimentally infested with *R. (B) microplus* were stalled and one was exposed to Fluazuron (Acatak®) using only one dose, according manufactures's recommendation. Hemolymph was collected from dorsal surface of *R. (B) microplus* engorged females at 4, 8, 11 and 15 days after treatment. Biochemical analyses were performed using spectrophotometry aiming evaluate glucose and glycogen concentrations and LDH activity.
RESULTS: Glucose concentration was significantly higher in ticks (40,66mg/dL±2,66) at the 15th day after exposure, when compared to control group (19,55mg/dL±10,59) (unexposed); glycogen concentration was higher at day 11th (12,14mg glucose/issue weight±0,05) and 15th (12,0mg glucose/issue weight±0,07) after exposure in comparison with control group (4,17mg glucose/issue weight±3,40). LDH activity showed increased activity during the evaluated period.
CONCLUSIONS: Increased levels of glucose in hemolymph suggested that Fluazuron might acts blocking chitin synthesis by prevention glucose utilization. The glycogenolysis was reduced at the final period of analysis (day 15). Anaerobic metabolism, expressed by LDH activity seems to not be affected by Fluazuron exposure. This drug didn´t affect lactic fermentative pathway and enhanced glucose amounts in the hemolymph. Investigations on these mechanisms are useful for finding new drug targets.

Hydatidosis in the Limarí Province: Ovalle, Río Hurtado and Punitaqui counties, IV Region, Chile.

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BACKGROUND: Hydatidosis is a parasitic disease caused by the cyst stage of *Echinococcus granulosus* that is a major public health concern. The Region of Coquimbo is endemic for human cystic echinococcosis. In the year 2011, 242 cases were reported in Chile; 7.8% from the IV Region, with the majority from the provinces of Elqui (47.5%) and Limarí (26.3%). The objective of this study was to determine the number of people affected by hydatidosis in the Province of Limarí.
METHODS**:** Random sampling was performed in the cities of Ovalle and Punitaqui, and in the town of Serón. Serum samples (99 from Ovalle, 40 from Punitaqui and 60 from Serón) were analyzed with the RIDASCREEN *Echinococcus* IgG Kit. Positive samples were read in triplicate.
RESULTS: Samples were taken from 199 people between 1 and 76 years of age; 31.2% had knowledge of parasitosis, 66.8% lived in rural areas, 43.2% lived in urban areas, and 16.1% were involved in goat livestock activity. Out of all the samples examined (n=199), 9.0% were positive for the ELISA assay of *Echinococcus granulosus*, out of which 55.6% corresponded to Ovalle, 27.7% to Punitaqui and 16.7% to Serón. Within this positive group, 44.5% were women, 55.5% were men, and 11.1% were minors (less than 18 years old). Also within this group, 22.2% were devoted to goat livestock, 33.3% had some degree of knowledge about hydatidosis, and 77.8% were dog owners.
CONCLUSIONS: These results demonstrate that a high percentage of the population is affected by hydatidosis due to their habits and involvement with goat livestock which favor the conditions for parasitosis. Currently, we are conducting epidemiological studies to have a better understanding of the situation in the Province of Limarí in order to develop new educative strategies for the control of hydatidosis.

Identification of candidate antigens for the development of a diagnostic test for cysticercosis

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BACKGROUND: Cysticercosis is a parasitic disease caused by the larval stage of *Taenia solium*, which affects both human health and the rustic pig. Currently, accurate and specific diagnostic tools for cysticercosis remain to be established. Using proteomic approaches, seven antigens were identified as specific for porcine cysticercosis. The aim of this study was to identify which of these antigens are the most useful for the development of a more sensitive and specific assay for human and porcine cysticercosis.
METHODS: In this study the *T. solium* genome database was used for the analysis. The amino acid sequences were compared with homologues of related parasites to find differences between the sequences. In addition peptides with the highest antigenicity were identified. These peptides were synthesized and their diagnostic ability was evaluated by ELISA. The coding sequences for the antigens selected were also cloned in a prokaryotic system to evaluate the same diagnostic ability of the recombinant antigens.
RESULTS: The amino acid sequence of the seven antigens was identified. Annexin B1 and cAMP-dependent protein kinase, possessed highly specific regions in its amino acid sequences compared with orthologues. Seven peptides ranged between 10 to 12 amino acids were selected, with the highest antigenic index for each of the relevant proteins. Four synthetic peptides were evaluated by ELISA and it was observed that these peptides were recognized by antibodies present in the different serum samples. Finally, the coding sequence of B1 annexin protein was cloned into the pET23a vector and was expressed in *E. coli* BL21 cells.
CONCLUSIONS: The comparison with orthologue sequences indicated that the amino acid sequences of annexin B1 and cAMP-dependent protein kinase, possess highly specific regions and the ELISA with peptides, suggest that these antigens could represent new candidates for development of a specific diagnostic assay for porcine cysticercosis.

Characterization of *Hc-fau* and its function in *Haemonchus contortus* diapause formation

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BACKGROUND: In nematode parasite *Haemonchus contortus*, diapause is induced in the early fourth stage, which is a strategy to avoid hostile conditions. In this study, we identified a new gene *Hc-fau*, a homologue to human *fau* and *C. elegans Ce-rps30*, which may regulate *H. contortus* diapause.
METHODS: Diapausing larvae of the fourth-stage *H. contortus* were obtained according to Gibbs (1971). QRT-PCR was performed to determine the abundance of Hc-fau transcripts in different developmental stages throughout the life cycle of *H. contortus*. Full-length cDNA and complete gene were isolated by RACE and Genome Walking from *H. contortus* and identified by PCR. Feeding RNAi and microinjection, conducted in *C. elegans*, and Lipofection, in HEK293 were carried out to investigate Hc-FAU and *Ce-rps30* functions.
RESULTS: *Hc-fau* gene encodes two proteins through alternative RNA splicing, Hc-FAUA and Hc-FAUB consisting of 130 and 107 amino acids, respectively. Hc-FAU possesses a diverged ubiquitin-like (UBil) protein domain and a conserved ribosome protein S30 domain. The protein is ubiquitiously expressed, except in the gonad. However, abundance of *Hc-fau* transcripts decreases significantly in diapausing larvae. In *C. elegans*, Knockdown of *Ce-rps30* confers to extended lifespan, increased fat storage in the intestine and shortened body length. These morphological characteristics are comparable to dauer stage of *C. elegans*, while gonad is condensed considerably. In contrast, shortened lifespan is observed in *C. elegans* overexpressing *Hc-faua, especially in Hc-faub, where hatching failure is detected*. Compared with Hc-FAUA, results of UBiL domain and S30 domain overexpression indicate synergism between UBiL and S30 in regulating lifespan and reproduction
CONCLUSIONS: These results indicate that *Hc-fau* gene has great influences on growth and development in *Haemonchus contortus* and it may participate in regulating diapause by ubiquitin-like modification or protein synthesis.

Profiling of differentially expressed genes in sheep T lymphocytes response to *Haemonchus contortus* infection

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BACKGROUND: *Haemonchus contortus* is a common bloodsucking nematode causing widespread economic loss in agriculture. Once infected with *H. contortus,* resistant sheep arouse and mobilize effective immunological components, especially those relating to T lymphocytes immune response, to defend against invaders. This study is to gain a systematic transcriptome profiling of the T lymphocytes in *H. contortus* artificial-infected sheep.
METHODS: A sheep infection model with *H. contortus* was established, and then T lymphocytes were separated from peripheral blood at 0, 3-5, 25-30 and 60 days post-challenge. Microarrays were used to compare gene expression in T lymphocytes between 0 days post infection (dpi), 3-5 dpi, 25-30 dpi and 60dpi.
RESULTS: Microarray analysis showed that 853, 242 and 42 differentially expressed genes were acquired in the 3d vs. 0d comparison, the 30d vs. 0d comparison and the 60d vs. 0d comparison, respectively. Gene Ontology and pathway analysis indicated that the roles of modulated genes focused on metabolism, signaling, cell growth and immune system processes. Functional analysis of significant genes including SLC9A3R2, ABCB9, COMMD4, SUGT1, FCER1G, GSK3A, PAK4 and FCER2 displayed a crucial association with cellular homeostasis maintaining and immune response.
CONCLUSIONS: Our results provide a meaningful list of candidate genes in sheep T lymphocytes response to *H. contortus* infection as a new research object, and may contribute a novel insight into a whole immune response description.

Toxoplasmosis : new insights into serological diagnosis.

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Serological techniques are tools of paramount importance in the diagnosis of human toxoplasmosis, congenital or in immunocompromised patients. Briefly, these techniques allow the determination of the prevalence of the disease, detect, affirm or infirm primo or ancient infection in pregnant women or congenital disease in the newborn, and the risk of reactivation or transmission of the disease in immunocompromised patients (both HIV and transplant patient). They are thus at the basis of all diagnosis of *Toxoplasma* infection in humans. The concept of detection of specific antibodies has been used for decades, but it remains perfectible and numerous studies and publications are devoted to this question. Some of the improvements of serological techniques for toxoplasmosis will be presented and detailed here. One of the main problems in the field of congenital toxoplasmosis is to date the maternal infection during pregnancy. To achieve this goal new techniques have been developed using mix of different parasitic antigens that allow different antibodies detection kinetics, of great interest. Another important point in this field is the development of new *Toxoplasma* specific IgG avidity tests that could permit new and more precise interpretations of avidity indexes. One very interesting improvement in the field could be serotyping of *Toxoplasma* strains. However, this approach is not transferred to the field yet and remains a research topic. Taken as a whole these data underline the fact that serological diagnosis of toxoplasmosis is in constant evolution and remains a very important tool in the field at the worldwide level.

Correlation between immune response against BK-SE36 malaria vaccine and protective efficacy

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BACKGROUND: The malaria vaccine candidate BK-SE36, developed by our group, is based on the N-terminal domain of *Plasmodium falciparum* serine repeat antigen 5, a blood stage antigen abundantly expressed on the merozoite surface. A Phase Ib clinical trial of BK-SE36, a recombinant SE36 protein formulated with aluminum hydroxide gel, and the follow-up study of vaccinated volunteers were conducted in Uganda. We recently reported that immunogenicity of BK-SE36 was remarkably high in the 6-10 year age group, with immune response gradually decreasing with increasing age. Ancillary analysis showed a possible protective effect of BK-SE36 against symptomatic malaria.
METHODS: We monitored anti-SE36 antibody titer transition of the participants aged 6-20 years during the follow-up period, 130-365 days after the second vaccination, to investigate how the vaccination affects immune response to malaria infection and how the induced antibodies are retained in a malaria endemic area.
RESULTS: In the longitudinal study that monitored malaria incidence in volunteers aged 6-20 years, most responders to BK-SE36 had 0 or 1 episode of natural infection during the follow-up period, while non-responders or participants in the control group had higher risk of multiple infections. The responders without any malaria episode had relatively high anti-SE36 antibody titers after the second vaccination compared with those with malaria episode(s), suggesting the correlation of anti-SE36 antibody level induced by BK-SE36 with protection against malaria infection. Interestingly, anti-SE36 antibody titers of the responders with malaria episode(s) were remarkably boosted after episodes of natural infection. However, no obvious boosting effect was found in the non-responder or control group even after malaria infection.
CONCLUSIONS: The vaccine responders to BK-SE36 are expected to keep their anti-SE36 antibody levels high in a malaria endemic area by natural infection and have lower risk of re-infection for a longer period than the non-responders or the non-vaccinated people.

ICOPA XIII MÉXICO
13th INTERNATIONAL CONGRESS OF PARASITOLOGY
AUGUST 10th - 15th, 2014

Health education as a central intervention tool for control of *Taenia solium* cysticercosis/taeniosis in sub-Saharan Africa. Introduction to the e-learning programme: 'The Vicious Worm'

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BACKGROUND: *Taenia solium* cysticercosis is emerging in sub-Saharan Africa because the demand for pork is increasing while knowledge regarding *T. solium* cysticercosis/taeniosis is negligible, free roaming pigs common, meat inspection non-existent or inappropriate, open defecation highly prevalent, and personal- and meat hygiene poor. Internationally, five strategies have been suggested for elimination of *T. solium*, i.e. treatment of humans and pigs, vaccination of pigs, improved pig management and pork production, improved sanitation, and improved knowledge through health education. The aim of this project was to develop a computer-based educational tool advocating evidence-based prevention and control of *T. solium* cysticercosis/taeniosis – "The Vicious Worm Disease".
METHODS: The computer-based information tool targets three levels of stakeholders: lay people, professionals (medical doctors, veterinarians, meat inspectors and agricultural extension officers), and policy makers at ministerial level. The information is displayed using an interactive map showing a village, a town, and a city. At each level information is provided using different education materials including short stories, pictures, videos, quizzes, and scientific and political texts. At the village level, information about transmission and prevention of the Vicious Worm is shown in a simple way. At the town level, information about diagnosis, treatment and prevention of *T. solium* cysticercosis/taeniosis is provided in a technical way. At the city level, information for policy making is found. Additionally a library is available with central internet links and references. The project was developed as part of an EU-7th framework funded programme on Integrated Control of Neglected Zoonoses in Africa (ICONZ).
RESULTS: The information tool can be downloaded at: www.theviciousworm.org. We welcome everyone to test it and participate in its evaluation.
CONCLUSION: As lack of knowledge has been identified as one of the major risks for the spread of *T. solium* cysticercosis, health education should be a central component of any intervention strategy.

Functional dissection of *Toxoplasma gondii* armadillo repeats only (TgARO) protein and its interacting partners

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BACKGROUND: Rhoptries are secretory organelles that form part of the apical complex, which defines the phylum Apicomplexa. Release of rhoptry content is a crucial event that assists *Toxoplasma gondii* in host cell entry and establishment of a successful infection. We have been studying the role of an armadillo repeats containing protein named TgARO that is anchored to the cytosolic face of the rhoptry membrane via acylation. Based on conditional disruption of the *aro* gene, it emerged that TgARO is involved in the positioning of the rhoptries to the apical pole. In absence of TgARO the rhoptries are dispersed within the parasite cytosol, resulting in defective rhoptry secretion that severely impairs host cell invasion. Our data suggests that positioning of the rhoptries is an actomyosin-based process, implicating the association of TgARO with Myosin F. Besides, TgARO interacts with two other proteins, namely adenylate cyclase β (TgACβ) and an ARO interacting protein (TgAIP).
METHODS: reverse genetic approaches coupled to Immunofluorescence assay, western blot and Co-IP experiments were used to characterize the two partners and assess their mode of interaction with TgARO.
RESULTS: The soluble proteins TgAIP and TgACβ localize to the rhoptry neck with TgARO binding to TgAIP, which in turn recruits TgACβ to the organelle. Specifically, the armadillo repeats are necessary for TgARO function and for the distribution of TgAIP and TgACβ to the neck. Despite their presence and conservation across the Apicomplexa phylum, both *TgAIP* and *TgACβ* are dispensable for parasite survival.
CONCLUSIONS: Our results suggest that the three proteins TgARO, TgACβ and TgAIP interact with each other in a specific manner. The non-essential nature of *TgACβ* suggests possible functional redundancy between adenylate cyclases in *T. gondii*.

Proteomic and transcriptomic analysis of extracellular vesicles from parasitic trematodes

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BACKGROUND: Exosomes are small extracellular vesicles (30-100nm), which incorporate membrane and cytosolic proteins as well as RNA molecules, including mRNAs and miRNAs. Their presence in diverse parasites has been described recently, suggesting that they could play important roles in cellular communications, including host-parasite interactions. In this study, we present an overview of the characterization and comparison of the components of exosomes from the parasitic trematodes *Fasciola hepatica, Dicrocoelium dendriticum* and *Echinostoma caproni.*
METHODS: We isolated exosomes by differential ultracentrifugation and ultrafiltration of excretory/secretory products (ESP) from adult trematodes. Vesicles were characterized by electronic microscopy (EM) analyses (Scanning EM, Transmission EM and immunochemistry), and their content identified by proteomics (LC-MS/MS and database searches by MASCOT and ProteinPilot) and transcriptomics (miRNAs sequencing and database searches) techniques.
RESULTS: The proteins identified in the exosomes constitute more than 50% of the proteins previously identified in the secretome (ESP) of each helminth species and explains the secretion of atypical proteins with no canonical secretion signal peptides, transmembrane domains and/or GPI-anchors. The protein composition shows qualitative and quantitative differences in the exosomes among the three species analyzed. We have also studied the presence of miRNA in *F. hepatica* and *D. dendriticum* exosomes. Computational analysis of the detected miRNAs shows some differences in both species.
CONCLUSIONS: Our results suggest that exosomes constitute a major way of secreting proteins by parasitic helminths. They shuttle specific cargo (proteins and miRNA) which could regulate infection and host response, featuring these appealing candidates for diagnostics as well as vaccine and treatment of parasitic diseases.

Molecular epidemiological survey on piroplasmosis in Asia

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BACKGROUND: Piroplasmosis is a significant tick-born disease that causes great economic losses to animal industry worldwide. However, effective control measures are still underway. Molecular survey on piroplasmosis is especially important to develop attenuated or sub-unit vaccines. In the present study, we performed molecular epidemiological survey on bovine and equine piroplasmosis in Asian countries.

METHODS: We collected blood samples in cattle and horses in Mongolia, Sri Lanka, Thailand, and China. Serum and DNA samples were subjected to ELISA and PCR assay for examination of prevalence of piroplasmosis and molecular diversity of *Babesia bovis*, *B. bigemina*, *B. caballi* and *Theileria equi*.

RESULTS: For equine piroplasmosis, the prevalence of for *B. caballi* and *T. equi* infections were 51.16% and 11.51% in China, and 51.6% and 19.6% in Mongolia, respectively, by ELISA. *BC48* and *EMA-1* sequences suggested the presence of genetically diverse populations of equine piroplasms and a new clade in Mongolia. For bovine piroplasmosis, the prevalence of *B. bovis* was 8.8% and the positive rate was relatively higher among the animals of 1-5 years of age in Thailand. In Sri Lanka, the prevalence of *B. bovis* and *B. bigemina* was 13.9% and 30.1%, respectively. Sequence analysis of merozoite surface antigens (MSAs) the live attenuated vaccine (K-strain), introduced in the early 1990s to immunize cattle populations, and Sri Lankan isolates of *B. bovis* suggested that genetic diversity among is very high and that the sequences of field isolates diverged genetically from the K-strain.

CONCLUSIONS: The present study suggests the importance of molecular epidemiological survey for molecular diagnostics and vaccine development in the field application.

Toxoplasma gondii in livestock and food samples of Sicily, Italy

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BACKGROUND: *Toxoplasma gondii* is a coccidian protozoa that can infect different tissues in several warm blooded animals. Worldwide a seroprevalence from 20 to 80 % in humans and animals is reported. A serological screening on livestock in Sicily was performed to evaluate food risk transmission.
METHODS: A serology screening was performed from several Sicilian farms. A total of 52 cattle farms, 125 sheep, 9 goat and 94 mixed flocks (sheep and goat) were analysed. Farm with a single positive animal were considered positive. Autochthon swine breed farms, backyard animals and wild boars were also analysed. A Molecular screening on food samples and sausages was also performed by nested PCR targeting the rRNA locus.
RESULTS: Almost 68% of sheep and mixed farms resulted positive. At farm level the seroprevalence was decreasing in goats (40%) and in cattle (45%). At animal level seroprevalence of 54% for sheep, 35% for goats and 25% for cattle was observed. Toxoplasmosis in the autochthon breed farms (black swine of Nebrodi) was almost 60% whereas at animal level was almost 40%. Wild boars showed an average prevalence of 55%.
The molecular screening showed up to 10% PCR positive animals. One positive pork sausage, prepared with backyard pig meat, was responsible for one case of acute toxoplasmosis. In a random screening, other 2 out of 114 pork sausages resulted positive.
CONCLUSIONS: *T.gondii* is mainly a food-transmitted parasite and the high seroprevalence in livestock species in Sicily, suggest a potential risk for transmission through meat. Pork and cattle meat is often consumed grilled in barbecues that do not assure the high temperature to kill the parasite in the inner part of the steak. In Italy fresh sausages are frequently consumed raw or undercooked (e.g. grilled) and are the suspected etiological cause for several human toxoplasmosis cases.

The grant RCIZS Si15/2011 to M.V. from Italian Ministry of Health is acknowledged.

Cryptosporidiosis in Sicilian ruminant farms, South Italy. Serology and molecular analysis.

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BACKGROUND: *Cryptosporidium* spp. have been found in a variety of domestic animals as well as poultry, calves, lambs, and goats. In southern Italy cryptosporidiosis in animals has been estimated to reach level up to more than 70%. In Sicily the diffusion of the parasite was unknown, although diarrheic problems in the animals are present in ruminant farms (sheep, goat and cows) ,each year.
METHODS: In symptomatic lambs the analysis on rectal swabs and fecal samples by PCR on Glycoprotein 60 (GP60) gene confirmed the positivity to *Cryptosporidium* spp. Fecal, sera and milk samples of the respective mothers were also collected and analyzed from farms of different Sicilian provinces. A serology screening on 165 farms was conducted, by direct immunofluorescent assay and ELISA, to establish the seroprevalence of *Cryptosporidium* spp. Molecular analysis were also performed.
RESULTS: Cryptosporidiosis outbreaks were detected in five sheep farms by clinical signs on the animals and microscopic observations on faecal samples in 2009. The symptomatic animals were confirmed by microscopic observation, serology and molecular analysis. The general screening on a total of 165 ruminant farms (cattle and sheep) showed an average prevalence of almost 15,15% of *Cryptosporidium* spp. by IFA and 5.5% by ELISA. Almost 55% of seropositive animals resulted positive also by PCR. RFLP analysis on16S rRNA are ongoing to evaluate the zoonotic potential of the circulating species. Preliminary analysis showed that *Cryptosporidium parvum* is the mostly prevalent species.
CONCLUSIONS: The serology analysis confirmed that the prevalence of Cryptosporidiosis outbreaks are probably underestimated in Sicily since episode of diarrhea are frequently attributed to bacterial infections. The circulation of *C. parvum* in several districts of Sicily should pose a major concern on this parasitic zoonosis.
The scientific support of Dr. Maria Anna La Giglia is acknowledged. The work was supported by a grant from the Italian Ministry of Health to Agnello S. RCIZS Si/2010

Comparative proteomic study of the cysticercoid and adult stages of the cestode *Hymenolepis diminuta* by SDS-PAGE and 2-DE: preliminary results.

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BACKGROUND: *Hymenolepis diminuta* is one of the most important cestode species in studies of new therapeutics, biochemical processes, and the immune responses and other host-parasite interrelationships. The aim of the present study is to compare somatic proteomes of two consecutive developmental stages; cysticercoid (invasive stage) and adult parasite. Data about similarities and differences in the protein profiles between these two stages could be crucial for understanding the tapeworm adaptations to the parasitic way of life as well as revealing the specific mechanism involved in invasion of the vertebrate host.
METHODS: In this study we introduced 1-D and 2DE gel electrophoresis techniques to compare somatic proteomes of these two developmental stages. Results were analyzed using QuantityOne and PDQuest (Bio-RAD) software.
RESULTS: Present results show differences in both high and low-molecular weight areas between the protein patterns of the cysticercoid and adult parasite. Similarities in protein bands and spots are marked (blue rectangles) in both 1-D and 2DE gels. Cysticercoids may be characterized by relatively low number of expressed proteins, whereas in the adults numerous proteins are present. Also the level of signal intensity distribution is higher in adults.
CONCLUSIONS: Observed differences between these two developmental stages are supposed to be related to their functions in the cestode life-cycle. In future research we plan to analyze the aforementioned results by the LC-MS/MS technique to identify selected proteins, which is crucial for more detailed studies for understanding cestode biology.

This study was funded by the National Science Centre Poland (grant number: 2012/05/B/NZ6/00769)

Mass spectrometry identification of immunogenic excretory-secretory (E-S) proteins of the adult cestode *Hymenolepis diminuta*

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BACKGROUND: Adult cestodes are obligate parasites in the intestine of vertebrates. Because of complete lack of a digestive system, they are forced to rely on their hosts for nutrients, which are absorbed through the tapeworm's body surfaces. In fact, tapeworms possess a unique body surface called the tegument that, among other functions, is thought to aid in absorption of host nutrients and protect the parasite against adverse conditions of the surrounding environment. For these reasons the parasite produces numerous E-S proteins and a layer of glycocalyx forming a surface coat covering its body. Our goal is to identify immunogenic E-S proteins of *H. diminuta*, recognized by sera from infected rats.
METHODS: Adult parasites were collected from the experimentally infected rats and cultured *in vitro* for 5 and 18h. Immunoblot was used to verify which of the E-S proteins identified by SDS-PAGE react with specific antibodies from sera of rats infected with *H.diminuta*. Immunogenic proteins were analyzed by the LC-MS/MS technique.
RESULTS: A total of 10 immunoreactive bands were selected for the LC-MS/MS. 32 proteins belonging to 26 different families were identified. Among them one protein is of unknown function. Results of our study indicate the presence of antigenic proteins (*eg.* calpain, myoferlin, filamin), some of which are known as vaccine candidates in other flatworm species.
CONCLUSIONS: As the E-S products of adult *H. diminuta* activate immunoresponses of their vertebrate host, our results provide important information on the host-parasite interrelationships during cestodiasis.

This study was funded by the National Science Centre Poland (grant number: 2012/05/B/NZ6/00769)

Lyme disease animal model: an essential tool to study vaccine candidates against human borreliosis

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BACKGROUND: Lyme disease is caused by *Borrelia burgdorferi* sensu lato spirochetes and primarily transmitted by *Ixodes* spp. ticks. An intensive effort has been put toward elucidation the host-pathogen interactions during *Borrelia* infection. The main aim of this endeavor was to find out factors that could be used as vaccinogens to prevent *Borrelia* transmission. Better understanding of the mechanisms of Lyme disease transmission can be approached using laboratory animal models. Present transmission models employ *Ixodes scapularis* nymphs - *B. burgdorferi* sensu stricto - mice system. Our aim was to develop a transmission model concerning main European tick species Ixodes ricinus and European Lyme disease agents (*B. afzelii, B. garinii, B. burgdorferi* sensu stricto).
METHODS: Infectious *B. burgdorferi* isolates were injected into mice. Noninfected *I. ricinus* larvae were fed on *Borrelia* infected mice to generate infected nymphs. Presence of spirochetes within the ticks was tested by PCR. The infection rates in nymphs were evaluated by quantitative real-time PCR. To examine Borrelia infection in mice, biopsies from different organs (ear, heart, urinary bladder and joint) were screened by PCR and quantitative real-time PCR.
RESULTS: Our results showed that urinary bladder is the most reliable tissue for *Borrelia* detection during persistent murine infection. However, additional examination of heart muscle and/or joints is recommended for precise detection of spirochetal infection in mice. Optimized transmission model was further employed to test tick immune genes influencing *Borrelia* dissemination within the tick.
CONCLUSIONS: We have developed a potent animal model for testing vaccine candidates against Lyme disease.

Acknowledgements: GACR: 13-12816P, 13-27630P, 13-11043S, EU FP7 project MODBIOLIN No. 316304

Lymphocyte responses of rats induced by vaccination with cDNA encoding a phosphoglycerate kinase of *Fasciola hepatica* (FhPGK) and *F. hepatica* infection

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BACKGROUND: Among all immune cells, lymphocytes are of particular importance since they are involved in the development of specific immune responses. Here, lymphocyte responses in blood, peritoneal fluid and both mesenteric and hepatic lymph nodes (MLN, HLN) of cDNA-FhPGK/pCMV vaccinated and/or *Fasciola hepatica* infected rats of both sexes were investigated.

METHODS: Vaccine trial involved four groups: 1 immunized with cDNA-FhPGK/pCMV, 2 immunized with pCMV, 3 infection control, and 4 physiology control. Four days post infection, and 4 and 10 weeks post infection, blood, peritoneal fluid, MLN and HLN were collected from rats for cytometric analysis and lymphocyte cultures.

RESULTS: cDNA-FhPGK vaccinated females were partially protected against *F. hepatica* infection (52% reduction in fluke recovery), while more liver flukes were found in the livers and bile ducts of vaccinated male rats than in unvaccinated animals (increase of 11%). Different CD4+ and CD8+ T cell profiles were noted in the peritoneal fluid and lymph nodes, but not in blood, during acute and chronic fasciolosis. Immune responses of male and female rats were polarized towards Th2/Treg with lymphocytes isolated from male rats showing higher IL-4 and IL-10 production than females.

CONCLUSIONS: Rat gender not only affected the ultimate effectiveness of vaccination but also lymphocyte responses following vaccination and/or infection.It is still obscure as to how to successfully overcome the parasite defense strategies required to develop an effective vaccine against fasciolosis.

Research funded by NationalScienceCentre No.2011/01/N/NZ6/00892.

Subcutaneous immunization with freeze thawed promastigotes against *Leishmania donovani* infection along with different adjuvants against experimental murine visceral leishmaniasis

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Background: Visceral leishmaniasis (VL) caused by *Leishmania donovani* is a life-threatening disease involving uncontrolled parasitization of liver, spleen and bone marrow. Till date no effective anti-leishmanial vaccine is available for human use. Killed whole parasite vaccines reached upto phase 3 clinical trials but failed to show enough efficacies mainly due to the lack of an appropriate adjuvant. Therefore, it is essential to search for adjuvants to enhance the immunogenicity of killed vaccines and to induce protection against leishmaniasis.

Methodology: Indian strain of *Leishmania donovani*, viz; MHOM/IN/80/Dd8, was used for the present study. Mice were immunized with freeze thawed promastigotes alone and further formulated with different adjuvants. The adjuvant dosages were 100 µg saponin, 40 µg MPL-A and 100 µg alum for each mice. For preparation of cationic liposomes commercially available kit from Sigma was used. Inbred BALB/c mice were immunized thrice with respective vaccine formulation. Two weeks after last booster mice were challenged with 10^7 promastigotes. Mice were sacrificed on 30, 60 and 90 post challenge days. Protective efficacy of vaccines was analyzed by assessment of the hepatic parasite burden and generation of cellular and humoral immune responses.

Results: All the vaccine formulations were found to be immunogenic and imparted significant protection. However, level of protection varied with the type of adjuvant used. Protection in immunized mice was associated with significant reduction in parasite burden, elevated levels of IgG2a, IFN-γ & IL-12 in conjunction with low levels of IgG1, IL-4 and IL-10 as compared to infected controls.

Conclusion: Our findings suggest greater effectiveness of liposomal encapsulated antigens for protection against murine VL.

Immunization with protein cocktail antigens along with different adjuvants induces protection against murine visceral leishmaniasis.

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Background: Leishmanial parasites are responsible for a group of diseases, known as leishmaniasis, with discrete clinical features ranging from cutaneous lesions to fatal systemic diseases. It is a global public health problem with an estimated 350 million people at risk of infection. Current treatment relies on handful of drugs which often face serious limitations. Vaccination remains the best hope for control of all forms of the disease. Recently a multicomponent vaccine i.e. LEISH-F3+GLA-SE has reached clinical trials. Most of the studies have been focused on use of high molecular weight antigens, so the present study was designed to check the protective efficacy of low molecular weight antigens.

Methods: The 31and 51 kDa antigens were identified in the gel with the help of molecular weight markers. The desired bands of interest were electroeluted and the proteins were dialysed and suspended in PBS (pH-7.2). The eluted protein was also checked on SDS-PAGE gel. Inbred BALB/c mice of either sex, weighing 20–25 g were immunized with 10 µg of cocktail antigens alone and along with different adjuvants. Groups under present study were normal controls, infected controls, 31+51kDa, 31+51+ALD, 31+51+saponin and 31+51+cationic liposomes. Two boosters were given at an interval of 2 weeks and after last booster, mice of control and immunized groups were challenged with 1×10^7 promastigotes. Mice were sacrificed on 30, 60, 90 post challenge days for evaluation of parasite load and other immunological parameters.

Results: Protective efficacy of different vaccine formulations was revealed by significant decline in parasite burden and increased DTH responses. The antibody response was of IgG type with elevated IgG2a and decreased IgG1 whereas cytokine levels pointed towards the generation of protective Th1 type of immune response.

Conclusions: Among all vaccine formulations, cocktail of 31+51+liposome gave promising results and was found to be highly immunogenic.

Studies on the protective efficacy of immunochemotherapy in murine visceral leishmaniasis.

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BACKGROUND: Visceral leishmaniasis is a neglected but typically fatal vector-borne protozoan disease. As the disease causes immunosuppression, and the recognition that many anti-leishmanial drugs operate in synergy with host immune mechanisms led us to investigate the antileishmanial efficacy of immunochemotherapy in BALB/c mice.
METHODOLOGY: Animals were infected with 10^7 promastigotes of *L. donovani* and a month after infection, these animals were given specific immunotherapy (KLD or KLD+MPL-A or 78kDa or 78kDa+MPL-A) or chemotherapy (SSG or cisplatin) or immunochemotherapy (SSG+KLD or SSG+KLD+MPL-A or cisplatin+KLD or cisplatin+KLD+MPL-A or SSG+78kDa or SSG+78kDa+MPL-A or cisplatin+78kDa or cisplatin+78kDa+MPL-A). Animals were also tested with two different treatments that is one group of animals was treated with a single dose of all the different therapies and another group of animals was treated with two doses of all the above therapies with a gap of 14 days between two doses.
RESULTS: It was found that animals treated with two doses of immunochemotherapy significantly reduced the parasite load in comparison to the chemotherapy or immunotherapy treated animals. Immunological studies revealed that immunochemotherapy treated animals generated a strong Th1 type of protective immune response as observed by elevated levels of delayed type hypersensitivity (DTH) responses, higher IgG2a levels, greater IFN-γ and IL-2 concentrations. Immunochemotherapy with SSG+78kDa+MPL-A was found to be most effective in reducing parasite load against experimental VL, however cisplatin+78kDa+MPL-A was also found comparable to SSG treated animals and therefore can be an alternative option for treatment of VL.
CONCLUSION: The current study showed that the treatment of infected mice with immunochemotherapy was more effective than chemotherapy or immunotherapy alone in providing protection as well as generation of protective immune responses against experimental visceral leishmaniasis. Thus, it may provide a valid alternative treatment for those cases where conventional chemotherapy fails.

Molecular characterization of the echinostomid intestinal trematode, *Artyfechinostomum sufrartyfex*, using rDNA gene marker regions

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BACKGROUND: *Artyfechinostomum sufrartyfex* Lane, 1915 (Trematoda: Digenea), a common intestinal parasite of pigs, is the etiological agent of echinostomiasis- a food-borne (and snail mediated) trematodozoonosis, which is frequently reported from several parts of India, eastern and north-eastern regions of the country in particular. With a view to authentically differentiate this species from the other potentially zoonoses of fluke origin, we aimed to establish molecular characterization of the parasite using DNA-based PCR amplification techniques.
METHODS: DNA of the parasite (recovered from pig and human hosts) was isolated using standard phenol-chloroform technique. The rDNA coding larger subunit region (LSR or 28S) and non-coding inter transcribed spacer 2 (ITS2) region were PCR amplified using primers 3S/A28 and dig12/1500R respectively. For sequence and phylogenetic analyses, Bioedit and MEGA5 software were used. Analysis of ITS2 rDNA secondary analysis was done using Mfold, RNAfold and 4SALE programs.
RESULTS: The sequences obtained for 28S and ITS2 regions have a length of 461 bp and 1131 bp respectively. The specific primer designed from ITS2 of *A. sufrartyfex* showed no cross amplification when tested against the other enteric (*Fasciolopsis buski, Gastrodiscoides hominis) and hepatic (Opisthorchis noverca)* flukes of pigs, which are known to have zoonotic implications. In phylogenetic analysis, our query sequences claded with the Echinostomatidae group and were close to Echinostoma spp.
CONCLUSION: Our study is the first to provide molecular characterization for *A. sufrartyfex.* The specific primer designed in the study appears to be a reliable diagnostic tool for species identification in cases of multispecies infections by zoonotic flukes.

Recent progress in *Ostertagia* and *Cooperia* vaccine development

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BACKGROUND: With the increasing incidence of anthelmintic resistance worldwide, immunological control of worm infections through vaccination is often put forward as the most rational and cost-effective alternative for anthelmintics to control helminth infections in livestock. In recent years, our research group has developed 2 experimental vaccines against the most important mucus-dwelling parasites in cattle, i.e. the abomasal nematode *Ostertagia ostertagi* and the small intestinal nematode *Cooperia oncophora*. Both experimental vaccines are based on the use of activation-associated secreted (ASP) proteins, purified from the excretory-secretory material of adult worms. Intramuscular immunisation of cattle with these vaccines raises an effective immune response, resulting in a significant reduction in faecal worm egg shedding of 56-60 % and 90 % for *O. ostertagi* and *C. oncophora*, respectively, during a 2 month period. This is the highest level of protection ever raised by vaccination of cattle against these parasites and sufficient to protect calves against parasitic gastroenteritis during the whole grazing season.
METHODS: In order to unravel the mechanisms involved in the induction of the protective immunity, several vaccine trials were recently conducted to define the vaccine-induced immune responses.
RESULTS: These trials indicated that both vaccines induce an antigen-specific proliferation of natural killer cells, both systemically and mucosally. These findings are significant as they challenge our understanding of vaccine-induced immunological memory, traditionally been attributed solely to B- and T-lymphocytes.
CONCLUSIONS: The mechanisms by which NK cells are actually involved in the vaccine induced immunity remains unclear and form the basis of our current research.

Efficacy of current drugs against intestinal parasites

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Intestinal parasitic diseases are a public health problem in developing nations.Soil transmitted helminths (*Ascaris lumbricoides*,*Trichuris trichiura Necator americanus* or *Ancylostoma duodenalis*)afect more than 2 billions people and often occur in poorest communities.
Many classes of drugs are commonly used in treatment of most parasitic diseases and can result in benefit to patients and public health. These are: Metronidazole, Tinidazole, Secnidazole, Nitazoxanide, Ivermectin, Mebendazole, Albendazole, Cambendazole. Update of pharmacotherapy will be discussed against intestinal protozoa and helminths.

Screening for *Giardia duodenalis* in livestock and dogs in Sicily, Italy.

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BACKGROUND: The intestinal protozoan *Giardia duodenalis* is a widespread opportunistic parasite of humans and animals and the genetic characterization has important implications for its epidemiology and control. To evaluate the presence of *Giardia* in Sicily a serology screening in livestock farms and a molecular screening in household and kennel dogs was performed.
METHODS: Ruminant farms of different Sicilian provinces were assayed by Elisa, Direct Immunofluorescence and PCR. Molecular analysis were performed on kennelled dogs in Palermo The presence of the parasite on forty dogs was evaluated by nested PCR of the glutamate dehydrogenase (*gdh*) gene on DNA extracted from faecal samples. The positive samples were genotyped by PCR-RFLP using *Nla IV* and *Rsa I* restriction enzymes.
RESULTS: The serology screening showed an average seroprevalence of 41% by direct IF to 15% by ELISA in ruminant farms. 35% of seropositive animals were also positive by PCR.The highest seroprevalence were detected in humid areas. The results in kennelled dogs showed that 12 out of 40 were positive.. The genetic analysis in dogs showed that assemblages B, C and E, were present and no mixed infection was detected. One positive sample of *Giardia duodenalis* resulted in the zoonotic assemblage B. One showed the pattern of assemblage E and the other 10 samples were identified as assemblage C.
CONCLUSIONS: The seroprevalence of *Giardia duodenalis* is particularly elevated in livestock farms located close by ponds, creeks or lakes. The genotyping assay of *Giardia* from canine samples showed that dog specific genotypes are dominant in environments where dog-to-dog transmission is likely to occur. The presence of one Giardia zoonotic assemblage B support the growing evidence that, also in Sicily, the dogs could be the potential reservoirs of the zoonotic species, with a not negligible risk of infection for humans. The work was supported by a grant from the Italian Ministry of Health to Agnello S. RCIZS Si/2010

Provincia	Totale Aziende saggiate	Giardia	
		IFD pos(prev %)	Elisa pos(prev %)
AG	33	15(45,45%)	4 (12,12%)
CL	20	9 (45,00%)	3 (15,00%)
CT	9	3 (33,33%)	2 (22,22%)
EN	28	9 (32,14%)	4 (14,29%)
ME	19	7 (36,84%)	1 (5,26%)
PA	24	13(54,17%)	6 (25,00%)
RG	11	3 (27,27%)	2 (18,18%)
SR	8	5 (62,50%)	1 (12,50%)
TP	13	4 (30,77%)	2 (15,38%)
SICILIA	165	68(41,21%)	25(15,15%)

The *mak16* gene of *Entamoeba histolytica* and its identification in isolates from patients

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BACKGROUND: The molecules that are expressed during the development of amebic liver abscesses (ALA) are related as probable pathogenicity markers. The *mak 16* gene was obtained by hybridization subtraction of an invasive amoeba from another that is not, which is why it was considered interesting to know if this gene is present en isolates from patients.
METHODS: In order to identify sequences of *E. histolytica* associated with the development of amebic liver abscess (ALA) in the hamster, subtractive hybridization of cDNA was performed of *E. histolytica* HM-1:IMSS under two growth conditions: 1) cultured in axenic medium, and 2) isolated from experimental ALA in the hamster. For this procedure, eight sequences were obtained. Of these, the *mak16* gene was selected for amplification in 29 cultures of *E. histolytica* isolated from feces of ten patients with intestinal symptoms and 19 asymptomatic patients.
RESULTS: Only five out of ten isolates developed ALA and amplified the *mak16* gene; 19 isolates from asymptomatic patients did not amplify the *mak16* gene nor did they develop ALA.
CONCLUSIONS: Based on the analysis of Fisher's exact test (p <0.001), an association was inferred between the presence of the *mak16* gene of *E. histolytica* and the ability to develop ALA in hamster as well as with the patient's symptoms (p = 0.02). Amplification of the *mak16* gene suggests that it is one of the important genes of *E. histolytica* because it was present in isolates that developed liver damage in the hamster.

13th INTERNATIONAL CONGRESS OF PARASITOLOGY
AUGUST 10th - 15th, 2014
ICOPA XIII MÉXICO

Effect of tannin-rich plant fractions against *Haemonchus contortus* and *Trichostrongylus colubriformis*

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BACKGROUND: Gastrointestinal nematodes (GINs) are a limiting factor in small ruminant production. The conventional mode of control has relied on the repeated use of anthelmintics (AH), but nowadays widespread resistance to commercial AHs exists in worm populations. Plants containing condensed tannins (CTs) seem to have a promising role as an alternative to GIN control. Repeated *in vitro* and *in vivo* results suggest an anthelmintic activity for these plants. However, the quantity and/or the quality of CTs also seem to affect these properties. The aim of this study was to gain a better understanding of the relationship between CT structure and AH activity.
METHODS: Seventeen tannin-containing resources were selected, which varied in CT content, tannin size (mean degree of polymerization, mDP) and composition in terms of prodelphinidin/procyanidin (PD/PC) ratio and stereochemistry (*cis:trans*-flavanol ratio): *Corylus avellana*, *Onobrychis viciifoliae, Trifolium repens*, *Ribes nigrum*, *Salix caprea*, *Pinus sylvestris*, *Ribes rubrum, Salix babylonica* (leaves and twigs), *Junglans regia*, *Salix alba*, *Betula pendula*, *Tilia*, *Theobroma cacao*, and *Lespedeza sericea*. From each plant source, 2 CT fractions were isolated. The AH activity of these 34 fractions was measured using the Larval Exsheathment Inhibition Assay, which was applied to infective larvae of *Haemonchus contortus* (Hc) and *Trichostrongylus colubriformis* (Tc). The EC_{50} (effective concentration for 50% inhibition) was calculated for each fraction and each nematode species.
RESULTS: Calculation of correlation by Spearman rank test (df= 32) showed for both parasites a tendency to correlate the AH activity with CT quality: the AH activity against Hc tended to be associated with mDP ($p < 0.10$), whereas for Tc the AH activity was correlated to the %PD ($p < 0.05$).
CONCLUSIONS: These results suggest different modes of CT action for the different nematode species.

Molecular identification and phylogeny of *Fasciola* flukes from Bangladesh, determined based on spermatogenesis and nuclear and mitochondrial DNA analyses

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BACKGROUND: Fascioliasis is a devastating disease of livestock caused by *Fasciola* flukes. Reports from many Asian countries suggest that there is a genetic diversity among *Fasciola* flukes. Unfortunately, data on molecular characterization of this fluke from Bangladesh remains unclear. Therefore, this study aimed to identify Fasciola flukes from Bangladesh based on ITS1 types and spermatogenic status and to analyze phylogenic relation between the flukes from Bangladesh and other Asian countries on the basis of the sequences of *nad1* gene.
METHODS: We collected 150 adult flukes from the bile ducts of cattle, buffaloes, sheep, and goats from six different regions of Bangladesh. Spermatogenic status was determined by analyzing stained seminal vesicles. The ITS1 types were analyzed using PCR-RFLP method. *Nad1* haplotypes were identified based on PCR and direct sequencing and analyzed phylogenetically by comparing with *nad1* haplotypes of *Fasciola* spp. from other Asian countries.
RESULTS: 127 flukes were aspermic *Fasciola* with Fg type or Fh/Fg type in ITS1 and showed a single *nad1* haplotype (Fsp-NDI-Bd11) with nucleotide sequences identical to aspermic *Fasciola* sp. from Asian countries. These findings suggest that spermic Fasciola flukes might have been introduced into Bangladesh quite recently. 20 flukes were identified as *F. gigantica* based on their spermatogenic status and Fg type in ITS1 and showed 11 haplotypes with high haplotype diversity. The remaining 3 flukes could not be precisely identified because their spermatogenic status, ITS1 types, and *nad1* haplotypes were ambiguous. Therefore, developing a robust method to distinguish aspermic *Fasciola* sp. from other *Fasciola* species is necessary in the future.
CONCLUSION: This study suggests that *F. gigantica* population was introduced into Bangladesh considerably earlier than the aspermic *Fasciola* sp. Aspermic *Fasciola* flukes from Bangladesh and other Asian countries are the descendants of a common maternal lineage because they displayed identical nucleotide sequence in *nad1* gene.

In Vitro trypanocidal activity of a Lectin isolated from _Bothrops diporus_ snake venom

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BACKGROUND: Chagas disease affects more than 18 million people in Latin America. The treatment is based on two drugs that have limited efficacy and significant collateral effects; therefore, the development of new treatments for this sickness is a priority. The aim of this study was to evaluate the *in vitro* trypanocidal activity of a Lectin, isolated from *B. diporus* snake venom.
METHODS: The venom was obtained from Paraguayan Bottrop's specimens kept in captivity. A Lectin was purified from the crude venom by affinity chromatography on an agarose galactose column and C18 (reversed phase) columns. The relative molecular mass was determined by SDS-PAGE and confirmed by mass spectrometry. To evaluate the trypanocidal activity, epimastigote forms of *Trypanosoma cruzi* (strain CL-B5) were previously cultivated in LIT broth with 10%fetal bovine serum at 28°C. For the screening, *T. cruzi was* incubated with the Lectin, (100-6.25 µg/ml). After 72 hours, a solution of 200 µM CPRG was added and the plate was incubated for 6 hours at 37ºC. After this, the absorbance was read at 595 nm. The efficacy of each concentration was estimated by calculating the percentage of anti-epimastigote activity as compared with the control.
RESULTS: The Lectin was isolated with a high degree of purity after two chromatographic steps and showed a dimeric molecule with molecular mass of 14 kDa in each monomer. The trypanocidal activity of the Lectin at concentrations of 100 and 50 µg/ml reached percentages of inhibition of 75 and 50%, respectively.
CONCLUSION: The Lectin isolated from *B. diporus* snake venom showed a specific trypanocidal activity. Snake venoms are an important source of molecules with pharmacological activities and with potential applications in chemotherapy for Chagas disease.

Discovery and animal atudies of new antigiardiasis drugs

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BACKGROUND: Treatment of giardiasis with standard care drugs such as Metronidazole, Tinidazole, and Albendazole fail at ~20% rate and the unpleasant side effects of these drugs lead to non-compliance and the spread of strains resistant to currently available drugs. For these reasons, we have undertaken studies to discover alternative antigiardiasis drugs that are not subjects to current resistance mechanisms.
METHODS: We screened the NIH Chemical Genomics Center Pharmaceutical collection library of approved drugs and identified 11 novel anti-*Giardia* agents. Next, we developed an adult mouse model coupled with *in vitro* proliferation assay, using a simple method to obtain axenized *G. lamblia* GS isolate cultures. This mouse model was used to study drug efficacy *in vivo.*
RESULTS: Four drugs used in human or veterinary medicine cured mice with efficacy better or comparable with Metronidazole. These drugs retained their *in vitro* potency in metronidazole-resistant *G. lamblia* isolates, suggesting that they act by a different mechanism than that of Metronidazole. In addition, we discovered that Disulfiram, a drug used to treat chronic alcoholism, targets the essential *Giardia* arginine dehydrolase pathway enzyme, carbamate kinase. We employed single-crystal X-ray crystallography to understand the structural basis for enzyme inactivation.
CONCLUSIONS: Further preclinical and clinical studies aimed at repurposing the four approved drugs will provide new therapeutic options to treat metronidazole-sensitive and metronidazole-resistant giardiasis cases. Moreover, carbamate kinase has been established as an excellent target for future drug development.

A novel monoclonal antibody-based immunoenzimatic assay for the epidemiological surveillance of the vector snails of *Fasciola hepatica* (Trematoda: Digenea)

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BACKGROUND: Fasciolosis is a worldwide-distributed snail-borne disease that has increasing prevalence in the human population and an enormous impact on veterinary medicine and the economy. The dissemination and successful transmission of this trematodiosis ultimately depends on the existence of susceptible snails that acts as intermediate hosts of the parasites. Therefore, in order to accomplish a plausible control of the disease, the control of the snails is essential. However, the surveillance and detection of infected intermediate hosts tackle this objective. The screening of trematodes within the snails has the handicaps of the classical parasitological examination of the larvae (variable sensitivity and specificity depending on the time of the infection and on the experience of the observer) or hit the expensiveness of molecular biology methods.
METHODS: We propose a novel monoclonal antibody-based immunoenzimatic assay to detect ongoing *Fasciola hepatica* infection in lymnaeid snails. Mouse monoclonal antibodies anti- *F. hepatica* rediae were generated and used for the development of a double monoclonal antibody based-ELISA for parasite detection. The performance of the ELISA was assessed with *F. hepatica* -infected and uninfected-lab-reared *Galba cubensis* and *Pseudosuccinea columella*. Parasitological examination of the snails subjected to experimental infection was provided as the gold standard method.
RESULTS: Sensitivity resulted in 100% for both snail species, while specificity was 98.04% in *G. cubensis* and 100% in *P. columella*. The infection was detected at day 8 post-infection in *G. cubensis* while in *P columella* it was noted as earlier as day 4. No cross-reactivity was detected in lymnaeids infected with *Trichobilharzia* sp. and *Cotylophoron* sp.
CONCLUSIONS: Since no previous immunoassays are reported to detect helminthes-infected snails, these results constitute the first experience on this field. The developed sandwich ELISA is prompted for validation with natural populations of lymnaeid snails.

Malaria parasitaemia and treatment-seeking behavior in Egbormung town, Andoni local government area, Rivers state, Nigeria.

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BACKGROUND: Malaria is a febrile disease caused by parasitic protozoa of the genus *Plasmodium*. It is a public health priority especially in the sub-Saharan Africa. The aim of this study is to know the prevalence of malaria in Egbormung town. It is also to determine the treatment-seeking behavior of the members of this community.
METHODS: Blood samples were collected from 71 individuals in the community. Thick film method was used to examine the blood samples microscopically. Questionnaires were used to obtain useful information from those sampled. Age groups of close ranges, sex, occupation, control method and treatment-seeking behavior were recorded.
RESULTS: The infection occurred in 69(97.2%) individuals out of the 71 randomly examined. Infection was significantly higher in females than males with a prevalence rate of 100% and 92% respectively. All the age groups were exposed to the risk of the infection, with 0-5(100%) years having the highest risk of the infection. About 65(92%) individuals own an insecticide treated bed-net but only 19(29%) individuals slept under the bed-nets. Fishermen and women were examined and had 100% infection. To seek treatment, 31(44%) go to herbalist 9(13%) go to the hospital, 20(28%) go to chemists and 11(15%) go to health centres for treatment.
CONCLUSION: The result suggested that malaria parasite is still a major public health problem in Egbormung town. This high prevalence may be due to the poor environmental conditions of the community, the poor compliance of the indigenes to control measures such as insecticide treated bed-nets, their occupational choices and also their treatment-seeking behavior.

RCB20, an experimental benzimidazole derivative, alters the *in vitro* energetic metabolism of *Taenia crassiceps* cysticerci

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BACKGROUND: *Taenia crassiceps* human cysticercosis is rare but of zoonotic risk. New benzimidazole compounds, such as RCB20, present enhanced antiparasitary activity and have been synthesized through modification of the benzimidazole ring. This drug showed good activity against *Trichinella spiralis* and *Hymenoleps nana.* The aim of this study was to *in vitro* analyze the effect of RCB20 on the energetic metabolism of *T. crassiceps* cysticerci.
METHODS: The cysticerci were cultured in RPMI supplemented culture medium and exposed to albendazole sulphoxide (ABSO) and RCB20, both at 6.5 and 13µM. Control groups were formed by cysticerci not exposed to drugs. After 24h of exposure the culture medium was separated from the cysticerci and both were frozen in liquid N_2. The culture medium was analyzed through HPLC to determine the secretion/excretion (SE) of organic acids related to the energetic metabolism.
RESULTS: The cysticerci showed a preference for the aerobic pathway of energy production due to the non-detection of lactate. It was possible to observe a decrease in the SE of pyruvate in the RCB 20 treated cysticerci when compared to the other groups. The SE of citrate, oxaloacetate, succinate and propionate was increased in the RCB20 treated group, which showed a preference for the partial reverse of the tricarboxilic acid (TCA) cycle. Also there was an increase in the SE of beta-hydroxybutyrate and acetoacetate in the RCB20 treated group indicating the increase in the fatty acid oxidation. All these results indicate a metabolic interference of the RCB20 formulation in comparison to the ABSO and control groups.
CONCLUSIONS: The treatment with low dosages of RCB20 induced a partial reverse of the TCA cycle as well as the increase in the fatty acid oxidation, inducing greater metabolic effects on *T. crassiceps* cysticerci than the treatment with ABSO, indicating that it is a promising new anthelminthic drug.
FINANCIAL SUPPORT: National Counsel of Technological and Scientific Development, CNPq

Specific histone modifications play critical roles in the control of encystation and antigenic variation in *Giardia lamblia*

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BACKGROUND: Since the early days of eukaryotic evolution, parasitic microorganisms have confronted the challenges of sensing and adapting to environmental insults to survive, colonize, and proliferate in a variety of hosts. *Giardia lamblia*, an early-branching eukaryote and a common cause of intestinal disease worldwide, has developed fascinating strategies to adapt both outside and inside the hosts´ intestine, such as the encystation-excystation processes and the continuous switching of their major surface antigens (antigenic variation). How gene expression during these events is regulated remains controversial. Early reports suggest that posttranscriptional and translational control of gene expression is crucial for *Giardia* differentiation. However, epigenetic factors may also play important roles at the transcriptional level.
METHODS: The GiardiaDB was searched for histone modifying enzymes, candidate ORFs were cloned and transfected into *Giardia* trophozoites and their localization was visualized by confocal microscopy. Commercial antibodies to specific histone modifications were screened for reactivity to *Giardia* histones and validated by Western blotting and microscopy, in the presence or absence of particular inhibitors.
RESULTS: Here, we describe the presence of common post-translational histone modifications; identify, localize, and characterize several enzymes involved in these modifications and, in particular, analyze their association with *Giardia* differentiation processes. We also present evidence that the inhibition of NAD^+-dependent and NAD^+-independent histone deacetylases abolishes encystation and increase the rate of switching of the Variant-specific Surface Proteins (VSPs) during antigenic variation.
CONCLUSIONS: Our results show the complexity of the gene expression machinery in this important pathogen.

Polymorphisms of cytokines genes in patients with Ocular Toxoplasmosis

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BACKGROUND: Toxoplasmic retinochoroiditis (TR) is the major cause of visual impairment in western countries and represents between 30 to 50% of all cases of posterior uveitis. TR is a consequence of congenital or acquired postnatal infection with *T. gondii*. *Toxoplasma* induces an inflammatory response that is important to control the infection but is detrimental in some patients that develop ocular lesions. Functional genetic polymorphisms in cytokines genes may interfere with the expression of such molecules and play a role in the genetic regulation of the inflammatory response and resistance or susceptibility to TR.
METHODS: Genomic DNA was obtained from 67 patients with ocular toxoplasmosis confirmed by ophthalmic evaluation and the detection of anti-*toxoplasma* IgG antibodies; and 46 controls without ocular lesions and presence of specific IgG antibodies. Genotyping was done by using the mini-sequencing technique or "ddNTP primer extension". The following polymorphisms were evaluated: IL-1β (-511C/T, +3954C/T, -31T/C); IL-1α (-889C/T); IL-10 (-819C/T, -1082G/A); IFN-γ (+874A/T); TNF-α (-1031T/C, -308G/A, -238G/A, -857C/T,); IL-12 (+169774A/C) and IL-17R (+18661C/T). Amplifications products were analyzed by capillary electrophoresis in the ABI Prism 3100-Avant sequencer.
RESULTS: These preliminary results indicate that the studied polymorphisms are found in our population. The polymorphism -308G/A of the TNF-α gene was associated with susceptibility to TR whereas the -238G/A polymorphism of the TNF-α gene, the -511C/T polymorphism of the IL-1β gene and the +874A/T polymorphisms of the IFN-γ gene were associated with resistance to TR.
CONCLUSIONS: The development of TR is associated with genetic polymorphisms in the TNF-a, IL-1β and the IFN-γ genes in a *T. gondii* infected population in Colombia.

This work was supported by Universidad Tecnológica de Pereira, Universidad del Quindío and COLCIENCIAS (Contrato 469-2013).

Impact of dietary zinc supplementation on growth performance, serum zinc status and humoral immune response of *Giardia lamblia* infected mice

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BACKGROUND: Zinc (Zn) supplementation is a critical new intervention for treating diarrheal episodes in children although the effects of this strategy may depend on the etiological agent. *Giardia lamblia* is a common intestinal-dwelling protozoan and cause of diarrhea in humans and animals worldwide. It was of interest to evaluate the effect of different levels of dietary Zn intake on growth performance, Zn status and immune response during infection.
METHODS: CD1 mice were fed 1 of 4 experimental diets: low-Zn (ZnL), adequate-Zn (ZnA), supplemented-Zn (ZnS1) and highly supplemented-Zn (ZnS2) diet containing 10, 30, 223 and 1383 mg Zn/kg respectively. After a 10-day feeding period, mice were orally inoculated with $5x10^6$ *G. lamblia* trophozoites and followed on the assigned diet during the course of infection (30-days). Body weight gain was recorded and blood samples were collected throughout the study. Diets and serum zinc levels were assessed by atomic absorption spectrophotometry; serum anti-*G. lamblia* IgG was measured by ELISA.
RESULTS: *Giardia*-free mice fed ZnL or ZnA diet were able to attain normal growth while *Giardia*-infected mice presented significant growth retardation and lower serum Zn levels. ZnS1 and ZnS2 diets avoided this weight loss during infection, improved growth rate and serum Zn levels, and up-regulated the host's humoral immune response by enhanced production of anti-*G. lamblia* IgG. Maximum growth rate and antibody mediated response were attained in mice fed ZnS1 diet with no further increases by feeding higher Zn levels (ZnS2).
CONCLUSIONS: These findings reflect biological effect of zinc that could be of public health importance in endemic areas of infection.

Proteomic characterization of the subpellicular cytoskeleton of *Toxoplasma gondii* tachyzoites

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BACKGROUND: *Toxoplasma*, the causative agent of toxoplasmosis in animals and humans, has a subpellicular cytoskeleton that is involved in motility, cell shape and invasion. Knowledge of components of the cytoskeleton is necessary to understand the invasion mechanisms as well as for the identification of possible therapeutic targets. To date, most cytoskeletal components of *Toxoplasma* remain unidentified due mainly to the lack of reproducible methods for their isolation.
METHODS: The isolated subpellicular cytoskeleton was separated by one dimension SDS-PAGE and analyzed by tandem mass spectrometry.
RESULTS: The proteomic subpellicular cytoskeleton of *Toxoplasma* is formed by 95 cytoskeletal proteins. By bioinformatic analysis of the data, proteins were classified as: Eighteen conventional cytoskeletal proteins; 10 inner membrane complex proteins, included 7 with alveolin repeats; 5 new proteins with alveolin like repeats; 37 proteins associated with other organelles and 25 novel proteins of unknown function. One of the alveolin like protein not previously described in *Toxoplasma* named as TgArticulin was partially characterized with a specific monoclonal antibody. Presence of TgArticulin was exclusively associated to the cytoskeleton fraction with a cortical distribution.
CONCLUSIONS: The successful isolation of the subpellicular cytoskeleton together with the tandem mass spectrometry analysis, allowed the characterization of the proteome of this subcellular fraction, which consisted of 95 proteins whose function remain to be determined.

This study was supported by grant #155459 and CTGL by scholarship #324983 from CONACyT.

Frequency of Congenital Chagas Disease in Chiapas State, México.

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Keywords: *Trypanosoma cruzi*, congenital transmission, Chagas disease.

INTRODUCTION: *Trypanosoma cruzi* is the etiologic agent of Chagas disease, it affects between 8 and 11 million people in Latin America. The congenital way is a mechanism of transmission where the newborn is infected by their mothers during pregnancy. In Mexico, the latest report of congenital transmission of *T. cruzi* was made in Oaxaca and Jalisco in 2012; it was 4.08% prevalence with variation according to geographical region studied.
The purpose of this study was to determine the prevalence of *T. cruzi* infection in 1125 pregnant women, the frequency of vertical transmission to their children and associated risk factors in two geographic areas in the state of Chiapas, México.
METHODS: The study was experimental, longitudinal and descriptive. It included 1125 pairs of pregnant women-newborns; of them, 600 were from Tapachula and 525 from Palenque, Chiapas. In both groups, the maternal prevalence was determined by the search of *T. cruzi* antibodies in serum with Immunochromatography (Stat-Pak) and ELISA test with total antigen. In newborns, the parasite DNA was detected by PCR in umbilical cord blood and eight months later; the anti-*T. cruzi* antibodies were shown in serum by two ELISA tests, which one total antigen and the other recombinant antigen. Nifurtimox was administered to seroreactive children until seroconversion.
RESULTS:. The total maternal seroprevalence was 1.6% (18/1125), in Tapachula was 1.3% (8/600) and Palenque was 1.9% (10/525). The newborns of seroreactive mothers that were positive to PCR are 9/18 (50%), of these, 2/8 (25%) are from Tapachula and 7/10 (70%) are from Palenque. The transmission frequency in Tapachula was 12.5% (1/8).The seven children of Palenque don't have own antibodies.
CONCLUSIONS: Our results show that the frequency of transmission was variable, children were asymptomatic and there are association between maternal seroprevalence and the number of deaths and RPM.

Gnathostomiasis: Clinical and immunological correlation in the diagnosis

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BACKGROUND: In 2000, thanks to the research of Dr. Akahane *et al*, was started to look for larvae of *Gnathostoma sp*. in muscle of fish from the Daule river (Guayas,Ecuador), for further epidemiological and etiological study. Where it was confirmed that the specie that found in Ecuador is *Gnathostoma binucleatum* (published in The Bulletin Central Research Institute, Fukuoka-University, 2003). Consequently, these larvae were conserved to develop the indirect immunofluorescence technique and test its effectiveness in diagnosing (published in ICOPA 2002). In this study we performed a retrospective analysis of positive cases that was attended to our center in the period 2005-2013, in order to relate the clinical symptoms, immunological findings before, during and after treatment.
METHODS: Clinical diagnosis of the patient with compatible pathologies. Serological confirmation through indirect immunofluorescence technique. The antigen was obtained from larvae that were found in muscles of contaminated fishes. Patients with positive clinical-serological diagnosis were given treatment with thiabendazole and ivermectin.
RESULTS: During the period of study, medical consultation was sought for 62 cases, of which 77.42% exclusively suffered pathological skin problems, while 22.58% had many different ailments in extremities, joints and other parts simultaneously. In serological diagnosis, positive results were obtained with IgG titers ranging from 1/160 to 1/1600, whereas IgM from 1/360 to 1/1800. Some patients expressed that they had a recurrence in three months after treatment, so new treatment protocol was established, its evolution was observed and serological control was performed during the third, sixth and twelfth month post treatment. Immunological and clinical cure was achieved in 40.32% of cases.
CONCLUSIONS: Lower titres of IgG 1/20 and negative IgM show that a clinical and immunological cure exists.

Marked anti-giardial activity of *Yucca baccata* extracts in gerbils (*Meriones unguiculatus)*

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BACKGROUND: *Giardia lamblia* infection is a global public health problem that includes Mexico, particularly the northwest Mexico. At present, the chemotherapy of selection is expensive and accompanied with side effects. Natural plant products represent an excellent alternative of anti-parasitic treatment. This study assessed the anti-giardial activity of *Yucca baccata* butanolic extracts using the gerbil as an experimental model.
METHODS: Thirty five females were distributed randomly into 5 groups. All gerbils were inoculated with $5x10^6$ trophozoites. Afterwards, the assigned treatment was administered once a day for 3 days. Extract concentrations for the exposed groups were 24.4, 12.2 and 6.1 mg/mL (8.61, 4.30 and 2.15 mg of saponins per dose, respectively). A treated group with metronidazole (2 mg/mL) and a control group (untreated) were included in the bioassay. On day 10 post-infection, gerbils were euthanized and the trophozoite counts were performed for duodenum and proximal segments of the small intestine.
RESULTS: The orogastric administration of 3 butanol extract treatments reduced but not significantly the trophozoites counts in the duodenum segment of infected gerbils in comparison to the control group. In contrast, the administration of butanol extract with different saponin concentrations reduced significantly the trophozoite counts in the proximal segment of infected gerbils in comparison to the control group. No difference was found in the geometric means of trophozoite counts among the 3 extract-treated-groups. The geometric mean of trophozoite counts remained unchanged in the untreated infected gerbils. On the contrary, difference was found in the trophozoite counts for the metronidazole treated groups between the duodenum and proximal segments.
CONCLUSIONS: Extracts with saponins of *Y. baccata* showed anti-giardial activity using gerbils as experimental model. Additional studies are required to uncover more about it. They may represent a more effective and an economic future alternative as anti-parasitic treatment for human and animal production.

Revealing native proteins that include the S3Pvac anti-cysticercosis vaccine

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BACKGROUND: Vaccination against *Taenia solium* porcine cysticercosis is a potential tool for transmission control and eventually to reduce the incidence of human neurocysticercosis. The S3Pvac anti-porcine cysticercosis vaccine, composed of three peptides, was developed in Mexico. S3Pvac efficiently prevents naturally acquired porcine cysticercosis. Considering that an effective vaccine requires the generation of a robust immune response against target proteins that are crucial for parasite survival, the location of the B and T mimotopes/epitopes included in the three vaccine components (GK-1, KETc1, and KETc12) within native parasite proteins are herein revealed.
METHODS: Proteins including B and T cysticercal epitopes and mimotopes were screened in the *T. solium* genome database, through a proteomic and computational approach.
RESULTS: A 264-amino-acid native protein with 30 predicted glycosylation sites, isoelectric point of 7.77, hydrophobicity level of 0.42, and no evidence of trans-membranal regions comprises GK-1. It presents two conserved functional domains: one HGRAM-domain towards the amine end, and a WWbp-domain in whose carboxyl end GK-1 is located. Different proteins from the cytoskeleton, in metabolic pathways or the mitochondria, all of them of potential interest as vaccine targets, were identified as including KETc1 and KETc12 B mimotopes by using specific antibodies against these peptides. The location of target vaccine proteins in cysticerci was revealed by electronic microscopic analysis in the different life stages of the parasite. Overlapping T cell epitopes of high MHC-I association under different mice haplotypes with high immunogenic scores were predicted, pointing to the inclusion of universal epitopes in their sequences. Only GK-1 includes T cell epitopes for MHC-II recognition.
CONCLUSIONS: These findings support the use of the S3Pvac synthetic peptides to promote a protective acquired immunity against critical proteins for *T. solium* survival.

Search of antibodies to *Trichinella* spp. in wildlife Procyonidae living at La Venta Park-Museum, Tabasco, Mexico

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BACKGROUND: Trichinellosis is a worldwide zoonosis caused by the nematode *Trichinella,* which life cycle is well known between domestic and feral animals but, in Mexico, data in wildlife animals are scarce. Raccoons (*Procyon lotor*) and coatis (*Nasua narica*) are *Procyonidae* carnivores, which are ones of the most preferred bushmeat at Southeastern Mexico. The aim of this work was to search for antibodies to *Trichinella* in wildlife *Procyonidae* living at La Venta Park-Museum; in addition, rodents (*Rattus rattus*, *R. norvegicus* and *Mus musculus*) were analyzed to search for *Trichinella* muscular larvae.
METHODS: Serum samples were obtained twice a year from summer of 2009 to winter of 2011 and, after bleeding, animals were released. IgG antibodies were determined by ELISA using the excretory secretory antigen of *Trichinella spiralis* MSUS/ME/92/CM-92 strain muscular larvae. Reactivity of ELISA positive serum samples was corroborated by Western blotting. Rodents were captured since 2013; diaphragm muscles were collected and analyzed by trichinoscopy.
RESULTS: Serum samples of 171 coatis and 60 racoons were collected. Prevalence in 2009 and 2010 was 0% but, in 2011, prevalence in coatis was of 6.9% during summer and 14.9% in winter whereas in raccoons prevalence was of 9.1% in summer and 7.14% in winter. Regarding rodents a total of 19 muscles samples were analyzed and the presence of parasite was absent.
CONCLUSIONS: We determined contact between *Trichinellidae* parasites and *Procyonidae* carnivores. Further studies are needed to identify the presence of the muscle larvae and all the hosts involved in the *Trichinella* wildlife cycle.

Rhodnius prolixus fitness variation when infected with different strains of *Trypanosoma cruzi and Trypanosoma rangeli*

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BACKGROUND: In the study of vector-borne diseases, it is critical to know the effect of an infectious agent on its insect vector to understand disease transmission dynamics and design disease control measures. *Trypanosoma cruzi*, the causative agent of Chagas disease, is a highly variable parasite with ranging effects on its mammal hosts. We asked if there is also variation in the fitness of its triatomine vector when infected with different strains of *T. cruzi, T. rangeli* or both, and if this variation is related to the insect's parasite burden.
METHODS: We compared the survival and reproduction of over 700 *Rhodnius prolixus* infected with five strains of T. cruzi I and three strains of *T. rangeli*, both alone and in co-infections. Insects were infected orally infected and insect survival and reproduction was monitored 50-90 days post-infection. Parasite burden was estimated using QPCR. Survival curves were analyzed using cox proportional hazards models.
RESULTS: We found high variation in insect survival and reproduction depending on the strain of *T. cruzi* and parasite composition of infection. Insects infected with just *T. cruzi* had significantly different survival, ranging from 20-95% of individuals alive after 90 days, depending on *T. cruzi* strain. T.*cruzi-T.rangeli* co-infection significantly reduced insect survival in the first 30 days post-infection but increased reproduction. *T. rangeli* infections alone had no significantly different effect on insect survival or reproduction regardless of strain. Finally, insects with higher parasite loads generally had higher reproduction rates.
CONCLUSIONS: It is currently accepted that *T. cruzi* has a variable effect on its vertebrate hosts, as reflected in variable host clinical manifestations and parasitemias. Our results suggest that this variability extends to *T. cruzi* invertebrate hosts as well. Furthermore, we present evidence that *T. cruzi* can more detrimentally affect *R. prolixus* fitness than *T. rangeli*, depending on *T. cruzi* strain.

Simultaneous development of ivermectin resistance amongst gastrointestinal nematodes and *Rhipicephalus microplus* populations from tropical cattle farms

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BACKGROUND: Ivermectin (IVM) have been considered a valuable tool for the control of gastrointestinal nematodes (GIN) and ticks. The viability of IVM has been hindered by the emergence of parasite populations resistant to this endectocide. it is unknown whether the use of IVM as an anthelmintic could select IVM resistant *R. microplus* populations. Therefore, this study determined the simultaneous development of IVM resistance amongst GIN and *R. microplus* ticks from cattle farms where IVM is used only as an anthelmintic drug (AH).
METHODS: Twelve cattle farms were studied, six had frequent use of IVM (≥ 4 times per year) and six had low frequency of IVM use (1-2 times per year). The fecal egg count reduction test and the larval immersion test were used to determine the resistant status of GIN and *R. microplus* against IVM respectively.
RESULTS: The results indicated that 100 % of the surveyed farms had IVM resistant GIN (reduction % from 0 to 67%). The genera involved were *Haemonchus, Cooperia, Ostertagia, Trichostrongylus* and *Oesophagostomum*. Although the IVM was never used for the control of ticks, 50% of the surveyed farms presented GIN and *R. microplus* simultaneously resistant to IVM. Furthermore, two *R. microplus* populations showed high resistance ratio (RR) to IVM (farm TAT: $RR_{50\%}$ = 7 and $RR_{99\%}$ = 40.1; and farm SLS: $RR_{50\%}$ = 2.4; $RR_{99\%}$ = 11.0). A high frequency of IVM use (≥4 times per year) seemed to promote IVM resistance amongst *R. microplus* ticks compared to the farms with low frequency of IVM use (1-2 times per year) (66.6 % *vs.* 25.0 % respectively). However, the number of surveyed farms was insufficient to show clear statistical inferences.
CONCLUSIONS: The use of IVM for the control of GIN promoted simultaneously the development of IVM resistance in the GIN and R. microplus populations of the cattle herds surveyed.

Characterization of four monoclonal antibodies directed to Tv LEGU-1, a legumain-like CP involved in *Trichomonas vaginalis* cytoadherence

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CONACYT Grants # 153093 and 162123 (to R.A.); ICYTDF Grant # ICyT-186 (to R.A.).

BACKGROUND Trichomoniasis is a sexually transmitted infection caused by the flagellate protist parasite *Trichomonas vaginalis*. This parasite has multiple proteinases; the majority of them are of the cysteine type (CPs). CPs are grouped into families and clans; in *T. vaginalis* the majority belong to the cathepsin-L subfamily of clan CA and the legumain family of clan CD. We have identified and characterized different CPs as parasite virulence factors. Some are involved in cytoadherence such as TvLEGU-1 or in cytotoxicity such as TvCP65 and TvCP39 to host cells. TvLEGU-1 is up-regulated by iron, localized in lysosomes, Golgi and on the parasite surface, participates in cytoadherence, and is one of the most immunogenic CPs of *T. vaginalis*. Thus, TvLEGU-1 is a virulence factor and a potential biomarker for trichomoniasis. The aim of this study was to characterize four anti-TvLEGU-1 monoclonal antibodies (mAb).
METHODS: Balb/c mice were immunized with *T. vaginalis* protease-resistant extracts and cell fusion of spleen cells was performed with X-63Ag8 myeloma cells. Hybridoma selection to obtain mAbs to TvLEGU-1 was performed by ELISA using recombinant TvLEGU-1 as antigen. Cloning by limiting dilution 22 unique clones producing mAbs to TvLEGU-1 were obtained. Four of them were selected for characterization by Western blotting (WB) and indirect immunofluorescence assays (IFA).
RESULTS: By IFA each mAb detected different location of TvLEGU-1 in *T. vaginalis* and by WB each anti-TvLEGU-1 mAb reacted with different size protein band using total protein or protease-resistant extracts.
CONCLUSIONS: The results obtained by IFA and WB suggest that the four anti-TvLEGU-1 mAbs analyzed have different recognition epitopes to TvLEGU-1. These may be useful to study processing and target identification of this CP as well as a diagnostic tool for patients with trichomoniasis.

Entamoeba histolytica interaction with human neutrophil extracellular traps

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BACKGROUND: Neutrophil granulocytes are the most abundant cells of the peripheral blood leukocytes, first to arrive to the site of infection as part of the innate response to pathogens. Neutrophils act against foreign agents by phagocytosis, degranulation and the formation of extracellular traps. The last process consists in the release into the extracellular space of networks of DNA containing antimicrobial peptides and proteins. Traps represent a defense strategy to prevent microbial spread, and concentrating the action of microbicides. Several factors induce neutrophil extracellular traps formation, such as bacteria, fungi, lipopolysaccharide, lipophosphoglycan from *Leishmania sp*, interleukin 8 and other immune factors and *in vitro* phorbol myristate acetate. The aim of this study was to determine if lipopeptidophosphoglycan from *Entamoeba histolytica* induces the formation of neutrophil extracellular traps and the outcome of their interaction with the parasite.
METHODS: Peripheral blood neutrophils were isolated from human healthy volunteer donors. The formation of extracellular traps with phorbol myristate acetate was standardized. Lipopeptidophosphoglycan from *E. histolytica* was isolated and used to induce neutrophil extracellular traps. After 1-h interaction of neutrophil extracelular traps with *E. histolytica*, the parasites were grown under habitual culture conditions and trophozoites were counted.
RESULTS: Lipopeptidophosphoglycan induced the formation of neutrophil extracellular traps. In addition, we observed a decrease in the growth of *E. histolytica* trophozoites after their interaction with neutrophil extracellular traps.
CONCLUSIONS: Lipopeptidophosphoglycan from *E. histolytica* induces *in vitro* the formation of neutrophil extracellular traps and after their interaction with *E. histolytica* the parasite grows less. These results suggest that neutrophil extracellular traps are important factors of the innate immune response against *E. histolytica*.

Protective effect of *Trichinella spiralis* excretion-secretion products absorbed in nanoparticles.

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BACKGROUND: The excretory and secretory products (ESP) of *Trichinella spiralis* muscular larvae have been wildly study as strategy to prevent and control trichinellosis. Indeed, the ESP has been employed as protective approach in combination with substances that have adjuvant properties; however, partial protection is obtained as result of treatment. Recently, chitosan has been used in the development of nanoparticles to administer medicaments. Thus, the aim of this work was analyze the protective effect of ESP absorbed in nanoparticles to immunize mice by oral.
METHODS: Three formulations of antigen (Ag) and nanoparticles, the firts formulation contained antigen inside (D) of nanoparticle (AgD), the second formulation was prepared with Ag outside of nanoparticle (AgF) and the third, with antigen in- and outside the nanoparticle (AgDF). Mice were immunized by oral during twenty days. The kinetic of antibodies was analyzed by ELISA and, at day 30 post immunization, mice were challenged with 500 muscle larvae. Adult worms (GA) were recovered at day 7 post infection and the number of recovered worms was compared with infected naïve mice
RESULTS: Antibodies were observed in groups AgD and AgDF but no in the AgF. The protection against a challenge infection was different between groups, a reduction of 34% was observed in the AgD group, 26% in AgDF and 44% in AgF
CONCLUSIONS: Immunization by oral with chitosan nanoparticles enhance the humoral response but further studies are need to determine the adjuvant potential of chitosan and new studies are required to evaluate the immune response.

Recovery of antigenic proteins in crude extracts of *Taenia solium* cysts using detergents

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BACKGROUND: The *Taenia solium* cysticerci are macroscopic larvae formed by several cell types; crude extracts resulting from the homogenization of whole larvae make complex mixtures of proteins, including significant amounts of host proteins. Detecting antigenic proteins in such parasite crude extracts is highly influenced by the physicochemical properties of individual proteins and by the formation of aggregates. Here we describe a systematic testing for the solubilization of antigenic proteins in crude extracts of *T. solium*.
METHODS: We evaluated the effect of different individual or combined detergents (CHAPS, ASB-14 and Triton x-100), on the solubilization of antigenic cysts proteins evaluated by 2D-PAGE, using a pool of sera from naturally infected pigs. Some antigenic protein spots in the gels were identified through LC-MS/MS sequencing and carbohydrate epitopes were tested through periodate oxidation.
RESULTS: Each detergent solubilized a different number of protein spots, including antigenic spots. However, combined detergents increased the number of total and antigenic spots. The optimal combination of detergents was 2% CHAPS, 1% Triton X-100, 1% ASB-14, resulting in the solubilization of 368 protein spots, including 99 antigenic ones and over 15-30% more than any single detergent. Increasing detergent concentration over commonly used levels appeared detrimental to the number of antigenic spots detected. Finally, some of the proteins solubilized with mixtures of detergents were glycoproteins with carbohydrate epitopes. As an example, antibody recognition of GP50 was highly reduced after periodate oxidation. In contrast, the soluble fraction of our larval crude extracts was specially rich in host proteins.
CONCLUSIONS: Our results showed that combining detergents is a good strategy to improve detection of total and antigenic protein spots in crude extracts of *T. solium* cysticerci. The more insoluble proteins included some glycoproteins with carbohydrate epitopes.

Proteomics of the *Taenia solium* cysticerci in the skeletal muscle and in the central nervous system of naturally infected pigs.

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BACKGROUND: Cysticercosis is caused by the larval stage of *Taenia solium*. The larvae of this tapeworm parasite (cysticerci) can be located in several tissues of their host: skeletal muscle, eye, brain, etc. Preferential tissue localization of certain pathogens has been associated with the expression of particular protein patterns. Thus, the aim of this study was to explore the protein patterns associated with the localization of cysts in the central nervous system (CNS) or skeletal muscle (SM) of infected pigs.
METHODS: Cysticerci were dissected from the SM and CNS of four naturally infected pigs. Protein extracts of the vesicular fluid and the tissue were obtained using 5 cysticerci from each location. Protein extracts were resolved using 2D-PAGE, silver stained and the images were analyzed using PDQUEST ™. A database of protein spots was built in order to determine the protein patterns associated with the tissue localization of the cysts.
RESULTS: 2D-PAGE maps of vesicular fluid and tissue samples from both tissue localization, detected an average of 300 and 304 protein spots, respectively. There were not significant differences in the number of total spots between SM and CNS cysts from different pigs. However, a Principal Components Analysis and Canonical Discriminants allowed identification of 19 spots associated with the SM localization of the cysts and 12 spots with the CNS localization. We are currently working in the identification of these spots through LC-MS/MS.
CONCLUSIONS: The vesicular fluid and the parasite's tissue are complex mixtures of proteins. Interestingly, multivariate analysis showed that reproducibility of the protein extracts from vesicular fluids and larval tissues. Only few spots were specific for the tissue localization of the cysts in the pig.

Intensity of infestation of chewing lice (Phthiraptera) and fleas (Siphonaptera) on poultry (*Gallus gallus domesticus)* in a region of Southern Mexico

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BACKGROUND: Chewing lice (Phthiraptera, Ischnocera) are economically important poultry ectoparasites that live mainly on the skin. Amblyceran lice may cause irritation of the skin, restlessness, overall weakening, cessation of feeding, loss of weight, reduced laying capacity and skin lesions that may become sites of secondary infection. The fleas (Siphonaptera) are obligate parasites that live, feed and shelter beneath the surface of their host`s epidermis, hair or feathers. The aim of this study was to determine the frequency of ectoparasites in poultry in Oaxaca, Mexico.
METHODS: The work was carried out in Oaxaca, Mexico. A total of 45 adult hens, reared in family farms, with makeshift facilities and floor were analyzed. Ectoparasites were collected after slaughter, using a swab with alcohol on the feathers, body, head and feet. The collected parasites were placed in vials with 70% alcohol, and were identified using a progressive number. The number of ectoparasites was estimated, as not all observed parasites were collected.
RESULTS: All hens tested positive for ectoparasites. In chickens, four species of chewing lice were identified: *Menopon gallinae* (86%), *Menacanthus stramineus* (88%), *Chelopistes meleagridis* (64%), *Lipeurus caponis* (33 %) and three species of fleas, *Echidnophaga gallinacea* (75 %), *Ctenocephalides felis* (66 %), *Ctenocephalides canis* (60 %).
CONCLUSIONS: This is the first study on the frequency and intensity of infestation of lice in chickens of backyard farms located in, Oaxaca, Mexico. The most frequent species were *Menacanthus stramineus, Menopon gallinae* and *Echidnophaga gallinacea*.

Formation of tissue cyst of *Toxoplasma gondii* is related with changes in the host cell cytoskeleton

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BACKGROUND: *Toxoplasma gondii* presents 3 infectious stages: sporozoite, present in the mature oocyst, tachyzoite, a highly dynamic and replicative form and bradyzoite, a slowly replicative form contained in cysts. The tissue cyst is surrounded by a protector wall against effector cells, molecules of the immune response and parasiticidal drugs. The encystment process can be initiated *in vitro* by exposure to alkaline pH, thermal shock, nutritional depletion or by the presence of interferon gamma (IFN-γ), but these conditions require long time to get a tissue cyst with a low frequency. In our laboratory, we induced the encystment of *Toxoplasma* in a high frequency by exposure of tachyzoites to an inhibitor of Inosine Monophosphate Dehydrogenase (IMPDH) and evaluated the changes in the organization of actin cytoskeleton during this process.
METHODS: HEp-2 cells were infected with tachyzoites and treated with the inhibitor at different times. Detection of the cyst was evidenced by the expression of CST1 with lectin-FITC from *Dolichos biflorus* and BAG-1, using immunofluorescence confocal microscopy (IF). Re-organization of actin cytoskeleton of the host cell was studied during encystment labeling actin filaments with phalloidin-TRITC.
RESULTS: Our results showed that treatment of tachyzoites with the enzyme inhibitor induced the differentiation to bradyzoites and parasitophorous membrane transformation to precyst wall. Encystment induced rearrangements in the actin filament cytoskeleton which modified the host cell shape.
CONCLUSIONS: Inhibition of IMPDH induced tachyzoites differentiation to bradyzoite and formation of the tissue cyst with changes in the host actin cystokeleton.

This study was supported by grant #155459 and KNCE by scholarship #261878 from CONACyT.

Prevalence of gastrointestinal parasites in adult and young goats in Oaxaca, Mexico

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BACKGROUND: Gastrointestinal parasitic infections are one of the most important causes of porduction losses in ruminants. The aim of this study was to estimate helminth and protozoan infections in adult goats, kids and dams with kids during the preweaning period.
METHODS**:** Fecal samples from forty 2.5-year-old goats, seven 6-month-old kids, and 7 dams with kids in Oaxaca, Mexico were examined using coprological techniques. Spearman correlation coefficient (rs) was used to evaluate the relationship between parasite burden and age.
RESULTS**:** Twenty percent of adult female goats were found to be passing coccidian, being *Eimeria alijevi* the most frequent species (40%). The frequency of *Haemonchus contortus*, *Bunostumum* spp., *Chabertia ovina*, *Cooperia* spp., *Oesophagostomum* spp., and *Trichostrongylus* spp. were 26.38%, 18.05%, 18.05%, 16.66%, 12.5%, and 8.33%, respectively. *Trichuris ovis* was detected in 2.5% of adult goats, and 20% of them were infected with *Moniezia* spp. Kids were positive to *Eimeria*, and the most prevalent species was *E. caprina* (20%). The infection rates of *Haemonchus contortus, Strongyloides*, *Bunostomum, Cooperia, Oesophagostomum*, and *Chabertia* in kids were 22.52%, 22.52%, 17.11%, 16.21%, 13.51%, and 8.1%, respectively. Dams with preweaned kids harbored 50% of *E. alijevi* as the most found species. These same adult goats hosed and infection consisting of 53.16% *Strongyloides*, 31.64% *Haemonchus contortus*, and 15.18% *Cooperia* spp. A negative and significant ($p \leq 0.05$) correlation was found between parasite burden and age.
CONCLUSIONS: The present study demonstrated that gastrointestinal parasitic infections occur frequently in young and adult goats around Oaxaca, Mexico, especially coccidian infections. Parasite burden and age are negatively correlated and this could be perhaps due to a stronger immunity in older animals.

Identification of *Habronema* spp in horses slaughtered in the wake of San Vicente Chicoloapan, Texcoco

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BACKGROUND: The parasites in horses (Equus caballus) are evident, producing colic, which often generate the animal's death. The aim of this study was the identification of adult parasites *Habronema* spp found in horses slaughtered on the trail of Texcoco, Edo de Mexico.
METHODS: During the months June-November of 2011, 6 stomachs of horses slaughtered were collected. Was conducted reviewing the contents of the stomach mucosa and submucosa, to identify the presence of parasites. Parasites were stained by Mayer Haemalumbre technique and were mounted for microscopic observation and identification.
RESULTS: Two stomachs were positive *Habronema* spp. One stomach analyzed in October, a total of 155 parasites were collected, 76 specimens were identified as *Habronema muscae*; of which 41.3% were female and 7.7% males, moreover, 44 organisms of the species *H. microstoma* were found: 22.6% females and 5.8% males, 22.6% parasites was not possible to identify species. in the stomach collected in September the presence of 170 specimens *Habronema* of which 126 belonged to the species H. muscae was determined, 43.5% females and 30% males. Were identified 27 specimens of *H. microstoma*: 10.5% females and 5% males, 10% could not identify the species.
CONCLUSIONS: We identified two species of parasites belonging to the genus *Habronema*; obtaining a greater frequency compared *H. microstoma* and *H. muscae*.

Update in giardiasis and cryptosporidiasis

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The Global Enteric Multicenter Study (GEMS) for diarrheal diseases made the surprising finding that cryptosporidiosis is one of the most important causes of infectious diarrhea in children in developing countries. In addition the Global Burden of Disease Study 2010 (GBD 2010) found, that together, cryptosporidiosis and amebiasis exceed the disease burden – as measured in disability-adjusted life years (DALYs) or in deaths- of any helminths infection now currently being targeted for preventive chemotherapy. Nitazoxanide, the first of thiazolides, is the only existing drug approved in the United States and in many countries of the world for the treatment of cryptosporidiosis. In addition the drug is also effective against other protozoa such as *Entamoeba histolytica* and *Giardia intestinalis*. It was recently suggested that nitazoxanide would be added to other essential medicines for integrated neglected tropical disease control and elimination. Research are continuing to identify new chemical compounds able to treat *Cryptosporidium parvum* using a cell culture assay of the parasite and a reliable *in vivo* model of intestinal and biliary cryptosporidiosis in jerbils experimentally infected. Assays against amebiasis and giardiasis *in vitro* and *in vivo* in experimentally infected mice are known for many years being proven reliable to predict a clinical activity of a test compound against these two protozoa.Two new thiazolides (RM-5038 and RM-5060) were recently identified for the treatment of the three protozoa with a significant higher activity than nitazoxanide for the treatment of *Cryptosporidium parvum* suggesting a potential use of these compounds in a single dose. RM-5038 and RM-5060 underwent a complete preclinical development in animal pharmacokinetics and toxicology and are now entering phase 1 clinical testing in the United States.

Novel drugs against protozoal diseases

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With the exception of malaria not reviewed in this abstract other protozoan diseases affecting a significant number of people could be reduced to ten types of diseases. African and American trypanosomiasis, cutaneous and visceral leishmaniasis, toxoplasmosis, uro-genital trichomoniasis and intestinal protozoan infections caused *Cryptosporidium parvum*, *Giardia intestinalis*, *Entamoeba histolytica*, *Blastocystis hominis* and *Balantidium coli*. Thiazolides such as nitazoxanide and valoxanide or RM-5060 are the only drugs effective *in vitro* or in cell culture assays against the five intestinal protozoa as well as *Leishmania, spp*, *Trichomonas vaginalis, Toxoplasma gondi* and *Trypanosoma spp* while clinical trials were only conducted against six of them. While several chemical compounds are in early preclinical development against these protozoa, only one new drug, miltefosine underwent clinical trials against both cutaneous and visceral leishmaniasis and is under clinical investigation in the treatment of Chagas disease while a new nitroimidazole derivative, fexinidazole, somewhat related to benznidazole, is being investigated in the treatment of *Trypanosoma cruzi*. Finally a new thiazolide derivative, RM-5038 is entering phase 1 clinical trial in the United States and will be tested against *Cryptosporidium parvum*. In the jerbil animal model of intestinal and biliary cryptosporidiosis, RM-5038 was twice as effective as nitazoxanide used as positive control. While there were several chemical compounds effective against several protozoa *in vitro* or in cell culture assays there were no new compounds in preclinical or clinical development for the treatment of other protozoa.

Landlocked mariner? *Gyrodactylus tomahuac*, a parasite from endemic freshwater fish in the Mexican highlands with affinities to marine monogeneans

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BACKGROUND: Freshwater fish in Mexico contain families with clear Nearctic (e.g., Ictaluridae and Cyprinidae) and Neotropical origins (e.g., Characidae and Cichlidae), as well as endemic elements (e.g., Atherinopsidae and Goodeidae). Similarly, fish families harbour characteristic parasite core faunas, which share the biogeographic origin of their hosts. Undoubtedly, helminths are the best known parasite group infecting vertebrate hosts in Mexico: in particular, the inventory of parasites of freshwater fish in the country is comprehensive and nearing completion, with the exception of the Monogenea, which remains the least studied group.
METHODS: We collected monogeneans from the genus *Gyrodactylus* from the blackfin goodea, *Goodea atripinnis*, an endemic fish of the Mexican highlands. Parasites were evaluated by morphology and molecular-based methods: sclerotised structures of their adhesion organ (haptor) were characterized and the ITS1, 5.8S and ITS2 region of the rDNA was sequenced. Molecular data were used to generate phylogenetic and ancestral area trees.
RESULTS: We describe a new species, *Gyrodactylus tomahuac*. Interestingly, although *G. tomahuac* was collected from a freshwater fish, it bears no morphological similarity to any known freshwater gyrodactylid. Phylogenetic analyses show that *G. tomahuac* is most closely related to gyrodactylids infecting cods (Gadiformes) and marine populations of sticklebacks (Gasterosteiformes). Ancestral area analyses indicate that the ancestor of *G. tomahuac* had a 50% probability of living in fresh or brackish water.
CONCLUSIONS: We discuss the hypothesis that *G. tomahuac* may be an ancestrally marine parasite that was caught “high and dry” when the landmass of what today is Mexico was gradually uplifted during the Cenozoic. If this hypothesis is true, *G. tomahuac* would join the relatively short list of ancestrally marine organisms known to inhabit the Mexican highlands; and this would suggest that a marine ingredient should be added to the mix of elements resulting in the very rich biodiversity of Mexico.

A novel ABCG-like transporter of *Trypanosoma cruzi* is involved in benznidazole natural resistance

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BACKGROUND: Benznidazole (BZ) and Nifurtimox (NFX) are the only two drugs available for the treatment of Chagas disease. Therapeutic failures in the chronic phase may be due to differences in drug susceptibility among *Trypanosoma cruzi* strains. The genetic diversity of *T. cruzi* is categorized into six discrete typing units (DTUs), TcI–TcVI.
METHODS: We employed DNA microarrays to assess the differential gene expression between BZ-susceptible and BZ-naturally resistant strains.
RESULTS: Among the probes up-regulated in the resistant strain, one ABCG transporter gene, *Tc*ABCG1, was detected. This result was confirmed by real-time RT-PCR with a panel of strains. Particular ABCG transporters have been implicated in drug resistance. The sequence of *TcABCG1* single copy gene was determined in fourteen strains belonging to different DTUs and with different degrees of BZ sensitivity. Phylogenetic inference supported hybridization patterns in the evolutionary history of TcIII, TcV and TcVI DTUs. *Tc*ABCG1 protein of TcI strains BZ-resistant *in vivo* and *in vitro* displayed fifteen amino acid variations as compared to CL Brener (CLB) BZ-susceptible strain. To investigate the influence of the structure of *Tc*ABCG1 transporter in BZ-sensitivity, genes of two TcI strains were cloned in the pROCK integrative vector and transfected in CLB. Around 45% increase of the IC_{50} value to BZ was verified in the TcI gene-transfected parasites as compared to CLB WT. Transfection of CLB with the homologous gene promoted 16% increased resistance. In all the transfectants 2.7-fold raise of the relative abundance of *TcABCG1* transcripts was observed. *TcABCG1* gene also improved resistance to NFX by 30-40%.
CONCLUSIONS: The data suggest that the ABCG transporter is one of the elements involved in *T. cruzi* resistance to nitroheterocyclic compounds. Orthologous proteins were found in *Leishmania* species and African trypanosomes. The characterization of the substrate specificities of the ABCG proteins might provide clues about their potential physiological role in pathogenic trypanosomatids.
Support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP);
Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Genotyping of *Blastocystis* sp. isolates from infected individuals in a rural community of Morelos, Mexico: is the infection associated with irritable bowel syndrome?

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BACKGROUND
Blastocystis is an enteric protozoan. Many genotypes exist in nature, and recent observations indicate that humans are in reality host to numerous zoonotic genotypes. Such genetic diversity has led to a suggestion about its pathogenesis are due to pathogenic and nonpathogenic genotypes. *Blastocystis* has been associated to diarrheal disease, and there are reports that also suggest an association between *Blastocystis* and irritable bowel syndrome (IBS). The objective was to perform the estimation of the molecular prevalence of infection of *Blastocystis* sp. in a rural population of women in Xoxocotla Morelos.
METHODS
The molecular characterization was using as molecular target the Propionyl CoA gene, PCR products were sequenced and the sequences were used to establish the phylogenetic relationship between local isolates. Besides, the correlation between the infected condition and the genotype, and the presence of gastrointestinal disorders was performed.
RESULTS
Ninety individuals were included in the study, about 50% aged between 13 to 25 years old. The prevalence of parasites was 60%, by PCR *Blastocystis* sp. was identified in the 52%.
Based in ROME III diagnostic questionnaire for the adult Functional Gastrointestinal Disorders (FGIDs), the IBS was not identified neither in parasite or non-parasite individuals.
CONCLUSIONS
Undoubtly the high prevalence of *Blastocystis* in the studied population was remarkable; however we did not find correlation between *Blastocystis* and irritable bowel syndrome (IBS).

Grants: PAPIIT, UNAM: IN226511; CONACYT: 210C01-140990 and PAPIIT, UNAM: IN218214

Epidemiologic research methods for congenital Chagas disease field studies

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BACKGROUND: Large population-based studies are needed to better measure the frequency of congenital *T. cruzi* infection, its risk factors, and its impact on health outcomes.
METHODS: We will compare epidemiologic designs used to study congenital *T. cruzi* infection at population level.
RESULTS: Approaches used to identify *T. cruzi*-infected mothers include:

- Cohort studies measuring maternal *T. cruzi* antibodies during prenatal care;
- Cohort studies measuring *T. cruzi* antibodies in umbilical cord blood;
- Case-control studies measuring *T. cruzi* antibodies in maternal blood;
- Household cross-sectional studies measuring *T. cruzi* antibodies in maternal blood.

Point-of-care rapid tests and tests performed on dried blood spots on filter papers facilitate the serological testing in the field.
Once seropositive mothers have been identified, challenges to identify *T. cruzi*-infected infants include:

- Having trained personnel available 24/7 to perform direct parasitological examination of cord blood and of infants' blood obtained by heel prick within a few hours after blood collection;
- Organizing follow-up household visits to perform *T. cruzi* serology after the disappearance of maternal antibodies after 8 months of age.

Mobile Health (mHealth) approaches to contact mothers by cell phone and geographic information systems are helpful to facilitate household visits.
CONCLUSIONS: Available epidemiologic study designs need to be compared while planning for population-based congenital Chagas disease studies.