Human Toxoplasmosis: an Update in Malaysia

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BACKGROUND: This prospective case-control study was carried out to determine the seroepidemiology of *Toxoplasma* infection among people having close contact with animals. A total of 312 blood samples were collected from veterinarians (38), veterinary technicians (45), veterinary students (194) and pet owners (35) from various veterinary hospitals and clinics in Klang Valley, Malaysia.
METHODS: About 4cc of blood samples was withdrawn from agreed participants were processed for measurement of anti-*Toxoplasma* IgG and IgM antibodies as well as avidity test of *Toxoplasma* IgG by ELISA. At the same times, the demographic profiles and possible risk factors of these participants were also recorded in the standardized data collection sheets.
RESULTS: Overall seroprevalence of toxoplasmosis was 19.9% (62/312) found among these participants being; 57 (18.3%) for IgG, 3 (1.0%) for IgM and 2 (0.6%) for both IgG and IgM antibodies. In univariate analysis, age group, gender, study population, task performance, working duration and gardening were statistically significant with *Toxoplasma* infection. Multivariate analysis showed that age group of equal or more than 30 years old (OR=0.34, 95% CI=0.18-0.63, P<0.001) and working or study duration of more than 10 years in handling with animals (OR=5.07, 95% CI=1.80-14.24, P<0.002) were the only two identified as significant factors associated with *Toxoplasma* acquisition.
CONSLUSION: Results suggest that *Toxoplasma* infection is surprisingly low in this study population. However, findings highlight the need to increase awareness about toxoplasmosis acquired throughout life and to improve our understanding on *T. gondii* in any given environments. To the best of our knowledge, this is the first ever reported study on toxoplasmosis among people having close contact with animal in this country.

Plasmodium vivax invasion: fine specificity and rapid remodelling of nascent reticulocytes.

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BACKGROUND: Malaria red cell tropism is an important characteristic of certain species of *Plasmodium spp.* as it defines the course of invasion and the pathology of the resultant disease. Although it is well understood that *P.vivax* (an important cause of human malaria) only invades reticulocytes, it is still not clear what receptor modulates this specificity to reticulocytes (certainly not DARC as this is also present on mature red cells).
METHODS: To facilitate the discovery of the reticulocyte receptor targeted by P.vivax, we used a flow cytometry approach to sample the different subsets of reticulocytes from cord blood based on the expression of CD71 (Transferrin receptor) to define the fine scale tropism of *Plasmodium vivax*. Different microscopy techniques (Scanning Electron Microscopy, Atomic Force Microscopy, immuno-fluorescence assay) were used to describe the phenotypic modifications of *Plasmodium vivax* infected reticulocytes. We also utilized microfluidics and micropipette aspiration to investigate the biomechanical implications of *P.vivax* development in the reticulocyte.
RESULTS: *Plasmodium vivax* preferentially targets immature reticulocytes (CD71 positive). This invasion is associated with a rapid modification of membrane structure and cytoplasm of the nascent reticulocyte. Within hours the shear modulus, host membrane antigens and nanostructures of the infected reticulocyte is significantly altered.
CONCLUSIONS: The restricted selectivity of *P.vivax* for nascent reticulocytes has important consequences for understanding the pathobiology of vivax malaria. Importantly nascent reticulocytes are rarely present in the peripheral blood, suggesting a cryptic role for bone marrow where such target cells are abundant.

Phenotypic convergence between *Rhodnius neglectus* and *Rhodnius nasutus* (Hemiptera, Reduviidae) from *Copernicia prunifera* palm trees in Northeastern Brazil

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BACKGROUND: Triatomines, vectors of *Trypanosoma cruzi* (the etiologic agent of Chagas disease), are able to develop morphological changes in response to adaptation to new habitats. Different triatomine species might converge phenotypically when inhabiting the same ecotope. In this study we describe this phenomenon for two *Rhodnius* species often found occurring in the palm tree *Copernicia prunifera* in northeastern Brazil.
METHODS: 117 palm trees from 19 municipalities of six northeastern Brazilian states were inspected for the presence of triatomines. Sampling covered almost the entire geographic distribution of *C. prunifera* in Brazil. Insects were collected from palm tree crowns. Species-level identification of triatomines was achieved via morphological and molecular taxonomy based on a 630-bp fragment of the mitochondrial cytochrome *b* gene (mtCytb).
RESULTS: Seventy-four palm trees (63%) were infested with 389 triatomines of the following species: *Rhodnius nasutus*, *Rhodnius neglectus*, *Triatoma sordida*, and *Triatoma pseudomaculata*. *R. nasutus* and *R. neglectus* were by far the most frequently captured species (51 and 46%, respectively). The molecular analysis revealed inconsistencies in the morphological identification of *R. neglectus* and *R. nasutus*. Specimens morphologically identified as *R. nasutus* clustered together with *R. neglectus* reference sequences (Posterior Probability = 1.0). These molecularly identified *R. neglectus* specimens displayed a pale yellowish-brown color without prominent darker connexivum spots, as observed for *R. nasutus*.
CONCLUSIONS: Our results indicate that specimens of *R. neglectus* found in *C. prunifera* were phenotypically similar to *R. nasutus* hindering species identification. The lighter color of *R. neglectus* specimens from *C. prunifera* may be the result of natural selection acting in favor of the better adapted phenotypes to a new ecotope.

Duplicated energy metabolism in hydrogenosomes and cytosol of *Mastigamoeba balamuthi*

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BACKGROUND: *Mastigamoeba balamuthi* is a free-living protist closely related to the human parasite *Entamoeba histolytica*. Although both organisms are adapted to oxygen-poor environment, mastigamoeba inhabits anaerobic freshwater, while entamoeba underwent more recent adaptation to parasitic life-style. In entamoeba, the adaptation resulted in reduction of mitochondria to mitosome, the organelle which lost majority of mitochondrial functions including ATP synthesis and FeS cluster biosynthesis. *Mastigamoeba* possess the hydrogenosome, an anaerobic type of mitochondrion that produces hydrogen.
METHODS: We investigated enzymes of extended glycolysis pyruvate:ferredoxin oxidorectutase (PFO), hydrogenase and acetyl-CoA synthetase (ACS) in subcellular fractions using enzymatic assays and immunoblot analysis. The cell localization was confirmed by imunofluorescence microscopy. The function of mitochondrial targeting sequences (MTS) of selected proteins was tested by overexpression in *Saccharomyces cerevisiae*.
RESULTS: In *M. balamuthi* we detected the typical hydrogenosomal enzymes PFO and hydrogenase in hydrogenosomes as well as in the cytosol. Accordingly, we identified genes of PFO and hydrogenase with and without MTS. Similarly to *E. histolytica*, ATP is synthesized from acetyl-CoA by ACS. Interestingly, this enzyme is also duplicated in mastigamoeba and displays dual localization. All three enzymes (PFO, hydrogenase, and ACS) have significantly higher activity in cytosol than in the hydrogenosomes. Dual localization of FeS proteins (PFO, hydrogenase) corresponds to the dual localization of NIF machinery that mediates FeS cluster assembly. Similarly to entamoeba*, M. balamuthi* posseses sulfate activation system in hydrogenosomes.
CONCLUSIONS: Our results indicate that *M. balamuthi* possesses duplicated pathways of extended glycolysis including ACS-catalyzed ATP synthesis. Although hydrogenosomes can synthesize ATP, their contribution to the cell energy metabolism seems to be marginal. Genes coding for PFO, hydrogenase and ACS were likely acquired by lateral gene transfer, duplicated, and subsequently, the hydrogenosomal forms were equipped with mitochondrial targeting sequences.

Porcine cysticercosis control strategies in Tanzania

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BACKGROUND: Currently, Tanzania has approximately two million pigs of which >90% are reared in rural areas. Prevalence of porcine cysticercosis of >30% have been reported in southern Tanzania based on antigen enzyme-linked immunosorbent assay (Ag-ELISA) while in northern Tanzania, an incidence rate of approximately 69 (95% CI: 65, 72) per 100 pig-years was estimated. One clinical study in northern Tanzania found significant association between neurocysticercosis and epilepsy ($P < 0.0001$; $n = 212$ people with epilepsy, 198 people without epilepsy). In southern Tanzania, a community based study found a human cysticercosis sero-prevalence of 16.7% based on Ag-ELISA ($n = 830$). Among 55 Ag-ELISA positive persons, 30 (54.6%) had brain structures suggestive of neurocysticercosis on CT scan. Various *T. solium* control strategies have been tried. Here we report results of pig chemotherapy and health education trials.
METHODS AND RESULTS: A controlled randomized treatment trial on 61 naturally infected pigs found that oxfendazole at a single oral dose of 30 mg/kg was able to kill muscular cysticerci but not those in the brain. A randomized community based health education of smallholder pig farmers in 42 villages in Mbulu district of northern Tanzania estimated a reduction of approximately 43% in the incidence rate of cysticercosis in pigs based on Ag-ELISA. A five-year projection found that the health education would be beneficial to the farmer (net present value US $3507, 95% CI: 3421-3591; internal rate of return 370%). A health education intervention randomized in 60 schools in Mbulu district, found significant improvements in knowledge regarding *T. solium* in both primary and secondary school students. The improvement was persistent during the 12 months of evaluation.
CONCLUSIONS: Health education should be incorporated in any control programme for *T. solium*. Combined interventions are necessary for ultimate elimination of *T. solium* in an endemic situation.

A tegument-specific venom allergen-like protein of the Chinese liver fluke *Clonorchis sinensis*

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BACKGROUND: Venom allergen-like (VAL) protein, a member of the larger sperm coating protein (SCP) superfamily, is a multifunctional protein found in eukaryotes. Although various VAL proteins have been reported, little is known about their biological and immunological function in parasitic helminths. In this study, a VAL protein of the Chinese liver fluke *Clonorchis sinensis* was cloned and characterized.
METHODS: To characterize a VAL protein of *C. sinensis*, DNA sequencing, multiple sequence alignment, production of recombinant protein, generation of immune sera and immunohistochemical staining were performed.
RESULTS: A cDNA encoding 25kDa protein was identified from EST database of *C. sinensis.* BLAST search revealed that the protein had 46% of sequence identity with *Schistosoma mansoni* VAL 13 protein, and thus the protein was named as CsVAL13. Multiple sequence alignment indicated that CsVAL13 shared 39~46% of sequence identity with VAL proteins of parasitic helminths. Histidine and tyrosine residues contributing structural stabilization were conserved well across the VAL protein sequences. Phylogenetic analysis showed that CsVAL13 clustered together with other VAL proteins of parasitic helminths. Three-dimensional structure of CsVAL13 modeled according to the golgi-associated plant pathogenesis-related protein1 of human (PDB ID 4aiw) as a template contained alpha-beta-alpha sandwich characteristic in the VAL protein. Recombinant CsVAL13 protein (rCsVAL13) was produced bacterially and purified by Ni-NTA affinity chromatography. Immune sera were obtained from BALB/c mice immunized with rCsVAL13. Immunohistochemical localization using the immune sera indicated that CsVAL13 is distributed mainly in the tegument and eggs of adult *C. sinensis*.
CONCLUSIONS: These findings suggest that CsVAL13 located specifically in the tegument of adult worm may have roles in host-parasite interactions and contribute immunological stimulation on the surrounding host environments.

The role of the interferon-gamma in experimental encephalitis in C57BL/6 mice

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BACKGROUND: Responsible for over than 50 thousand annual deaths neurocysticercosis (NCC) is one of the main parasitosis of the central nervous system (CNS) and the main cause of active epilepsy in patients with neurologic clinics in development countries such Brazil. Studies demonstrate that C57BL/6 mice are more resistant to experimental intraperitoneal infection with *Taenia crassiceps* cysticerci as they present a predominance of type 1 immune response. This study aimed the evaluation of IFNγ role in the inflammatory response of conventional C57BL/6 mice and C57BL/6 mice lacking IFNγ gene (KO- IFNγ) inoculated via intracranial with *T. crassiceps* cysticerci.
METHODS: Those mice were inoculated via intracranial with inicial stage T. crassiceps cysticerci and euthanized at 7, 30, 60 and 90 days after the infection (DAI). Their brains removed and histopathologically analyzed.
RESULTS: In conventional animals the presence of IFNγ induced ventriculomegaly with greater intensity due to the chronification of the inflammatory response which was more intense with greater microgliosis, gliosis, ependymitis and precocious destruction of the cysticerci. While in the KO-IFNγ animals the inflammatory response was less intense with prevailance of polimorphonuclear cells. The increase in the mononuclear cells occurred only at 90 DAI when the destruction of the parasites occurred.
CONCLUSIONS: In conclusion, this results show that IFNγ plays a fundamental role in the stimulus of the inflammatory response against the intraventricular *T. crassiceps* cysticerci and stimulates the microglia cells and the intensity of the inflammatory response in the host which induces the precocious destruction of the parasite.

Modulation of haemocyte activities in *Radix lagotis* (Lymnaeidae) snails infected with the bird schistosome *Trichobilharzia regenti*

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BACKGROUND: The bird schistosome *Trichobilharzia regenti* is transmitted *via* lymnaeid snails of the genus *Radix*. In other snail genera, the parasite is likely eliminated by innate immune reactions in which haemocytes represent the main cellular effectors. Therefore, it is hypothesized that a compatible trematode modulates haemocyte activities of susceptible snail host in order to ensure its survival and replication. The mechanisms by which the compatibility is achieved in *T*. *regenti-R*. *lagotis* combination remain largely unknown.
METHODS: Light and electron microscopy observations were combined to evaluate histological sections of *R*. *lagotis* snails experimentally infected with *T*. *regenti*. Haemocyte numbers and cell activities (phagocytosis, hydrogen peroxide (H_2O_2) production, activities of protein kinase C (PKC) and extracellular-signal regulated kinase (ERK) in *T*. *regenti* infected and uninfected snails were compared by use of Bürker haematocytometer, fluorometry and western blotting.
RESULTS: Histological observation disclosed the capability of *R. lagotis* haemocytes to surround but not destroy *T*. *regenti* larvae. Microtubular aggregates likely corresponding to the remnants of phagocytosed ciliary plates were observed within haemocytes by use of TEM. Haemocyte abundance increased in the infected snails when compared to the uninfected counterparts. By contrast, haemocyte phagocytic activity and H_2O_2 production were significantly decreased in infected snails. At the molecular level, both of these haemocyte activities were controlled (at least partly) by PKC and ERK. These kinases were less active in haemocytes from infected snails.
CONCLUSIONS: Our observations suggest that *T*. *regenti* larvae avoid elimination in *R*. *lagotis* snails which results in subsequent parasite development. As for haemocyte activities, decreased phagocytosis and H_2O_2 production in infected snails might correspond with lowered PKC and ERK activities in response to infection.

Identification and isolation of local *Toxoplasma gondii* strain

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BACKGROUND: *T. gondii* is an intracellular obligate protozoan parasite causing toxoplasmosis, which targets almost all warm-blooded vertebrates including mammals and birds. Humans are infected by the parasite after eating poorly cooked meat containing cysts of the parasite or by contact with cat feces. So, meat is the main source of toxoplasmosis in humans especially among people who consume poorly cooked meat in the form of barbecues, beefsteaks, kebabs, burger and shawarmas.
METHODS: The study was designed to isolate locally prevalent strains of *T. gondii*. The collected fecal samples from cats brought to pet clinics of UVAS, were analyzed for oocyst presence using compound microscopy. The oocysts were incubated for sporulation. After that mice were infected for *in-vivo* cultivation to form the tissue cyst. Outer and inner sets of primers were designed by Primer-Blast software of NCBI and Primer-Select software of Lasergene DNA star. DNA was isolated from tissue cysts of mice and cat feces and was further used for optimization of nested PCR. Sequencing of amplicons was done.
RESULTS: 3 out of 200 fecal samples from cats were found positive for *T. gondii* oocysts. The unsporulated oocysts were poorly sporulated then mice were infected orally. DNA extracted from the brains of mice showed no PCR amplification product. DNA extracted from fecal oocysts showed PCR amplification product, which was further extracted through gel extraction kit. The amplified product was further subjected for nested PCR by inner primers. The DNA was confirmed to be *T. gondii* by nested PCR method.
CONCLUSIONS: The optimization of Nested-PCR for diagnosis of toxoplasmosis is useful in the early detection of parasite infection. The DNA should be characterized for genotyping of the parasite by RFLP method at SAG2 locus.

New species in parasite fauna of Santer Seabream *Cheimerius nufar* (Valenciennes, 1830) (Sparidae)

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BACKGROUND: Santer Seabream (*C.nufar*) is an important fish species for local artisanal fisheries in the Sultanate of Oman and a promising candidate for the development of aquaculture industry in the country. This fish is mainly distributed in the Indian Ocean and its highest concentration is found in South-West Africa. Parasite fauna of *C.nufar* is one of the least studied among other species of bream (Bray,1986). The Sultanate of Oman is set to establish aquaculture industry which initiated an intensive research project in Oman in 2012 aiming to study the parasites fauna of several fishes which are considered as promising targets for aquaculture. *C.nufar* being among the selected fish species, the investigation resulted in recording 17 parasite taxa including microsporidia, trematodes, monogeneans, cestodes, nematodes, parasitic copepods and isopods. Some of the detected parasites were already described as new species to science. While the work is underway to identify the others and to recognize the ones that might be harmful to their hosts.
METHODS: This paper discusses a new species of monogenean in the parasite fauna of *C.nufar*.
RESULTS: The structure of the atrium revealed the similarity of this monogenea to the genus *Lutianicola* Lebedev, 1970 (Microcotylidae Taschenberg, 1870). Mamaev (1990) described two new microcotylid species of *Lutianicola vittae* Mamaev, 1990 and *Lutianicotyle indica* Mamaev, 1990 from the *Lutjanus vitta*, *L. fulviflammus* and *Lutjanus malabaricus, L.sanguineus,* accordingly. A study of the descriptions of these three species showed the similarity of the monogenean species in *C. nufar* with the atrium structure and shape, number of hooks to *Lutianicola haifonensis*. However, some morphological and anatomical differences were also revealed suggesting that the monogenea in *C. nufar* could be a new species.
CONCLUSIONS: Characteristically monogeneans of genus *Lutianicola* are exclusively found in Snappers, members of another family of demersal fish Lutjanidae Bloch 1790.

Toxoplasmosis: new understanding of an old disease

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Known for over a century, *Toxoplasma gondii* is a globally distributed parasite that infects one third of the world population. The clinical significance of toxoplasmosis has long been defined by the disease it may cause in the developing fetus in case of maternal infection in pregnancy, and as an opportunistic infection in immunosuppressed individuals. However, a body of data that has emerged in the past decades suggests the time has come to re-define some existing concepts. First, ocular toxoplasmosis (OT), once considered to develop as a consequence of congenital infection, is now known to occur as frequently after acquired infection. Next, the *T. gondii* population structure, characterized by clonal lineages designated as types I, II and III predominating in Europe and North America (and a fourth one in the latter), and by a higher frequency of non-clonal, atypical strains in South America and Africa, has been shown to be related to the clinical presentation. Atypical strains have been associated with more severe OT, atypical presentations and even life-threatening disease in both immunocompetent and immunosuppressed individuals. Reinfection with atypical strains has explained cases of CT in babies born to immunized mothers, leading to a changing concept of congenital toxoplasmosis. Finally, the WHO and FAO have recently established toxoplasmosis as a foodborne infection of global concern, with its disease burden, similar to that of salmonellosis and campylobacteriosis, the greatest of all parasitic infections. This, along with the widely varying geographically dependent prevalence of infection and the geographic differences in the *T. gondii* population structure, determine toxoplasmosis as a travel risk. Moreover, globalization of food including importation of meats from areas of a highly divergent population structure may also present a risk factor for severe infections. This changing understanding of *T. gondii* infection calls for new strategic approaches in both management and prevention.

The molecular basis of parasitism in the nematode *Strongyloides ratti*

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BACKGROUND: The *Strongyloides* lifecycle includes a parasitic female-only stage, which inhabits the small intestine of its host, and a facultative, dioecious free-living adult generation. These adult life-cycle stages are genetically identical, so that comparing parasitic and free-living stages offers an almost unique opportunity to discover the molecular adaptations required to be a successful parasitic nematode.
METHODS: We have used quantitative mass spectrometry and RNAseq analyses to compare the proteome and transcriptome of parasitic and free-living females of *S. ratti,* a parasite of rats.
RESULTS: We find that 15% of genes are differentially expressed between these two life stages. Many of the genes with upregulated parasitic expression are physically clustered in the genome. Clusters comprise 2-18 adjacent genes, mostly from the same gene families and therefore with likely similar functions. Approximately 20% of the genes in these clusters code for astacins of the zinc metalloproteases family. The largest clusters are mainly CAP domain-containing genes. These gene families are therefore likely to be key to parasitism in *Strongyloides* and possibly other parasitic nematodes. We further compare RNAseq data for parasitic and free-living females of the closely related species – *S. stercoralis*, a parasite of humans' parasite.
CONCLUSIONS: Together with comparisons of other parasitic nematode species, we have identified genes and proteins important for parasitism that are (i) unique to *S. ratti* and *S. stercoralis* (ii) unique to the *Strongyloides* genus, and (iii) common across many parasitic nematodes.

Assessment of safety and anthelmintic efficacy of *Oroxylum indicum*, a medicinal plant of Northeast India

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BACKGROUND: The bark decoction of *Oroxylum indicum* (L.) Kurz. (Bignoniaceae) has long been used as a folk medicine for the treatment of intestinal worms by the native tribes in Northeast India. However, no evidence is available regarding its efficacy and safety, as claimed by tribal people. The present study was, therefore, undertaken to investigate the safety and anthelmintic efficacy of this plant.
METHODS: The anthelmintic efficacy of plant's bark extract was tested *in vitro* at a concentration of 10, 20, and 30 mg/ml employing *Raillietina echinobothridia* as a model test parasite. The toxicity of extract was monitored by OECD 407 guidelines, where Wistar rats were administered 250, 500 and 1000 mg/kg p.o. single doses of plant extract for 28 days. Two additional satellite groups were also maintained for another two weeks to monitor/detect any delayed occurrence or persistence of, or recovery from toxic effects.
RESULTS: Of the three test concentrations of extract, 30 mg/ml concentration revealed significant *in vitro* anthelmitnic effects. At this concentration, the test worms became paralysed in 1.28±0.49 h and then showed mortality in 2.46±0.57 h. The activity of extract was quite comparable with that of a reference drug, praziquantel (1 mg/ml). In the sub-chronic toxicity study, the extract did not produced any mortality or adverse symptoms in extrat-treated animals.
CONCLUSIONS: This study suggests that *O. indicum* possesses significant anthelmintic properties and its testing in rats show no evidence of adverse effects. Therefore, the use of bark decoction of this plant as anthelmintic remedy may be considered as reliable and safe in local folk medicine.

Inhibition of *Trypanosoma cruzi* growth by derivatives of komaroviquinone in vitro and in vivo

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BACKGROUND: *Trypanosoma cruzi* is a protozoan parasite that causes Chagas' disease in Central and South America, where an estimated 8-11 million people are infected. Despite the large number of deaths each year, established treatments for Chagas' disease are limited. An efficacious therapeutic agent is urgently required. It has been reported that a diterpene, komaroviquinone, was a trypanocidal agent. We focused on the chemical structure of komaroviquinone and synthesized its derivatives.
METHODS: Previously, to search for potential anti-trypanosomiasis drugs, we established a culture system of host cells infected with *T. cruzi.* We investigated the effects of the synthesized derivatives on infection rate, growth and trypanocidal activities of *T. cruzi* by using the in vitro assay system utilizing host human fibrosarcoma HT1080 cells. We also examined the derivatives in C57BL/6 mice. *T. cruzi* and the derivatives were intraperitoneally administered to mice and parasitemia was measured at day14 post infection.
RESULTS: Using in vitro assay system, the derivatives with a quinone structure showed inhibitory effects on infection rate, amastigote growth. Among the derivatives, GTN024 including a quinone structure and a triazole group in its chemical structure, showed the killing activity against *T. cruzi* at micro-molar levels. GTN024 also inhibited infection rate and growth of amastigotes at concentration as low as 1 μM. In mouse model, GTN024 (20 mg/kg) significantly suppressed parasitemia with no apparent adverse effects.
CONCLUSIONS: Our results suggest that the derivatives with quinone structure could be new lead compounds in the development of anti-trypanosomiasis drugs.

Mitochondrial COI sequence motifs for identification of morphologically difficult amphistomid taxa (Trematoda: Paramphistomoidea) - aetiological agents of amphistomiasis

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BACKGROUND: Amphistomiasis is a widespread parasitic infection of ruminants which causes high morbidity and mortality in tropical and subtropical areas. The disease is caused by several morphologically similar species of amphistome parasites belonging to the superfamily Paramphistomoidea. In the present study, short DNA motifs in the mitochondrial cytochrome oxidase c subunit I (mtCOI) gene were evaluated for their role in identification of seven amphistome species plausibly implicated in amphistomiasis, the occurrence of which is commonly reported in Northeast India.

METHODS: Adult flukes were recovered from freshly slaughtered livestock animals at local abattoirs from different parts of Northeast India. Total genomic DNA was isolated from individual flukes and mtCOI region was PCR-amplified using universal trematode primers. Short sequence motifs (50 bp in size) were identified from aligned COI sequences of each species using PRATT software and evaluated for their species specificity through BLAST analysis against the nonredundant GenBank database of NCBI.

RESULTS: A total of 50 motifs were generated for each species. The BLAST analysis revealed a number of motifs to be specific for their target species, of which the three best motifs were selected for their use in species identification. The selected motifs were found to show high specificity for their respective species with higher scores and lower expect (E) values than any other species in the entire GenBank database.

CONCLUSIONS: The mtCOI motifs generated in the present work allowed accurate *in silico* identification of the fluke species. The study strongly suggests the use of these motifs as a potent tool for delineation of amphistomid taxa.

Unique cocoon-like envelope in the intrauterine eggs of the pleurogenid digenean *Prosotocus confusus*

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BACKGROUND: Helminth eggs play a critical role in movement of the parasite from definitive to intermediate host. Eggs of the pleurogenid digenean trematode, *Prosotocus confusus* (Looss, 1894), a parasite of frogs (Amphibia: Anura) in Europe, are described here for the first time.
METHODS: Intrauterine eggs of *P. confusus*, collected from *Rana lessonae* in Belarus, were examined in situ by standard methods for transmission electron microscopy (TEM).
RESULTS: Each embryonating egg is composed of an early embryo surrounded by a four-layered egg wall: (1) an outer, anucleate layer external to the eggshell, which forms a thick cocoon; (2) the operculate egg-shell; (3) not fully formed, a differentiating outer embryonic envelope containing large nuclei of macromeres; and (4) situated below, undifferentiated layer of the future inner embryonic envelope containing mesomere nuclei. Layers enveloping the egg apparently play an important role in the protection, metabolism and storage of nutritive reserves for the developing miracidium. The outer anucleate layer, or cocoon, is situated externally to the eggshell and composed of an electron-lucent substance with numerous dense osmiophilic islands attached to its peripheral membrane.
CONCLUSIONS: A cocoon envelope such as this has never been seen in previously TEM studies of the eggs of parasitic platyhelminths, with the exception of another pleurogenid *Brandesia turgida*. The origin, formation, functional ultrastructure and chemical composition of this peculiar layer remain enigmatic, although its function appears to be protective. The thick, electron-dense eggshell resembles that of other trematodes, exhibiting a characteristic fissure zone around the operculum. Numerous lysosome-like structures observed in some eggs may be involved in the autolysis of both the embryonic envelopes (in particularly the early degeneration of macromere nuclei of the outer envelope, characteristic for this species) and in the disintegration of several early micromeres. The inner envelope, which forms later from mesomeres, persists longer during embryogenesis.

Development of an *in vitro Plasmodia* parasite killing assay for the evaluation of cell-mediated immune responses following vaccination with pre-erythrocytic malaria vaccine candidates.

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BACKGROUND: Vaccination against liver stage malaria antigens can induce T cell-mediated immunity to the disease. A viral vector vaccination regime undergoing Phase 2b clinical testing uses chimpanzee adenovirus 63 (ChAd63) and modified Vaccinia virus Ankara (MVA) encoding liver stage antigen Thrombospondin-Related Adhesion Protein (TRAP) fused to a malaria multi-epitope string (ME). This regime induces high frequencies of antigen-specific T cells, providing 21% sterile protection and a delay to patent parasitaemia in a further 36% of vaccinees, following controlled human *Plasmodium falciparum* infection. Monofunctional IFNγ-producing $CD8^+$ T cells correlate with vaccine-induced protection but the associated protective mechanisms remain unidentified. Development of an *in vitro* hepatocyte assay is underway, to quantify cell-mediated parasite killing and investigate mechanisms of vaccine-induced protection.
METHODS: Human hepatoma cell lines were infected with transgenic *P.berghei* sporozoites expressing TRAP from *P.falciparum.* After infection, separated peripheral blood mononuclear cells (PBMC) from partial-HLA matched ChAd63.MVA ME-TRAP vaccinees were added. Intrahepatic sporozoites were quantified using immunofluorescence.
RESULTS: Transgenic *P.berghei* sporozoites infected human hepatoma cells, even with full replacement of the wild type TRAP gene. Parasite infectivity was calculable through staining with an anti-heat shock protein 70 antibody with fluorophore-conjugated secondary antibody, or alternatively through expression of mCherry or GFP under a *P.berghei* promotor. Quantification of parasite killing following addition of vaccinee PBMC is pending.
CONCLUSIONS: Employment of transgenic parasites in this system should provide proof of assay concept and sufficient protocol optimization to allow progression towards the investigation of cell-mediated parasite killing in *P.falciparum*-infected primary human hepatocytes.

Socio-economic and demographic impact on prevalence of malaria in Akoko South-west of Ondo state, Nigeria

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BACKGROUND: Malaria is an endemic disease prevalent in the tropical and sub tropical region of the world. About 216 million people are still affected by malaria yearly killing about 650,000 people with children under five and pregnant women mostly affected. Many people had died unnecessarily due to malaria due to their inability to procure hospital prescribed drugs especially where free malaria drugs are not available. Some do not even have enough money for transport to the clinic to obtain free treatment especially in impoverished countries of the world.
METHODS**:** Two hundred and four pregnant women were involved in the study. The pregnant women were grouped into two based on their age. Primary, secondary and tertiary data were collected for this study. Socioeconomic and demographic factors such as age, educational, occupational and income were considered. This study was carried out via the administering of well structured questionnaires on the demographic and socio-economic status of pregnant women and malaria prevalence.
RESULTS**:** Of the 204 pregnant women who administered the questionnaire, 136 had malaria infection. The prevalence of infection among younger age range (15-35 years) was significantly higher (25.89%), than those with middle age group (10.3%). The prevalence of infection was higher among those with secondary education (54.4%) when compared with those with tertiary education (14%). The prevalence of malaria infection was higher among those who engaged in craft work and those without job (22.8% and 22.1% respectively) than the civil servants (8%). Based on income per capital the study also revealed that the prevalence of infection was highest among those with lowest income per capital (43.4%) and 5.1% in highest income earners.
CONCLUSION**:** This study shows that socioeconomic and demographic factors play a significant role in the prevalence of malaria infection in Nigeria according.

Bioinformatic identification of cytochrome *b*5 homologues from the parasitic nematode *Ascaris suum* and the free-living nematode *Caenorhabditis elegans* highlights the crucial role of *A. suum* adult-specific cytochrome *b*5 in parasitic adaptation

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BACKGROUND: Adult *Ascaris suum* possesses NADH-metmyoglobin and NADH-methaemoglobin reductase systems that are located in the cells of the body wall and in the extracellular perienteric fluid, respectively, which helps them adapt to environmental hypoxia by recovering differential functions of myoglobin and haemoglobin. *Ascaris suum* cytochrome *b*5, an essential component of the NADH-metmyo (haemo) globin reductase system, has been extensively studied, and its uniqueness has been demonstrated. However, the relationship between *A. suum* cytochrome *b*5 and the canonical cytochrome *b*5 proteins from the free-living nematode *Caenorhabditis elegans* and *A. suum* are unclear. Here, we characterized 4 cytochrome *b*5-like proteins from *C. elegans* (NCBI Protein Data Bank (PDB) accession numbers: CAB01732, CCD68984, CAJ58492, and CAA98498) and 3 from *A. suum* and compared them with *A. suum* cytochrome *b*5 *in silico*.
METHODS and RESULTS: Bioinformatic and polymerase chain reaction analyses showed that the *C. elegans* equivalent of *A. suum* cytochrome *b*5 is CAA98498, which was not expressed as a mature mRNA.
CONCLUSIONS:This result suggested that this free-living nematode, which, unlike the adult *A. suum*, primarily lives under normal atmospheric conditions, does not need a haemoprotein like *A. suum* cytochrome *b*5. These results highlight the crucial function of this *A. suum* adult-specific cytochrome *b*5 in parasitic adaptation.

Characterization of *Babesia rossi* genotypes in dogs diagnosed with canine babesiosis

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BACKGROUND: *Babesia rossi* is a virulent tick-transmitted parasite responsible for causing canine babesiosis in dogs. Canine babesiosis still remains the cause of mortality and morbidity in dogs in South Africa. Preliminary results have suggested that there might be a link between the parasite genotypes and the disease phenotypes. Therefore, the aim of this study was to determine *Babesia rossi* genotypes in dogs diagnosed with canine babesiosis.
METHODS: Ten blood samples were collected from sick domestic dogs presented at the Onderstepoort Veterinary Academic Hospital. Six dogs were clinically classified as uncomplicated and four as complicated cases. DNA was extracted from the blood samples and *Babesia rossi* infections were confirmed using the Reverse Line Blotting assay. Only 7 *Babesia rossi* genotypes were amplified from 7 blood samples using real-time PCR, followed by sequencing of these samples.
RESULTS: Based on the sequence analysis, *Babesia rossi* genotype 28 was identified in 5 dogs and genotypes 19 and 29 were identified in two dogs respectively.
CONCLUSIONS: Our results are in agreement with previously published findings that *Babesia rossi* genotypes are associated with disease phenotypes in dogs diagnosed with canine babesiosis.

A web-based analysis platform for comparative functional genomics and proteomics in eukaryotic pathogens: *Trichomonas vaginalis* as an example

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BACKGROUND: Recent advances in high-throughput research technologies such as new-generation sequencing and multi-dimensional liquid chromatography makes it possible to dissect the complete transcriptome and proteome of many eukaryotic pathogens in a single run for the first time. However, it is almost impossible for many laboratories to handle and analysis these “BIG” data without the support from a bioinformatics team. We aimed to provide a web-based analysis platform for users with only limited knowledge on bio-computing to study the functional genomics and proteomics in eukaryotic pathogens.

METHODS: We use the *Trichomonas vaginalis G3* genome that contain more than 90,000 predicted genes as an example to demonstrate the power of the web-based analysis platform: TvXpress. TvXpress takes a simple text file that contain the gene or protein ID (TVAG_XXXXX) and expression levels (rpkm or fold) as input file to generate a distribution map of gene/protein expression levels in a heatmap diagram organized by color gradients. The diagram is hyper-linked to a dynamic html table that allows the users to filter the datasets based on more than 20 different levels of annotation.

RESULTS: We implemented an integrated database that contain pre-defined annotations such as gene/protein properties (ID, name, length, MW, pI, signal peptide, transmembrane domain, paralog); pathways based on KEGG and GO biological process; subcellular localization based on GO cellular component and known hydrogenosome/exosome proteins; functional classification based on GO molecular function, kinase, peptidase and transporter. Multiple ways of sorting of column and rows is also provided for comparative analysis and visualization of multiple samples.

CONCLUSIONS: We introduced a new platform to compare and visualize differential gene/protein expression levels cross multiple datasets. This is particularly useful in an exploratory stage to grasp the characteristic of the dataset, which can be applied in the subsequent discovery stage to prioritize candidate targets of interest.

Preliminary evaluation of the safety of a new benznidazole for treatment of chronic Chagas disease

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BACKGROUND: Actually the most used treatment for Chagas' disease is benznidazole (BZN). Since 2012 its supply by Roche laboratories to Argentina was suspended and Elea laboratories started its production in March 2012. Side effects (SE) due to BZN-Roche were common in adults. The SE of new BZN should be monitored.
METHODS: A retrospective analysis of medical records of adults chronic Chagas disease patients treated with BZN-Elea from June 2012 to December 2013 was done. Data about demography, clinical aspects, treatment duration and SE was registered. Results were compared with data from a double-blind randomized controlled trial (TRAENA) with 352 patients treated with BZN-Roche.
Fisher's exact test was applied, significance test was considered at $p < 0.05$.
RESULTS: Seventy patients between 18 and 57 years old (media 36.3± 9.1) were treated. The 67% (47) were female. The adherence to treatment was completed in 48 (68.5%); in 10 (14%) was suspended by physician and 12 (17%) dropped out.
Two (2.9%) serious SE were detected, requiring hospitalization due to neutropenic fever. The most common SE were dermatitis 51% (36), digestive symptoms 16% (11), fever and arthralgia 11% (8), eosinophilia and increase of liver enzymes 7% (5), leucopenia 5.7% (4), adenopathy 4% (3) and neuropathy 2,9% (2).
Statistical significance between BZN-Elea and BZN-Roche was found in SE related to arthralgia ($p = 0.0001$), liver enzymes increase ($p = 0.008$), leucopenia ($p = 0.008$), fever ($p = 0.0416$) and a tendency to serious SE (Elea 2/70 vs Roche 1/352, $p= 0.07$). There were no differences due to dermatitis, adenopathy and neuropathy
CONCLUSIONS: The evaluation of the new BZN suggests more frequency of some SE; even this information should be confirmed due to the little number of patients. This preliminary data shows the relevance to close monitoring of patients under treatment with BZN.

Identification of *Leishmania* in semen of individuals from Amazonia

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BACKGROUND: Leishmaniasis is a tropical infection caused by the protozoan, belonging to the genus *Leishmania* which causes a vector borne neglected disease. These are divided into cutaneous, mucocutaneous, visceral, and disseminated disease. World Health Organization estimates 12 millions of infected people, yearly incidence of 500,000 new cases. The Amazon is an endemic area of leishmaniasis, containing a large diversity of species of *Leishmania* spp. and phebotomine sandflies. The diagnosis is made by parasitological and immunologic assays; however conventional tests may presents limitations. Molecular methods based on PCR emerge as a powerful tool for screenings, showing high sensitivity and specificity. In this study, we evaluate the performance of PCR assay to identify *Leishmania* DNA in semen from a study population resident at Brazilian Amazonia.

METHODS: Semen samples from 51 donors selected by Health Bureau of Pará State, were evaluated to detect *Leishmania* DNA. A universal PCR method targeting the internal transcribed spacer 1 (ITS1) region between the SSU and 5.8S rRNA genes were utilized and Southern hybridization of the PCR product with was used to validate the molecular tests.

RESULTS: The PCR amplicons were detected in 49% (25/51) of cases and specific bands were confirmed by *Leishmania* ITS labeled probe. In 64% (16/25) the presence of PCR amplicons agrees with positive results of serology assay.

CONCLUSIONS: The experiments carried out on semen samples from individuals residents in Amazonia, reveals the presence of *Leishmania* DNA in 49% of cases. The presence of parasite nuclear DNA in semen of infected persons suggests a potential role of sexual transmission route in leishmaniasis.

The knowledge of medicine students on host-parasite interactions

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BACKGROUND: The sophisticated molecular analyses as genomics, proteomics and metabolomics of parasites have changed the understanding of parasitic relationship. These analyses have shown that current phenotypes of hosts and parasites resulted from interactions between the two genomes. However, this conception hasn't been changed in textbooks, which interferes directly in the teaching of Parasitology. In this context, the present study aims to analyze the knowledge of medicine students on the basic concepts in Parasitology and associate them with current vision of the parasitic relationships.
METHODS: This is a qualitative research that verified the concepts of parasite and host and their interactions described by 156 medicine students at an University of state of Rio de Janeiro. It was used a questionnaire with open questions and the answers were analyzed according to the method of critical discourse analysis.
RESULTS: Of the 156 answers, 24 (15.4%) were unspecific and incomplete. The majority of the parasite's concepts (69.7%) described the idea of metabolic dependence of parasites, 15.1% associated the parasites with parasitic disease, 12.1% reported that the parasites result in injury or damage to the host, 2.3% considered the parasite an aggressor and only 1 answer (0.75%) presented the word balance when referencing to the parasite. No answer related the importance of the parasite in the evolutionary process of the host. In relation of host, 85.6% of the answers described that "The host houses the parasite" and 13.6% that the host is harmed by the parasite. Only 1 (0.75%) doesn't associated disease when the host presents parasite.
CONCLUSIONS: We concluded that students still have a restricted vision of the parasitic relationship. In Parasitology, methodologies have evolved and the basics concepts remained the same. It's necessary a revision of basic concepts in Parasitology.

Influence of different concentrations of calcium on the TCA cycle of *Biomphalaria glabrata,* the main of intermediate host of *Schistosoma mansoni*

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BACKGROUND: Calcium is considered an essential element of the metabolism of aquatic snails such as *Biomphalaria glabrata* and has been described as a limitant factor on its distribution and adaptation to the environment. This study aimed the evaluation of the influence of the calcium bioavailability on the concentration of glucose, organic acids such as pyruvate and the ones from the tricarboxilic acid (TCA) cycle and calcium in the hemolymph of *B. glabrata* exposed to different concentrations of $CaCO_3$ in the different time intervals.
METHODS: Snails with 60 days of life were distributed into six groups from which five were exposed to the different concentrations of $CaCO_3$ (control, 20,40,60,80 and 100mg/L) and analysed in the different time intervals (1, 14, 21 and 30 days). The concentrations of calcium and glucose in the hemolymph (collected of cardiac puncture) were determined through a diagnostic kit (Liquiform LABTEST®). The organic acids were extracted from the hemolymph through an ionic exchange column (Bond Elut® – Varian) and the resulting sample was analyzed through HPLC.
RESULTS: No difference significant in the calcium concentration among the control and the test groups. The glucose concentrations from the snails exposed to 20 and 40 mg of calcium was significantly lower than the others. The organic acids pyruvate, oxaloacetate, citrate, succinate and fumarate presented an increase in their concentrations when compared to the control group, while propionate presented a decrease in its concentration.
CONCLUSIONS: Different concentrations of $CaCO_3$ influence the glucose concentrations and its homeostasis and the running of the TCA cycle, maintaining the aerobic metabolism in energy cost and allowing the inversion the cycle. The snails may be used as models for the study of physiological markers of stress.

Chagas disease diagnosis in school children from Puebla Mexico as a longterm platform of health surveillance

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BACKGROUND: Chagas disease remains the most important infectious disease in South America with in excess of 8 million infected patients, but is also present in Meso-America.
METHODS: To redress the dearth and potential public health importance of childhood infection of Chagas disease in Mexico a blood survey was performed in 5 localities for 244 school children (8-12 years) in the municipality of Huaquechula, Puebla between October and December, 2012.
RESULTS: PCR diagnosis for Chagas disease for school children in Huaquechula gave 9% positivity (22 patients). However, Chagas disease diagnosis using enzyme-linked immunoabsorbent assays (ELISA), gave sub-threshold positivity in comparison to the requirements of clinical diagnosis (<0.9 OD): 'sub-threshold' ELISA positive children were congruent with indirect immunofluorescence (IFAT) and half of the subthreshold ELISA positive samples were a subset of the PCR positive children. Twenty of the 44 subthreshold ELISA positive children demonstrated cardiac irregularities when subject to electro-cardiogram (ECG), predominated by T-wave inversion and could be indicative of early stage cardiomyopathy.
CONCLUSIONS The high prevalence of ECG irregularities we observed in chagasic children is compatible with previous reports in Mexico. Unexpectedly low IgG in chagasic infection we detected by ELISA is compatible with serosurveys performed in Peru. The study provides a platform to assess the longterm impact Chagas disease has on a rural community in Mexico.

Etiological Treatment of Women Infected with Trypanosoma cruzi and its Effect on Preventing Congenital Chagas

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BACKGROUND:
With the control of the vectorial and transfusional routes of infection with Trypanosoma cruzi, congenital transmission has become an important source of new cases.This study evaluated the efficacy of trypanocidal therapy to prevent congenital Chagas disease and compared the clinical and serological evolution between treated and untreated infected mothers.
METHODS: We conducted a multicenter, observational study on a cohort of mothers infected with Trypanosoma cruzi, with and without trypanocidal treatment before pregnancy. Their children were studied to detect congenital infection.
RESULTS:
Among 354 "chronically infected mother-biological child" pairs, 132 were treated women and 222 were untreated women. Among the children born to untreated women, we detected 34 infected with Trypanosoma cruzi (15.3%), whose only antecedent was maternal infection. Among the 132 children of previously treated women, no infection with T. cruzi was found (0.0%) ($p < 0.05$).
Among 117 mothers with clinical and serological follow up, 71 had been treated and 46 were untreated. The women were grouped into three groups. Group A: 25 treated before 15 years of age; Group B: 46 treated at 15 or more years of age; Group C: untreated, average age of 29.2 ± 6.2 years at study entry. Follow-up for Groups A, B and C was 16.3 ± 5.8, 17.5 ± 9.2 and 18.6 ± 8.6 years respectively. Negative seroconversion: Group A, 64.0% (16/25); Group B, 32.6% (15/46); Group C, no seronegativity was observed. Clinical electrocardiographic alterations compatible with chagasic cardiomyopathy: Group A 0.0% (0/25); B 2.2% (1/46) and C 15.2% (7/46).
CONCLUSIONS:
The trypanocidal treatment of women with chronic Chagas infection was effective in preventing the congenital transmission of Trypanosoma cruzi to their children; it had also a protective effect on the women's clinical evolution and deparasitation could be demonstrated in many treated women after over 10 years of follow up.

Comparative transcriptome analysis of East Coast fever and Corridor disease parasites

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BACKGROUND: In cattle, *Theileria parva* infections cause two recognized disease syndromes, East Coast fever (ECF) and Corridor disease (CD). Not much is known about the underlying phenotypic strain differences contributing to the different disease syndromes.
METHODS: High-throughput transcriptome profiling of two *T. parva* isolates, Muguga (ECF isolate) and 7014 (CD isolate) maintained in lymphoblast cultures, was performed using next generation sequencing.
RESULTS: Using Muguga genome sequence as a reference, 98.4% (3969 genes) of the 4034 predicted coding genes were mapped from the two transcriptomes, 3916 detected in 7014 and 3925 from Muguga. Differential expression was identified in 1109 transcripts (27.9%), mostly up-regulated in 7014 (757, 68.3%). Included in the differentially expressed transcripts (DETs) were antigenic genes including CD8 T-cell antingens and gene families of TA9/TP9, subtelomeric variable secreted protein (SVSP) and TashAT proteins. The top twelve of the most common assigned GO terms of DETs were the same for both isolates except for immune system and biological adhesion processes identified in Muguga and reproduction and growth processes identified in 7014. Pathway enrichment analysis for up-regulated transcripts revealed 32 pathways involving products encoded by some of the DETs. The reference assembly statistics for SNP detection showed a total of 49016 SNPs in 7014 transcriptome compared to 54 in Muguga, and 99.9% of 7014 SNPs were genotype specific.
CONCLUSION: Findings of this study provides evidence of differential expression between transcriptomes of ECF and CD parasites, including genes implicated in host invasion. Transcriptome differences identified here will be highly valuable for further genetic studies to elucidate phenotypic strain differences contributing to the different disease syndromes in cattle theileriosis; however, these findings need to be confirmed in clinical cases.

Expression of recombinant Trypanosome antigens in *Leishmania tarentolae*

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BACKGROUND: Many members of the kinetoplastid family contain members which are parasitic on animals of commercial importance, and on humans, and have major socio-economic impact on populations in affected areas. In particular the trypanosome family are responsible for human sleeping sickness, Chagas disease, surra and nagana in animals. Most diagnostic methods rely on microscopic identification of the parasite or tests made from crude lysates of the organism. When large populations require screening these methods may be time consuming and the latter immunological methods may lack specificity and sensitivity.

METHODS: Using information from proteomics, and evidence from related family members', antigens from *Trypanosma brucei gambiense* (Tbg) were expressed in the recombinant expression system of *Leishmania tarentolae*. (Lt). By using the related organism *Leishmania tarentolae* for recombinant expression it was expected that post-translational modifications would occur in a similar way to *Trypanosma brucei gambiense.*

RESULTS: Fragments of protein representing cell surface and intracellular highly repetitive moieties expressed in the recombinant Lt cells were purified using nickel affinity chromatography. They were tested against panels of sera from people exposed to *Tbg* and control sera. Some antigens show potential to be developed further for diagnostic purposes.

CONCLUSIONS: In this study a range of recombinant antigens were assessed and their recognition by sera examined. Their potential suitability for development into a diagnostic lateral flow device, rapid diagnostic test (RDT) will be discussed

The role of SUMOylation in the cell biology of *Giardia lamblia*

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BACKGROUND: The addition of post-translational modifications (PTMs) usually results in alterations of the biochemical properties and functions of then target protein. In the latter case, ubiquitination (addition of a ubiquitin) and SUMOylation (addition of a SUMO) are well known PTMs occurring in eukaryotic cells. Modification of a protein by SUMO (Small Ubiquitin Modifier) is known to play a role such a in many cellular processes such as nuclear-cytosolic transport, transcriptional regulation, progression through cell cycle, protein stability and protein cellular localization. While in mammal and yeast models the SUMO pathway is a well known system, in parasites there is not enough information about how SUMO interacts with proteins or what the consequences of the modification are.
METHODS: Trophozoites and cysts of *Giardia lamblia* (WB strain) were used as a model to investigate the presence of the SUMO protein and SUMO targets by western blotting and indirect immunofluorescence using an antibody against SUMO.
RESULTS: *Giardia lamblia* possess a unique SUMO gene, codifying for a SUMO protein with 47% identity with the human SUMO-1. The protein is differentially distributed in cyst and trophozoites with the latter showing an intense label in the median body. Upon incubation of trophozoites with an inhibitor of the E1 enzyme (ubiquitin-activating enzyme 1) parasites showed a dramatic morphological change from a pear-shaped to a rounded cell. Moreover, inhibition of SUMO resulted in a reduction of tubulin acetylation.
CONCLUSIONS: Our results suggest that SUMOylation may play an important role in *Giardia lamblia* cytoskeleton probably through changes in the tubulin PTMs.

Identification of Risk Factors for Leishmaniasis in Formiga, Minas Gerais, Brazil, Using Spatial Analyses

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BACKGROUND: Geoprocessing tools have been used to elucidate the influence of ecological and environmental aspects in the transmission of infectious diseases. In this study, this approach was used to create thematic maps that characterize the most vulnerable regions to the occurrence of leishmaniasis in a potential area of recent transmission of Minas Gerais state, Brazil.
METHODS: We used data from systematic captures and canine serological survey conducted in the municipality of Formiga, Minas Gerais state, Brazil. The total number of *Lutzomyia longipalpis*, *Lutzomyia whitmani* and positive dogs found was used to construct Kernel density maps. Thematic vegetation and hydrography maps were analyzed by the signature method to identify correlations between spatial characteristics and the occurrence of vectors, infected dogs and human cases.
RESULTS: The density maps revealed that *L. longipalpis* is more dispersed by the municipality while *L. whitmani* concentrated at the periphery. The vegetation and hydrology variables can influence the distribution of vectors and positive dogs: 99.8% of *L. whitmani* and 94.2% of *L. longipalpis* were captured approximately at 200m from the forest and 99.1% of positive dogs were located at 200m from the river. The overlapping human cases of leishmaniasis with the density maps of sandflies and infected dogs was most evident in the northeast, southeast and south regions of the city.
CONCLUSIONS: The overlapping between vectors, positive dogs and human cases suggest the active transmission cycle in Formiga, especially in the Northeast, Southeast and South regions, which makes such areas a priority for the implementation of control measures. The presence of vegetation seems to influence in the distribution of *L. longipalpis* and *L. whitmani.* Hydrography seems to relate to the positive dogs. However, the importance of these findings in the epidemiology of the disease in the study area requires further study.

Clinical findings in Congenital Chagas disease in Bolivia during the implementation of a Program for the National Control of Congenital Chagas Disease (2004-2010)

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BACKGROUND
Chagas disease (CD) or American trypanosomiasis, is among the neglected diseases in Latin America and its congenital transmission is the hidden way of transmission from mother to child. Bolivia is one of the most endemic countries for Chagas disease, and consequently, congenital Chagas disease represents a vast public health problem.
METHODS
Starting in 2004, a National congenital Chagas program was implemented in 90 health facilities of Bolivia following the PAHO recommendations. Briefly, serological tests were performed to pregnant women during prenatal controls, and children born from positive mothers were followed up until 12 months of age, with controls at birth, between 1 and 2 months and after 6 months. When a child proved positive, treatment was started with Benznidazole, 10 mg/Kg/day/30 days with one post treatment control 6 months later.
RESULTS
Between 2004 and 2010, a total of 1392 children were diagnosed with congenital Chagas disease. Out of those 1392 children, we retrieved clinical data from 893 children diagnosed until 12 months of age in 23 health facilities from 4 departments of Bolivia.
The principal findings were: 19 % were symptomatic (at least one symptom, mainly hepato-esplenomegalia, and respiratory distress), 13 % were premature (< 37 weeks) , 3% of deliveries presented premature rupture of membranes (PRM) and 3 % had low weigh for gestational age (LWGA measured with Lubchengo scale).
77% of babies finished the treatment, and >90% of them were cured.
CONCLUSIONS
These results show that diagnosis must be based on laboratory and not clinical findings, and that efficacy of treatment is really high in babies who are less than one year old.

Profiling the humoral immune responses to Plasmodium vivax infection and identification of candidate immunogenic rhoptry-associated membrane antigen (RAMA).

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BACKGROUND: Completion of sequencing of the *Plasmodium vivax* genome and transcriptome offers the chance to identify antigens among > 5000 candidate proteins. To identify those *P. vivax* proteins that are immunogenic, a total of 152 candidate proteins (160 fragments) were expressed using a wheat germ cell-free system.
METHODS: In this study, high-throughput screening assays have been applied to investigate blood stage-specific immune proteomes from *P. vivax*. An indirect immunofluorescence assay (IFA) was performed to determine the subcellular localization of PvRAMA in *P. vivax* blood-stage parasites by confocal microscopy.
RESULTS: The results of Western blot analysis showed that 92.5% (148/160) of the targets were expressed, and 96.6% (143/148) were in a soluble form with 67.7% of solubility rate. The proteins were screened by protein arrays with sera from 22 vivax malaria patients and 10 healthy individuals to confirm their immune profile, and 44 (27.5%, 44/160) highly reactive *P. vivax* antigens were identified. Overall, 5 candidates (rhoptry-associated membrane antigen [RAMA], Pv-fam-a and -b, EXP-1 and hypothetical protein PVX_084775) showed a positive reaction with >80% of patient sera, and 21 candidates with 50% to 80%. More than 23% of the highly immunoreactive proteins were hypothetical proteins, described for the first time in this study.
CONCLUSIONS: One of the top immunogenic proteins, RAMA, was characterized and confirmed to be a serological marker of recent exposure to *P. vivax* infection. These novel immunoproteomes should greatly facilitate the identification of promising novel malaria antigens and may warrant further study.

Parasite burden and genotypes in patients with chronic Chagas cardiopathy of Chile by NYHA

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BACKGROUND: Up to date no biomarkers exist to know which patients with chronic indeterminate period will remain in this asymptomatic state for life and who will develop cardiopathy. The objective of this study was to understand the relationship of the parasite burden and *T.cruzi* genotypes in patients with chronic Chagas cardiopathy (CChC) versus chagasic individuals without cardiac involvement (CChD).
METHODS: Patients with chronic Chagas disease confirmed by serology, ELISA and IFT were submitted to anamnesis, physical examination and ECG. The patients were randomly divided in group A, 50 patients with CChC and B, 50 individuals with CChD. Echo-doppler was performed to group A discarding all known causes of cardiopathy. To both groups, xenodiagnosis (XD), conventional PCR and quantitative PCR (qPCR) were performed. To 23 patients of both groups *T.cruzi* genotypes were done.
RESULTS: No statistical difference was observed between the average age of both groups. In group A according to NYHA 17 cases were classified in type I and 33 in type II by ECG; only 1 case had grade III by echo-doppler. No significant difference was found between average parasites/mL in both groups. *T.cruzi* V was the genotype more frequently observed isolated or mixed in both groups. The mixture of TcI, TcV and TcVI was found in 5 cases of group A and 2 in group B.
CONCLUSIONS: The number of parasites and the *T.cruzi* genotype does not correlate with type or number of alterations that appear in ECG. The findings show that ECG alteration has more complex relation than a simple cause effect relation on the number or genotypes of the parasites.

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Biomarker analysis revealed distinct profiles of innate and adaptive immunity in infants with lesions of congenital toxoplasmosis

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BACKGROUND: *Toxplasma gondii* is the main infectious cause of human posterior retinochoroiditis, the most frequent clinical manifestation of congenital and acquired toxoplasmosis.
METHODS: This is an investigation performed after neonatal screening for congenital toxoplasmosis aiming to identify biomarkers of innate and adaptive immunity associated with immunopathological features of the disease by flow cytometry. The study included infected infants without (NRL) and with active retinochoroidal lesions (active-ARL, active/cicatricial-ACRL and cicatricial-CRL) besides non-infected individuals (NI).
RESULTS: Our data demonstrated that leukocytosis with increased monocyte and lymphocyte is relevant hematological biomarkers of ARL. Immunophenotypic analysis also revealed an expansion of $CD14^{+}CD16^{+}HLA\text{-}DR^{high}$ monocytes along with $CD56^{dim}$ cytotoxic NK-cell in ARL. Moreover, augment of $TCRgd^{+}$ and $CD8^{+}$ T-cells along with activated $CD4^{+}$ T-cells seem to be good biomarkers of active lesions. Biomarker network analysis revealed a complex and imbricate networks underscore the negative cross talk of monocytes with NK and B-cells in NRL. Outstanding was the remarkable lack of connections involving B-cell and a relevant shift of NK-cells connections from B-cells toward T-cells observed in ARL. A stronger biomarker network was the hallmark of resolute immunity observed in CRL with relevant connections of NK cells and $CD8^{+}$ T-cells with a broad range of cell subsets.
CONCLUSION: Our findings added novel elements to the current knowledge of the innate and adaptive immune responses in congenital toxoplasmosis.

Structural and functional characterization of *Hc-daf-2*, an insulin-like receptor kinase encoding gene of *Haemonchus contortus*

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BACKGROUND: Little is known about the molecular mechanisms regulating the development of parasitic nematodes. As the infective third-stage larvae (iL3) of parasitic nematodes share many common characteristics with dauer larvae of the free-living nematode *C. elegans*, it is hypothesized that the development of the dauer stage in *C. elegans* and the third-stage larvae (iL3) of parasitic nematodes are regulated by similar molecular mechanisms. One of the key molecules regulating the dauer development in *C. elegans* is the insulin-like receptor (designated DAF-2) encoded by the gene *daf-2*. However, nothing is known about DAF-2 homologues in most parasitic nematodes.
METHODS: using PCR-based approaches, we identified and characterized a *daf-2* homologous gene (*Hc-daf-2*) and its inferred product (*Hc*-DAF-2) from *Haemonchus contortus*, a socioeconomically important parasitic nematode infecting ruminants.
RESULTS: The full-length *Hc-daf-2* cDNA was 5463 bp in length, consisting of a 5'-UTR of 697 bp, a 3'-UTR of 551 bp and a 4215 bp region encoding a predicted protein of 1404 aa. *Hc*-DAF-2 displays significant sequence homology to insulin receptors from various metazoan species, and contains conserved structural domains. *Hc-daf-2* is transcribed in all life stages of *H. contortus*, with a highest level in the iL3. In addition, the *Hc-daf-2* promoter drove GFP expression in amphidial neurons of wild-type *C. elegans*, similar to the expression pattern of *Ce-daf-2*. Furthermore, heterologous expression of *Hc-daf-2* in a *daf-2*-deficient strain of *C. elegans* (CB1370) effected partial functional rescue.
CONCLUSION: Overall, these findings provide the first insight into the roles of *Hc-daf-2/Hc*-DAF-2 in regulating the development of *H. contortus*.

Giardia lamblia infection: study of parasite adhesion to intestinal mucus and mucolytic activity as a mechanism of infection

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BACKGROUND: *Giardia lamblia*, a flagellated parasite protozoan, attaches to enterocytes from the small intestine to survive and multiply, causing the disease known as giardiasis. As a mechanism of defense, the inner wall of the small intestine is covered by a mucus layer composed mostly by highly O-glycosilated (50-80% w/w) mucin glycoprotein (MUC2). It is widely known that most intestinal pathogens possess mechanisms of trespassing the MUC2 layer. This study investigates the ability of *Giardia lamblia* to degrade this mucus layer, allowing the protozoan to adhere to enterocytes.

METHODS: Intestinal mucus was obtained from BALB/c mice by mechanical extraction with a glass microscope slide into a 4M guanidine-HCl solution. After ultracentrifugation and dialysis, the material was checked for the presence of glycoproteins, mucin, glycosaminoglycans and proteoglycans by dot-blottings, stained with PA-Schiff, toluidine blue and revealed with α-MUC2. Degradation assays with live trophozoites and excretory-secretory products (ESP) were performed with the purified mice intestinal mucus (MIM), using bovine submaxilary (BSM) and porcine gastric mucins (PGM) as controls. Migration assay was perfomed by covering transwell inserts with MIM, BSM and PGM as controls, given a total of 5 hours for the trophozoites to trespass it.

RESULTS: In this study it is shown that live trophozoites and ESP degrade considerably more MIM compared to BSM and PGM. These data was corroborated in the migration assay where the number of trophozoites that trespassed the MIM-coated insert was greater than with BSM and PGM-coated inserts, respectively.

CONCLUSIONS: The data suggest that degradation of the mucus layer may be a mechanism exploited by G. lamblia trophozoites to penetrate the secreted mucus layer, allowing the contact with enterocytes. More assays must be performed, however, for a better comprehension of this mucolytic capacity of G. lamblia.

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The periodic oscillation of clinical parasitaemia for *Trypanosoma cruzi* across a longitudinal cohort

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BACKGROUND The PCR diagnosis of *Trypanosoma cruzi*, the causative agent of Chagas disease, in known chagasic patients is 40-60% efficient and parasite isolation is far lower. PCR technology in *T. cruzi* diagnosis is advanced including qPCR, dip-stick technology and numerous methodological improvements. To investigate a rationale for variations in PCR positivity in human Chagas disease during a longitudinal study we reassessed the assumption of a constant level of *T. cruzi* parasitaemia throughout infection by considering the alternative hypothesis of periodic oscillations.
METHODS We examined a cohort of chagasic patients over 1 to 2 years using longitudinal qPCR comprising 5 persistently positive patients, 11 persistently negative samples and 22 patients with both positive and negative results across the study. We observed a single periodic oscillation in *T. cruzi* parasitaemia derived using qPCR across the combined cohort analysed using two independent statistical analyses, which, to our knowledge, is the first application to model parasitaemia since germ theory.
RESULTS We demonstrate the robustness of the methodology by modelling periodic oscillations in *T. brucei* parasitaemia in two patient infections half a century apart and observed a single oscillation of 7 days in the combined data set. Interestingly, the *T. brucei* data harboured a longterm, but non-significant period. The periodic oscillation in *T. cruzi* showed a highly statistically significant single longterm oscillation. Moreover, the entire data set of persistent positive and negative patients could be modelled using two independent approaches resulting again in same longterm periodic oscillation. We conclude periodicity of parasitaemia is compatible with a 40-60% threshold of PCR positivity in *T. cruzi* diagnosis.
CONCLUSION We hypothesise the longterm periodic oscillations of *T. cruzi* result from the (intracellular) amastigote stage causing clinical persistence of infection and not blood-borne trypomastigotes, allows the complete clearance of the parasitaemia without threatening the parasites survival, but the parasitaemia periodically re-establishes to facilitate transmission. In contrast, in*T. brucei* the persistence of the parasitaemia is central to the survival of the host infection, resulting in strong selection pressure for short-term periodic oscillations. Periodic oscillations in *T. cruzi* parasitaemia infer PCR diagnostic efficiency of chagasic patients would be increased by longitudinal sampling.

BERENICE (BEnznidazol and triazol REsearch group for Nanomedicine and Innovation on Chagas diseasE). A new treatment for Chagas disease

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BACKGROUND: Chagas disease is an important health problem in Latin America and a challenge in non endemic countries. Its prevalence is estimated between 8 and 10,000,000 people infected and approximately 14,000 deaths/year, representing the second highest burden of disease among Tropical Diseases in the Americas. Although Chagas disease has been identified and described for more than 100 years, the therapeutic alternatives are limited: benznidazole and nifurtimox are the only 2 drugs available for treatment. Both benznidazole and nifurtimox have frequent side effects, especially in adults, requiring discontinuation in up to 10% of patients. The aim of BERENICE consortium is to obtain a more effective, better tolerated and cheaper formulation of a drug with trypanocidal activity to cure Chagas disease.
METHODS: The encapsulation of benznidazole using nanotechnology will generate a new drug delivery system. This new approach will allow a release of medication directly into the intracellular space, therefore increasing tissue drug concentration and avoiding side effects. A better toxic profile will be obtained because of the reduced amount of benznidazole used. BERENICE Consortium is created to carry out this task as a European research network (Collaborative Project) coordinated by Vall d' Hebron University Hospital.
RESULTS: BERENICE brings together 8 European and Latin American partners. Starting in September 2012, this 5-year project is supported by the European Commission under the Health Innovation Work Programme of the 7th Framework Programme.
CONCLUSIONS: BERENICE project will develop a low-cost intervention with an important cost effective impact that can be implemented during the project period and thereby have an immediate effect on the control of Chagas diseases in endemic and non endemic countries.

Dissecting the roles of selected PI3K genes in *Haemonchus contortus* using integrative molecular tools

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BACKGROUND: Phosphoinositide 3-kinases (PI3Ks) are important intracellular lipid kinases that phosphorylate the 3'-OH of phosphatidylinositol and phosphoinositide. The second messengers generated by this reaction leads to the activation of many signalling pathways that regulate a variety of biological responses. In *Caenorhabditis elegans*, the heterodimeric PI3K consists of a catalytic subunit AGE-1 and a regulatory subunit AAP-1, which is a key component of the insulin-like signaling pathway, which plays a key role in dauer regulation. The "dauer hypothesis" have proposed that similar molecular mechanisms regulate the resumption of the third-stage larvae (iL3) in parasitic nematodes and the recovery from dauer in *C. elegans*. However, nothing is known about PI3Ks in parasitic nematodes during the transition from free-living to parasitic stages.
METHODS: Using PCR-based approaches, transgenesis in *C. elegans* and yeast two-hybrid methods, we characterized *age-1* and *aap-1* homologous gene (*Hc-age-1* and *Hc-aap-1*) from *Haemonchus contortus*, a socio-economically important parasitic nematode of ruminants.
RESULTS: *Hc-age-1* and *Hc-aap-1* contain conserved structural domains. Both genes are transcribed in all life stages, with higher levels in the eggs, iL3s and adult females. Their promoter regions drove GFP expression predominantly in the gut or neurons of *C. elegans*. Yeast two-hybrid experiments showed that the p85 binding domain of *Hc*-AGE-1 interacts strongly with the *Hc*-AAP-1 in yeast cells. However, *Hc-age-1* did not rescue the function of *Ce-age-1* in *age-1*-deficient *C. elegans* (CY246).
CONCLUSIONS: Taken together, these results provide some support for the "dauer hypothesis" and insights into the role of PI3Ks in *H. contortus*.

Optimizing the inhibition of a uniquely composed *Trypanosoma brucei* F_1-ATPase

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BACKGROUND: Two different environments encountered by *Trypanosoma brucei* in its insect and vertebrate hosts are reflected by substantial changes in bioenergetic pathways. Oxidative phosphorylation is restricted to the procyclic form (PS) as glycolysis of virtually limitless glucose is sufficient to produce ATP in the bloodstream form (BS). Thus, in the absence of proton-pumping respiratory complexes the mitochondrial membrane potential is uniquely maintained by the hydrolytic activity of the essential F_oF_1-ATPase. We study the parasite-specific features of the F_1-ATPase and its natural protein inhibitor ($TbIF_1$), which is expressed in PS and its ectopic expression is lethal in BS.
METHODS: The F_1-ATPase from PS was purified by two-step chromatography and subunits were characterized by LC/MS. RNAi cell line was generated to address function of unique F1-ATPase subunit. Recombinant TbIF1 variants were used to study the inhibition of the F_1-ATPase.
RESULTS: The purified F_1-ATPase contains besides the conserved eukaryotic components an additional protein, p18. Together with the α subunit cleavage, occurring presumably between the crown and NTP-binding domains, this represents unique features of the F_1-ATPase in Kinetoplastids. We knocked down the p18 to analyse its function within the F1-ATPase. We defined the regions of TbIF1, which are crucial for the inhibition of the F_1-ATPase and described the link between TbIF1 inhibitory capacity, oligomerization, and pH sensitivity. While the C-terminal deletion of TbIF1 prevents homodimerization, it doesn't disrupt the pH sensitivity as it does in bovine IF_1. Importantly, $TbIF_1$ can't inhibit bovine F_1-ATPase, strengthening the differences between the parasite and mammals.
CONCLUSIONS: The F_1-ATPase purification is suitable for structure resolution by X-ray crystallography. Given the enzyme's extraordinary composition, its non-conventional function in BS, and the F_1-$TbIF_1$ binding data, we propose that the structure could be exploited to design specific inhibitors for potential use in chemotherapeutics.

Intestinal parasites and environment in a settlement on the coast of the “Rio de La Plata”

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BACKGROUND: The settlement "El Molino" is on the Coast of La Plata River, in Ensenada district, Buenos Aires province, Argentina. The coastal environment, population precarized with inadequate sanitary behaviors (overcrowding and promiscuity with animal excreta in the open, high density of dogs and rodents, human feces coprophagy in dogs, feeding fish and frogs) favor the presence of zoonotic diseases. The team is composed by members of a University Volunteer project composed by teachers and students of 4 schools of the National University of La Plata. The objective was contributing to the prevention, diagnosis and health improvement of that community. The aim was to diagnose intestinal parasitoses in the settlement and to analyze its relationship with lifestyle of families.
METHODS: Fecal samples and anal swabs from 386 people from 0-80 years were collected. Fecal samples were processed by techniques Telemann and Sheather and swabs were centrifuged at 400 g.
RESULTS: Of the 497 fecal samples tested, 316 (63.6%) were positive, in order:
Blastocystis sp. (32,3%), *Enterobius vermicularis* (28,8%), *Giardia intestinalis* (19%), *Entamoeba coli* (8,7%), *Ascaris lumbricoides* (5,9%), *Endolimax nana* (5,7%), *Hymenolepis nana* (5,1%), *Enteromonas hominis* (2,6%), *Trichuris trichiura* (2%), Uncinarias (1,6%), *Iodamoeba butschlii* (0,6%), *Dientamoeba fragilis* (0,8%), *Strongyloides stercoralis* (0,4%), *Taenia* sp. (0,2%). Male and children of 5-9 years were the most parasitized. Parasitized families inhabited houses of metal and wood with dirt floors, defecated in latrines, consumed unsafe water, lived with animals and waste thrown open sky. Geohelminths were more closely associated with those conditions.
CONCLUSIONS: The results confirm that the neighborhood "El Molino" has eco-epidemiological and cultural characteristics risky to human health.

Prevalence of *Blastocytis* spp. among patients referred to Eskisehir Osmangazi University Hospital during the 2010-2014 years

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BACKGROUND: *Blastocystis spp*. are ubiquitous parasites with a worldwide distribution. These pleomorphic intestinal parasites are transmitted by fecal-oral route and are reported to be associated with many health problems. We determined of in this retrospective study; the prevalence of *Blastocystis* species among different patient groups attended to our hospital for the last four years.
METHODS: A total 5946 stool specimens were referred to Eskisehir Osmangazi University Department of Parasitology laboratory in a period 2010-2014 years. Diarrheal specimens were examined microscopically using normal saline and iodine wet mount preparation. Soft or formed species were concentrated by ethyl formalin method. Microscopic examination was identified of different forms of the parasite; vacuolar, cyst, granular and amoeboid seen under 40X magnification were reported.
RESULTS: A total of 5946 stool specimens referred to parasitology lab from different clinics for testing. 619(10.4%) were positive for parasites, of all these 274(4.6%) were positive for *Blastocystis spp*. In 31 cases were detected *Blastocystis spp*. along with other parasites. *Blastocystis* positive specimens were mainly from pediatrics, internal medicine and dermatology clinics. There were no differences between the sexes, but in recent years is determined a significant increase in *Blastocytis* cases. Clinical recorts revealed that most of these patients suffer from general abdominal symptoms, diarhea and a variety of allergic symptoms.
CONCLUSIONS: According to our results is a significant increase in *Blastocystis* spp. cases in recent years. The establishment of such data will be beneficial for the public health authorities in the planning and implementation of specific prevention and control strategies of this infection.

Cultural barriers to effective communication between Indigenous communities and health care providers in Northern Argentina: an anthropological contribution to Chagas disease prevention and control

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BACKGROUND: Ninety percent of the aboriginal communities of Argentina are located in areas of endemic vectorial transmission of Chagas disease. The goal of this research was to explore the role played by beliefs, habits, and practices of Pilaga and Wichi indigenous communities in their interaction with the local health system in the province of Formosa. This article contributes to the understanding of the cultural barriers that affect the communication process between indigenous peoples and their health care providers.
METHODS: Twenty-nine open ended interviews were carried out with members of four indigenous communities located in central Formosa. We described and compared these communities' approach to health and disease as they pertain to Chagas as well as their perceptions of Western medicine and its incarnation in local health practice.
RESULTS: Five key findings are presented: 1) members of these communities tend to see disease as caused by otherpeople or by the person's violation of taboos instead of as a biological process; 2) while the Pilaga are more inclined to accept Western medicine, the Wichi often favour the indigenous approach to health care over the Western approach; 3) members of these communities do not associate the vector with the transmission of the disease and they have little awareness of the need for vector control activities; 4) indigenous individuals who undergo diagnostic tests and accept treatment often do so without full information and knowledge; 5) the clinical encounter is rife with conflict between the expectations of health care providers and those of members of these communities.
CONCLUSIONS: Our analysis suggests that there is a need to consider the role of the cultural patterning of health and disease when developing interventions to prevent Chagas disease among indigenous communities in Northern Argentina. This is especially important when communicating with these communities about prevention and control.

Diversity of endoparasites in bats from preserved, rural and urban areas in the state of Espirito Santo, southeastern Brazil.

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BACKGROUND: The Chiroptera belong to the mammals class and is considering the second largest order in number of species. In Brazil, about 170 species have been described, of which 90 are found in southeastern region. Although they can act as a host for helminths and protozoa parasites, their endoparasites diversity is still little known. The aim of this study was to compare the diversity of endoparasites in bats captured in conservation, urban and rural areas in the state of Espírito Santo, southeastern Brazil.
METHODS: In this work, 254 bats were captured from 39 urban, rural and preserved areas in the Atlantic Forest, which is among the 34 global hotspots of endoparasites. Helminths were harvested in the digestive tract and fixed in 10% hot formalin. Trypanosomatids were collected by cardiac puncture and cultivated in NNN culture medium.
RESULTS: Out of 254 bats evaluated, grouped in 29 genera and 53 species, 75 specimens (29.52%) were infected with trypanosomatids, nematodes, trematodes or cestodes. Nematodes, cestodes, trematodes and trypanosomatids were found in 64, 10, 9 and 4 animals, respectively. Among the identified parasites were *Tricholeiperia* sp., *Litomosoides* sp., *Pterygodermatites* sp., *Stilestrongylus* sp. and *Biacantha* sp. Ecological indices indicated that preserved regions have higher richness of parasitized hosts. *Trachops cirrhosus* showed a higher diversity of parasites among the hosts.
CONCLUSIONS: The preserved areas, which suffer less human interference, represent more stable environments with higher diversity of microclimatic conditions, habitats and hosts, promoting the heterogeneity of parasites. The higher amount of parasites in *T. cirrhosus* appears to be associated with their habitat and feeding habits. This study has contributed significantly to the knowledge of the biodiversity of parasites in wild animals in the state of Espírito Santo, southeastern Brazil.

Toxoplasma gondii contamination of imported horse meat

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BACKGROUND: *Toxoplasma gondii* can be classified as an environmental born parasite where the food, the soil, the water or the cat spreading oocysts can transmit at various rate the infective form of the parasite according to the geographic area. In order to perform risk analysis, it is necessary to define the level of the contamination for each "compartment": food, water, soil, and cat population. Several fatal human cases have already been described in France, incriminating the imported horsemeat of South America. Therefore a wide study was conducted to evaluate the prevalence of *T.gondii* in freeze horsemeat imported in France thru the Roissy, CDG inspection-point.
METHODS: Muscle tissues (50g) from 247 horse carcasses, were collected, during May to December 2012. Tissue fluids were tested serologically by the Modified Agglutination Method (MAT) following thawing. Since the carcasses were frozen, the detection of parasites was performed by quantitative PCR, following a trypsin digestion.
RESULTS: Most of the tested samples were originating from Mexico (222/247), having as a final destination the Switzerland (126/247). The overall estimate of *Toxoplasma gondii* seroprevalence is 34%. Only two carcasses show positive qPCR amplification. No genotyping was possible since the DNA quantity was low (39.8 CT)
CONCLUSIONS: These results confirm the potential risk associated with the consumption of horse meat imported into France, especially due to the national habit of eating raw horse meat.

Identification and characterization of energy-conserving acetyl-CoA synthetase in the hydrogenosomes of *Trichomonas vaginalis*.

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BACKGROUND: *Trichomonas vaginalis*, a protozoan parasite that causes trichomoniasis, is the most common non-viral sexually transmitted pathogen. Every year, trichomoniasis affects more than 280 million people worldwide. *T. vaginalis* lacks typical mitochondria and instead possesses mitochondrion-related organelles, the hydrogenosomes, which produce hydrogen and ATP. So far, the only ATP-synthesizing enzyme recognized in hydrogenosomes was a two-subunit succinyl-CoA synthetase similar to the Krebs cycle enzyme. The annotation of *T. vaginalis* genome revealed the presence of a putative fatty acyl-CoA synthetase, similar to one-subunit acetyl-CoA synthetases (ADP forming) of diplomonads *Giardia* and *Spironucleus* and various bacteria. Analysis of hydrogenosomal proteome identified this putative acetyl-CoA synthetase in the organelle, consistently with the presence of a hydrogenosomal targeting signal on the amino-terminus of the predicted protein.
METHODS: Putative *T. vaginalis* acetyl-CoA synthetase (ADP forming) has been overproduced in *E. coli*, purified using affinity chromatography and characterized. It was also episomally expressed in *T. vaginalis* to verify its subcellular localization.
RESULTS: We have confirmed the hydrogenosomal localization of *T. vaginalis* acetyl-CoA synthetase (ADP forming). Activity studies indicate that this protein indeed functions as a thiokinase and forms ATP using acetyl-CoA, Pi and ADP.
CONCLUSIONS: We have identified another ATP-forming enzyme in *T. vaginalis* hydrogenosomes. In contrast to long-known succinyl-CoA synthetase, it utilizes directly acetyl-CoA as a substrate. This finding substantially extends the knowledge of energy metabolism of trichomonad hydrogenosomes.

Advances in Leishmaniasis diagnosis. Cutting out cross reactions.

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Background: Leishmaniasis are considered emerging diseases, due to, among other factors, climatic changes and migrations. Clinical diagnosis is complicated, different antigenic fractions of parasites have been studied, but no one has reached the goal. The use of a purified Iron Superoxide dismutase secreted (Fe-SODe) by *Leishmania* sp. as a specific antigen has been demonstrated with good results. Fe-SOD activity represents an important virulence factor, which allows the parasites to proliferate in the host. The aim of this study is to demonstrate the absence of cross-reactivity between different *Leishmania sp.* and other trypanosomatids.
Methods: we tested 113 sera from Peru from two groups of leishmaniasis patients: 45 sera with mucocutaneous Sylvatic Leishmaniasis (SL) and 68 sera with Andean Cutaneous Leishmaniasis (ACL), and 10 sera from individuals diagnosed for Chagas' disease from a no-endemic area in ELISA technique using the purified Fe-SODe by four *Leishmania* sp. as antigens and we have compared these results with the results obtained using the crude protein preparation.
Results: 105 sera were positive to *L. peruviana* purified Fe-SODe (Fe-SODe-Lp) while only 70 sera were positive to *L. braziliensis* purified Fe-SODe (Fe-SODe-Lb). Out of 68 patients with ACL 26 sera were positive to Fe-SODe-Lb and 67 sera were positive to Fe-SODe-Lp. Testing the 45 sera from patients with SL, 21 were positive to Fe-SODe-Lp and 44 to Fe-SODe-Lb. When we tested 113 sera with the purified Fe-SODe of *L. amazonensis* (Fe-SODe-La) and *L. infantum* (Fe-SODe-Li), we observed 35 and 7 positive sera, respectively. Out of 10 sera from Chile none was positive.
Conclusion: We confirmed that there is no cross-reactivity between different species of *Leishmania* as well with other trypanosomatid family members using purified Fe-SOD secreted by *Leishmania* spp., indicating that secreted Fe-SOD is highly specific and sensitive antigen that could be successfully used in diagnosis of leishmaniasis.

γδ T cell-involved protective immunity against *Plasmodium berghei* XAT

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BACKGROUND: It has been shown that γδ T cells play important roles in immune responses against various infectious diseases caused by protozoan parasites, bacteria, and viruses. Our previous report showed that γδ T cells are essential for protection against blood-stage *Plasmodium berghei* XAT infection. However, mechanisms of the γδT cell-involved protective immunity are largely unknown.

METHODS: We infected γδ T cell-deficient (TCR-δ KO) mice and control wild-type (WT) mice with *Plasmodium berghei* XAT, which is a non-lethal strain, by intravenous inoculation of 10^4 infected red blood cells (iRBCs). To examine whether activation of dendritic cells and $CD4^+$ T cells are altered by deficiency of γδ T cells, we carried out flow cytometric analysis

RESULTS: TCR-δ KO mice could not control *P. berghei* XAT parasites. We showed that IFN-γ production in splenic $CD4^+$ T cells are significantly reduced in TCR-δ KO mice on day 7 to 9 after infection than that in WT mice. Next, we compared the activation states of dendritic cells (DCs) in WT mice and TCR-δ KO mice. The number of DCs and expression levels of their activation indicators, such as CD40, CD80, CD86 and MHC-II, in spleen were transiently increased on day 5 after infection. Expression levels of the activation indicators were reduced in *P. berghei* XAT-infected TCR-δ KO mice, as compared with WT mice. Upregulation of IFN-γ and CD40L expression in γδT cells was observed on day 5 after infection that was earlier than that in splenic $CD4^+$ T cells.

CONCLUSIONS: Our results suggest that γδ T cells would directly activate DCs through IFN-γ and CD40L-CD40 signaling after *P. berghei* XAT infection, resulting in enhancement of Th1 differentiation and clearance of the parasites.

In vivo analysis of the control of *Entamoeba histolytica* glycolysis points out to the first reactions as the main controlling steps

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BACKGROUND: Entamoeba histolytica lacks functional mitochondria and have neither Krebs cycle, nor oxidative phosphorylation enzyme activities, thus glycolysis is the main pathway of ATP production for cellular work. We here identified the main enzymes/processes that control the pathway flux in live amoebal trophozoites by applying the elasticity analysis, an experimental approach of the Metabolic Control Analysis Theory.

METHODS: The glycolytic metabolites were gradually titrated in amoebal trophozoites by feeding glycolysis with different glucose concentrations and by adding variable amounts of inhibitors of the last pathway reactions; in parallel, the ethanol flux was determined at each metabolite variation. The elasticity coefficients, for individual or group of enzymes around a common pathway intermediary, were determined from plots of metabolite concentration versus ethanol flux. With these coefficients the degree of control on the glycolytic flux of different pathway reactions was determined.

RESULTS: The results indicated that the glucose transport/hexokinase/glycogen degradation are the main controlling steps (72%-78%) followed by the bifunctional aldehyde-alcohol dehydrogenase (ADHE, 18%). Further, when the first pathway steps were inhibited with 2-deoxyglucose (2-DOG), the pathway flux decreased to 30% whereas ATP and cell viability decreased to 70%, in comparison tocontrol amebas. Moreover, 2-DOG in combination with disulfiram, an ADHE inhibitor, decreased viability and ATP content at 50% and 45%, respectively. Amino acids did not prevent cell death indicating that glucose is the main nutrient for amoebal ATP production and survival.

CONCLUSIONS: The results indicated that amebal glycolysis is mainly controlled by the first pathway reactions and that their inhibition promotes ATP decrease and cell death.

Unprecedentedly reduced respiratory chain and minimalistic genome in the mitochondrion of *Chromera velia* - a photosynthetic alveolate closely related to apicomplexan parasites

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BACKGROUND: *Chromera velia* is the closest known photosynthetic relative of Apicomplexa, which mt genome contains only 3 protein-coding genes (cox1, cox3, cyb).
METHODS: *C. velia* mitochondrial genome was sequenced using a combination of single 454 reads, paired-end and mate-pair Illumina reads, and assembled using Newbler. Mt contigs were identified using the apicomplexan mt proteins and rRNA fragments. Measurement and lipid identification was achieved by means of HPLC MS.
RESULTS: Mitochondrial genome of *C. velia* carries two protein-coding genes (cox1 and cox3). Consequently, using genomic BLAST we identified highly reduced respiratory chain (RC) of *C. velia* lacking respiratory complexes I and III. The novel combination of L- and D-lactate:cytochrome c oxidoreductases is capable of transferring electrons from lactate to cytochrome c, thus bypassing the missing complex III in *C. velia*.
CONCLUSIONS: Absence of complex III is likely driven by the need to lower reactive oxygen species (ROS) production. *Chromera* contains high amount of storage fatty acid (FA). They are used to generate energy in mitochondrion by β-oxidation known to produce ROS. However, ROS are produced also by RC itself; complex III is the biggest producer of ROS, followed by complex I. We assume that elimination of complex III from RC of *Chromera* helped to reduce ROS production in mitochondrion energetically dependent on the decomposition of FA. Alternatively, reduced RC allows the utilization of FA in *C. velia*. This strategy is further enhanced by production of benzenepropanoic acid 3,5-bis(1,1-dimethylethyl)-4-hydroxy methyl ester, a powerful antioxidant protecting stored FA against oxidation.
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Quantitative metabolic analysis of the *Trypanosoma cruzi* peroxide detoxification pathway identifies tryparedoxin as a suitable drug target

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BACKGROUND: The *Trypanosoma cruzi* tryparedoxin-dependent peroxide detoxification pathway is the principal system to contend oxidative stress. The participating enzymes, trypanothione reductase (TryR), tryparedoxin (TXN), tryparedoxin peroxidase (TXNPx) and tryparedoxin-dependent glutathione peroxidase A (GPxA) have been widely studied in *T. cruzi* and other trypanosomatids, each of them have been proposed as drug targets. However, to rationally prioritize the most adequate drug target(s) within the pathway, Metabolic Control Analysis (MCA) was applied to identify the enzymes that mainly control its flux.
METHODS: An *in vitro* reconstituted pathway was set up with the recombinant enzymes which had similar properties regarding enzyme activities and metabolite concentrations found in the parasite. Individual titrations of the pathway enzymes or substrates were performed and the changes in the flux of peroxide reduction were in parallel monitored. From the plot of pathway flux *versus* enzyme activity, the degree of control of each enzyme was determined.
RESULTS: The TXN-TXNPx and TXN-GPxA redox pairs controlled by 90-100% the pathway flux, whereas TryR controlled by ≈10%. It was determined that the NADPH supply may contribute to control the pathway flux due to limitation, whereas for $T(SH)_2$ or H_2O_2, the pathway had the capacity to respond at its full rate at low concentrations of these metabolites.
CONCLUSIONS: The kinetic analyses indicated that TXN is the less efficient enzyme in the pathway, which may be the reason for its high control on the pathway flux. This result in addition to its role as provider of reducing equivalents to the two main peroxidases in the parasite, identifies TXN as a convenient drug target.

Different phenotypic susceptibility between *Haemonchus contortus* Isolates suggests resistance to plant secondary compounds

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BACKGROUND: Evidence of different phenotypic susceptibilities to tannin rich plant (TRP) extracts has been found in *H. contortus* isolates from temperate and tropical regions. The larval exsheathment test suggested that *H. contortus* isolates from Yucatan, Mexico, show less susceptibility to TRP extracts compared to other isolates. This study determined the susceptibility to a TRP extract on the egg eclosion of *H. contortus* isolates from different regions.
METHODS: Egg Hatch Inhibition Assay was used to determine the susceptibility to the acetonic extract of *Acacia pennatula*. Fresh eggs from 6 isolates of *H. contortus* were tested: FES-C & CENID (temperate, Mexico), PARAISO & FMVZ (Tropical, Mexico), France1 (south of France) and CAVR (southeast Australia). Eggs were incubated at 28°C during 48 h at eight concentrations (from 150 to 6000 µg/ml PBS) to determine the effective concentration 50 (EC_{50}) for each isolate. The EC_{50} value of the most susceptible isolate was used as reference to calculate Resistant Ratio (RR).
RESULTS: France1 (EC_{50} 2971.6 µg) and FESC (EC_{50} 3539.9 µg, RR 1.2) were recorded as susceptible and CAVR as moderate resistant (EC_{50} 5456.5 µg, RR 1.8). PARAISO and CENID were both recorded as resistant (EC_{50} (7714.2 µg, RR 2.6 and EC_{50} 9225.7 µg, RR 3, respectively). The FMVZ had the highest EC_{50} (15215.7 µg, RR 5.1).
CONCLUSIONS: The results of the FMVZ isolate suggest an adaptive phenomenon to plant secondary compounds (PSC) in *H. contortus*. The FMVZ isolate was obtained from goats browsing tannin rich plants in the tropical forest. The continuous ingestion of these plants might favor resistance to PSC.

Evaluation of biosolid composting from a wastewater plant from Colombia and its effect en the elimination of protozoan and helminth parasites

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BACKGROUND: Biosolids are organic material produced during wastewater treatment and could be employed as soil amendment. However, because of the high concentration of pathogens, their use without a prior stabilization process is considered as a health risk. Composting is a suitable way of biosolids recycling that can deactivate pathogens. Helminth and protozoan are the best indicators of this effect because of their high resistance to environmental stress.
METHODS: Three composting process were done during 34 days. Duplicate piles were prepared with wood sawdust. Biosolids were mixed with sawdust at two ratios (50:50 and 75:25 sludge:sawdust). A control pile was also included in the study with a 100% composition of biosolids. Chemical, physical and microbiological tests were accomplished following the protocol described by the Colombian technical guideline 5167. The identification and viability of protozoan and helminths was performed according to ROBERTSON et al. (2000) and the Mexican official Norm 004 with some modifications.
RESULTS: Biosolid had viable *Ascaris* spp. eggs, *Giardia* spp. cysts and Enterobacteria at the beginning of composting process. In the second week of composting, we found a gradual decrease of pathogens in all piles. At the end of the study we only detected two viable *Ascaris* spp. eggs in the control pile and in the 75:25 ratio pile; while in the 50:50 pile there were 0,6 *Ascaris* spp. viable eggs. *Giardia* spp. cysts were eliminated in the 50:50 pile.
CONCLUSIONS: Our results suggest that composting (50:50 ratio) allows the elimination of pathogens in biosolids and generates a high quality compost that can be used as soil fertilizer.

In vitro susceptibility to tannin rich extracts differs amongst _Haemonchus contortus_ isolates from tropical and temperate regions.

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BACKGROUND: The *in vitro* anthelmintic (AH) effect of tannin containing extracts on some biological processes seems less evident in tropical *Haemonchus contortus* isolates compared to temperate isolates. This study compared the sensitivity of *H. contortus* isolates originated from tropical or temperate regions when exposed to plant and by-product tannin rich (TR) extracts.
METHODS: Three Mexican *H. contortus* isolates (CENID-INIFAP, FESC-UNAM, Poxila-UADY), and three French isolates (INRA, FARM1, FARM2) were used to perform respective larval exsheathment inhibition assays (LEIA). Three water-acetone extracts from leafs (*Lysiloma latisiliquum*, *Havardia albicans* and *Acacia pennatula*) and three extracts from agroindustrial by-products (*Theobroma cacao* seeds and husk, *Coffea arabica*, *Juglans regia*, *Carya illinoinensis* and methanolic extracts of *Corylus avellana* (UAE3 and M1) were used. Increasing concentrations (0, 150, 300, 600 and 1200 µg/ml) were used for each bioassay to estimate lethal concentration 50% (LC50) and resistance ratio (RR).
RESULTS: The most sensitive *H. contortus* isolates were FARM1 and FARM2 (inhibited exsheathment at the lowest concentrations). The Yucatán's Poxila-UADY isolate was the most resistant isolate (higher RR). The *C. avellana* extracts affected all isolates at the lowest dose irrespective of origin. Based on PVPP results, extracts were categorized into: (i) effect is related to tannins, (ii) effect was partially due to tannins, (iii) effect was not due to tannins.
CONCLUSIONS: Clear differences in the sensitivity against the tannin rich extracts were evident between different *H. contortus* isolates. The Poxila-UADY isolate was resistant to most extracts tested.

The whipworm genome and dual-species transcriptomics of an intimate host-pathogen interaction

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BACKGROUND: Whipworms are common soil-transmitted helminths that cause debilitating chronic infections in man. These nematodes are distantly related to *Caenorhabditis elegans* and have evolved to occupy an unusual biological niche, tunneling through epithelial cells of the large intestine. Here we present the genome sequences of the human-infective *Trichuris trichiura* and the murine laboratory model *T. muris*.
METHODS: We identified the sex chromosomes and find high-level synteny both between these species and in comparison to the related parasite *Trichinella spiralis*.
RESULTS: Whole transcriptome analyses in *T. muris* based on RNAseq detect many genes and protein families that are expressed in a gender-, life cycle stage-, or morphological region-specific manner, such as WAP domain-containing and DNase II-like proteins. In particular, they characterise the transcriptional landscape of a morphological region with unique biological adaptations, namely bacillary band and stichosome, found only in whipworms and related parasites. To investigate the host response to infection, we generated RNAseq data from chronically whipworm-infected mouse caecum and used this to describe a caecum-wide regulated Th1-like immune response in unprecedented detail. We further used these data to explore the similarities between chronic whipworm infection and immune-related diseases such as ulcerative colitis.
CONCLUSIONS: Together, these genomes and associated functional data elucidate key aspects of whipworms and their molecular host-parasite interactions and provide a solid foundation for future studies, facilitating the development of novel anthelmintic interventions and the exploitation of unique whipworm biology.

Helminths Associated with *Sterna* spp. (Aves: Laridae) of the Atlantic Coastal Region in Southern Brazil

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BACKGROUND: *Sterna* belong to the family Laridae and has about 30 species. Due to the wealth of the Brazilian fauna, for many species no studies have been conducted regarding the parasitic fauna. Moreover, the species to be studied are migratory, and therefore the knowledge of the parasitic fauna associated with them and thus disseminated along its route is of the utmost importance, parasites with zoonotic potential can be transported by long distances facilitating the spread of disease between countries, eliminating geographical barriers and can be transmitted to other birds and get to the human being.
METHODS: So far were analyzed 25 birds belonging to the genus *Sterna*, captured with permission of the Ministry of Environment. The capture occurred on Cassino beach, municipality of Rio Grande, state of Rio Grande do Sul, Brazil. After capture, the birds were sedated and euthanized following the rules of the Federal Board of Veterinary Medicine. After necropsy and analysis of all organs was performed.
RESULTS: Helminths were found in 92% of the birds, phylum Nematoda was present in 80%, represented by the families Capillariidae (64%), Anisakidae (12%) and Acuariidae (12%). The class Trematoda in 84% with representatives of the families Renicolidae (52%), Echinostomatidae (64%), Stomylotrematidae (8%) and Diplostomidae (4%). The phylum Acanthocephala was present in 36% of birds, represented by the families Pomphorhynchidae (12%), Polymorphidae (12%) and Neoechinorhynchidae (4%). Specimens belonging to the class Cestoda were also found in 56% of birds.
CONCLUSIONS: Although these are preliminary results of the study, we can observe the presence of a variety of helminths associated with *Sterna* spp. in southern Brazil, being registered for the first time in these birds the families Polymorphidae and Neoechinorhynchidae (Acanthocephala), Capillariidae and Anisakidae (Nematoda) and the family Stomylotrematidae belonging to class Trematoda.

Effect of tannin containing extracts on the hatching process of *Haemonchus contortus* eggs under *in vitro* conditions

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BACKGROUND: Tannin containing extracts impair the egg hatching process but the mechanism involved in this anthelmintic (AH) effect is still unknown. The effect of different plant and by-products acetonic extracts on the development of larvae within the eggs and the eclosion (L1 emergence) was evaluated under *in vitro* conditions.
METHODS: Respective egg hatch assays were used to determine the AH effect of tannin containing extracts on *Haemonchus contortus* eggs. Water-acetone extracts from *Lysiloma latisiliquum*, *Laguncularia racemosa*, *Rizophora mangle*, *Avicennia germinans* and by-products of *Theobroma cacao* seed and husk, and *Coffea arabica* were obtained. Fresh *H. contortus* eggs were incubated with increasing concentrations of each extract (0, 600, 1200, 2400 and 3600 µg/ml PBS). A general linear model was used to determine the dose effect of each extract.
RESULTS: The *C. arabica* extract did not show any AH effect. The *T. cacao*, seed and husk, extracts showed ovicidal effect from 600µg/ml to 2400 µg/ml. The other plant extracts did not show ovicidal activity, but blocked larvae eclosion in a dose-dependent manner ($P<0.05$). Extracts of *R. mangle* and *T. cacao* husk blocked eclosion at 1200 µg/ml or above. Meanwhile, extracts of *L. racemosa*, *A. germinans* and *T. cacao* seed affected eclosion at 2400 µg/ml and the *L. latisiliquum* extract at 3600 µg/ml.
CONCLUSIONS: Plant and by-product acetonic extracts affected egg hatching either by an ovicidal effect or by causing the failure of larvae to complete their eclosion process.

Peptidases involved in blood digestion in monogeneans of the family Diplozoidae

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BACKGROUND: Mechanisms of digestion have been reported for a few monogeneans, but these were based mainly on ultrastructural studies. Only little information exists about the biochemistry of digestion. Thus, the aim of our study was to identify and characterize peptidases that are responsible for processing of blood in selected species of diplozoid monogeneans.
METHODS: Peptidolytic activities were measured in excretory/secretory products and in protein extracts from adult *Paradiplozoon bliccae* and *Eudiplozoon nipponicum* using fluorogenic substrates and a set of specific inhibitors. For detection of cysteine peptidases in gels and on blots, a specific probe DCG-04 was applied, followed by mass spectrometry. Transcriptomic data from adult *E. nipponicum* were obtained by 454 sequencing. *E. nipponicum* cDNA coding for cysteine cathepsins was amplified by PCR using either specific or degenerate primers, the latter derived from conserved sequences of cysteine peptidases; 3´RACE-PCR was applied to obtain 3´ ends of genes where needed. Two recombinant cysteine peptidases (cathepsins L and B) were expressed in *Pichia pastoris* yeasts.
RESULTS: Cysteine peptidase activities predominated in all samples. DCG-04-labelled bands/spots after 1D/2D electrophoresis of worm protein extracts contained peptide sequences of cathepsin L of *E. nipponicum* identified in the transcriptome. Moreover, other 30 peptidase genes have been predicted in *E. nipponicum* transcriptome; 9 of them may be likely involved in digestion of host´s blood proteins. Recombinant cathepsins L and B are being biochemically and functionally characterized and localized by immunohistochemistry.
CONCLUSIONS: On the basis of our results, we assume that blood digestion in diplozoid monogeneans is based on cysteine peptidases; particularly cathepsin L seems to represent the major proteolytic enzyme. The exact involvement of other peptidases identified in the transcriptome requires further research.

Cryptosporidium occurrence in captive asymptomatic canaries (*Serinus canaria*) from Brazil

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BACKGROUND: Many studies have described the occurrence of *Cryptosporidium* species in birds, worldwide, but none has been conducted in a population of captive canaries. This paper aims to identify *Cryptosporidium* parasites in captive canaries from several Brazilian states by microscopy and PCR.
METHODS: Feces excreted by 394 birds were collected every 24 hours for seven consecutive days. The feces were concentrated by diethyl ether centrifugation method and used for microscopic analysis by Ziehl-Neelsen staining method. Positive samples were subjected to SSU rDNA nested-PCR and screened for *Cryptosporidium galli* and *Cryposporidium* avian genotype III DNA by a duplex real-time PCR. A nested-PCR screening was also performed on 70 negative samples for microscopy. DNA extraction was performed using the QIAamp DNA Stool Mini Kit (Qiagen). All the amplicons were purified and sequenced on both strands.
RESULTS: Eight samples were positive by microscopy, none of which was positive by nested-PCR whereas five were positive by real time PCR: *C. galli* in four birds and one sample as *Cryptosporidium* avian genotype III. The molecular screening on the microscopic negative samples showed six PCR-positive samples: three *C. galli*, two avian genotype I and one avian genotype V. The frequency of microscopic positive samples was 2,03% (8 in 384), whereas the prevalence increased up to 14,1% (11 in 78) by using molecular techniques.
CONCLUSIONS: These results suggest the importance and the need of molecular methods to obtain a more reliable diagnosis.

Emerging Zoonoses in Romania due to Climate Changes

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BACKGROUND: Dirofilariasis in humans is considered an emerging zoonosis, this is due to a recent increase of diagnosed and published cases in areas where this nematode used to be reported only in cats, dogs and foxes. Infection with another "exotic" nematode, *Gongylonema pulchrum (G.pulchrum)* may also be due to climate changes that favor the life cycle of intermediary hosts (cockroaches).
METHODS: We describe two human cases diagnosed in our parasitology laboratory as infection with *Dirofilaria repens (D.repens)* and *G.pulchrum* respectively. In both cases the diagnosis was only reached after parasitological examination of the nematodes extracted by surgery. The first patient was operated with a diagnosis of soft tissue benign tumor above the pectoral muscle. The second patient was hospitalized and investigated by endoscopy, which revealed a worm-like structure embedded in the esophageal mucosa, removed by the endoscopist.
RESULTS: The first sample was a white, translucent, viable, round structure, size: 12/0.1 cm, with a slightly bent posterior end, with a well developed, unsegmented muscle wall. Based on patient history (living in Southern Romania, close to the Danube river, working in disinfection of buildings) as well as macroscopic and microscopic examination, the structure was identified as being an immature adult of *D.repens*. Blood smears did not reveal the presence of microfilaria, and blood-count/blood smear did not demonstrate any eosinophilia. No parasitic treatment was prescribed and a 6 month follow-up did not demonstrate any recurrence.
The second sample was identified based on patient history (alcoholic with unhygienic ways of nutrition - cockroaches present in food, hepatocarcinoma and mycotic esophagitis), physical examination, macroscopic and microscopic examination of the nematode (*G.pulchrum*).
CONCLUSIONS: Due to climatic changes, the number of cases with so-called "exotic" parasites may increase in Romania, leading to the need of better awareness among doctors for optimal management of cases.

Genetic diversity of *Fasciola hepatica* isolated from three definitive hosts in Cajamarca's valley, Perú.

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BACKGROUND: Fasciolosis is highly prevalent disease in Cajamarca, Perú, affecting livestock and humans. It was apparently introduced in America by imported European cattle during colonization. This work deals with the molecular characterization of *F.hepatica* from different definitive hosts (DH) in order to understand the current population structure which could give new insights about the epidemiology of this parasitosis and its humans' transmission in this endemic region.
METHODS: DNA was extracted from 15 flukes obtained from cattle (5), sheep (5), and swine (5). ITS1, ITS2 rDNA, NADI and COI mtDNA were PCR-amplified, sequenced and then analyzed, aligned and compared. Haplotypes number, diversity (Hd), Fu's Fs neutrality test, fixation index (Fst) and gene flow (Nm) were also calculated.
RESULTS: We obtained a total of 1759 bp. No differences among sequences were observed for ITS1 (433 bp) and ITS2 (366 bp). We found high genetic diversity in mtDNA over all samples analyzed, evidenced by high number of haplotypes in both NADI (3) (559 bp) and COXI (6) (401 bp) markers, the genetic structure across the DH species was not significant ($Fst=0.03$; $p<0.05$) and Fu's statistic was significant for both markers ($Fs=-0.48$ and -1.33) suggesting that some historical mechanisms of selection (like purifying) is operating.
CONCLUSIONS: rDNA's ITS markers reinforced the species identity in Cajamarca and the analyzed mtDNA markers reveals many lineages but no structure. So, additional molecular markers will be addressed searching for relationships among genetic features and the DH that flukes belong to.

Occurrence of *Isospora* Schneider, 1881 (Protozoa: Apicomplexa) in a population of asymptomatic captive canaries, *Serinus canaria* Linnaeus (Passeriformes: Fringillidae) from Brazil

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BACKGROUND: *Isospora* are widespread in wild passerine birds, but few data are available for captive canaries. This work focuses on the occurrence of *Isospora* spp. in a population of asymptomatic captive canaries from several Brazilian states.
METHODS: The feces excreted by 394 birds were collected every 24 hours for seven consecutive days and were processed according to the Sheather method. For the molecular analysis, 32 samples were selected. DNA extraction was performed using the QIAmp DNA Stool Mini Kit (Qiagen). The SSU rDNA gene was amplified by nested PCR using new primers. PCR reactions were checked by electrophoresis and positive sample purified by QIA Quick PCR purification kit (Qiagen), and sequenced on both strands.
RESULTS: Of the 394 samples analyzed by microscopy, 176 (44,6%) were positive for *Isospora* spp. Among the 32 microscopically positive samples, 26 (81.2%) tested positive by PCR and sequencing revealed that 23 had 100% homology with deposited sequences of *Isospora* sp (JX984669, JX984668) and *Atoxoplasma* (AY331571, AY331569), whereas the remaining three samples showed novel sequences having 99% homology with the sequences reported above.
CONCLUSION: The occurrence of *Isospora* in captive canaries is higher than expected and the appearance of new sequences shows the need for further studies to clarify the molecular epidemiology.

Parasite load in chronic Chagas disease patients treated with Nifurtimox. Preliminary results

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BACKGROUND: Up to day, the parasitological methods to evaluate the therapeutic effect of drugs in the chronic Chagas disease (CChD) are qualitative. The objective of this study was to determine by Real-time PCR (qPCR) the parasite load of *T. cruzi* in Chilean individuals treated with Nifurtimox (NFX).
METHODS: 86 persons with CChD, proceeding of rural (51.2%) and urban (48.8%) endemic areas of Chile (13 man and 76 female with age average 44,9 years), were treated under Inform Consent with NFX according the currents protocols. qPCR *T. cruzi* TaqMan® system was applied in DNA samples extracted of peripheral blood taken in pre and post-therapy conditions. The follow-up average post-therapy was 23.6 months.
RESULTS: In pre-therapy conditions, 50% and 33.7% had a parasite load between <0.1-1.0 and 1-100.000 parasites/ml, respectively. In 16.3% of the cases, *T. cruzi* was not detected. On the contrary, in post-therapy conditions, 62.8% and 9.3% had a parasite load between <01-1.0 and 1-100.000 parasites/ml, respectively. In 27.9% of the cases, *T. cruzi* was not detected.
CONCLUSIONS: The parasite load of *T. cruzi* in persons with CChD treated with NFX show statistical differences between the results of qPCR pre and post-therapy in <0.1-1.0, 1-100.000 parasites/ml and negative groups (p values <0,003). 78 cases (90.7%) have very low parasite load (between <0.1-1.0 parasites/ml) or negative results at end of follow-up period. All the cases continue in prolonged follow-up.

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Small molecule analogues of an immunomodulatory helminth product provide a novel approach to dissecting dendritic cell signal transduction pathways

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BACKGROUND: ES-62 is a phosphorylcholine (PC) containing glycoprotein secreted by the rodent filarial nematode *Acanthocheilonema viteae.* ES-62 modulates the function of antigen presenting cells, rendering them hypo-responsive to stimulation with LPS, with a reduced capacity to produce pro-inflammatory cytokines such as IL-12, IL-6 and TNF-α. The unusual post-translational addition of PC appears to be responsible for many of the immunomodulatory properties of this molecule as PC conjugated to ovalbumin can mimic ES-62 action and therefore a library of small molecule analogues (SMAs) of ES-62 based on its PC moiety has been synthesised. The aim of this project is to investigate the effects of these SMAs on dendritic cells to identify any that mimic ES-62.
METHODS: Bone marrow-derived dendritic cells were pre-treated with SMAs for 18 hours before stimulation with LPS, BLP or CpG and cytokine production measured by ELISA and RT-PCR.
RESULTS: Of the 80 SMAs tested, 6 were found to significantly down-regulate LPS-induced IL-6 and TNF-alpha production. These SMAs appear to have selective effects on PAMP responses as they vary in ability to down-regulate cytokine production following BLP or CpG stimulation. The SMAs also differentially act on the mRNA expression level of the p40 subunit of IL-12 in order to inhibit this pro-inflammatory cytokine.
CONCLUSIONS: Due to their ability to selectively inhibit cytokine responses PC-based SMAs could be used as novel tools to dissect the key signalling pathways involved in PAMP-induced dendritic cell inflammatory responses.

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Presence of *Leishmania* spp . in different organs of *Rhipicephalus sanguineus*

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BACKGROUND: About 10 % of the 896 species of ticks (Ixodida) are involved in the transmission of pathogens. Why are hematophagous , these mites are predisposed to eat many kinds of microorganisms in their blood meal. Thus , the role of *Rhipicephalus sanguineus* in the epidemiology of Canine Visceral Leishmaniasis has been of interest to many researchers.
METHODS: Thus , in order to detect the diagnostic yield of *Leishmania* ticks in endemic areas, 10 copies of *Rhipicephalus sanguineus* of each 66 dogs from endemic zoonosis for the area in question were collected , totaling 660 ticks . These were dissected , and separate the intestine , ovary and salivary gland. In each organ ,PCR was performed using real-time RV1/RV2 primers.
RESULTS: The positivity of the parasite in the gut of the tick was 96.97 % (640/660) , and this in the ovaries was 43.94 % (290/660) with significant differences ($p < 0.001$) . Regarding the salivary glands this value was 37.88 % (250/660) is also statistically different ($p < 0.001$) when compared to the intestine , but not getting the difference ($p > 0.001$) in relation to the ovaries analyzed .
CONCLUSIONS: In conclusion , we detected the presence of this pathogen in different organs of ticks , with a predominance of the guts , but further research is needed to establish the possible transmission capacity of these ectoparasites .

Detection of *Entamoeba histolytica*, *Giardia lamblia*, *Dientamoeba fragilis* and *Cryptosporidium spp*. in clinical stool samples by using Multiplex Tandem Real-Time PCR

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BACKGROUND: Diagnosis of intestinal parasites is usually provided by microscopy. However, this method is usually known to have a low sensitivity and is unable to discriminate some protozoa. To overcome all of these limitations, a Multiplex Tandem Real-Time PCR (MT-PCR) was evaluated as an alternative method for diagnosis. Our study evaluated the frequency of detection and identification of four common intestinal pathogenic protozoon parasites in human clinical samples.
METHODS: Stool samples were collected at Eskisehir Osmangazi University Parasitology Laboratory. We analyzed 1500 stool samples by microscopy (Saline solution and formalin ethyl acetate methods). We selected suspicious 450 stool samples after the microscopic examination. Selected samples were studied by MT-PCR. DNA extraction was performed from all unfixed stool samples by using the QIAamp mini kit (Qiagen). MT-PCR primer design to determine suitable areas for primer production consensus. Additionally, permanent smears stained with modified Ziehl-Nelsen and Trichrome were prepared for all samples.
RESULTS: A total of 80/450samples were positive by MT-PCR. The parasites defined were as 59 cases of *D. fragilis, 7* cases of *Giardia lamblia,* 5 cases of *E. histolytica,* 2 cases of *Cryptosporidium spp.* in clinical samples. In 5 patients two different parasites were detected. *E. histolytica* was identified with PCR test cases, 4 of 5 were identified by direct microscopy and Trichrome staining. PCR results were consistent when permanently stained by direct microscopy used together.
CONCLUSIONS: We found that MT-PCR for the detection of *E*. *histolytica*, *G. lamblia*, *Cryptosporidium spp*. and *D. fragilis* presented in this study is a useful alternative method for these individual additional methods. There was no difference in the performance of the amplification of the specific targets in the individual assays compared with the multiplex PCR. The presence of intestinal parasites in the population indicates that more epidemiological studies are needed.

Comparative study of transcriptome profiles of cercariae/schistosomula of bird schistosomes *Trichobilharzia regenti* and *T. szidati*

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BACKGROUND: Bird schistosomes *Trichobilharzia regenti* and *T. szidati* are the causative agents of cercarial dermatitis. They belong to the same family, Schistosomatidae, like the serious human pathogens, e.g *Schistosoma mansoni*, but their life strategy is different. Cercariae released from intermediate host – snail, actively penetrate into the final host – water fowl where immediately transform to schistosomula. In the case of *T. szidati* the schistosomula use the bloodstream as a migratory route to reach the final location – intestinal wall, while schistosomula of *T. regenti* uniquely migrate through the nervous system up to the nasal cavity. The aim of our study is to compare the transcriptome profiles of cercariae and schistosomula of these two trematodes with emphasis on proteolytic enzymes (peptidases) facilitating the penetration of cercariae and migration feeding and immune response evasion of schistosomula.
METHODS: Cercariae were released from infected snails and schistosomula were collected from experimentally infected ducks dissected after 90 and 162 hours post infection. Cercariae of both species (minimally transcriptionally active stages) were used as a baseline for estimation of the transcribed genes and their relative expression level. All investigated biological samples of total RNA were processed in 4 replicates. In total 16 samples were obtained and in the form of cDNA pyro-sequenced by using Illumina HiSeq™ 2500 (Yourgene Bioscience, Taiwan).
RESULTS: In the case of *T. szidati* schistosomula/cercariae 98 mil./77,9 mil. and for *T. regenti* 99,7 mil./122,3 mil. reads were generated with average length of 149 bp. The results were bioinformatically analyzed.
CONCLUSIONS: This comparative study of the *T. regent*/ *T. szidati* transcriptomes would elucidate the crucial phase of ontological development of bird schistosomes such as fast transformation from cercariae to schistosomula and their further migration, with respect to spectrum of involved biomolecules.

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Role of eosinophils in polarization of T- and B-cell responses during intestinal nematode infection.

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BACKGROUND: Infection with the intestinal nematode *Heligmosomoides polygyrus* result in a Th2 skewed immune response and tissue eosinophilia. Eosinophils have the ability to influence early immune events through the rapid secretion of pre-formed cytokines and have been shown to contribute to T-helper 2 (Th2) immune induction through early secretion of IL-4. Furthermore, eosinophils have recently been shown to play a role in plasma cell maintenance in the bone marrow and in IgA class switching in the gut associated lymphoid tissue (GALT).
Thus, eosinophils have the ability to influence humoral and cellular adaptive responses and it is conceivable that they contribute indirectly to anti-helminth immunity.
METHODS: Eosinophil deficient ΔdblGATA-1 and BALB/c control mice were infected with *H. polygyrus* for 14 days. The differentiation of Th2 and Th1 cells was analysed based on expression of the lineage transcription factors GATA-3 and t-bet as well as the respective cytokines IL-4/-5/-13 and IFN-γ. The relative abundance of IgA^+ and $IgG1^+$ B-cells in Peyer's patches (PP) was also analysed and worm counts and individual female fecundity was recorded.
RESULTS: Although infection with *H. polygyrus* resulted in Th2 induction in both mouse strains, ΔdblGATA-1 mice showed an unexpected augmentation of local and systemic Th2 responses while the Th1 reactivity was unaffected. Similarly, both strains expanded $IgG1^+$ B-cells following infection but ΔdblGATA-1 mice did so to a significantly higher degree. In contrast, ΔdblGATA-1 mice displayed an inherent impairment of IgA^+ B-cell levels in PP, which was accompanied by severely reduced IgA secretion from intestinal tissues. Crucially, eosinophil deficiency impaired parasite fecundity significantly, possibly as a result of the strong Th2 induction.
CONCLUSIONS: While eosinophils have been associated with early Th2 induction, our results suggest that their absence may augment nematode-induced Th2 responses. Thus, eosinophils in the intestine may have a regulatory role, preventing excessive Th2 inflammation.

Comparative proteomic profile of cysts, cyst-like structures and trophozoites of the human parasite *Entamoeba histolytica*

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BACKGROUND: Entamoeba histolytica is a protozoan parasite that has two stages in its life cycle: the trophozoite and the cyst. The study of the biology of this parasite has remained limited to trophozoites, mainly due to the fact that the study of cysts has been hampered by the absent of a culture medium to maintain cysts or a method for *in vitro* encystment. In recent years, our research group proposed a method for induction of cyst-like structures (CLS). In this study we determine whether CLS are more like to trophozoites or to cysts based in their proteomic profiles, or if CLS are an intermediate state between both.
METHODS: Protein extracts of trophozoites, CLS and cysts were prepared. Samples were prepared for digestion using the filter-assisted sample preparation (FASP) method. Then portion of the digested peptides, were desalted using C18 stop-and-go extraction (STAGE) tips. Each digestion mixture was analyzed by UHPLC-MS/MS.
RESULTS: From the three samples, a total of 1944 entries were identified in the available *E. histolytica* databases. A total of 1023, 1070 and 333 proteins were identified for trophozoites, CLS and cyst samples, respectively. A comparative analysis of the identified proteins in each sample showed that over 774 of these proteins were shared between trophozoites and CLS. In contrast, only around 120 proteins identified in cysts were shared with trophozoites and CLS, suggesting that nearly 60% of proteins identified in cysts were exclusively expressed in this stage.
CONCLUSIONS: Thus, the protein profile suggest that CLS are more related to trophozoites than cysts and their differences lies in protein abundance; however, functional analysis suggests that CLS are metabolically more related to cyst. In addition, a number of hypothetical proteins were identified whose role in cyst biology and encystment is largely unknown.

Identification of *Trypanosoma cruzi* Discrete Typing Units (DTUs) in bloodstream of individuals with chronic Chagas disease: new genotypes not described in Chile

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BACKGROUND: The *Trypanosoma cruzi* discrete typing units (DTUs) more frequently found in bloodstream of individuals with chronic Chagas disease (ChD) in Chile are TcI, TcII and TcV. Because there are important biological differences between these DTUs, the objective of this study was to identify *T. cruzi* DTUs with a simple and applicable typing method.
METHODS: We evaluated 20 blood samples of individuals with ChD positive to minicircle-based PCR assay. DTUs were identified using improved PCR strategies using oligonucleotides previously described on the basis of spliced-leader gene polymorphisms, 24Sa-ribosomal DNA, and heminested-PCR targeted to RAPD fragment A10e. The results were verified by sequencing data.
RESULTS: We detected mixtures of *T. cruzi* DTUs in most of the cases (90%) concordant with other studies. However, we detected TcIII (50%) and TcIV (75%) furthermore the presence of TcI (25%), TcII (45%), TcV (10%) and TcVI (70%).
CONCLUSIONS: Our results evidence the presence of TcIII, mostly associated with the sylvatic cycle in Brazil and TcIV, a secondary cause of ChD in Venezuela, both not described in individuals with chronic ChD in Chile; and the presence of TcVI, only found through typing assays applied to samples of xenodiagnosis. The application of new methodologies of typing, allow detect *T. cruzi* DTUs not previously described.

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Identifying novel trafficking components of the *Plasmodium falciparum* virulence factor PfEMP1 through QTL analysis

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BACKGROUND: The primary virulence factor of *Plasmodium falciparum* is the erythrocyte surface-displayed adhesin *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1). The central role of PfEMP1 in mediating the cytoadherence of infected red blood cells (iRBCs) to the host microvascular endothelium makes it an attractive therapeutic target; however, this approach is limited due to the protein's hypervariable nature, which gives rise to ≈60 different variants. Targeting the intracellular trafficking of PfEMP1 may be a more successful strategy, as hypervariability becomes inconsequential and previous studies have demonstrated that reduced surface PfEMP1 levels significantly weaken cytoadherence, likely lessening the severity of malaria symptoms and permitting parasite clearance by the spleen. Interestingly, the *in vitro* culture-adapted parasite line 3D7 has a natural PfEMP1 trafficking defect. Presuming that PfEMP1 export from the parasite to the iRBC surface is controlled at the genetic level, we hypothesized that 3D7 harbors one or more genetic determinants of impaired PfEMP1 trafficking.
METHODS: Using Western blotting and a two-color, triple-layer flow cytometry assay with plasma from malaria-immune Malian adults, we established surface-PfEMP1 display phenotypes for 17 progeny clones from the genetic cross between 3D7 and HB3, a 'trafficking-competent' parasite line. These phenotypes were combined with 3,597 genome-wide SNP markers in R/qtl to identify loci contributing to the defective PfEMP1 trafficking in 3D7.
RESULTS: Normalized to HB3, we found that 3D7 displays 75% less PfEMP1 on the iRBC surface, with progeny phenotypes ranging from 37% more to 88% less PfEMP1. QTL analysis identified a significant locus on chromosome 12 with a LOD score of 4.963 that explains approximately 50% of the phenotypic variance. Refining this locus revealed a single gene, *Pf3D7_1245600*, containing several known polymorphisms between 3D7 and HB3.
CONCLUSIONS: The varying levels of surface-displayed PfEMP1 seen across 3D7, HB3, and their progeny may be explained by genetic differences in the putative kinesin *Pf3D7_1245600*. Knock-out and allele-exchange experiments are currently underway to confirm the role of this gene in the trafficking of PfEMP1.

Serological screening of *Trypanosoma cruzi* infection in peri-urban communities from Chaco, Argentina.

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BACKGROUND: Chagas disease (CD) is associated with poverty in Latin America. The affected communities have little access to health services. More research is needed in order to find strategies to integrate vectorial control actions with access to diagnosis and treatment to these vulnerable communities.

METHODS: We performed *T.cruzi* serological screening in Pueblo Viejo, a peri-urban area from Pampa del Indio (PDI), an endemic location under vectorial surveillance. The study and a brief summary about CD were explained to community. Participants signed an informed consent and asked a questionnaire about demographics and exposure to CD risks. Samples were collected by venous puncture. Diagnosis was performed using ELISA Chagatest and Recombinant v3.0 Chagatest(Wiener). A sample was considered positive with two reactive tests and discordant samples were retested at the reference Central Laboratory of Province.

RESULTS: 834 individuals (416 creoles, 399 Qom) were tested. The seroprevalence was 20.0% (2.8% of discordant results); without significant differences between Creoles (17.3%) and Qom (23.0%); women (23.3%) have significantly higher seroprevalence than men (15.8%). The seroprevalence increased markedly with age: no child under 5-years-old showed positive diagnosis and seroprevalence over 40% were detected in people older than 40-years-old. The history of residence in houses infested was strongly associated with positive diagnosis (X^2test, $p<0.01$).

CONCLUSIONS: There is a high human seroprevalence in peri-urban communities of PDI. A bug exposure and residence questionnaire could be helpful for detection of highly seroprevalence groups in peri-urban areas. The performance of *T.cruzi* screening in communities of endemic areas allowed the access not only to diagnosis but also to early treatment.

Automation of Diagnosis of Human Enteric Parasites by Computerized Image Analysis

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BACKGROUND: Intestinal parasitosis constitute a Public Health problem in most tropical developing countries. Their laboratory diagnosis usually relies on the visual analysis of stool samples using optical microscopy, which can be seriously compromised by excess of fecal debris in the microscope slide, use of improper techniques, and lack of human knowledge. To overcome these problems, we propose a solution to automate the diagnosis of the 15 most common species of enteric parasites in Brazil.
METHODS: The proposed system uses a sensitive parasitological technique (*TF-Test Modified*), a motorized microscope with digital camera for automatic image acquisition and focus, a computer and fast image analysis methods. The parasitological technique produces microscope slides with high parasite concentration and low amount of debris, and the computer controls the microscope for automatic image acquisition and processes the images to detect the presence of parasites. The prototype in development can scan a slide in about 3 minutes, and we performed an experiment to evaluate the classification accuracy using an image dataset with 1,791 parasites and 6,068 debris.
RESULTS: Using the dataset with images acquired automatically, the system achieved 93.00% of mean sensitivity, 99.17% of mean specificity and mean *kappa* of 0.84.
CONCLUSIONS: Our results suggest that the system in development is a viable solution to improve the diagnosis of human enteric parasites, producing diagnostic results with images of detected parasites in an unprecedented manner.

Chemosensory behavior in the filarial worm, *Brugia malayi*

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BACKGROUND: Lymphatic filariasis (LF) is a disease caused by mosquito-borne filarial nematodes including *Wuchereria bancrofti* and *Brugia malayi*. Although over 120 million people suffer from this disfiguring disease, chemotherapeutic options for LF are limited to three drugs: diethylcarbamazine citrate, albendazole and ivermectin. The threat of drug resistance combined with the inefficacy of these drugs against adult parasites highlights the need for new anthelmintic drugs. Chemosensation is an essential behavior used by multi-cellular organisms to interact with the environment. In the free-living nematode, *Caenorhabditis elegans*, chemosensation plays a crucial role in finding food and mates, avoiding noxious conditions and in development. Little is known, however, about the chemosensory system of parasitic nematodes, such as *B. malayi.*
METHODS: Scanning electron microscopy was used to visualize the amphids of juvenile and adult stages of the parasite and potential chemosensory genes were identified based on sequence homology to known genes in *C. elegans*. Chemotaxis assays were used to identify compounds that were either attractive or repellent to *B. malayi*.
RESULTS: These studies demonstrate that amphids, the major chemosensory organs of nematodes, are present in both juvenile and adult stages of *B. malayi*. In addition, orthologues of several genes that are known to be involved in chemosensory behavior in *C. elegans* were identified in *B. malayi*. Finally, over 10 compounds were shown to elicit a chemotactic response in *B. malayi* L3 stage parasites.
CONCLUSIONS: This research represents the first study to demonstrate that *B. malayi* has a responsive chemosensory pathway. In addition, the results obtained indicate that the chemosensory response in *B. malayi* plays an important role in host-seeking and host-invasion behavior, thus making the proteins involved potential candidates for chemotherapeutic intervention.

Status of helminthiasis among schoolchildren in Ziracuaretiro, Michoacán, 2013.

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BACKGROUND: Helminthiasis morbidity has remained in some populations, while others have declined mainly because of deworming interventions and health education, improvement of environmental sanitation, water supply and sewage disposal. In Mexico mass deworming was implemented in 1993, starting to intervene municipalities with highest rates helminth infection in the country, being Ziracuaretiro one of the selected towns. The students between 5 and 14 years of age who were given anti-parasite. After 20 years this strategy is still in effect during the National Health Weeks (a Government health campaign), three times a year, offers people of albendazole.
METHODS: During September 2013, schoolchildren in Ziracuaretiro were chosen. Information was collected for the risk factors associated with parasitosis by a questionnaire and a fecal sample was analyzed with direct examination, quantitative Kato, Formalin-ether and permanent stainings.
Children in whom was identified helminths, albendazole 400 mg was administered as a single dose. After two weeks the control sample was analyzed.
RESULTS: We studied 409 children 1% had *Hymenolepis nana*, 25% had protozoan pathogens, other 25% had commensal-pathogens, 4% only with commensals and 45% negatives. The most common parasite was *Blastocystis* sp. (45.7%) followed by *Endolimax nana* (17.8%), *Entamoeba coli* (13.0%) and *Giardia lamblia* (9.8%). The 4 children who had H. *nana* were prescribed with albendazole.
In 1993, 504 students were studied, finding that 26% had helminths, including *A. lumbricoides* (41.9%), *T. trichiura* (50.4%) and *Hymenolepis nana* (29.8%), while for the year 2013, no cases were identified the first two, only 0.7% were infected with *H. nana*.
CONCLUSIONS: There are significant differences in the frequency of helminth infections attributable to mass deworming campaigns, however it is necessary to conduct impact assessment studies, as well as considering other schemes for introducing preventive and prophylactic treatments to decrease the protozoan parasite load.

The opossum *Didelphis virginiana* support a spatial focus of infection with *Trypanosoma cruzi* in a rural village of Yucatan.

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BACKGROUND: Synanthropic mammals play an important role as hosts of *Trypanosoma cruzi* in the peridomestic transmission cycle of Chagas disease. *Rattus rattus*, *Mus musculus* and the opossum *Didelphis virginiana,* have been frequently infected with *T. cruzi* in domicile and peridomicile of several rural villages of Yucatan. As previously reported, *D. virginiana* has high infection prevalence in the peridomicile of rural areas of the region. The objective of the present study was to document the contribution of infected opossums to support a local spatial focus of infection with *T. cruzi* in the peridomicile of a rural village of Yucatan.
METHODS: Synanthropic small and medium mammals were trapped in the peridomicile of 40 households in the rural locality of Molas from October 2010 to april 2011, Yucatan. *T. cruzi* infection was determined by PCR, both in blood and tissue samples. Each domicile was georeferenced for a spatial auto-correlation analysis and clustering (Moran's index). Hot spot analysis and IDW interpolation for presence/absence and abundance data of opossum catches, were carry out.
RESULTS: An autocorrelation was founded in a cluster of 369 m of amplitude ($p<0.05$) reaching an important focus of *T. cruzi* infected opossums involving eleven houses at the southwest of the village.
CONCLUSIONS: Our results confirm the role of opossums as the main contributor in the establishment of peridomicile focus transmission of *T. cruzi* in the rural area of Yucatan. The daily pattern of dispersion (more than 1 kilometer/night), the population density (0.77/ha) and the increased colonization of peridomiciles during the reproduction period (march to june), are three factors that could influence the presence of the infection focus in the Yucatan rural peridomicile, which expose to human population a higher risk of *T. cruzi* transmission.

TcArrestin and TcPTS-1 of *Trypanosoma cruzi:* functional characterization.

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BACKGROUND: *Trypanosoma cruzi*, the causal agent of Chagas disease, has a complex life cycle that expose the parasite to multiple environmental conditions that are linked to its differentiation process, allowing the adaptation and survival of the parasite. Differentiation implicates morphological and physiological modifications that undoubtedly involve changes in signaling pathways and gene expression of the parasite. With limited therapeutic options and no vaccines available, research efforts are focused on elucidating cellular mechanism essential for parasite survival. Understanding how the parasite adapts its gene expression profiles during differentiation could provide valuable information regarding potential drug targets. Here we report the characterization of TcArrestin and TcPTS-1 of *T. cruzi* obtained from a differential expression library of trypomastigotes and intermediary forms derived from an *in vitro* secondary amastigogenesis.
METHODS: TcArrestin and TcPTS-1 clones of *T. cruzi* were characterized using a reverse genetic strategy with antisense mRNA. Knock down parasites were evaluated by growth curves, invasion and infection efficiency in NIH/3T3 fibroblasts and in vitro differentiation.
RESULTS: TcArrestin and TcPTS-1 knock down parasites showed a decrease in their infection and differentiation capacity with respect to transfected control parasites. Furthermore, TcArrestin knock down parasites have an strong defect in their ability to invade target cells.
CONCLUSION: Our results suggest that TcArrestin and TcPTS-1 may play an important role in the intracellular differentiation of *T. cruzi* and that TcArrestin also participates in the invasion process of target cells.

Study of Canine *Leishmania* Infection in Area of Recent Transmission of Minas Gerais state, Brazil.

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BACKGROUND: Visceral leishmaniasis is a parasitic disease which present the dog as the main reservoir in urban areas. Therefore, this study aimed to identify the rate of canine infection in a city of recent transmission for visceral leishmaniasis in the state of Minas Gerais, Brazil.
METHODS: The canine blood samples was performed in veterinary clinics and in Defense of Animal Life Center of Formiga, Minas Gerais, Brazil. Blood samples were collected for identification of *Leishmania* spp infection by serological techniques (ELISA and DPP®) and molecular tests (Nested_PCR). The positive samples on the Nested_PCR were submitted to the sequencing technique for species identification.
RESULTS: Out of 570 samples analyzed, 13.7% (n=78) were positive by serological tests. The Brazilian Ministry of Health determines the euthanasia of dogs positive in both DPP® and ELISA, which represented 29 dogs (infection rate=5.1%). Forty five samples were positive by Nested PCR, 26 (4.6%) only in this method and 12 also in both serological tests (2.1%).The sequencing technique allowed to confirm the species *L. infantum* and *L. braziliensis* circulating among the dogs.
CONCLUSIONS: Our results revealed that the municipality of Formiga shows a higher canine infection rates (5.1%) than that established by the Ministry of Health (2%) to initiate control actions. Furthermore, it was confirmed that dogs are infected by *L. infantum*. These findings corroborate the suspicion of a transmission cycle of visceral leishmaniasis in the municipality and indicate the need for monitoring the canine infection.

The expression of TcTASV-C, a trypomastigote-specific protein family of *Trypanosoma cruzi*, is upregulated in bloodstream parasites.

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BACKGROUND: The TcTASV is a recently-identified multigene family in *T. cruzi* that is conserved in the different lineages of the parasite and has no orthologues in other trypanosomatids. The TcTASV-C subfamily is composed by ~15 genes and one of them has been identified among a pool of protective antigens. TcTASV-C is expressed at the surface of trypomastigotes and shed into the medium. However, studies carried out with cell culture-derived trypomastigotes indicated that TcTASV-C is not a major component of the parasite. In the present study we evaluate the expression of TcTASV-C in blood-derived trypomastigotes.
METHODS: *T. cruzi* strains of lineages I,V and VI were used. Infected mice were bled at the peak of parasitaemia and circulating trypomastigotes purified. Trypomastigotes from *in vitro* cultures were obtained from monolayers of infected Vero cells grown in MEM-4%FBS. Purified blood-derived parasites were used to infect Vero cells. TcTASV-C expression was analyzed by western blot using anti-TcTASV-C antibodies.
RESULTS: TcTASV-C is expressed in blood-derived trypomastigotes in all the strains analyzed, in contrast with culture-derived trypomastigotes where TcTASV-C was expressed in few strains. Moreover, the lysate from 10^5 bloodstream parasites was enough to detect its expression, while the equivalent of 2x10^7 parasites was necessary to detect TcTASV-C expression in culture-derived trypomastigotes. When bloodstream trypomastigotes were used to infect cells, the TcTASV-C expression in culture-derived trypomastigotes dropped dramatically after the second *in vitro* passage.
CONCLUSIONS: The expression of TcTASV-C in bloodstream trypomastigotes is 100-fold higher than in culture-derived trypomastigotes, suggesting that a factor present in the host triggers its expression.

Amoebicidal activity of the essential oils from *Lippia spp.* against *Acanthamoeba polyphaga* trophozoites

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BACKGROUND: *Acanthamoeba* species are free-living amoebae that constitute the etiological agent of *Acanthamoeba* keratitis, a potentially devastating and sight-threatening infection of the cornea. Disease eradication is difficult because of the amoebal encystment which makes them highly resistant to conventional drugs. Plants of the genus *Lippia* are used in Brazilian folk medicine, especially in respiratory and gastrointestinal disorders. This study aimed to evaluate the *in vitro* amoebicidal activity of the essential oils from three *Lippia* species on *A. polyphaga* trophozoites.
METHODS: *Lippia* leaves were collected and the essential oils were extracted using the hydrodistillation technique. The chemical constituents of these oils were previously determined by means of gas chromatography-mass spectrometry. In order to evaluate the *in vitro* amoebicidal activity of the essential oils on *A. polyphaga* (ATCC 30872) trophozoites, amoebae (8 x 10^4 trophozoites) were incubated with different concentrations of *L. sidoides* and *L. gracilis* (5, 10, 20, 30, 40, and 50 µg/mL), and *L. alba* (10, 20, 30, 40, 50, and 100 µg/mL) in 2 mL of PYG medium for 24 hours. The major compounds rotundifolone and carvone (10, 20, 30, 75, and 100 µg/mL), and carvacrol (10, 25, 50, 75, and 100 µg/mL) present in the essential oils were further tested. After an incubation period, 100 µL of each medium was evaluated in a hemocytometer and the 50% lethal concentration (LC_{50}) was established. The cytotoxic effect of the essential oils was also tested in mammalian cells using MTT assay.
RESULTS: *Lippia* essential oils and the major compounds showed significant amoebicidal activity on *A. polyphaga* trophozoites as compared to the non-treated control, and the inhibition was found to be dose dependent. A LC_{50} value of 18.19 µg/mL, 10.08 µg/mL and 31.79 µg/mL was obtained for *L. sidoides*, *L. gracilis* and *L. alba*, respectively. The major compounds rotundifolone, carvone, and carvacrol exhibited LC_{50} of 18.98 µg/mL, 43.62 µg/mL and 24.74 µg/mL, respectively. During the cytotoxicity assay all essential oils exhibited moderate to high cytotoxic activity against tumor cell line NCI-H292.
CONCLUSIONS: Essential oil from *Lippia* species showed substantial amoebicidal activity against trophozoites of *A. polyphaga*. Further studies with the major components of the essential oil are under consideration.

Polymorphisms and Ambiguous Sites Present in DNA Sequences of *Leishmania* Clones: Looking Closer

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BACKGROUND In genetic studies of *Leishmania* parasites, co-dominant markers are chosen for their ability to detect heterozygous, infer the occurrence of inbreeding and resolve genetic variability. The majority of DNA sequence based reports perform conventional dye terminator sequencing where perfectly double peaks in the chromatogram are interpreted as heterozygous strains. Nonetheless, molecular peculiarities of *Leishmania* such as aneuploidy, mixed populations and preponderant clonal structures with recombination events advise that data from regular DNA sequence analysis should be carefully evaluated.
METHODS Six *L.* (*Viannia*) strains presenting double peaks in 6*pgd* DNA sequences were retrieved from Coleção de *Leishmania* do Instituto Oswaldo Cruz, cultivated and divided for biological cloning and direct DNA isolation. 10^4 parasites, added to 2.4 ml of Low Melting Point Agarose 1% were distributed over NNN medium in a Petri Plate. After seven days, colonies were harvested, cultured, processed for DNA isolation and isoenzyme assay. DNA isolation, PCRs and purification were carried out. Sequences were obtained from non-cloned and cloned culture, and PCR products were either subjected to direct sequencing or molecular cloning. The sequences were edited, checked for ambiguous sites and aligned.
RESULTS 286 DNA sequences from biological and molecular clones of *L.* (*Viannia*) were generated and different sequence types detected within each strain. Loss of ambiguous sites, detection of less frequent alleles, recombination and mutation could be distinguished as events that contribute to genetic variation in *Leishmania*.
CONCLUSIONS The variety of DNA sequences within a strain demonstrates how diversity might not be completely represented through regular DNA sequence analysis and signals the importance for molecular epidemiology research to be aware of such possibilities while choosing the samples for studies. Moreover, to exclude samples with multiple peaks from analysis is not the best way to deal with the occurrence of infra-populations in *Leishmania* because such a phenomenon occurs frequently.

Susceptibility of M-10 strain of *Entamoeba histolytica* to several antibiotics and antiamoebic drugs

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BACKGROUND. Amoebiasis is a parasitic disease caused by protozoan *Entamoeba histolytica*. The parasite infects the large intestine of man where it can produce an asymptomatic infection or invade the intestinal mucosa producing clinical amoebiasis. If the production of amoebic ulcers occurs, the infection can be disseminated to the liver and other organs. Treatment of amoebiasis is necessary to eradicate amoebas, reduce the severity of illness, and decrease transmission and it is achieved by using drugs for either luminal infection or tissular invasion. Although no clinical resistance to parasitic drugs has been reported in amoebiasis, there had been some experimental approaches to obtain drug-resistant amebas for emetine and metronidazole. In this study we investigated if a strain of *E. histolytica* resistant to neomycin (G-418) could be also resistant to other antibiotics or antiamoebic drugs.

METHODS. We used the HM1 strain of *Entamoeba histolytica* and a mutant derived from this strain called M-10 which was obtained in our laboratory and is resistant to G-418. We tested the susceptibility in vitro of these two amoebic strains to antibiotics (kanamycin, penicillin, neomycin) or parasitic drugs (emetine, chloroquine, metronidazole, tinidazole, and nitazoxanide) at different concentrations and IC_{50} was determined. Likewise, the effect of either drug on growth curve was observed. In some experiments, neomycin was added in addition to a specific drug.

RESULTS. HM1 strain was susceptible to G-418 but M-10 was resistant to the same drug. Both amoebic strains showed susceptibility to other tested drugs, being inhibited in its growth almost in the same degree. When G-418 was added to test tubes in addition to specific drug, the effect was the same.

CONCLUSIONS. M-10 strain of *E. histolytica* is not resistant to other antibiotics or antiamoebic drugs.

Lutzomyia longipalpis intestinal microbiota and vector competence for Leishmania infantum chagasi

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BACKGROUND: Leishmaniasis are diseases caused by protozoa of the genus *Leishmania* and they are transmitted by the bite of sandflies. The disease presents different clinical manifestations, such as visceral leishmaniasis (VL). In Brazil, VL is caused by *Leishmania (L.) infantum chagasi* and it is transmitted by the vector bite *Lutzomyia longipalpis.* Sandflies, during blood feeding, can ingest the parasite that goes to the insect midgut, where it gets in contact with the microbiota. The microbiota activity over the parasite was evaluated *in vitro* demonstrating cell walls lysis of *Leishmania (L.) infantum chagasi* and *L. braziliensis* caused by *Serratia marcescens* but their role in the vector is not clear yet. This work identified bacterial isolates from the *L. longipalpis* midgut from two different places in Brazil, one in Minas Gerais state (Lapinha Cave) and Bahia state (Cavunge), by sequencing DNA.
RESULTS: Our results showed the presence of bacteria from the Lapinha Group: Unfed - Enterobactereaceae family; *Providence sp* and *Erwinia sp.*; Post digestion - *Enterobacter sp*, *Providencia vermicola, Providencia burhodogranariea* and *Providencia sneebia.* Cavunge group: Unfed - *Enterobacter ludwigii*, *Klebsiella pneumonia;* Post digestion - *Klebsiella pneumonia, Enterobacter asburiae*. In both locations showed a majority of Gram-negative bacilli. In order to analyze the influence of bacteria in the parasite development, *L. longipalpis* were infected with *L. infantum chagasi* and treated with three antibiotics: carbenicillin, gentamicin and Pen-strep plus gentamicin. After treatment, we identified by DNA sequencing of bacteria isolates.
CONCLUSION: Parasites of the group treated were able to establish themselves in sandflies after digestion had an increase in their number. By incubating the bacteria identified by us with *L. infantum chagasi* in vitro we could see that bacteria interferes in the growth of the parasite.

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Involvement of CD200 in the infectivity of *Leishmania (Leishmania) amazonensis* isolates associated to Localized Cutaneous Leishmaniasis (LCL) and Diffused Cutaneous Leishmaniasis (DCL)

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BACKGROUND: Leishmaniasis is a tropical disease caused by the intracellular obligate protozoan from the genre *Leishmania*. Depending on the *Leishmania* species and the immunological state of the host, it could be established different manifestations of the disease. Recently, it was described the role of CD200 expression in macrophages infected by *L. (L.) amazonensis*. In this study, we examined the difference in infection rate and expression of CD200 in infected macrophages of two isolates of *Leishmania amazonensis* associated to Localized Cutaneous Leishmaniasis (LCL) and Diffused Cutaneous Leishmaniasis (DCL).
METHODS: Bone marrow macrophages of C57BL/6 mice were infected with axenic amastigotes in different time points and samples of each point were prepared for quantification of parasites growth index and CD200 levels by qPCR and western blotting assays. Also, *in vivo* infections were made by injection of 10^6 stationary phase promastigotes from each isolate in the footpad of mice. Parasite load and lesion size were measured.
RESULTS: There was a significant difference ($p<0.05$) in growth between the *L. (L.) amazonensis* isolates. These results were accompanied with a differential expression of CD200, 1 h post-infection of macrophages *in vitro*. Also, it was demonstrated that the course of infection *in vivo* in C57BL/6 mice was different between LCL and DCL. Although the lesion size was apparently similar, the number of parasites in the lesion was significantly lower in the DCL rather than LCL isolate. All these results were compared to the reference isolate of *L. (L.) amazonensis* IFLA/BR/67/PH8, in which the rate of infection and CD200 expression was previously demonstrated.
CONCLUSIONS: Our results suggest that the isolate of *L. (L.) amazonensis* associated to DCL isn´t capable of growing inside the parasitophorous vacuole of macrophages, although it persists up to 96 hours post-infection. This is accompanied by the expression of CD200, which is absent in the early stages of infection.

Highly water-soluble benzimidazole derivatives useful for the treatment of fasciolosis

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BACKGROUND: *Fasciola hepatica* is the causative agent of fasciolosis, a foodborne zoonotic disease affecting grazing animals and humans worldwide. Triclabendazole (TCBZ) has been the drug of choice for the treatment of liver fluke infections for over 20 years. More recently, it has been approved to treat human cases of fasciolosis. Resistance to triclabendazole in farm animals in Australia and in a number of European countries has already been developed, hence the need of new fasciolicides. One product in particular, Compound Alpha is an experimental fasciolicide that has a range of activity against *F. hepatica* similar to that of TCBZ. In this study we present the synthesis and activity of compound Alpha and TCBZ water-soluble derivatives.
METHODS: Compound Alpha or TCBZ (1a, 1b) was treated with sodium hydride in DMF, then with di-*tert*-butylchloromethyl phosphate to give the corresponding esters (2a, 2b), that upon hydrolysis and neutralization gave the water-soluble salts (4a, 4b). These compounds were tested in vitro against recently excised metacercarias.
RESULTS: Two highly water-soluble compounds were obtained in good yields. The in vitro fasciolicidal activity obtained at concentrations of 10 or 50 mg/L for Compound Alpha or TCBZ range from 95 to 100%.
CONCLUSIONS: The high activity shown by compounds 4a, 4b is a good signal to test then in vivo.

EPIDEMIC OUTBREAKS OF CUTANEOUS AND VISCERAL LEISHMANIASIS

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Leishmaniasis transmission occurs inside of natural foci of infection which are geographical areas where vector and infected reservoirs coexist.

Vectors presence and their density determine the epidemiological risk of infection. Infected reservoirs complete the necessary elements for Leishmania transmission.

Reservoirs are mammals that are hosting the parasite enabling the transmission. There are **primary reservoirs** (or primary hosts) that maintain endemically a continuous transmission of the disease. There are also **secondary reservoirs** which depending of different variables could permit the vector infection generating epidemic outbreaks, however in these cases the disease doesn't maintain endemically in absence of a primary host.

Sometimes, reservoirs are domestic or wild animals but also in classic zoonotic leishmaniasis they could be humans.

During the last 25 years, a significant number of epidemic outbreaks of both cutaneous and visceral leishmaniasis have been documented in America and in the Old World

Occasionally, the triggering element of the outbreak is the arrival of a secondary infected reservoir, often a human, as it was observed in the Inter-Andean valleys with outbreaks of CL caused by *L braziliensis, L panamensis and L guyanensis*, as well as for *L donovani* in Buthan. The increases in the density of primary reservoirs, as a consequence of climatic changes, explain the CL outbreaks due to *L major* in the south of Morocco. Another triggering element are the changes in human behavior that allow the increase in the density of reservoirs, this is the case of CL and VL due to *L. infantum* in Madrid. People migration from non-endemic areas to transmission areas explain the bigger CL outbreak in Colombia where more than 40.000 cases, occurred in the army, mainly due to *L braziliensis*.

The establishment of an early warning system based on the eco-epidemiological approach developed by Pr Rioux from Montpellier, allows the determination of the epidemiological risk of infection, the prediction of outbreaks and the establishment of effective prevention and control measures.

Strongyloides stercoralis: Baermann modified method as coproparasitary routine tests.

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BACKGROUND: *Strongyloides stercoralis* is a helminth of worldwide distribution, particularly in tropical and subtropical areas , usually produces an asymptomatic infection in humans , it could become a serious disease in immunocompromised patients. This disease is often under-diagnosed because to observe the larvae is necessary to use special methods. Due to the above, in our laboratory located in the Guayaquil city (Guayas-Ecuador), in addition to the direct smear, and Willis-Molloy technique we have included the modified Baermann method routinely in coproparasitary examinations. Achieving important information on the prevalence of the parasite in patients referred from private clinics, from medium and high social status, that could be at risk to get hyperinfection at short-term, if they infected with any associated pathology that triggers the disease.
METHODS: A retrospective study of coproparasitary tests that were analyzed during the period 2011-2013 was performed. The techniques used were, direct smear, Willis-Molloy and modified Baermann
RESULTS: Were analysed 2321 coproparasitary test, where 69.96% of fresh examinations were positive for protozoa and only 5.98 % for helminth and tapeworms eggs.
In concentration technique (Willis-Molloy) was obtained 64.97% positive samples for geohelminth eggs and 11.97 % to tapeworm's eggs. While, in the method of Baermann was found 19.99% of the samples positive for larvae of *Strongyloides stercoralis*.
CONCLUSIONS : Perform a routine study of stools using the modified Baermann method is essential to prevent hidden infections of *Strongyloides stercoralis* for the sake of the patient and the treating physicians , that they could obtain a significant clinical improvement.

Paragonimiasis due to the consumption of wild boar meat in Japan: Contamination levels of *Paragonimus* larvae in wild boar muscle samples

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BACKGROUND: Paragonimiasis occurs due to the consumption of raw or undercooked second intermediate hosts such as freshwater crabs or crayfish. Paratenic hosts such as wild boars are other important sources of infection. Cases of paragonimiasis in humans often occur in the western part of Japan, especially in Kyushu Island, where hunters eat raw or undercooked wild boar meat and become infected. This mode of infection has not attracted sufficient attention considering the aspect of food hygiene; one of the main reasons is obviously the lack of studies about the contamination levels of *Paragonimus* larvae in wild boars.
METHODS: The muscle tissues of 22 wild boars (140–1,300 g per boar) from Kyushu were examined for the presence of lung fluke larvae.
RESULTS: *Paragonimus westermani* larvae were detected in 7 samples (32%); as less as 215 g of a single sample of wild boar meat showed the presence of up to 8 larvae.
CONCLUSION: We should conduct health education campaigns to emphasize the risk of paragonimiasis due to the consumption of raw or undercooked wild boar meat.

Changes in gene expression during *Giardia* differentiation.

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BACKGROUND: Differentiation into infectious cysts through the process of encystation is crucial for transmission and survival of *Giardia intestinalis* parasites in the environment. Hitherto the majority of efforts to explain the encystation process have focused on the early events of the process like formation of encystation-specific vesicles (ESVs), leaving many aspects of late encystation poorly defined.
METHODS: We developed a new encystation protocol that produces larger numbers of complete cysts. Transcriptional changes during the full encystation process were studied using RNA sequencing. Protein expression and localization was verified using epitope-tagged proteins.
RESULTS: We tested different published encystation protocols and developed a protocol using a modified high-bile encystation medium. This protocol generated a much higher yield of mature, 16N cysts after 24hrs, compared to the standard two-step encystation method. Transcriptional changes during differentiation from trophozoites to cysts in the new medium were studied using RNA sequencing (RNA seq). The two early time points (1.5 and 7 h p.i) were compared to existing transcriptional data generated by microarray and SAGE technology, thereby generating a consensus table of up-regulated genes using different encystation protocols and techniques. The consensus table showed that only 13 of the around 6000 *G. intestinalis* protein encoding genes are consistently up-regulated early during encystation. Five genes with unknown function found in the consensus table were further specifically characterized by localization and expression studies.
The largest transcriptional changes were seen in the late part of encystation (22 h p.i.) and the majority of the highest up-regulated genes at this time point encode hypothetical proteins. Several of these were epitope tagged and localized to gain information of new important proteins involved in the differentiation process. We found three proteins that localizes to the nuclei during the late stage of encystation, four proteins localizes to the cyst wall membrane and another three proteins seem to localize to a cytoskeleton-associated structure in cysts.
We also detected a switch of variant surface proteins (VSPs) in the late phase of encystation. This occurred at the same time as nuclear division and DNA replication, suggesting a potential link between the processes.
CONCLUSIONS: Our data gives a starting point from which it is possible to further explore important processes occurring in the later part of encystation to gain crucial information about this vital process for parasite survival.

Economic costs and benefits of morbidity management and disability prevention for lymphatic filariasis

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BACKGROUND: Lymphatic filariasis (LF) is endemic in 73 countries, with 120 million people infected, of whom 40 million suffer serious disability, including lymphedema of the leg (15m), hydrocele (25m), and acute attacks of fever and disabling pain (adenolymphangitis—ADL), causing worsening lymphedema and substantial productivity loss. The Global Programme to Eliminate Lymphatic Filariasis (GPELF) has two goals: interrupting LF transmission by 2020 and caring for those already infected through morbidity management and disability prevention (MMDP). While 53 countries have ongoing MDA, only 27 had begun MMDP by 2013.
METHODS: We estimate global costs of MMDP for those affected and at risk. Program costs reflect community-based care (CBC), referral to primary care clinics, and specialty clinics. We calculate societal costs of morbidity from acute attacks, chronic lymphedema, hydrocele, lost wages, and other costs.
RESULTS: Research suggests societal cost of untreated LF far exceeds cost of MMDP. In India, patients in CBC morbidity management averaged 29 fewer lost work days annually. In Haiti, simple training in self-care reduced ADL substantially. A 3-year MMDP program in Togo reported stabilization of lymphedema stage and slight decrease in ADL. In Ghana, patients reported substantial improvement in work capacity after hydrocele surgery.
CONCLUSIONS: We will combine results from these and other countries and adjust costs and benefits to each context, to estimate savings in direct costs, productivity loss, and marginalization that provide economic rationale in addition to the ethical mandate for MMDP, the second pillar of GPELF.

Synthesis and activity of 1*H*-benzimidazole derivatives, designed by docking, against *Giardia intestinalis*

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BACKGROUND: Common antigiardial drugs have been employed for decades, but little practical progress has been made in expanding and improving the existing drug arsenal, hence the need to find new giardicidal agents. An approach could be to have drugs that act on targets that exist in the parasite but not in humans. An example of this is the enzyme arginine deiminase (ADI), which plays an important role for the energy generation in *Giardia intestinalis*, making this enzyme an attractive target for drug development.
METHODS: Based in the amino acid sequence of ADI (from *Giardia intestinalis*), the tridimensional structure of this enzyme was obtained by a homology modeling study. Considering that ADI has arginine as a natural substrate, isosteres of this amino acid were docked in the enzyme and allowed to select six compounds with high probability to inhibit this enzyme. These compounds, benzimidazole derivatives, were synthesized by conventional synthetic procedures and characterized by spectroscopic and spectrometric methods. The novel benzimidazole compounds were evaluated in vitro against *G. intestinalis* trofozoites.
RESULTS: Results on the molecular modeling studies, synthetic procedures, as well as the biological activity in vitro against *Giardia intestinalis* of these compounds will be presented. However, studies on the recombinant arginine deiminase enzyme are still in progress.
CONCLUSIONS: The development of drugs against known *Giardia* targets that are important to parasite survival is in the early stages. This new technique, named target-centered approach, is a promising way that can lead to viable drug candidates.

Redox system gene modulation and biochemical analysis in the cattle tick *Rhipicephalus microplus*

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BACKGROUND: The immune response mediated by Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are crucial components of host-pathogen interaction. In vector insects ROS and RNS are involved in the control of human pathogens and in the maintenance of the natural microbiota. However, our knowledge regarding the role of the redox system in tick-pathogen interactions is limited.
METHODS: The embryonic cell line BME26 from *R. microplus* was used for a transcriptomic study of the genes involved in the oxidative stress and antioxidant defenses. The BME26 cells were exposed to different immune stimuli (heat-killed Gram- positive/negative bacteria and yeast) as well as intracellular *Anaplasma marginale* and *Rickettsia rickettsii*. Using a high-throughput qPCR approach, the expression levels of 16 genes involved in the redox system were analyzed at different post-challenge time points. In addition, hydrogen peroxide (H_2O_2) production was measured by the Amplex Red assay.
RESULTS: qPCR analysis revealed pathogen specific gene expression signatures. In both *A. marginale* and *R. rickettsii*-infected cells the antioxidants transcript abundance was significantly induced, including *peroxidase* and *catalase*. Conversely, the expression of *dual oxidase 1* and *2* involved in ROS production was down-regulated by *A. marginale* infection. Additionally, biochemical assays indicate the accumulation of ROS upon inactivated bacteria, yeast and *R. rickettsii* challenge, whereas H_2O_2 production was not detected when BME26 was challenged with *A. marginale*. This lack of H_2O_2 production by infected BME26 cells can be the result of gene modulation by *Anaplasma*. Further experiments are being conducted to elucidate the redox modulation by *Anaplasma*.
CONCLUSIONS: The differential expression of the analyzed genes suggests the redox system involvement in tick-pathogen interactions. Particularly, *A. marginale* inhibited pro-oxidant genes and increased the antioxidant genes, indicating that this suppression mechanism could be the key for its intracellular survival.

Occurrence of tick-transmitted pathogens in dogs in Jos, Plateau State, Nigeria

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Canine babesiosis caused by *Babesia rossi*, is the most common and economically important tick-borne disease in South Africa and has also been reported from Nigeria. The primary objective of this study was to detect and characterise tick-borne pathogens in dogs presented to a veterinary hospital in Jos, Plateau State, Nigeria. In *B. rossi*-positive specimens, we aimed to determine whether the *BrEMA*1 gene occurred. Lastly, we wished to identify the tick species that were recovered from the sampled dogs.

Blood specimens (n=100) were collected (Jan-March 2010) from domestic dogs presented at the hospital. They were screened for the presence of *Babesia*/*Theileria* and *Ehrlichia*/*Anaplasma* genomic DNA using PCR and RLB assays. Positive *B. rossi* specimens were tested for the presence of the*BrEMA1*gene using RT-PCR. In addition, ticks were collected from dogs found to be infested during sampling.

On RLB, 72 (72%) of the specimens were positive for one or more haemoparasites. Of the positive specimens, 38 (53%) were infected with *B. rossi;*9 (13%) with *T* sp.(sable); 5 (7%) with either *E. canis* or *Anaplasma* sp. Omatjenne, respectively;3 (4%) with *T. equi*; and 1 (1%) with *B. vogeli* and *E. ruminantium*, respectively. Co-infections were detected in 13 (18%) of the specimens. Results of RT-PCR screening for the *BrEMA1* gene were negative. A total of 146 ticks belonging to 8 species were collected and identified: *R. sanguineus* 107 (73%), *H. leachi*(sensu stricto) 27 (18%), *R. turanicus* 3 (2%), and *A. variegatum*, *H. elliptica, R. lunulatus, R. muhsamae* and *R. senegalensis* 1 (1%), respectively.

There appears to be numerous tick-borne pathogens circulating in dog populations at Jos, with *B. rossi* being the most prevalent. The absence of the *BrEMA1* gene suggests that *B. rossi* occurring in that area may be less virulent than South African isolates.

Low sensitivity of microscopical observation for the detection of trypanosomatids in synanthropic triatomines in the Federal District of Brazil

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BACKGROUND: In public health services, the detection of trypanosomatids in synanthropic triatomines (ST) is determined by microscopical observation (MO). However, PCR has been proposed as a promising method for detecting ST infection. In this study we compared the rates of detection of trypanosomatids in ST by PCR and by MO.
METHODS: STs were captured in the Federal District of Brazil (FD) and classified by species, sex, nymphal instar and habitat. ST infection was detected by MO of flagellates in fresh and stained feces. PCR was performed on DNA samples of each insect using polymorphic D7 domain of $24S_{\alpha}$ rDNA to detect trypanosomatids.
RESULTS: Overall, 237 STs were analyzed: *Panstrongylus megistus* (n=207), *P. geniculatus* (n=3), *Triatoma pseudomaculata* (n=26) and *Rhodnius neglectus* (n=1). MO revealed 8 STs infected by *Trypanosoma cruzi* (3%) and 13 by *Blastocrithidia* (5%). PCR showed 94 STs infected by *T. cruzi* (40%) and 59 by *Blastocrithidia* (25%). Two STs showed mixed infection.
CONCLUSIONS: The results show a low sensitivity of MO for the detection of trypanosomatids in STs in the FD. Moreover, results show high frequency of *Blastocrithidia,* which reinforces the need for high specificity methods for the development of appropriate control strategies. PCR is a powerful tool for the detection of *T. cruz*i in triatomines and could be applied to insects from domestic colonies to better assess the risk of Chagas disease transmission.

Host-parasite cross-talk during giardiasis.

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BACKGROUND: The non-invasive protozoan parasite *Giardia intestinalis* is a major cause of diarrhea worldwide. The mechanisms of disease remain poorly defined and; no major intestinal tissue destruction and inflammation is induced. To better understand the crosstalk between *G. intestinalis* and human intestinal epithelial cells, and especially the initial response in human cells, we studied gene expression in human intestinal epithelial cells and the parasites during interaction *in vitro*.
METHODS: RNA sequencing was performed upon *in vitro* interaction of human intestinal epithelial cells (Caco2) and parasites. We used the *in vitro* adapted *G. intestinalis* isolate WB (assemblage AI) as well as the clinical isolate AS175 (assemblage AII) that was established recently from a human patient sample. Results were confirmed with specific experiments to verify the results from RNA sequencing.
RESULTS: The two *Giardia* isolates lead to highly correlated (r=0.93) response in human intestinal epithelial cells after 1.5 hours, dropping at later time points of 3 and 4.5 hours. Gene network analysis revealed that *Giardia*-infection leads to the immediate activation of chemokines (CCL2, CCL20, CXCL1, CXCL2, CXCL3) and cytokines (IL8) on the RNA level but the level of secreted cytokines is low. Further, regulatory proteins of apoptosis and proliferation as well as cell adhesion molecules were induced after 1.5 hours of host-parasite interaction. Most of the early induced genes were down-regulated on transcript-level before 3 hours. Data analysis suggested that this was due to RNA decay of AU-rich element-containing transcripts. In the parasites the expression of high-cysteine rich membrane proteins and genes related to protection against oxidative stress was induced.
CONCLUSIONS: Interactions between *Giardia* trophozoites and host intestinal epithelial cells induce specific gene expression changes in both cell types. These gene expression changes can partly explain the low levels of inflammation and the disease mechanism during giardiasis.

Trypanosoma cruzi infection in _Panstrongylus megistus_ (Hemiptera: Reduviidae: Triatominae) and callitrichids (Primates: Cebidae) at the Brasília Zoo, Federal District of Brazil.

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BACKGROUND: *Trypanosoma cruzi* has been detected in non-human primates, which play an important role in the maintenance of this parasite in nature. In this study we report *T. cruzi* infection in triatomines and callitrichids at the Brasília Zoo (ZooB), Federal District of Brazil.
METHODS: The insects were captured in a captive primate unit at ZooB and classified by species, sex and nymphal instar. *T. cruzi* infection was detected by microscopical observation of fresh and stained feces. Blood samples from 25 callitrichids and tissue samples of 33 insects were used for DNA extraction. PCRs were performed using TCZ1/2 primers specific for *T. cruzi*. PCR products were validated by Southern hybridization. Additional nested qPCR was performed for the blood samples.
RESULTS: Triatomines were identified as *Panstrongylus megistus*. Microscopy revealed two females, one nymph and one male infected with *T. cruzi* (12%) which were confirmed by PCR hybridization. qPCR identified *T. cruzi* in 19 callitrichids (58%). One specimen of *Saguinus imperator* with high parasite load died during the research.
CONCLUSIONS: Our results show potential vector borne transmission of *T. cruzi* at ZooB. High parasite loads suggest an authoctonous acute non-human case. The geographical proximity with a gallery forest where sylvatic *T. cruzi* cycle was already described may explain the presence of *T. cruzi*-infected triatomines at ZooB.

Evidence of enhanced infectivity of the North American Type IV strain of *Trypanosoma cruzi* for human placental syncytial trophoblast cells.

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BACKGROUND: *Trypanosoma cruzi* represents a genotypically diverse family of organisms with documented differences in tissue tropism and pathogenicity. Our previous breeding experiments comparing the relative abilities of Type I and Type IV isolates of *T. cruzi* from the southeastern United States to be congenitally transferred in mice suggests that the Type IV may have adaptations that increase its rate of congenital transfer.
METHODS: We sought to determine whether a strain-specific placental tropism existed using BeWo cells, a human derived placental syncytial trophoblast cell line. Cultures of BeWo cells were exposed to isolates of either the Type I or Type IV strains of *T. cruzi*. Cells were fixed and stained at 48, 72, and 96 hours post-exposure and microscopically assessed for the percentage of cells infected and average number of intracellular amastigotes. The mechanism of invasion was assessed using cytochalasin-D in culture media.
RESULTS: BeWo cultures exposed to the Type IV isolate had significantly higher percentages of infected cells, as well as a higher average number of intracellular amastigotes, at all time points tested. Control cultures carried out in DH-82 canine macrophages found that the two isolates invaded and reproduced similarly, supporting an enhanced tropism of the Type IV isolate for the placental cells. Invasion of BeWo cells was found to be independent of the presence of cytochalsin-D, confirming that invasion is an active and parasite specific ATP-dependent process. CONCLUSIONS: These results confirm that significant differences exist in the ability of these two isolates to invade and replicate in placental syncytial trophoblast cells. Tit also adds additional support to the hypothesis that the Type IV strain may possess adaptations to facilitate placental transmission.

The Onchocerciasis Vaccine for Africa Initiative: Recent Advances

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BACKGROUND: Human onchocerciasis is caused by *Onchocerca volvulus* and an important cause of blindness and chronic disability. Although mass drug administration of ivermectin has had a profound effect on control of the disease, additional tools are critically needed including the need for a vaccine against infection.
METHODS: Eight vaccine antigens were studied under controlled conditions and their vaccine efficacy was evaluated using a single harmonized immunization protocol using two animal models: mouse *O. volvulus* larvae chamber and the *Brugia malayi* infection in gerbil. The use of the two animal models increases the probability that results obtained will reflect what will be elicited in vaccinated humans.
RESULTS: Three antigens with alum as the adjuvant, 103, RAL-2 and CPI-2M, repeatedly induced significant reductions in establishment of larvae (up to 40%) or adult worms (up to 70%) in the *O. volvulus* or *B. malayi* models, respectively. The *Litomosoides sigmodontis* mouse model confirmed that these proteins alone or in combination also can reduce microfilarial burdens.
CONCLUSIONS: We are now ready to move forward through the pre-clinical product development stages. For this purpose four American, six European and three African research groups have now joined forces under the umbrella of The Onchocerciasis Vaccine for Africa (TOVA) Initiative, which aims to take a vaccine through Phase I human safety trials by 2016. Modelling analyses have shown that an onchocerciasis vaccine would markedly reduce microfilarial load in those under 20 years of age, and have the beneficial impact of reducing onchocerciasis-related disease burden in these populations.

Macrofilaricidal drugs for the treatment of onchocerciasis

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BACKGROUND: Although mass drug administration of ivermectin has had a profound effect on control of onchocerciasis, additional tools are needed, especially macrofilaricidal drugs that kill adult filarid worms wiithout cross reactivity against *Loa loa* microfilariae.
METHODS: Two libraries of compounds have been screened: a compound collection from Anacor Pharmaceuticals Inc., which uniquely incorporates a single boron atom into drug candidate molecules, and FDA-approved drugs that can be repurposed. A screening funnel was designed to most efficiently identify promising leads and allow for rapid attrition of compounds that do not meet target product profile criteria. Because of the close similarities of *Onchocerca* and *Brugia,* we screened the drugs with adult worms of both parasites *in vitro*. Their inhibitory activity was also assayed with *O. volvulus* L3 (molting), and with *O. ochengi* and *L. loa* microfilariae (viability). Confirmatory *in vivo* screening was done using *Brugia* infected gerbils.
RESULTS: Over 2,000 FDA drugs were screened using the “Worminator” and adult female *Brugia* worms; 24 of the compounds inhibited worm motility with IC50s<3μM. Two of these, auranofin (a gold-containing compound used in the treatment of rheumatoid arthritis) and pyrvinium pamoate (an anthelminthic) were found to have potent (nanomolar to picomolar) activity, killing adult worms of *O. ochengi* and inhibiting the molting of *O. volvulus* L3 to the L4 stage. *In vitro* screening of more than 2000 Anacor compounds has focused attention on several novel scaffolds.
CONCLUSIONS: *In vitro* and *in vivo* screens of two non-overlapping compound libraries have identified promising candidates for further development as macrofilaricidal drugs for human use. This work was funded by the Bill & Melinda Gates Foundation.