Role of TLRs on the host CD200 induction by *L. (L.) amazonensis*

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Leishmaniasis is caused by infection with parasite of the genus *Leishmania*, an intracellular protozoan that infects phagocytic host cells. Recent work showed that *L. (L.) amazonensis*, a causative agent of the cutaneous leishmaniasis, is capable of avoiding macrophage responses through the induction of CD200 in the host cell. CD200 is a membrane glycoprotein that, when in contact with its receptor CD200R, inhibits macrophage activation. Toll like receptors (TLRs) play a central role in macrophage activation and control of parasitic infection. TLR-ligand interaction initiates signal transduction through the adaptor proteins MyD88 and/or TRIF, resulting in immune response production. Early studies in other pathogen models showed the link between TLRs and CD200, and the participation of MyD88 in this event. In this context, the aim of this work is to investigate if the immunomodulation of CD200 molecule in macrophages induced by *L. (L.) amazonensis* is TLR-dependent. For this, bone marrow macrophages (BBMs) from wild-type (WT) or MyD88$^{-/-}$ mice were infected with axenic amastigotes of *L. (L.) amazonensis*. Preliminary results showed that the number of intracellular parasites 96 h after infection increased in BMMs from WT mice, while no significant growth was observed in BMMs isolated from MyD88$^{-/-}$ mice. Western blot analysis showed an increase in CD200 protein levels in BBMs from WT and MyD88$^{-/-}$ mice, 1 and 96 h after infection. These results suggest that *L. (L.) amazonensis* induces CD200 in macrophage for a mechanism MyD88-independent. Further experiments will be made to demonstrate the role of TRIF adaptor on the induction of CD200 in macrophages infected by *Leishmania*.

Opisthorchis viverrini secreted exosomes promote cholangiocytes to adopt a tumorigenic phenotype

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BACKGROUND: A combination of processes is thought to drive *Opisthorchis viverrini* infection towards cholangiocarcinoma, including chronic biliary inflammation caused by resident flukes and active release of excretory/secretory proteins by the parasite and their subsequent entry into biliary epithelial cells. However, the molecular mechanisms by which these processes occur remain mostly unknown.
METHODS: We combined cell biology and proteomics (including xCELLigence®) approaches to observe the internalization of *O. viverrini* secreted microvesicles by human cholangiocytes and assess the impact on their uptake on host cell protein expression.
RESULTS: *O. viverrini* adult flukes secrete microvesicles containing proteins that are diagnostic of exosomes, including tetraspanin transmembrane proteins. Internalization of exosomes resulted in cholangiocyte proliferation and secretion of IL-6, and induced major changes in expression of proteins associated with processes such as phagocytosis, wound healing and cancer. We showed that antibodies to a recombinant *O. viverrini* surface tetraspanin blocked the uptake of *O. viverrini* exosomes by cholangiocytes, highlighting a novel potential approach to vaccine development for this chronic infectious cancer.
CONCLUSIONS: These findings are the first to implicate parasitic helminth exosomes in the disease process, and reveal novel molecular mechanisms of immunopathogenesis and tumorigenesis.

Association between antigen-presenting molecules and activation of double-negative T cells in Chagas disease

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BACKGROUND: $CD4^-CD8^-$ (double-negative [DN]) T cells have recently been shown to be important sources of immunoregulatory cytokines in Chagas disease. They express αβ or γδ T-cell receptors that recognize lipid/glycolipid antigens presented via the nonclassical major histocompatibility complex molecules of the CD1 family. In this study, we determined the association between different antigen-presenting molecules expressed on the surface of monocytes and the activation/functional state of DN T cells from individuals with different clinical forms of Chagas disease.
METHODS: Peripheral blood samples from healthy donors and chagasic patients were collected and submitted to *in vitro* infection using *T. cruzi* trypomastigotes. After 18 hours of culture, immunophenotyping of leukocytes was performed by flow cytometry. Expression of the antigen-presenting molecules CD1 family and HLA-DR by monocytes, as well as activation markers and cytokine expression in DN T cells were evaluated.RESULTS: Cells from chagasic patients showed higher intensity of expression of antigen-presenting molecules (CD1 family) by monocytes after *in vitro* infection with trypomastigotes forms of *T. cruzi,* as compared to cells from healthy donors. In the same assay, we evaluated the effect of stimulation on DN T effector cells. Only cells from chagasic patients showed increased frequency in the expression of the activation molecules ($CD69^+$), as well as of the regulatory ($IL\text{-}10^+$) and proinflammatory cytokines ($IFN\text{-}\gamma^+$). Finally, association analysis regarding the data for monocytes and T DN cells from chagasic patients showed that $CD1d^+$ monocytes exhibit positive correlation with $IFN\text{-}\gamma^+$ T (αβ and γδ) cells and negative correlation with DN T cells $IL\text{-}10^+$(γδ).
CONCLUSIONS: This study suggest that during *T. cruzi* infection the effector activity of DN T cells may be associated with antigen recognition via the CD1 family molecules, mainly CD1d. This molecule plays an important role in the presentation of self-antigens and therefore may be related to the pathogenesis of Chagas disease.

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Adverse Pregnancy Outcomes Associated with Malaria and Typhoid co-infection in Pregnant Women attending antenatal Clinics in Anambra State, South- Eastern Nigeria.

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BACKGROUND**:** Malaria in pregnancy has been associated with adverse pregnancy outcomes. Its co-infection with typhoid fever is indeed a double tragedy.
OBJECTIVE**:** The present study was designed to evaluate the pregnancy outcome in malaria and or malaria- typhoid co-infected pregnant women attending antenatal clinics in Anambra State, South Eastern Nigeria.
METHODOLOGY**:** This was a cross sectional study involving 700 pregnant women recruited during routine antenatal visits by voluntary participation between January 2012 and March 2013. Blood samples were collected under aseptic conditions. Malaria infection was determined by immunodiagnostic methods and confirmed by Giemsa staining of thick and thin smears. Typhoid infection was determined by stool culture for Salmonella Typhi. Pregnancy outcomes were obtained from hospital records in the various hospitals used for the study.
RESULTS**:** The result showed that the preterm delivery rate (PTD) was 6.3%, while 6 (0.9%) of the women had spontaneous abortions. Four of the women (0.57%) had intra-uterine deaths (IUDs) while 46 (6.6%) had low birth weight babies (LBW) indicating LBW rate of 6.6%. All the women with these complications were either malaria infected or malaria-typhoid co-infec ted.
CONCLUSION AND RECOMMENDATION**:** Malaria and its co- infection with typhoid are responsible for most of the adverse pregnancy outcomes in the study area. Use of insecticide treated bed nets (ITNs), environmental sanitation and provision of basic amenities including portable water will go a long way to reverse this trend.
KEY WORDS: Malaria, typhoid, co-infection, pregnancy outcome, Southeast, Nigeria.

Multi-component integrated approach for the elimination of schistosomiasis in the Peoples' Republic of China: design and baseline results of a 4-year cluster-randomised intervention trial

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BACKGROUND: Schistosomiasis japonica continues to be a public health problem in the People's Republic of China (P.R. China) despite major success in its control over the past 50 years. Major endemic foci occur in the lakes and marshlands along the Yangtze River, areas where transmission interruption has proven difficult. The current endemic situation may change due to the closure of the Three Gorges Dam. Considerable environmental and ecological changes are anticipated that may result in increased habitats for the intermediate snail host of *Schistosoma japonicum*, increasing the risk of human disease. The current national strategy for P.R. China employs a multi-component integrated approach, but despite targeting multiple transmission pathways, challenges remain as the Chinese government pushes for elimination and there is a need for other tools such as vaccination for long-term prevention. Whereas the zoonotic nature of schistosomiasis japonica adds to the complexity of control, it provides a unique opportunity to develop a transmission blocking bovine vaccine to help prevent human infection and disease. Mathematical modelling has shown that a control approach targeting the various transmission pathways of schistosomiasis japonica and incorporating bovine vaccination, mass human chemotherapy and molluscicide could lead to its elimination from P.R. China.
METHODS AND RESULTS: Here we present the study design (double-blind cluster-randomised controlled trial, using a multi-factorial randomised design) and baseline results of a 4-year intervention trial around the Dongting Lake in Hunan Province to determine the impact on schistosome transmission of multi-component integrated intervention strategies that include bovine vaccination using a heterologous "prime-boost" delivery platform based on the previously tested SjCTPI vaccine. Specific strategies include: 1) human mass chemotherapy + bovine vaccination/placebo; 2) molluscicide + bovine vaccination/placebo; 3) bovine vaccination/placebo. Statistical modeling will be able to quantify the effect of molluscicide + human mass chemotherapy + bovine vaccination. Baseline results show that the characteristics of the selected village pairs and the human and bovine prevalence within pairs were similar, indicating our success in carefully matching the pairs and subsequently reducing confounding.
CONCLUSIONS: The results of the 4-year multi-component intervention trial (comprising: human PZQ treatment, bovine vaccination and molluscicide of *Oncomelania* snails) we are undertaking will provide insight into the feasibility of using a bovine vaccine; and will have important implications for the design of this elimination strategy in P.R. China and possibly serve as a model for global control efforts.

Current perspectives on comparative genomics of three human *Taenia* tapeworms: *Taenia solium*, *T. saginata* and *T. asiatica*

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BACKGROUND: Human infecting *Taenia* tapeworms are *Taenia solium*, *T. saginata* and *T. asiatica* that cause taeniasis in humans and cause cysticercosis in intermediate host animals. Molecular identification of human *Taenia* tapeworms has been reported in aspects of differential diagnosis, epidemiology, geographical distribution, and genetic diversity.
METHODS: Molecular approaches to differential diagnosis of human *Taenia* tapeworms have been developed, including nucleotide sequencing of amplified PCR product of mitochondrial cytochrome c oxidase (cox1), cytochrome b (cob), NADH dehydrogenase subunit 1 (nad1), 12s ribosomal RNA, and nuclear ribosomal RNA (i.e., 18S rRNA, 5.8S rRNA, internal transcribed spacer 2 (ITS2) and 28S rRNA), elongation factor-1-alpha and ezrin/radixin/moesin-like protein (elp). Other molecular diagnostic assays have been also reported in PCR based on sequence-specific DNA probes, PCR-restriction fragment length polymorphisms (PCR-RFLP), Random Amplified Polymorphic DNA analysis (RAPD), Base Excision Sequence Scanning Thymine-base analysis (BESS T-base), multiplex PCR, and Loop-Mediated Isothermal Amplification (LAMP). Molecular methods have been developed for distinguishing *T. asiatica* from other human *Taenia* tapeworms. The complete sequence of the human *Taenia* tapeworm mitochondrial genome was determined and its complete sequence data was available.
RESULTS: The mitochondrial genome of human *Taenia* tapeworms was 13,703 bp (*T. asiatica*), 13,670 bp (*T. sagianta*) and 13,709 bp (*T. solium*) long, containing 36 genes, i.e., 12 protein-coding genes, 22 tRNA genes, and 2 rRNA genes. The overall nucleotide sequence difference in full mitochondria DNA between *T. asiatica* and *T. saginata* was 4.6%, while *T. solium* differed by 11%. The sequence data of nuclear gene was available. The 18 kDa/HP6 protein-coding gene showed a similarity of 95.5% between *T. asiatica* and *T. saginata*, while those of *T. solium* and *T. saginata* was 61.5%. The low genetic diversity between *T. saginata* and *T. asiatica* was identification of hybrids based on the mitochondrial cox1 gene, elp gene and ef1 gene. The mitochondrial/nuclear discordance was observed in China, Japan and Thailand isolates.
CONCLUSION: Molecular diagnostic tools of human *Taenia* tapeworms have been developed for accurate detection and applied for identification of their species.

Molecular approach for Controling schistosomiasis japonica through snail monitoring and evaluation

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BACKGROUND: Snail monitoring and control is one of the key elements in schistosomiasis control. Recent situations of Asian schistosomiasis is facing “disease elimination”, which means the necessity of more sensitive surveillance tools. Various scientific information should be utilized to implement more effective control activities. Application of molecular approaches in surveillance are urgently considered.
METHODS: Application of LAMP method was tested for laboratory and field assessment of snail vectors with/without schistosome infection. Results of LAMP testing were compared with conventional parasitological methods, and tested their applicability in evaluation of snail infection. For the probability of LAMP in field use, we tested various protocols for LAMP testing to get estimated infection rate of field snails. At the same time, lyophilized LAMP reagents were checked to test the applicability even in the poor conditions of cold chain. For laboratory study, LAMP was tested for detection of infected snails even in the early stage of milacidial infection.
RESULTS: Sensitivity of LAMP testing was enough to detect only 1 infected snails in 99 uninfected snails. This means that infection rate is <1% when LAMP testing using 100 field snails was negative. Considering situation of the endemicity of test areas, we can change the number of snails in one LAMP tube. Lyophilized reagents functioned in LAMP testing, indicating that cold chain is not essential at the on-site testing in the local endemic area. LAMP method is also useful in the study of snails infected with S. japonicum miracidia; in the early phase of snail infection, we can chase miracidia invaded to the snails and their survival or death in the vector snails. It was interesting to note that schistosome DNA was not detected if snails killed invading miracidia even 24 hr after the contact. Molecular detection is, thus, enough accurate to make “real-time” evaluation.
CONCLUSIONS: LAMP method is a useful tool to monitor the infected snails in endemic fields, especially of low endemicity situation. It seemed likely that LAMP testing could be done even under poor laboratory condition and availability of cold chain. LAMP was also useful to analyze time-course change of parasites in the intermediate snail hosts. Molecular detection of infected parasites in snail hosts is essential tool applicable for “low endemic conditions” in many endemic foci of Asian schistosomiasis.

Identification of potent and selective inhibitor allows chemical validation of Trypanosomatid 12-subunit respiratory Complex II as drug target.

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BACKGROUND: Respiratory complex II (Succinate-quinone oxidoreductase: SQR) connects TCA cycle and oxidative phosphorylation through electron transfer between succinate and ubiquinone. We have previously reported that the SQR from *Trypanosoma cruzi* (TcSQR) possesses 12 subunits including heterodimeric Ip subunit. Genomic analyses show that all of 12 subunits from TcSQR were only conserved among Trypanosomatid species. The insensitivity of TcSQR to classical SQR inhibitors indicates the structural divergence within the active site. Thus, SQR from Trypanosomatid has great potential to be a drug candidate.
METHODS: The non-pathogenic *Leishmania tarentolae* was chosen since large scale culture was already established, a prerequisite for large-scale purification required for crystallization and screening of inhibitors. The LtSQR was purified, characterized and the used for screening of inhibitors. The identified LtSQR inhibitor was further evaluated against *T. cruzi*, *T. b. brucei*, *L. donovani* and *L. major* at enzyme and cellular levels.
RESULTS: The biochemical property and the subunit composition are conserved among Trypanosomatid species. Screening of inhibitors using LtSQR resulted in the discovery of a potent inhibitor. The Trypanosomatid specific SQR inhibitor did not inhibit NADH and glycerol-3-phosphate dependent respirations. Instead, it specifically inhibited succinate-dependent respiration from *L. tarentolae* mitochondria. This inhibitor also inhibited SQR from *T. brucei*, *T. cruzi* and *L. donovani* with no effect over mammalian SQR, showing a specificity index of 4500-fold over Trypanomatid SQRs. This inhibitor showed mixed-type inhibition versus ubiquinone with K_{i1} of 39 nM. Furthermore, the Trypanosomatid SQR specific inhibitor suppressed not only the growth of *L. tarentolae* promastigote form, but also the intracellular amastigote form of *T. cruzi* and the blood stream form of *T. brucei brucei*.
CONCLUSIONS: Our results represent the first chemical validation of SQR as drug target for Trypanosomatid and provide a specific and potent lead candidate to combat Leishmaniasis, Chagas disease and African Trypanosomiasis.

Validation of housekeeping genes as internal control for differential gene expression studies in *Theileria parva* by real-time PCR

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BACKGROUND: The reliability of any relative real-time PCR experiment can be improved by including an invariant endogenous control (reference gene) in the assay to correct for sample to sample variations in real-time PCR efficiency and errors in sample quantification. Real-time RT-PCR-specific errors in the quantification of mRNA transcripts are easily compounded with any variation in the amount of starting material between the samples, e.g. caused by sample-to-sample variation, variation in RNA integrity, reverse transcription efficiency differences and cDNA sample loading variation. This is especially relevant when the samples have been obtained from different individuals, different tissues and different time courses, and will result in the misinterpretation of the derived expression profile of the target genes. Therefore, normalisation of target gene expression levels must be performed to compensate intra- and inter-kinetic RT-PCR variations (sample-to-sample and run-to-run variations).
METHODS: In this study candidate housekeeping genes for the East Coast fever causing tick-borne parasite *Theileria parva* were validated for expression stability using real time-PCR. The genes are GAPDH, cytochrome b, beta-actin, F6P and 28S rRNA.
RESULTS: The expression of these genes was analyzed between two *Theileria parva* isolates being; *T. parva* muguga and *T. parva* 7014 and their expression varied considerably with GAPDH and beta-actin being the most stable.
CONCLUSIONS: These genes are suitable candidates to be used for normalization of real-time PCR results for gene expression studies in a wide variety of *T. parva* samples.

Detection of anti-*Trypanosoma cruzi* antibodies in mothers and their babies during the first year of life

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BACKGROUND. Chagas disease is caused by *Trypanosoma cruzi* which is endemic in Latin America. Human infection is mainly spread by Triatominae insects, but other routes of transmission are: from mother to child (congenitally), blood transfusion and organ transplantation. The congenital transmission of *T. cruzi* has been poorly studied in Mexico, but has been determined that this form of transmission occurs in 2-12% South America.

METHODS. Anti-T.cruzi antibodies were determined by ELISA-WB in 155 sera samples from mothers and your babies. IFI and Commercial kit was also used to validate efficacy of specific ELISA-excreted Superoxide dismutase (SODe) assay. Sera from babies were collected at 6 and 12th months while mother sample were only obtained after giving birth but also calostrum and umbilical cord samples were simultaneously obtained.

RESULTS. Anti-T. cruzi antibodies were detected in 8 (5.8%) mothers by ELISA-WB, in 7 (4.51%) using IFI and in 1 (0.64%) by commercial kit. Nine (5.8%) 6-month children were positive in ELISA-WB, 7 (4.51%) by IFI and no one when de commercial kit was used. At 12 month of age, 15 (9.67%) children by ELISA-WB, 13 (8.38%) by IFI and 1 (0.64%) were positive. Antibodies were detected in 5 children since 6-month through 12-month of age without positive values in the mothers, suggesting an early exposure to pathogen. Also, antibody-levels were detected in 4 mothers, however, their children gave negative results during the study. In other 4 samples, positive results were observed both in the mother and the children, thus assuming vertical transmission.

CONCLUSIONS. The use of SODe as antigen in serological tests for detection of antibodies to T. cruzi suggest promising results as diagnostic procedure. Also, evidence of early exposure to the parasite is shown as well as the probably vertical transmission of the infection by congenital route.

Helminth parasites associated with 21 species of Leptodactylidae amphibians (ANURA) in the Brazilian Midwest.

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BACKGROUND: The comprehension of how the species and what species are parts of an ecosystem is one of the most important issues of ecology. Such questions can be answered through surveys of diversity in macroecological scales. The objective of this study is to characterize the component helminth parasite communities associated with the amphibians from four ecosystems in the Brazilian Midwest.
METHODS: We studied the helminth fauna of 668 frogs belonging to 21 leptodactylid species collected from 1987 to 2010, in Mato Grosso State, Brazil, in four different ecosystems: Tropical Rainforest, Savannah, Hyper seasonal Savanna in the Brazilian Pantanal Wetlands and transition areas between the first two ecosystems.
RESULTS: A total of 535 (80%) amphibians were parasitized and 31,580 helminths were found, representing eight superfamilies, 16 families, 21 genera, and 40 species. The following helminths were recovered: 3,124 nematodes (*Brevimulticaecum* sp., *Aplectana* sp., *A. crossodactyli*, *A. pintoi*, *A. crucifer*, *Cosmocerca brasiliensis*, *C. parva*, *C. podicipinus*, *Falcaustra mascula*, Cosmocercidae gen. sp., *Oswaldocruzia* sp., *O. lopesi, O. mazzai*, *Oxyascaris oxyascaris, Ochoterenella digicauda*, *Physaloptera retusa*, *Physaloptera* sp. *Physalopteroides venancioi*, *Pteroxyascaris caudacutus*, *Rallietnema minor*, *R. spectrans*, *Rhabdias* sp., *Schrankiana* sp., *S. formosula*, *S. freitasi*, *S. schranki*, *Schrankianella brasili*, and unidentified larvae and cysts of nematode); 463 trematodes (*Catadiscus marinholutzi*, *C. propinquus*, *Glyphtelmins* sp., *Gorgoderina parvicata*, *Mesocoelium monas* and *Plagiorchis* sp.); 10 monogenean (*Polystoma cuvieri*, *P. naponensi* and *Polystomatidae* gen. sp.); two cestodes (*Ophiotaenia ecuadoriensis* and unidentified fam. gen. sp.); and 66 cystacanths of Acanthocephala.
CONCLUSIONS: The results enabled a new host record for South America, an occurrence record and four new records for Brazil, 103 new host records and 25 new records for Mato Grosso State, widening the distribution from type locality of seven helminth species.

Ticks of dogs: from ecology to disease

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Dogs are domestic animals with a great impact on human health and welfare but also with a unique ecology. Their close contact with humans, their sporadic or continuous roaming behavior, the great spectrum of zoonotic pathogens they carry, as well as the lack of constant veterinary care mostly in free-roaming pets and ownerless animals confer dogs an important epidemiological sentinel species status. The ecology of tick communities found on dogs and the interspecific associations are significantly dependent on the geographical area and are related to climatic factors and habitat type. In Europe, around 20 species of ticks have been reported from dogs, but only few of them seem to be more common. In central parts of Europe, the most common species infecting dogs are *Ixodes ricinus* and *Dermacentor marginatus*, while in the Mediterranean countries *Rhipicephalus sanguineus* s.l. is the dominant species. Dogs from northern Europe carry mainly *I. ricinus* and *I. hexagonus*. In North America eight species of ticks are particularly prevalent on dogs: *Dermacentor variabilis*, *D. andersoni*, *Amblyomma americanum*, *A. maculatum*, *Ixodes scapularis*, *I. dammini*, *I. pacificus* and *R. sanguineus* s.l. South American dogs are infected principally with *R. sanguineus* but also *Amblyomma cajannense* or *A. ovale*. Predominant ticks of dogs in sub-Saharan Africa include *R. sanguineus* s.l., *R. pulchellus* and *Haemaphysalis leachi*. In the case of certain tick species (i.e. *R. sanguineus*), despite their extensive or almost worldwide distribution, the spectrum of associated tick-borne pathogens is rather different geographically. This may be an indication for a multispecies structure of certain groups of ticks or a complex ecology of the tick-borne pathogen and its circulation between different natural reservoir hosts. In the case of other pathogen-tick associations, genotype-based epidemiological studies (i.e. *Anaplasma* spp.) are required for a better understanding of their ecology and codistribution with the vectors.

Ascofuranone, a specific and potent inhibitor of cyanide-insensitive alternative oxidase of *Trypanosoma brucei*

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BACKGROUND: The cyanide-insensitive respiration in plants has long been recognized since 1920s. Intensive biochemical studies revealed that the mitochondrial membrane enzyme, alternative oxidase (AOX), is responsible for the cyanide-insensitive respiration. AOX, which is cyanide-insensitive and salicyl hydroxamic acid (SHAM)-sensitive, is a non-proton-pumping ubiquinol oxidase catalyzing the 4-electron reduction of dioxygen to water. AOX has been found in higher plants, algae, yeast, slime molds, free-living amoebae, eubacteria and nematodes, as well as protozoan including trypanosomes.
T. brucei, which causes African sleeping sickness in human and Nagana in livestock, those are serious health and economic problem in sub-Saharan Africa had been known to show the cyanide-insensitive respiration. For this parasite cyanide-insensitive respiration, the trypanosome alternative oxidase (TAO) functions in the African trypanosomes as a cytochrome-independent terminal oxidase, which is essential for their survival in the mammalian host. TAO has been thought as a good target for the anti-trypanosomal drugs because mammalian hosts do not possess this protein.
METHODS: we found that the ascofuranone, isolated from pathogenic fungus, specifically inhibits the quinol oxidase activity of TAO and rapidly kills the parasites. In addition, we have confirmed the chemotherapeutic efficacy of ascofuranone *in vivo*.
RESULTS: IC_{50} of ascofuranone for quinol oxidase activity of recombinant enzyme was 0.13 nM. Structure activity relationship analysis revealed the essential structure in the ascofuranone for its potent inhibition of quinol oxidase activity. Furthermore, our first 3D structure study provided a conclusive information of inhibitor-binding mechanism.
CONCLUSIONS: The result mentioned here leads to a rational design of more potent and safe anti-trypanosomal drugs. In fact, at least, 3 completely synthetic derivatives of ascofuranone cured the infected mice.

Hormonal and behavioral changes induced by acute and chronic experimental infestation with *Psoroptes cuniculi* in the domestic rabbit *Oryctolagus cuniculus*

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BACKGROUND: Parasitic diseases are important in animal production because they cause high economic losses. Affected animals often exhibit stereotypical behavioral alterations such as anorexia and inactivity. Among the diseases that commonly affect domestic rabbits is mange, caused by the mite *Psoroptes cuniculi*. Therefore, within the context of the host-parasite relationship, understanding the mechanisms involved in the alteration of host behavior is critical in order to better utilize sick animal behavior as a strategy for diagnosis and treatment of disease.
METHODS: Rabbits were infested placing mites in the ear conduct. We characterized changes in exploratory behavior and scent marking evoked by acute and chronic experimental infestation. Locomotor activity, chinning and rearing behavior were recorded during ten minutes in a 120 cm × 120 cm open field arena. Serum cortisol was measured individually using radioimmunoassay kits.
RESULTS: We observed a significant decrease in rearing behavior as early as two days post-infestation, while chinning and locomotor activity were significantly decreased four days post-infestation. Chronic infestation was associated with decreased food intake, significant weight loss, and a trend toward increased serum cortisol levels, while no changes were observed in body temperature.
CONCLUSIONS: The presence of visible lesions within the ear canal is commonly used to detect mite infestation in rabbits, but this is possible only after chronic infestation. The behaviors described here can be a useful and economic tool in guiding the early diagnosis of parasitic infestation by *Psoroptes cuniculi*, allowing for early treatment and the application of control measures before significant weight loss occurs, thereby avoiding economic losses.

In vitro ovicidal and cestocidal effects of toxins from Bacillus thuringiensis on the canine and human parasite Dipylidium caninum

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BACKGROUND: *Bacillus thuringiensis* (*B. thuringiensis)* is a gram-positive soil-dwelling bacterium that is commonly used as a biological pesticide. This bacterium may also be used for biological control of helminth parasites in domestic animals. In this study, we evaluated the possible ovicidal and cestocidal effects of a total protein extract of B. thuringiensis native strains on the zoonotic cestode parasite of dogs, *Dipylidium caninum* (*D. caninum*).
METHODS: Dose and time response curves were determined by coincubating *B. thuringiensis* proteins at concentration ranging from 100 to 1000 μg/mL along with 4000 egg capsules of *D. caninum*. Egg viability was evaluated using the trypan blue exclusion test. For morphologycal studies the parasite was processed according to the paraffin embedded tissue technique, and histological sections stained to observe the general histological structure and measure the thickness of the integument.
RESULTS: The lethal concentration of toxins on eggs was 600 μg/mL, and the best incubation time to produce this effect was 3 h. In the adult stage, the motility and the thickness of the tegument were used as indicators of damage. The motility was inhibited by 100% after 8 hours of culture compared to the control group, while the thickness of the cestode was reduced by 34%.
CONCLUSIONS: Conclusively, proteins of the strain GP526 of B. thuringiensis directly act upon *D. caninum* showing ovicidal and cestocidal effects. Thus, B. thuringiensis is proposed as a potential biological control agent against this zoonosis.

In vitro acaricidal effect and histological damage of *Bacillus thuringiensis* on the mite *Psorotes cuniculi*.

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BACKGROUND: The mite *Psoroptes cuniculi* (P*. cuniculi*) is a common worldwide parasite and the most frequently found in rabbit farms. It causes significant losses associated with poor leather quality, reduced conception rates, weight loss, poor growth and death. Several strategies have been proposed for the treatment of mange caused by this mite, ranging from the use of environmental acaricides, entomopathogenic fungi and essential oils, to vaccines, however, therapy and control of both human scabies and animal mange are still based mainly on the use of drugs and chemicals, mainly Ivermectin, which involves disadvantages including genotoxic and cytotoxic effects, drug resistance and environmental damage.
METHODS: Dose and time response curves were determined by coincubating B. thuringiensis proteins at concentration ranging from 100 to 1500 μg/mL along with mites *P. cuniculi*. Mortality was analyzed and quantified microscopically at different times, taking as dead mites those with persistent immobility and lacking of reaction to stimulation with a brush for 15 seconds. For histological analysis the mites were fixed in 10% formalin and processed according to the paraffin embedded tissue technique.
RESULTS: The strain 532 of *Bacillus thuringiensis* has an *in vitro* acaricidal effect on the mite *P. cuniculi*, with an LC50 of 1.3 mg/ml and an LT50 of 68 hrs. The histological damage caused by B. thuringiensis on this mite includes diffuse perforations in the peripheral matrix and morphological alterations in columnar cells of the intestine. In ivermectin treated mites we did not observe the changes induced by B. thuringiensis, and opposite than expected, in the mite synganglion we did not observe alterations.
CONCLUSIONS: Since this mite is an obligate ectoparasite that affects rabbits, goats, horses, cows and sheep, *Bacillus thuringiensis* is proposed as a potential treatment for biological control of mange in productive species.

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

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BACKGROUND: Schistosomiasis is an important public health problem, with high morbidity and mortality in endemic countries. This study analyzes the epidemiological characteristics and time trends of schistosomiasis-related mortality in Brazil.
METHODS: We performed a nationwide study based on official mortality data obtained from the Brazilian Mortality Information System. We included all deaths in Brazil between 2000 and 2011, in which schistosomiasis (ICD-10: B65) was mentioned on the death certificate as underlying or associated cause of death (so-called multiple causes of death). We calculated crude and age-adjusted mortality rates (per 100,000 inhabitants), and proportional mortality rates. Trends over time were assessed using Joinpoint regression.
RESULTS: Over the 12-year study period, 12,491,280 deaths were recorded in Brazil. Schistosomiasis was mentioned in 8,756 deaths (0.07%), 6,319 (72.2%) as underlying cause and 2,437 (27.8%) as associated cause. Average annual age-adjusted mortality rate was 0.49 deaths/100,000 inhabitants. Mortality rates were highest in males (0.53 deaths/100,000 inhabitants), ≥70 years-old (3.41 deaths/100,000 inhabitants), brown race/color (0.44 deaths/100,000 inhabitants), and residents in the Northeast region (1.19 deaths/100,000 inhabitants). Age-adjusted mortality rates showed a significant decrease in the country (Annual Percent Change [APC]: -2.8%; 95% confidence intervals [CI]: -4.2 to -2.4) over the entire period. We observed decreasing mortality rates in the Northeast (APC: -2.5%; 95% CI: -4.2 to -0.8), Southeast (APC: -2.2%; 95% CI: -3.6 to -0.9), and Central-West (APC: -7.9%; 95% CI: -11.3 to -4.3) regions, while the rates maintained stable in North and South regions.
CONCLUSIONS: Despite of the reduced mortality, schistosomiasis is still an important but neglected cause of death in Brazil, with considerable regional differences. Sustainable control measures should focus on increased coverage, and intensified and tailor controlled actions, to prevent the occurrence of severe forms and deaths.

Characterization of the role of the PDI gene in the invasion process of *Toxoplasma gondii*

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BACKGROUND: Protein Disulphide Isomerase (PDI) is one of the proteins of *Toxoplasma gondii* apparently involved in the initial phase of the host cell invasion. In *T. gondii* the PDI protein was identified as the major antigen recognized by IgA antibodies in human tears. In *Neospora caninum* it was identified as involved in the interaction with the host cell. The aim of this study was to identify if the PDI promotes invasion and if has any effects on the invasion and intracellular division of *T. gondii* within Müller cells.
METHODS: RNA isolation of *T. gondii* was performed to amplify a Tg-pdi transcript to generate expression vectors that produce a purified recombinant PDI (rPDI). Müller cells were infected with *T. gondii* (RH strain) tachyzoites synchronized in G1 phase with PDTC and exposed to rPDI. Percentage of invasion was determined by flow cytometry and the number of vacuoles per cell was determined by fluorescence microscopy.
RESULTS: PDI protein was cloned and purified successfully in a concentration of 120 µg/ml. We found a mean of parasites per vacuole of 140±15 in PDI treated vs. 92±17 in control cultured wells ($P < 0.001$). Percentage of invasion was higher (8.4%) in parasites incubated with rPDI compared to unstimulated parasites (4.3%) after 1 hour of infection.
CONCLUSIONS: Recombinant PDI facilitates invasion and intracellular division of *T. gondii* in Müller cells when it is added to a cell culture.

A strategy for accelerated vaccine development against Chagas disease

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BACKGROUND. Chagas disease is caused by the protozoan parasite Trypanosoma cruzi and affects 8-10 million people in Latin America and elsewhere. Current drug treatments have limited efficacy for patients with indeterminate (seropositive patients without signs or symptoms of disease) and symptomatic (seropositive patients with clinical disease) status. As an alternative (or complement) to chemotherapy, an injectable therapeutic Chagas disease vaccine is under development to prevent or at least delay Chagasic cardiomyopathy in patients with indeterminate or symptomatic status.
METHODS. The bivalent vaccine is based on two recombinant *T. cruzi* antigens, Tc24 and TSA-1, formulated with a Th1 adjuvant such as E2060 or MPLA. These antigens have been found effective in pre-clinical studies for both the prevention and therapy of T. cruzi infection in mouse and dog models. We have formed a Product Development Partnership with the nonprofit Sabin Vaccine Institute, Texas Children's Hospital Center for Vaccine Development in collaboration with an international consortium of academic and industrial partners in Mexico, Germany, Japan, and the USA, to develop this vaccine.
RESULTS. Production and process development of the two recombinant antigens is underway, as well as the pre-clinical evaluation of formulations in mice. Technology transfer is also been prepared together with initial economic modeling.
CONCLUSION. We expect that this initiative will contribute to the accelerated development of a Chagas disease vaccine and lead to an improved control of the disease for patients.

Status of Canine Babesiosis in domestic dogs in three localities of the Ecuadorian coast.

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BACKGROUND: Canine babesiosis is a disease of cosmopolitan type, being very common to find in daily veterinary practice, as one of the most frequent causes of death in dogs. An analysis of the situation of canine babesiosis in domestic dogs living in three regions of the Ecuadorian coast was performed, the city of Guayaquil, the rural parish El Morro (Guayas province) and the city of La Libertad (Santa Elena province).
METHODS: In total 980 canine´s blood samples was analyzed, 260 from dogs living in El Morro, 400 in the city of Guayaquil and 320 of La Libertad City. The thin blood smear was stained with Giemsa, were processed in the laboratory of Microbiology, of veterinarian faculty of Agrarian University of Ecuador from July to October 2012.
RESULTS: We found that in El Morro 18% of the samples were infected with parasite *Babesia sp*, with 58% in the city of Guayaquil, and 47.19% in La Libertad. Clinical symptoms were detected in 65.22% of the positive cases in El Morro, 43.10% in Guayaquil city, and 60.26% in La Libertad. According to age group, 48.25% of positive dogs were in the range of 1-3 years. There was no statistical significance according to the sex of the animal.
CONCLUSIONS: A high rate of domestic dogs living in the Ecuadorian coast has canine babesiosis, being more susceptible in the range of 1-3 years of age.

Seroprevalence of *Toxoplasma gondii* in a poor rural community from Yucatan, Mexico

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BACKGROUND: The parasite Toxoplasma gondii (*T.gondii*) affects more than two-thirds of the world population. This parasite is widely distributed across the planet and the infection is usually asymptomatic. In Mexico, the parasite is widely present, particularly in tropical areas where have been described high frequencies of infection in different population groups as well rural as urban areas. The aim of this study was to determine the seroprevalence of specific IgG-antibodies against *T.gondii* in one village from Yucatan with a low Human Development Index (term used to measure the level of development, wealth etc)
METHODS: A random sample of 360 people who attended the health center in the village and hence for obtaining blood samples were collected serum was performed. These samples were subsequently evaluated in duplicate by indirect semi-cuantitative ELISA (ToxoIgG, Human).
RESULTS: It was determined that 87.3% of participants had IgG antibodies vs. *T.gondii*, 10% were negative and 2.7% were indeterminate cases (gray zone), 6.06% positive of cases had high antibody concentrations (in IU/mL), the average concentration of 88.22 % of the same and only 5.7% of positive showed a low concentration of specific antibodies against the parasite.Several reports suggest that a high concentration of antibodies indicates an active infection, when it is just expected that about 1 % of the population could be in this condition. No association between seropositivity and risk factors evaluated were found (by univariate analysis) but stressed that households with overcrowding, rodents and where no pets deworm, higher frequency of antibodies (*p* 0.06) was recorded.
CONCLUSION: The results described suggest that in the town there is a high circulation of the agent, so it is necessary to implement specific control measures in this population in order to interrupt the transmission dynamics of the causative agent.

Molecular frequency of *Sarcocystis* spp and other Sarcocystidae coccidian in rodents and marsupials in two Brazilian biomes.

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BACKGROUND: Recent studies have demonstrated the importance of wildlife in the epidemiology of coccidian parasites in Brazil, however the approach of the studies has been an epidemiological unidirectional focus (host-parasite). The parasitic cycle in the wild is much more complex and requires new approaches that allow inferences about the biological and ecological factors.
METHODS: The eco-epidemiology of Sarcocystidae coccidian parasites among different trophic levels of the food chain, specifically inside the taxon of small non-flying mammals (rodents and marsupials) and carnivorous predators of two different biomes of the state of Mato Grosso were evaluated by molecular methods (amplification of 18S locus).
RESULTS: DNA was extracted from 332 animals of the Amazon Legal biome (AmazB) and 117 of the Pantanal biome (PantB). In AmazB, the molecular detection of coccidia Sarcocystidae was obtained in 33.1% (110/332), corresponding to 11.6% of tissues examined (110/945). A total of 51 marsupials and 66 rodents were collected in PantB with positivity of 21.4% (25/117) of the animals and 7.5% (25/333) of the tissues sampled. In 30 of carnivorous mammals (18), primate (6) and xenarthra (6) orders, molecular amplification was 80% (24/30).
CONCLUSIONS: No significant differences in frequency of positive animals of the order Rodentia (29.4% AmazB, 28.0% PantB) and Didelphimorphia (37.5% AmazB, 52.0% PantB) were established. Noteworthy is the detection of *Toxoplasma gondii* in samples of skeletal muscle in *Cerdocyon thous* (Crab-eating Fox) and *Sapajus* sp. (Large-headed Capuchin), corresponding to the first records for these species in Brazil.

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Fasciola hepatica: Experimental exposure to miracidia modifies the life cycle of *Lymnaea (Fossaria) humilis*

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BACKGROUND. *Lymnaea* (*Fossaria)* are amphibian snails living near freshwater, with worldwide distribution. Forty-five species have been identified as intermediate hosts of *Fasciola hepatica*, an important parasite of cattle and sheep, and even humans, which produces economic losses. The aim of this work was to obtain snail cultures of *L. humilis* to compare growth rate and reproductive capacity among different lots and generations. Also, the effect of exposure to miracidia or infection by *F. hepatica* upon the mollusk life cycle was analyzed.
METHODS. Two field collections (August and November) of *L. humilis* were cultured on Petri dishes containing sterilized mud, *Oscillatoria* spp algae, commercial fish food, and calcium carbonate. Six to twelve uninfected young adults of each collection/generation were exposed to three, four or five miracidia each and life cycle parameters were determined again.
RESULTS. Nineteen and 15 generations of snails were obtained from the first and second collections, respectively. Snail growth rate and reproductive capacity were homogeneous among collections and generations. Exposure to miracidia without actual infection induced survival decrease, while infection caused dwarfism and significant decrease in the reproductive capacity. An inverse relation between number of parasites used to challenge and harvested metacercariae was observed.
CONCLUSIONS. Exposure of *L. humilis* to *F. hepatica* miracidia caused damage to an extent that it increased death probability; actual infection prevented it, but decreased shell growth and fertility.

Reactive oxygen and chlorine intermediates regulate the immune response and promote wound healing in cutaneous leishmaniasis

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BACKGROUND: Reactive oxygen intermediates (ROI) are known for their antileishmanial and immunoregulatory activities. We have recently shown that a preparation of pharmaceutical sodium chlorite (DAC N-055), which can give rise to a diverse set of chlorine-containing ROI, exerts a leishmaniostatic effect and promotes the healing of chronic cutaneous leishmaniasis caused by *Leishmania (L.) tropica* or *L. major* (Jebran et al., PloS Negl Trop Dis 2014; Stahl et al., submitted). We hypothesized that part of the effect of DAC N-055 results from the modulation of the antileishmanial immune response. Thus, we analysed the effects of DAC N-055 on both mouse and human immune cells.
METHODS: Mouse peritoneal exudate and bone marrow-derived macrophages, dendritic cells and $CD4^+$ or $CD8^+$ T cells as well as human peripheral blood mononuclear cells (PBMC) and monocytes were exposed to various cytokine stimuli or anti-CD3 in the absence or presence of non-toxic concentrations of DAC N-055. mRNA expression was evaluated by TaqMan PCR and by microarrays using Affymetrix Gene Chips 2.0. Cytokine levels were determined by ELISA, nitrite content was measured using the Griess assay and cell proliferation was assessed by CFSE labeling.
RESULTS: DAC N-055 enhanced the production of pro- and anti-inflammatory cytokines, the expression of type 2 NO synthase, and the generation of type I interferons by myeloid cells, which involved MAP kinase signaling pathways. DAC N-055 inhibited the IL-2 receptor expression on T cells, the production of IL-2 and the proliferation of T cells. The microarray analysis revealed a gene expresssion pattern compatible with a wound healing signature.
CONCLUSIONS: Our findings identify DAC N-055 as a potent immunomodulatory compound. Part of its wound healing effect is likely to result from switching on host-protective immune responses.

Morphological and genetic identification of nematodes parasites of the Argentine goatfish, *Mullus argentinae* (Perciformes, Mullidae) from Rio de Janeiro, Brazil.

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BACKGROUND: The Argentine goatfish, *Mullus argentinae*, is a small benthic fish distributed from Brazilian southeastern coast to northern Argentina. In Brazil, especially in the Rio de Janeiro state, is widely appreciated by consumers. Knowledge about fish parasite composition is important to determine impacts on fish's health, contributes with biodiversity information and alerts for zoonotic parasites that may cause human diseases. Until present, few studies aim to elucidate the composition and structure of parasite communities of *M. argentinae* and genetic characterization of its parasites was never done. The aim of this study was to investigate and characterize morphologically and genetically nematodes infecting the Argentine goatfish from the littoral of Rio de Janeiro, Brazil.
METHODS: Fishes (n=60) were collected during the summer and winter of 2013 in São Pedro Fish Market, Niteroi municipality, Rio de Janeiro state. Fishes were measured, weighed, necropsied and their nematode parasites investigated. Morphological analysis was performed according to taxonomic keys. DNA extraction was performed using the middle sections of 21 specimens by QIAamp® DNA Mini Kit (Qiagen). PCR reactions targeting the 18S rDNA and *cox*2 genes were done. Amplicons were directly sequenced and analyzed with Blast/NCBI command. Neighbor-Joining phylogenetic trees were constructed using MEGA 5 software, Kimura-2-parameter model and 1000 bootstrap replicates.
RESULTS: Nematodes (n=150) were found infecting intestine, mesentery, stomach, liver, lungs and/or muscle. The morphological analysis identified 146 *Hysterothylacium* sp. and 4 *Procamallanus* sp. The genetic analysis revealed species identification as *Hysterothylacium deadorffoverstreetorum* and *Procamallanus* (*Spirocamallanus*) *istiblenni*.
CONCLUSIONS: Because of *Hysterothylacium* sp. and *Procamallanus* sp. have been reported as causing lesions in fishes of high commercial value, and also, allergenic reactivity in *Hysterothylacium* sp. has been described, our results reinforce the importance of monitoring and surveillance of fish markets and highlights the usefulness of an integrative taxonomic approach, which provides a robust species identification.

Molecular epidemiology of cryptosporidiosis in cattle and other food animals

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Cryptosporidium spp. is an enteric protozoan parasite that infects a wide range of vertebrate hosts including humans. Cryptosporidial infection is known now as one of the most common causes of diarrhea in humans and livestock. Worldwide prevalence studies indicate that livestock has a high prevalence of *Cryptosporidium* infection. *Cryptosporidium* is a potential zoonotic pathogen and contact with animals, manure, or contaminated water and food is believed to lead to human infections. The epidemiology of cryptosporidiosis has received significant attention because of the public health and economic importance of this disease in humans and animals. Early studies using traditional diagnostic and epidemiological tools focused mostly on the prevalence, infection patterns, and risk factors. However, traditional microscopy techniques are insufficient for identifying species and/or genotypes of *Cryptosporidium*. Advances in molecular techniques have provided a basis for most recent studies for detection and characterization of *Cryptosporidium* at species, genotype, and subtype levels. Currently, with the help of molecular tools a large number of species and genotypes have been described and molecular techniques have proven to be essential for the detection and epidemiological tracking of *Cryptosporidium* improving our understanding of the transmission of this parasite in human and animals. The need to precisely identify *Cryptosporidium* as well as the risk of zoonotic transmission of *Cryptosporidium* will be discussed in greater detail.

Use of epimastigote secretion/excretion antigens (ESEA) from *Trypanosoma cruzi* in a Western blot assay for the diagnosis of Chagas disease

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BACKGROUND: Epimastigote secreted/excreted antigens of *T. cruzi* (ESEA) are released in axenic culture. These antigens are inexpensive and technically simple to produce therefore represents an alternative for the diagnosis of Chagas disease in laboratories with low resources in endemic areas. In this study, we developed a Western blot assay using ESEA from *T. cruzi* and compared the protein profiles and the immunogenicity of these antigens using different isolates of the parasite.
METHODS: A total of 155 serum samples with positive conventional serology (indirect immunofluorescence, indirect haemagglutination and ELISA) for *T. cruzi*, 148 serum samples from healthy individuals and 50 sera from patients with different parasitic diseases were used to evaluate the ESEA-based Western blot. *T. cruzi*-ESEA (RG1 isolate) was obtained in LIT medium. SDS-PAGE was performed to compare the protein profile among seven isolates of *T. cruzi* and Western blot assay to compare the antigenic recognition of individuals in different phases of chronic Chagas disease.
RESULTS: The test had an excellent sensitivity (100%), predictive negative value (100%), acceptable specificity (93.9%) and positive predictive value (95%). Cross-reactivity was observed in sera from subjects with *Leishmania* infections. The protein profile of *T. cruzi*-ESEA was similar among the seven isolates. The sera of all individuals with chronic Chagas disease in phase III recognized a band at 40 kDa.

CONCLUSIONS: These data suggest that a standardized Western blot based on ESEA antigens represents an excellent alternative for the diagnosis of Chagas disease. The ESEA proteins are conserved among isolates.This represents an advantage for the diagnosis of *T. cruzi* infection. The 40 kDa band detected for the sera of the chronic chagasic patients may be a clinical biomarker that can be used as a risk predictor.

Fish population studies using parasites from the Southeastern Pacific Ocean: considering temporal sources of variability

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BACKGROUND: The Humboldt Current Large Marine Ecosystem is one of the most important upwelling systems, with high rates of primary supporting some of the most massive fisheries worldwide. Large scale fisheries in Southeastern Pacific, especially in Chile, began in the 60`s. Since then, all major “resources” and probably long before had drastic increases and decreases in abundance, as reflected by the landings of anchovy, jack mackerel, sardine and hakes. Studies on parasites of marine fish have increasingly been incorporated into the normal protocols of fish population studies.
METHODS: We summarize studies using parasites as tools for fish population studies in the South Eastern Pacific, whether or not of economic importance and discuss on the importance of considering the compositional and aggregated variability in time and space of hosts and parasite communities
RESULTS: We summarized research using parasites in fish population studies. Those taken into account compare parasite prevalence, abundance, morphology, reproduction or assemblage composition between sampling localities and/or between sampling times. There are ca. 30 such studies and most are on economically important fish species though others on coastal and intertidal fish, or on less or non-commercial species, provide insights on scales of temporal and spatial variation of parasite infracommunities.
CONCLUSIONS: This case-by-case review shows that there has been a persistent effort to use parasites in fish population studies in SEP. Six out of the top ten Chilean fisheries, have been studied on its stock structure and/or migration. Main aspects considered were sampling site, a less frequent combination of sampling in time and space, and a lower frequency of those dealing with variability along time. Thus, we emphasize on the need for more long-term studies that will permit us to see the variability of these biological tags along time, such as in the jack mackerel.

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Effect of cholesterol accumulation in the *Entamoeba histolytica* virulence: possible participation of EhNPC1 protein

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BACKGROUND: Amoebiasis, caused by *Entamoeba histolytica,* is a disease with a high incidence in developing countries. *E. histolytica* trophozoites do not synthesize cholesterol, but is a fundamental compound for the parasite virulence. It is taken from the serum added to the culture medium or from the blood during trophozoites infection. The intracellular traffic of cholesterol in this parasite remains still unknown. In mammal cells, the U18666A, a cholesterol agonist has been employed to study cholesterol traffic. The target of U18666A and cholesterol is the NPC1 protein, involved in cholesterol transport. The aim of this work was to study the cholesterol traffic and the effect of its accumulation on *E. histolytica* virulence.

METHODS: Trophozoites were incubated for different times in serum-depleted medium with the U18666A and then, analyzed through confocal microscopy, using filipin to mark cholesterol. We evaluated their adherence efficiency, their rate of erythrophagocytosis and their cytopathic and cytotoxic effect on MDCK cells. By *in silico* analysis identified the *ehnpc1* gene and its expression was determined by RT-PCR, western blot and immnunofluorescence experiments, using a specific antibody that we produced.

RESULTS: Trophozoites treated with U18666A accumulated cholesterol in cytoplasmic vesicles. These trophozoites presented a higher rate of erythrophagocytosis, but similar adherence efficiency than untreated trophozoites, in a time dependent manner. They also produced more damage to MDCK monolayers, in comparison to control trophozoites. The *E. histolytica* genome has one *ehnpc1* gene and its amino acid sequence present one sterol-sensing domain, characteristic of the mammalian NPC1 proteins. The *Ehnpc1* mRNA was down-regulated in trophozoites where the cholesterol was accumulated.

CONCLUSIONS: In *E. histolytica*, the intercellular traffic of cholesterol is relevant for virulence processes and the EhNPC1 protein participates as a candidate to transport cholesterol.

Four Cases of *Taenia saginata* Infection in Korea with an Analysis of *COX1* Gene

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BACKGROUND: Human taeniases had been not uncommon in the Republic of Korea (= Korea) until the 1980s. The prevalence decreased and a national survey in 2004 revealed no *Taenia* egg positive cases. However, a subsequent national survey in 2012 showed 0.04% (10 cases) prevalence of *Taenia* spp. eggs suggesting its resurgence in Korea. We recently encountered 4 cases of *Taenia saginata* infection who had symptoms of taeniasis that included discharge of proglottids.
METHODS: We obtained several proglottids from each case. Because the morphological features of *T. saginata* are almost indistinguishable from those of *Taenia asiatica,* molecular analyses using the PCR-RFLP and DNA sequencing of the cytochrome *c* oxidase subunit 1 (*cox1*) were performed to identify the species.
RESULTS: The PCR-RFLP patterns of all of the 4 specimens were consistent with *T. saginata,* and the *cox1* gene sequence showed 99.8-100% identity with that of *T. saginata* reported previously from Korea, Japan, China, and Cambodia. All of the 4 patients had the history of travel abroad but its relation with contracting taeniasis was unclear.
CONCLUSION: Our findings may suggest resurgence of *T. saginata* infection among people in Korea.

Intestinal parasitism in pre Columbian people from Áspero, Perú (3000 - 1800 BC)

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BACKGROUND: The archaeological site of Áspero is located in the coast of the Barranca province, Department of Lima, Peru. During the Late Archaic Period (3000 - 1800 BC), people from Áspero developed their fishing activity, which allowed for economic exchanges amongst the towns of Caral Civilization. Ceremonial pyramids, residential buildings, coprolites, and remains of fishing tools have been found in the site. The present study was performed in order to detect and identify parasitic forms in coprolites of pre Columbian inhabitants of the Archaeological Site of Áspero.
METHODS: 20 human coprolites from Áspero were rehydrated, employing aqueous solution of trisodium phosphate 0.5%. Direct examination and the technique of spontaneous sedimentation in tube were used for parasitological examination. Microscopic identification was based on morphometric patterns observed.
RESULTS: The total frequency of intestinal parasites in human coprolites of Áspero was 20%. We identified vacuolar forms of *Blastocystis* sp. (15%), *Entamoeba coli* cysts (5%), *Entamoeba* sp. cysts (5%) and eggs of *Enterobius vermicularis* (5%).
CONCLUSIONS: These findings represent a valuable contribution to paleoparasitological studies in Peru. Based on the results, we can infer that pre Columbian population of Áspero was afflicted by intestinal parasitism as enterobiosis and blastocystosis. There might have been overcrowding and poor hygiene conditions, which could have facilitated the maintenance and dissemination of these intestinal infections.

Helminth fauna of *Merluccius gayi peruanus***, from the Peruvian sea**

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BACKGROUND: *Merluccius gayi peruanus,* commonly known as "Peruvian hake", is the most abundant and economically important species in the trawl fishery in Peru. Its common presence in the eastern Pacific goes from 0° 30 'S (Ecuador) to 10° 00' S (Peru), varying according to seasonal and interannual fluctuations in Cromwell Flow. This study aims to contribute to the knowledge of Helminth fauna in *Merluccius gayi peruanus.*
METHODS: 30 specimens of *Merluccius gayi peruanus* were collected in the Fishing Terminal of Ventanilla, Callao, between October and November 2013. Parasitological examination was performed on outer surface, gills, gut, visceral surface and muscle tissue of each sample. The parasites found, were fixed using AFA solution and preserved in alcohol 70°. Staining with acetic carmine and clearance with lactophenol were used for observation of internal structures.
RESULTS: 46.7% of the specimens were parasitized by helminths. We identified adults of *Clestobothrium crassiceps* (16.7%), *Aporocotyle wilhelmi* (20%), third stage larvae of *Anisakis simplex* (6.7%) and plerocercoid larvae of Tetraphyllidea Order (6.7%). There was no correlation between host length, weight, sex and parasitism.
CONCLUSIONS: These findings represent an update of the helminth fauna of *Merluccius gayi peruanus* from the Peruvian sea and they enable us to make recommendations about how this fish should be consumed to prevent zoonotic parasitic infections.

The study of the diversity of freshwater fish parasites from South America: A long hard walk.

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BACKGROUND: South America is a subcontinent that includes five of the 17 so-called megadiversity countries and a high species diversity of freshwater fishes. A substantial portion of these fish is distributed within the intricate organization of numerous river basins. Among them, the Amazon is the most emblematic and represents a vast ecosystem with high fish diversity and a high degree of endemism. However, regulatory and logistic difficulties in combination with the “taxonomic impediment” serve to make it impossible to quantify the, clearly underestimated, diversity of fish parasites (>80% of fish species do not have a unique parasite record). Unfortunately, the situation is similar in the majority of South American basins.
METHODS: A preliminarily quantification of an extensive database of freshwater fish parasites recorded in the main South American basins revealed the primary targets for future studies and geographic points which are at a critical stage in terms of studies on parasite diversity. Based on this information, historical aspects and developments from current studies were analyzed and evaluated with focus upon problems related to and possible solutions for deficiencies/gaps in parasite biodiversity studies, with emphasis on the Amazon basin.
RESULTS AND CONCLUSIONS: Data concerning freshwater fishes in South America revealed a worrying picture. In addition to the established problems associated with taxonomic studies, it is necessary to consider the dramatic increases in anthropogenic activities within the ecosystems. As such, the planning of extensive parasitological surveys focusing on selected locations, on key fish species and on fish of economic importance could be viewed as immediate priorities. An increase in taxonomic studies is highly relevant at this moment, but a complete inventory of freshwater fish parasites in South America is not a plausible goal. International collaborative efforts and interaction between local research groups, based upon a multidisciplinary approach, are urgently required to establish a long-term strategy for studies on diversity of freshwater fish parasites in South America.

NGS and its Imminent Impact on Diagnostic Parasitology

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Microscopic examination of smears continues to be the gold standard for laboratory diagnosis in parasitic diseases. PCR can also be used for confirmatory testing, especially in cases where an accurate identification cannot be made by microscopy, e.g. low parasitemia, poor preparation or staining, or when a dual infection is suspected. For a few decades diagnostic reference laboratories, such as the one the CDC's Division of Parasitic Diseases and Malaria has been developing and applying molecular methods to solve difficult cases of infections. In some cases the application of a diagnostic PCR can provide the level of confirmation required and this component has applied that rationale to the diagnosis of malaria, babesiosis, cryptosporidiosis, leishmaniasis, Chagas disease, cyclosporiasis, free-living amebic infections, amebiasis, giardiasis and toxoplasmosis. When a diagnostic PCR is not in place, the molecular identification can be performed by the use of primers that amplify a specific region of the parasite's genome and the identification can be obtained after DNA sequencing analysis of the amplified product. For that the primers have to be specific enough to avoid amplification of host DNA or other microorganisms that might be contaminating the sample, but which are not the etiologic agent. Recent advances in DNA sequencing with the use of platforms that can perform high-throughput parallel sequencing also known as new or next generation sequencing (NGS) have created a unique perception about how to detect microbes with a direct impact in the infectious diseases diagnosis. This was also driven by the continuous decreasing in cost of such applications in comparison to PCR and Sanger sequencing methods. Microbiologists have increasingly used approaches now known as clinical metagenomics worldwide. In this talk the potential of clinical metagenomic applications in diagnostic parasitology will be discussed.

Antinflammatory effect of the recombinant calreticulin from *Taenia solium* on monocyte-derived human cells

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BACKGROUND: Chronic inflammatory diseases are highly prevalent worldwide. Currently prescribed treatments present a number of adverse side-effects, making necessary the quest for better options. Helminth eggs and excretion/secretion (ES) products induce Th2-type regulatory responses which partly alleviate the symptoms in animal models and patients. However, details of the underlying mechanisms remain elusive, particularly for human cells. Calreticulin is a ubiquitous protein found in different locations within the cell and involved in several processes, e.g. protein folding, Ca^{2+} homeostasis, cell adhesion, etc. Our group has identified the calreticulin of *Taenia solium* (TsCRT) and demonstrated that it is a component of ES products. Additionally, we expressed TsCRT as recombinant protein (rTsCRT) and showed that it promotes the release of IL-4, -5 and -10 from hamster mesenteric lymph node and splenic cells. Therefore, the aim of this work is to address the consequences of the interaction between rTsCRT and human cells, specifically in the context of cytokine induction.
METHODS: rTsCRT was produced in *E. coli* BL21 cells transformed with the peT23a plasmid containing the coding region for TsCRT and the recombinant protein was purified by electroelution. Monocyte-derived human macrophages (MDMs) and dendritic cells (MDDCs) will be differentiated from buffy coats using the method reported by Tsang et al, 2009. The cells will be immunophenotypified by flow cytometry, staining for the following surface markers: CD14, HLA-DR (both cell types), CD68 (MDMs), CD1a and CD11c (MDDCs). Supernatants from rTsCRT-stimulated MDMs and MDDCs will be collected, and Th1 (TNF-a, IFN-g and IL-12), Th2 (IL-4, TGF-b) and regulatory (IL-10) cytokines ELISA measured.
RESULTS: rTsCRT has been successfully purified up to a single-protein solution, confirmed by silver-stained SDS-PAGE. Cytokine profiling results will be presented at the congress.
CONCLUSIONS: In agreement with previous publications, it is expected that rTsCRT will favour the production of Th2-type/regulatory cytokines by MDMs and MDDCs.

Reassessment of the potential economic impact of cattle parasites in Brazil

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BACKGROUND: The profitability of livestock activities can be diminished significantly by the effects of parasites that affect cattle. Brazil has the largest commercial cattle herd in the world that includes ~212 million head. The last time that an attempt was made to assess the economic impact of cattle parasitism was in 2002.
METHODS: Economic losses caused by cattle parasites in Brazil were estimated on an annual basis considering the total number of animals at risk and the detrimental effects of parasitism on cattle productivity. Estimates in U.S. dollars (USD) were based on reported yield losses among untreated animals and reflected some of the effects of parasitic diseases. Here, they are referred to as potential losses.
RESULTS: Relevant parasites that affect cattle wellbeing and productivity in Brazil, and their economic impact in USD billions include: gastrointestinal nematodes - $7.1; cattle tick (*Rhipicephalus* (*Boophilus*) *microplus*) - $3.2; horn fly (*Haematobia irritans*) - $2.5; cattle grub (*Dermatobia hominis*) - $0.3; New World screwworm fly (*Cochliomyia hominivorax*) - $0.3; and stable fly (*Stomoxys calcitrans*) - $0.3. The combined annual economic loss due to internal and external parasites of cattle in Brazil considered here was estimated to be at least USD 13.9 billion.
CONCLUSIONS: This analysis provides the foundation for discussions on methodologies and research that are required, like studies on the effects of resistance to veterinary parasiticides, to improve the accuracy of economic impact assessments. Such information needs to be taken into consideration by decision-makers to influence research and regulatory programs and develop sustainable policies for mitigating the impact of parasitism on the profitability of Brazilian cattle producers.

* USDA is an equal opportunity provider and employer.

Current situation of schistosomiasis and the success of the control program in the Dongting Lake, China

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Schistosomiasis is a chronic and debilitating parasitic disease that has been neglected because it is a disease of poverty, affecting poor rural communities in the developing world. Schistosomiasis japonica has long captured the attention of the Chinese authorities who have undertaken remarkably successful control programs and have substantially reduced the disease burden. Successful control of schistosomiasis in China has involved three stages: morbidity control, transmission control and transmission blocking. The final goal to eliminate schistosomiasis from China has been established and involves a program of integrated control involving mass treatment of humans and bovines, snail control through environmental change, safe water provision, improved sanitation and health education. This presentation will report on the current schistosomiasis situation and the success of the control program leading to its elimination in the Dongting Lake, China.

Purified antigens of *Haemonchus contortus* and *Ostertagia ostertagi* as possible candidates for vaccine designing

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BACKGROUND: Sheep being a close grazer is regarded as museum of parasites especially for helminths. Economic losses due to helminth parasites in sheep throughout the world are considerable. At the moment, control is almost exclusively based on preventive treatment with synthetic chemotherapeutic drugs, i.e. anthelmintics. Thus alternative effective strategies are required.
METHODS: For the isolation of the proteins of the parasites, a well defined methodology was adopted. The parasites were collected in normal saline, washed and stored in 0.05M PBS with pH of 7.4 at 0^{o}C. After refrigeration, frozen nematodes were thawed, homogenized, centrifuged (12000rpm for 20 minutes). The supernatant was then clarified by passing through a 0-22µm filter. This clear supernatant or filtrate was thus collected as a purified antigenic mixture and stored at 0^{o} Celsius.
RESULTS: Protein estimation of the samples was estimated to be 4.2 mg/ml in case of *Haemonchus contortus* and 3.9 mg/ml in case of *Ostertagia ostertagi*. *Haemonchus contortus*- Two distinct bands of approximately molecular weights of 55 kDa and 33 kDa were observed in PAGE analysis and 66 kDa, 40 kDa, 33 kDa and 26 kDa bands were observed in SDS-PAGE analysis. *Ostertagia ostertagi*. Two distinct bands were also observed in PAGE analysis which were approximately of the molecular weights of 67 kDa and 20 kDa. SDS-PAGE analysis revealed 6 prominent protein bands of molecular weights 60 kDa, 50 kDa, 45 kDa, 40 kDa, 32 kDa and 28 kDa.
CONCLUSIONs: Protein profiling is expected to multiply the number of known drug targets 100-fold. Currently, there is a paucity of information regarding the comparative protein profiling of *Haemonchus contortus* and *Ostertagia ostertagi* in ruminants of the study area. The current study would open a new area of research for application to livestock industry in Jammu and Kashmir in particular and the country in general.

Comparative study of four Rapid Diagnostic Tests (RDT's) for the detection of *Cryptosporidium* and *Giardia*.

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BACKGROUND: *Giardia* and *Cryptosporidium* are protozoan parasites which commonly cause gastrointeritis in human. For a rapid and accurate detection of these pathogens several simple antigen tests are currently available which require minimal equipment and training.
METHODS: We compared the performance levels of four RDT's : the *Crypto-Giardia* Duo-Strip (Coris ®), the *Giardia* K-set (Coris®), the *Giardia/Cryptosporidium* Quik Chek® (Alere) and the ImmunoSTAT! *Cryptosporidium/Giardia* (Meridian Bioscience, Inc.) using the Real-time PCR as a gold standard.
All RDT's were performed on fresh and frozen specimens in accordance with the manufacturer's guidelines. To determine the sensitivity and specificity of each RDT the Real-time-PCR was used as a Gold Standard, using a Taqman 5'-nucleasis assay. DNA isolation was done according the BOOM-Principe on the EasyMAG. The target genes were the gdh-gene for *Giardia* and the COWP-gene for *Cryptosporidium.*

RESULTS:

		Nr. of TN/TP samples	Nr. of FN/FP samples	Sensitivity	Specificity
Crypto-Giardia Duo-Strip (Coris®)	*Cryptosporidium*	75/58	32/17	64,4%	81,5 %
	Giardia lamblia	135/31	7/4	81,5%	97,1 %
Giardia K-SeT (Coris®)	*Giardia lamblia*	131/28	8/6	77,8%	95,6 %
ImmunoSTAT! (Meridian Bioscience, Inc.)	*Cryptosporidium*	79/65	17/1	79,3%	98,9 %
	Giardia lamblia	133/24	2/0	92,3%	100 %
Giardia/Cryptospor. Quik Chek (Alere®)	*Cryptosporidium*	77/74	6/1	92,5%	98,7 %
	Giardia lamblia	124/33	2/0	94,2%	100 %

CONCLUSIONS: The RDT *Giardia/Cryptosporidium* Quik Chek (Alere®) turns out the most sensitive rapid diagnostic test for *Cryptosporidium*. For *Giardia* the Quik Chek (Alere®) and the ImmunoSTAT! (Meridian Bioscience, Inc.) both have a high

sensitivity and are reliably tests for routine diagnosis of *Giardia* in human fecal samples.

Assessment of the Community-Led Total Sanitation approach for the control of *Taenia solium* and soil transmitted helminths in Eastern province, Zambia

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BACKGROUND: Diseases caused by *Taenia solium* and soil transmitted helminths (STHs) are strongly linked to poor sanitation and poverty. These infections are perpetuated by open defecation (OD). Community-Led Total Sanitation (CLTS) involves facilitating a process to inspire and empower rural communities to stop OD and to build and use latrines, without offering external hardware subsidies. This project aimed at assessing the implementation and the effects of CLTS on *T. solium* and STH infections in endemic rural communities of Zambia.
METHODS: The study was held in Katete district, Eastern Province of Zambia. In 20 eligible villages the study aims were explained to the communities, a census conducted, and the villages mapped. At baseline, stool and blood samples from 786 humans and 244 pigs were sampled and in-depth interviews on sanitation and hygiene were held. Villages were divided into geographical strata and randomly allocated to intervention or control groups. The implementation of the CLTS triggering process in the intervention villages was done by CLTS champions under the auspices of UNICEF. Triggering of the villages was monitored between the start of the intervention and the post-intervention sampling, 22 months after the start of the intervention. All the factors investigated at baseline were evaluated using the same tools: in-depth interviews, Ag-ELISA for human and porcine cysticercosis, and STH prevalence by coprology; in addition taeniosis prevalence was measured by copro-Ag ELISA.
RESULTS: At baseline the prevalence of STH in humans and of porcine cysticercosis ranged from 0-57.1% and 0-37.5%, respectively. Latrine construction in intervention villages was in general progressing slowly, hindered by heavy rains. Preliminary results on the CLTS implementation and the post-intervention monitoring (April 2014) will be presented at the conference.
CONCLUSION: To our knowledge, this is the first study to evaluate the impact of CLTS on the transmission of *T. solium* and STH.

Molecular epidemiology of selected invasive and not invasive *B. burgdorferi* s.s. strains: does the equal ability to disseminate in reservoir hosts responsible for systemic disease in human?

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BACKGROUND: Multiple studies showed that *ospC* alleles are linked with strain invasiveness: alleles A, B, I, and K are responsible for systemic disease in humans, alleles C, D, E, F, G, H, J, M, N, T, V- cause a local infection, but not invasive disease; alleles L, P, Q or S very rarely, if ever, cause human disease. Our goal was to clarify the molecular epidemiology of *Bb* s.s. *ospC* allele L strains in comparison with strains that more likely than others cause disseminated Lyme disease.
METHODS: Six weeks old female mice were infected with low passage *Borrelia* cultured in BSK-H. DNA was extracted from ticks and murine tissues (ear, heart, bladder, joint) with NucleoSpin Tissue Kit. Borrelia DNA was detected by amplification of 154 bp fragment of flagellin gene. Non-infected *I. ricinus* larvae were fed on infected mice until repletion. Infection rates were determined later in hatched nymphs. Infected nymphs were then fed on naïve mice; spirochete transmission from infected nymph to mice was screened. Spirochete load in positive samples was determined by qPCR using LightCycler 480 (Roche) and TaqMan chemistry with FlaF1A/FlaR1primers and FlaProbe1 probe. Spirochete burden in tissues was normalized to mouse actin according.
RESULTS: *OspC* alleles B, L and V strains were able to infect mice: B- 100%, host originated L- 80% & vector originated strain-60%, allele V-50%.Dissemination of *ospC* L strains into the host was confirmed by qPCR and was comparable to *ospC* B strains, responsible for systemic disease in human.
CONCLUSIONS: Xenodiagnosis has been successfully used in our study. The confirmed ability of *ospC* allele L strain to disseminate into vertebrate host in the same manner as invasive strains responsible for systemic disease in humans increases the possible disease risk to humans in the geographic regions where analyzed alleles are distributed.

Persistence of *Borrelia burgdorferi* sensu lato spirochetes in Lyme disease patients after multiple antibiotic treatments: abnormalities in post-treatment cultured spirochetes.

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BACKGROUND: The effectiveness of antibiotic treatment of patients diagnosed with Lyme disease is a subject of intensive discussion by a medical community and wide public. Multiple studies on laboratory animals, including nonhuman primates, confirmed that Lyme disease spirochete is able to survive antibiotic treatment in immunocompetent hosts after short-course therapy. It is worth to find if the same short term treatment may fail to eradicate *B. burgdorferi* infection in humans. The main task of our study was to optimize the conditions of *Borrelia* cultivation from different samples obtained from Lyme disease diagnosed patients, including those after antibiotic treatment.
METHODS: Serum and plasma from LD patients after antibiotic treatments were inoculated into MKP and BSK-II media and cultured for 8 to 12 weeks at 34°C. *Borrelia* species in positive cultures were identified by MLSA and MLST. *Borrelia* was prepared by negative staining and high pressure freezing/freeze substitution methods for further analyses. Electron tomography followed by 3D reconstruction enable to study number of flagella as well as architecture of cells especially cysts. ImageJ software was used to measure lengths and diameters of the cells.
RESULTS: Using MKP media prepared in the laboratory we cultured two species from *Borrelia burgdorferi* sensu lato complex from three LD patients after antibiotic treatment. Results of molecular analysis confirmed that 2 patients were infected with *B. burgdorferi* sensu stricto. The third cultured *Borrelia* was identified as *B. bissettii*. Using different microscopy techniques we confirmed the presence of significant abnormalities in cultured spirochetes.
CONCLUSIONS: Our results confirm that the causative agent of Lyme disease is able to survive in human after multiple antibiotic treatments. We confirmed that *B. bissettii* as well as *B. burgdorferi* sensu stricto is responsible for systemic disease in human.

Lyme disease – features of a common tick-borne infection

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Lyme disease is a tick-transmitted inflammatory disease induced by spirochetes of the *Borrelia burgdorferi* sensu lato complex. The infection of susceptible hosts is common in moderate climate regions of the Northern Hemisphere; specific antibody prevalence against borrelia organisms in dogs is in general up to 10 %, but regional prevalence may exceed this rate. Equine populations in Europe show to some extent higher antibody prevalence, but clinical signs are rarely seen in horses.
The infection is zoonotic and Lyme disease in humans is the most important reported vector-borne disease in Europe and the USA. The human disease was first identified in the north-eastern part of the USA in the town Lyme during the 1970s, but retrospective analysis revealed its presence since the late 19th century in Europe and the USA.
At least three closely interrelated elements must be present in nature to spread Lyme borreliosis: (i) the Lyme-disease-causing bacteria, (ii) *Ixodes* ticks as transmitting vectors for the pathogens, and (iii) mammals, birds and reptiles that provide a blood meal and transportation for the ticks through their various life stages.
Although a high proportion of dogs and other hosts are positive for specific antibodies in endemic areas, not all infected animals develop clinical signs. Therefore, clinical diagnosis of should be supported with serologic testing. Nowadays, a comprehensive selection of assays is available for specific antibody detection. However, antibody tests (Western blots, line immunoassays - LIA, rapid tests) which include VlsE or C6 as capture antigens are recommended for routine diagnostic procedures.
Therapy consists of antibiotic treatment with doxycycline or amoxicillin for four weeks. Vaccines for animals, which block the spirochetes' transmission from the tick to the host by the effect of antibodies directed against the bacterial outer surface protein A, are available for several European countries and the USA.

Gastrointestinal parasitic infections in yaks at different farms of Sikkim, a North-East humid Himalayan region of India

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BACKGROUND: Gastrointestinal parasitism represents an obstacle to yak rearing, causing severe damage to herds such as growth retardation, weight loss, low milk yield, and even death. The aim of the study was to determine the occurrence of gastrointestinal parasites in yaks managed in small scale farms of Sikkim, a humid eastern Himalayan region of India.
METHODS: The research material included faecal samples of yaks collected in spring, summer, autumn and winter seasons during 2005 to 2007. A total of 971 yaks from eight farms were systematically sampled in the study using standard parasitological procedure and samples were examined both by flotation and sedimentation techniques.
RESULTS: The study showed that the overall infection rate was 18.43 % in yaks, either in single (9.43 %) or mixed infection (14.83 %) with a mean number of eggs per gram of faeces (EPG) as 43.862. The incidence was significantly lower in Government yak farm (16.7 %) than that of privately managed (18.51 %). The highest prevalence was noticed in private yak farm (23.53 %), Yumthang in North Sikkim. Strongyles, *Strongyloides, Nematodirous* and *Ascaroid* spp. were the most prevalent parasites encountered in the area. The EPG count was low to moderate in all the farms with a significant difference in their incidence between the farms. A higher rate of gastrointestinal parasitic infections was also found in animals reared at Kupup village (22.22 %) than in yaks reared at Gnathang valley in East Sikkim, and a greater proportion of study animals had moderate EPG compared with study animals with low (7.21 %) to severe EPG (0.11 %). Age and sex were significant variables for the development of gastrointestinal parasites. 15.37 % of animals over 24 months of age and 19.8 % of females were infected. The seasonal distribution of gastrointestinal parasitism indicated a higher percentage of infection during summer (24.31 %) followed by autumn (19.92 %) and spring (18.27 %). The infection rate was significantly lower in winter (11.87 %).
CONCLUSIONS: The present results may be useful to formulate an appropriate management strategy for gastrointestinal parasites of yaks in Sikkim.

Effects of G-type immunoglobulin's from Chronic Chagasic Patients in a pharmacological model of drug-acquired Type-2 Long QT Syndrome

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BACKGROUND: The presence of functional autoantibodies (Ab) able to activate β-adrenergic (Ab-β), M2 muscarinic (Ab-M) and both (Ab-Mβ) G protein-coupled receptor in chronic chagasic patients (CChP) has been described. They were also associated with cardiac electrical disorders modulating different cardiac ion currents. Previous works of our groups shown the arrhythmogenic effect of these autoantibodies in health isolated rabbit heart.

METHOD: Hearts from male rabbits were cannulated in a Langendorff apparatus and perfused with modified Tyrode solution (Tyr). The electrocardiogram in this preparation was recorded in different conditions. The I(Kr) blocker, E-4031 (5 µM), was used to elicit LQTS2 model. Sera from 10 CChP previously functionally characterized as Ab-M (n=2) or Ab-Mβ (n=8) were tested in a LQTS2 model in isolated rabbit hearts to study ventricular repolarization. QT QTc and RR interval were measured. Statistical analyses were made using one-way ANOVA. The data are expressed as mean ± SEM.

RESULTS: In the presence of E-4031 was observed a significant longer QT and QTc interval when compared to control condition. Sera from 8 CChP with Ab-Mβ were tested in the drug-induced LQTS2 model. This serum did not induced any significant changes in QT, QTc and interval in this model. However, the preliminary data obtained with Ab-M (n=2) showed reversible increase of RR, QT and QTc interval, in the LQTS2 model.

CONCLUSIONS: In the present work was possible to induce a LQTS2 model in isolated rabbit heart. Also, was observed that, even in preliminary data, Ab-M reversibly increased ventricular repolarization duration reflected by longer QT and QTc interval in the same model. However, the Ab-Mβ did not modulate the electrocardiogram parameter in the LQTS2 model.

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ENZYMATIC ACTIVITIES IN MALARIA PARASITE AND HIV INFECTIONS AMONG PREGNANT WOMEN IN ABEOKUTA, NIGERIA.

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Abstract

Introduction: Malaria and Human Immunodeficiency Virus (HIV) infections are among the leading causes of morbidity and mortality during pregnancy in tropical and sub-tropical regions of the world. The study was carried out to determine the effects of malaria parasite and HIV infections on some biochemical parameters of pregnant women in Abeokuta, Ogun State.

Methodology: Two hundred and fifty one (251) pregnant women were enrolled for the study during their ante-natal clinic at the Federal Medical Centre, Idi-Aba and Ogun State General Hospital Ijaye both in Abeokuta. Blood samples (5ml) were collected from each consented pregnant women during ante-natal clinic for malaria test, HIV screening, Malonydialdehyde (MDA), Serum iron (Fe) and some enzymatic activities such as Gluthathione –S-transferases (GST), Superoxides dismutase (SOD), Peroxidase (PERO). M DA was estimated calorimetrically by thiobarbituric reactive substance (TBARS).

Results: The prevalence of malaria parasites in the study was 28.3%, 16.3% were HIV^{+} out of which 48.8% were malaria positive. Malaria prevalence increased with decrease in T helper cells (CD4) count. Fe levels reduced significantly ($p<0.05$) among the HIV^{+} group than HIV^{-} group. Levels MDA was significantly higher ($p<0.05$) among the HIV^{+} group compared to HIV^{-} group. GST, SOD and PERO concentrations were significantly higher among pregnant women with either HIV infection or malaria alone compared to subjects with co-infection of malaria and HIV ($p<0.05$), and significantly lower among malaria and HIV co-infection group compared to subjects without malaria or HIV ($p<0.05$).

Conclusion: It is recommended that biochemical parameters such as MDA and FE should be tested during ante-natal clinic to prevent anaemia and lipid peroxidation in pregnant women.

Comparison of InPouch TF kits and Diamond's Medium for *Trichomonas gallinae* identification in Domestic Pigeons

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BACKGROUND: *Trichomonas gallinae* is considered one of most serious pathogen in pigeons all over the world causing high mortalities and massive losses in bird fauna, especially in columbiformes. Limited information on the prevalence of *T. gallinae* infecting domestic pigeons is available all over the world including Pakistan. The aim of current research was to compare the sensitivity of the two methods InPouch TF kits and Diamond's Medium as well as finding most appropriate technique for identifying *T. gallinae* in domestic pigeons.
METHODS: We obtained 120 pigeons from local bird fanciers; they were tagged with different colored rings, according to their gender and weight. All subjects were oropharyngeal swabbed for presence of trichomonad parasites using imported InPouch TF kits and Modified Diamond's medium. Cultures were observed to check the growth of live parasites for the time intervals of 24 hours of post inoculation (PI) i.e. after 24 hours, 48 hours and 72 hours. The efficiency of different techniques was compared by using Chi square in SPSS for Windows.
RESULTS: Our results suggest that Diamond's medium is considered as "gold standard" for identification *T. gallinae* in animals. On the other hand, InPouch TF kit has plenty of benefits over Diamond's medium used in glass vials. Pouches neither break nor easily spill out. The pouch is quite lighter and easier to handle in the field than a usual glass culture tube in the laboratory. Another advantage is that, if pouches are kept vertically after inoculation, protozoans are concentrated by gravity at the bottom of the pouch which enhances the identification of trichomonads. Unlike culture tubes of Diamond's medium, once inoculated, an InPouch is not needed not be reopened for repeated examinations. Also, aseptic technique in examining established cultures is not required. The one year long shelf life at room temperature of InPouch allows the convenient keeping of a supply of pouches. So, it's more expenditure than the mount microscopy could be acceptable for these advantages along with its high sensitivity and potentially lower subjectivity in diagnosing infectious organisms.
CONCLUSIONS: We propose InPouch TF as more sensitive method as compared to Diamond's medium, which makes it as perfect technique for the diagnosis of *Trichomonas gallinae* in avian fauna especially the fancy bird in field conditions as well as in the laboratory.

Prevalence of Plasmodium species in domestic pigeons (Columba livia domestica)

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BACKGROUND: The Haemosporidians are transmitted by the mosquito vector culex in case of pigeons. They are as common in pigeons as insects are in fruits and vegetables. They cause little damage to feral pigeon population, but can become a causative agent of diseases in the fancy and racing pigeons which are greater importance, leading pathological changes in the pigeons or cause decrease in the fitness or reduction of the market value of the pigeon especially the fancy and racing ones. Despite the considerable progress in the parasitology in the last decades, there are still a number of gaps exists in the avian blood parasites knowledge of taxonomy and biology. The present study was designed to determine the prevalence of *Plasmodium species* (malaria) in the pigeons and identification of the causative agent (*Plasmodium species)* causing malaria in pigeons.

METHODS: This study was carried out on 120 pigeons (*Columba livia domestica)* weighing 300-400gms that were collected from the 6 different locations of Lahore and kept in the animal house of Zoology department GCU Lahore for six months. The blood samples were collected using an insulin syringe inserted through a brachial vein catheter. Each sample provided two blood smears, fixed with methanol and stained with field stain and Giemsa stain. The slides were analyzed for the presence of parasites under microscope using an oil immersion objective. The parasites appear more concentrated (denser under the microscope) in thick blood films as compared to thin blood films.

RESULTS: Haemosporidians includes three species, *Plasmodium*, *Leucocytozoon* and *Haemoproteus*. The presence of schizonts indicated infective birds with *Plasmodium species.* The incidence in males was noted 40.9% and in females 34.8% during the 6 months duration of study. In month wise incidence detection highest incidence rate in males was recorded in June i.e. 56.9% and in females in May i.e. 48.5%. There was no significant difference observe in different age groups. The highest incidence rate irrespective to sex, location and different age groups was recorded in June i.e. 48.5% and mortality rate was highest in May i.e. 9%.

CONCLUSIONS: The *Plasmodium species* causes malaria, cause a lower body fat, lower body mass, impair immunological responses, decrease nest success and reduction in the parental caring behavior typically limiting to unique settings such as captive or closely managed flocks.

Metronidazole and *Entamoeba histolytica*: new twists in an old story

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BACKGROUND: Metronidazole has been used successfully for 47 years to treat *Entamoeba histolytica* infections. Surprisingly, many aspects of its activity are not well understood. Some studies pointed to mechanisms generating oxidative stress and hitting the important thioredoxin system of the parasite. Using a mutant thioredoxin as bait, we had identified 234 proteins interacting with thioredoxin. It is unknown so far to what extent thioredoxin remains functional under metronidazole stress. Another phenomenon is the severe DNA degradation that occurs after treatment with metronidazole, hydrogen peroxide, nitric oxides or G418, and we investigated what could be the responsible DNase.
METHODS: The redox state of thioredoxin in the cells treated with metronidazole with or without additional oxidative stress was examined by means of the redox western blot method. Putative DNase genes were identified in the databases and examined biochemically and by qRT-PCR.
RESULTS: Unexpectedly, the redox western blots showed that during metronidazole treatment, thioredoxin remained predominantly in the reduced state. When the amoebae were exposed to metronidazole and additional oxidative stress, the reduced state of thioredoxin could no longer be maintained.
Database search revealed 58 nuclease candidates. First we characterised *E. histolytica* TatD, a homologue of the bacterial TatD nuclease, reported as a DNase associated with apoptosis. EhTatD displayed Mg-dependent endo-DNase activity and was found in the cytoplasm of the amoebae. qRT-PCR showed that, surprisingly, TatD mRNA was downregulated, whereas two DNA repair endonucleases were upregulated.
CONCLUSIONS: In the absence of additional oxidative stress, metronidazole-mediated inhibition of TrxR does not lead to severe overoxidation of thioredoxin which suggests that the disulfide reducing system might be dispensable under these conditions. Additionally, the results point towards the existence of further important metronidazole targets. There is a complex dynamics of DNA degradation under metronidazole action, but no evidence yet of programmed upregulation of non-repair endonucleases.

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A proteogenomic approach to investigating snail-schistosome interactions

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BACKGROUND: In order to begin dissecting the complex molecular interplay between early developing *Schistosoma mansoni* sporocysts and the immune system of the snail *Biomphalaria glabrata*, we are taking a proteogenomics approach in which host proteins with putative involvement in parasite immune interactions are isolated/enriched and subjected to proteomic analyses to identify the proteins contained within isolated samples. Since protein expression represents the molecular "phenotype" of specific genes expressed, identification of host proteins directly involved in immune interactions can then be traced back to their encoding genes within the host genome.
METHODS: We accomplished this by subjecting isolated interactive protein fractions or solublized cell populations to protease digestion, followed by nanoLC/MS/MS to yield peptide sequences. These sequences were then searched against the translated *B. glabrata* genome to confirm the existence of their encoding genes within the snail genome, and against the non-redundant NCBI protein database to provide putative identifications based on amino acid sequence homologies.
RESULTS: Two examples of how we applied this approach include: (1) Identification of snail plasma proteins with binding reactivity to the sporocyst surface tegument and larval transformation proteins (LTPs) and (2) comparison of cellular proteins expressed during *in vitro S. mansoni* sporocyst encapsulation by hemocytes of susceptible and resistant snail strains. Differential expression of various immune-related proteins (e.g., fibrinogen-related proteins, heat shock/stress proteins) between snail strains were consistently noted.
CONCLUSIONS: These examples illustrate how this "reverse genomics" approach can be valuable in not only identifying proteins with predicted functional relevance to snail-schistosome immune interactions, but also in its application as an important tool for assisting in the annotation of the *B. glabrata* genome.

Teaching medical Parasitology in Europe: which horizon?

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BACKGROUND: During EMOP X, in Paris, in 2008 the results of a survey on teaching Parasitology to medical students in Europe was presented and the following conclusions drawn: it is confirmed the previously described high discrepancy among different countries in the modalities to teach medical Parasitology, with some Countries where the discipline in some Faculties is completely inglobated in the Microbiology course, (Bruschi, 2009, Parasitol. Research, 105: 1759).
METHODS: During the next Symposium in Mexico City the results of a new survey will be presented with data from Italy, Spain, Romania, France, Germany, The Netherlands, Ireland and possibly, other Countries.
In particular, the following issues were analised through a questionnaire distributed around Europe: the allocation of basic and clinical Parasitology in the courses, the year in which the course is given, the number of hours, the main parasitological topics taught, the feasibility of practical works, possible experimental teaching activity, evaluation modalities, suggestions for improvement.
RESULTS: Unfortunately, the situation looks to be worsened since the last survey and the number of hours dedicated to parasitological subjects is decreasing more and more in many Countries, for example in Spain Parasitology does not appear any more in the programs of Medicine (Osuna, pers. comm).
In Romania, on the contrary, in Transylvania University in Brasov the situation is much better and 14 hours of lessons + 14 hours of practical works are taught to the second year students (Nemet, pers. comm). In Ireland, at the Trinity College 6 hours dedicated only to basic Parasitology are given (Holland, pers. comm).
CONCLUSIONS: There is no doubt that when it is present a group involved in Parasitological research it is more probable the existence of a course of Parasitology not embedded in other disciplines.

Leishmanicidal activity of hydroxyurea for treatment of disseminated cutaneous leishmaniasis in mice experimentally infected.

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BACKGROUND. The hydroxyurea (HU) is a drug used to decrease the growth of cancer cells. HU (100 ug/mL) inhibits the growth of promastigotes and amastigotes since the third day of exposition. The aim of this work was to study the leishmanicidal activity of HU through three schemes of treatment for the disseminated cutaneous leishmaniasis in a murine model.

METHODS. Mice experimentally infected with *L. mexicana* were divided into three groups (treatments). The first group was administered with HU 0.4 mg/kg/day/15 days, the second group with 0.02 and the third with 0.1. Mice were studied at short (2 weeks), medium (16 weeks) and long term (52 weeks). The size of the footpad, the physical status, the number of alive mice after treatment and, the amplification of a 121 pb kinetoplast fragment were studied as response to treatment.

RESULTS. The first treatment showed that leishmanicidal activity of HU, but clinical improvement was discrete. The second treatment showed that lower doses of HU rapidly deteriorated the general condition of the infected animals. The third group showed clinical improvement in the small and medium term but not for the long run; similar results were obtained using glucantime (treatment for cutaneous leishmaniasis).

CONCLUSIONS. The treatment with 0.1mg HU/day/15 days showed clinical improvement in the short and medium term. The disease remission in the long run was correlated with the *Leishmania* DNA amplification from different tissues obtained from treated mice. This phenomenon was attributed to the parasite load ablated by the HU but, not totally eliminated and the parasites surviving were sufficient for clinical remission. Further studies are needed to determine a treatment that eliminates the totality of parasites.

Electron Probe X-Ray Microanalysis of the Elemental Composition of the Tegument of *Schistosoma mansoni* developed from cercariae exposed to *Euphorbia milii* latex

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BACKGROUND: Schistosomiasis is one of the most important parasitic diseases in the world. The main structure responsible for the survival of *Schistosoma mansoni* and in modulating the host response is the tegument. Despite our extensive knowledge of tegumental function and biochemistry, little is known of its inorganic composition in relation to anthelmintic drugs. In this context, the present work aims to use Electron Probe X-Ray Microanalysis to evaluate the elemental composition of the tegument of adult *S. mansoni* developed from cercariae experimentally exposed to *Euphorbia milii* latex.
METHODS: Fifteen female Swiss-webster mice were infected with 150 cercariae exposed for one hour to LC_{50} of *E. milii* latex; a control group was infected with non-exposed cercariae. The parasites recovered were fixed in 70% ethanol, dehydrated in an ethanol series, critical point dried with CO_2 and coated with carbon to be observed in a Leo 1430VP Scanning Electron Microscope coupled to an electron probe X-ray (EDS). The lyophilized latex was also analyzed for the elements: N, Na, Mg, Al, K, Cr, Fe, Cu, Rh, Pd, Ag, Cd, Pt, Hg and Pb.
RESULTS: The exposure of the cercariae to *E. milii* latex significantly reduced the survival of the adult parasites and changed the tegument surface of male worms. The normal tegument of males exhibits tubercles and spines throughout most of the body, but these structures were scarce in the exposed group. It is known that the tegument of *S. mansoni* has an active Na-K-Mg-dependent ATPase. Although the latex presented high levels of K and Mg, their levels decreased in the exposed worms. However, although the Na level was low in the latex, it almost doubled in the exposed ones. Another element, Rh, exhibited high levels in the latex, and, although present in the control group, its level decreased slightly in the exposed worms.
CONCLUSION: The cercariae exposed to *E. milii latex* formed adults with a morphologically changed tegument. This may be related to the main changes in the composition of the elements Na-K-Mg induced by the exposure to the latex.

Comparison of extraction methods for the mass spectrometry analysis of *Schistosoma mansoni*

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BACKGROUD: Proteomic analyses are helpful tools for the identification of molecules of *Schistosoma mansoni* that may be new targets for vaccine or drugs. However, the most critical step in these studies is the efficiency of the protein extraction, as the total proteomics may be represented differently, depending on the method used. Thus more accurate methods that produce a greater number of extracted proteins and a more precise computation of the different gene expressions are required. In this work, we compare two methods of protein extraction and analyze the quantity and diversity of proteins.
METHODS: The protein extraction of 15 males of *S. mansoni* was evaluated using two solutions: 2DE standard lysis buffer (7M UREA, 2M THIOUREA and 2% CHAPS) and SDS (0.125M Tris-HCl, 4% SDS, 20% v/v Glycerol, 0.2M DTT, 0.02% Bromophenol blue) subjected to heating. Cell disruption and protein solubilization were obtained by ultrasonication. Extracted proteins were centrifuged to remove cell debris and the protein profile was analyzed by SDS-PAGE 1D. The optical densities of bands were analyzed by ImageJ (1.6.0_20) and the statistical analysis was made using GraphPad Prism5. Proteins were identified by MALDI-TOF/TOF.
RESULTS: The number of bands found in the 1-D electrophoresis analysis was nine and 12, respectively, and nine common bands were submitted for analysis. The samples extracted with SDS-heated showed bands of protein with a stronger intensity and density, differing up to 20% in comparison with the initial extraction solution. The SDS-heated protocol generated more information for MS/MS analysis and we included SDS 0.2% in 2DE buffer to improve the protein extraction without affecting the performance of the isofocusing IEF.
CONCLUSIONS: The addition of the detergent SDS-heated as an extraction buffer was more efficient for the protein extraction of *S. mansoni*, compatible with 2DE electrophoresis and increased the peptide and protein identification.

Protein extraction standardization from *Hysterothylacium* sp. (Nematoda: Anisakidae) larvae for mass spectrometry analysis

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BACKGROUND: *Hysterothylacium* is a zoonotical fish parasite reported as etiological agent of a human disease called Anisakiasis that has gained great relevance due to the popularization of food based on raw fish, being registered in several parts of the world, such as Brazil, Japan and USA. Larval stages of this parasite do not show morphological characteristics permitting the identification of individuals at species level. The identification and characterization of *Hysterothylacium* larval proteins by mass spectrometry aim to increase the knowledge about the biochemical protein profile of this helminth.
METHODS: To the standardization of *Hysterothylacium* larval protein extraction for mass spectrometry analysis we tested two different buffer solutions: (1) SDS-PAGE (0.125M Tris-HCl, 4% SDS, 20% v/v Glycerol, 0.2M DTT, 0.02% Bromophenol blue), and (2) CHAPS (7M Urea, 2M Thiourea and 2% CHAPS). Cell disruption and protein solubilization were done by ultrasonication. The extracted proteins were centrifuged to remove cell debris, been posteriorly analyzed by SDS-PAGE 1D. After reduction, alkylation and digestion enzyme, the proteins were identified by MALDI-TOF/TOF, followed by search in several proteins databases using MASCOT algorithm.
RESULTS: As preliminary results, we verified that SDS-PAGE buffer solution showed to be more efficient than CHAPS to the extraction of *Hysterothylacium* larval proteins for posterior mass spectrometry analysis. The most abundant identified proteins were actin and troponin, both involved in cell and muscle movements, and 40S ribosomal protein s19.
CONCLUSIONS: The proteins extraction under lysis using SDS-PAGE buffer followed by the mass spectrometry demonstrated to be as a powerful tool to detect the most abundant proteins in *Hysterothylacium* larvae.

Molecular diagnosis of intestinal parasitic infections in the clinical laboratory and epidemiology

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BACKGROUND: Shortly after the first publication of the in vitro DNA amplification in a polymerase chain reaction (PCR) a breakthrough in molecular parasitology and in the diagnosis of parasitic infections was predicted. In the last 10 years, many laboratories have been provided with facilities to perform molecular diagnostic tests.
METHODS: Molecular detection, differentiation, and genotyping methods have been described for a large number of parasites and carried out in both diagnostic and research settings.
RESULTS: The introduction of real-time PCR, greatly reduced earlier problems related to contamination with PCR products and made it possible to combine multiple DNA targets in a multiplex assay. In addition, the implementation of automated DNA isolation methods made it possible to perform DNA-based detection techniques in a high-throughput format.
CONCLUSIONS: In this presentation the implementation of molecular diagnostics for the detection of intestinal parasites in the clinical laboratory and examples how molecular diagnostics can change the perspectives in our understanding of parasite epidemiology will be discussed.

Intestinal parasitic infections and their relation to iron status in Kenyan pregnant women

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BACKGROUND: There is increasing evidence that iron supplementation can increase the burden of infections. We aimed to measure the prevalence and intensity of *Schistosoma* and intestinal parasitic infections before and after iron supplementation in pregnant Kenyan women. In addition, for infections associated with blood loss (*Schistosoma* spp., *Trichuris trichiura* and hookworm), we aimed to assess associations with iron status at baseline.
METHODS: 470 rural Kenyan women with singleton pregnancies, gestational age 13–23 weeks and haemoglobin concentration ≥90g/L were randomized to supervised daily supplementation with iron (60mg) or placebo until 1 month postpartum. To prevent severe anaemia during intervention, both groups were treated with praziquantel and albendazole at baseline but after first stool collection. They additionally received 5.7mg iron/day through flour fortification. Stool and blood were collected at baseline and at the end of intervention. Stool samples were tested semi-quantitatively for parasitic DNA by multiplex real-time PCRs.
RESULTS: Real-time PCR for intestinal parasites was performed in 404 (86%) and 316 (67%) women at baseline and at the end of intervention, respectively. At baseline, 29.7%, 14.4%,10.1%, and 5.9% of women were infected with *Schistosoma* spp., *T. trichiura, Giardia lamblia* and *Necator americanus*, respectively. *Ancylostoma* spp., *Ascaris lumbricoides, Strongyloides stercoralis*, *Cryptosporidium* spp., and *Entamoeba histolytica* were rare or absent. At the end of intervention a reduction in prevalence was observed for *Schistosoma* spp. and *T. trichiura* infections (10.1% and 8.9%). The prevalence of *G. lamblia* and *N. americanus* remained similar (7.6%). We found no evidence that iron supplementation increased the risk of (re-)infection by *Schistosoma* spp., *T. trichiura, N. americanus, or G. lamblia*. We found no evidence that *Schistosoma* spp. or *T. trichiura* were associated with haemoglobin concentration, plasma concentrations of ferritin, soluble transferrin receptor, C-reactive protein, or α_1-acid glycoprotein at baseline.
CONCLUSIONS: We found no support that *Schistosoma* spp., or *T. trichiura* infections affected iron status, or that iron increased the risk of re-infection with these parasites.

Prospective Cohort Study to Demonstrate Rapid Clearance of *Giardia lamblia* DNA From the Gut After Successful Treatment

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BACKGROUND: *Giardia lamblia* is a protozoan parasite causing diarrhea in individuals worldwide. Recently, PCR was introduced as a diagnostic option, significantly increasing the sensitivity of *G. lamblia* testing. Although highly relevant for patients with persistent symptoms, it is still unknown for how long parasitic DNA can be detected after successful treatment. We performed a prospective cohort study to determine when the real-time *G. lamblia* PCR on fecal samples becomes negative after successful treatment of the infection.
METHODS: Patients tested positive for *G. lamblia* by real-time PCR were requested to send a maximum of three follow-up stool samples to test for persisting *G. lamblia* DNA after treatment with Metronidazol. Patients Follow-up sample 1 (FU1) was to be sent as soon as possible after treatment, FU2 one week after treatment and FU3 one month after FU2, if positive. Our primary outcome was *G. lamblia* PCR negativity at FU2.
RESULTS: Hundred-eleven of all 2307 persons tested had *G. lamblia* DNA in their stool (positivity rate; 4.8%). Seventy-five patients were included, 36 patients were not willing to participate. The average CT value and age for both groups did not differ significantly (p=0.415 and p=0.818). In 30 FU1 samples *G. lamblia* DNA was detected (40.0%), all 65 returned FU2 samples were negative (100.0%). Because of this, no FU3 samples were requested. Ten patients did not return their FU2 sample (lost to follow-up). The median time between the end of treatment (EOT) and FU1 was 1 day (range 0-22 days) and the median time between FU1 and FU2 was 7 days (range 4-22 days).
CONCLUSIONS: We found that approximately one week after antibiotic treatment *G. lamblia* DNA became undetectable in 100% of our subjects. A positive test one week after treatment therefore is a strong indicator for on-going or renewed infection.

Helminth fauna associated with two anurans species of Microhylidade family from São Paulo State Northwest, Brazil

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BACKGROUND: The anurans species *Elachistocleis* cf. *bicolor* and *Dermatonotus muelleri* are included in the Microhylidae family and occupy similar habitats. This study aimed to compare the helminth fauna of these two anurans species and evaluate the ecological descriptors of parasitism, as component community, prevalence (P), mean intensity of infection (MII) and mean abundance (MA).
METHODS: The anurans were collected at the same pound in a biological reserve from São Paulo State, during the rainy season (January 2013). Twenty-four specimens of *E*. cf. *bicolor* and 19 of *D. muelleri* were surveyed for helminthes.
RESULTS: The helminth fauna of *E*. cf. *bicolor* was composed by the nematodes Cosmocercidae gen. sp. (n=26), encysted larvae of Centrorhynchidae gen. sp. (Acanthocephala) (n=3), and the digeneans *Glypthelmins* sp. (n=3), and unidentified metacercariae (n=9). *Dermatonotus muelleri* was parasitized only with the cosmocercid *Aplectana membranosa* (Nematoda) (n=678), representing overall prevalence of 15.79%, MII of 226 ± 165.69 and MA of 35.68 ± 29.31. The overall prevalence of *E*. cf. *bicolor* was 25%, and each host presented an average of 6.83 ± 1.85 helminthes, while MA was 1.71 ± 1.85. The comparison of P and MA of *E*. cf. *bicolor* and *D. muelleri* showed that these parameters were similar for both hosts (p=0.72 and p=0.693, respectively). Contrarily, MII was higher in *D. muelleri* (p=0.024).
CONCLUSIONS: The component community of the two host species showed differences. Although these anurans occupy the same habitat, intrinsic factors of species can be more determinant for helminthes infection than external factors, as the environment (FAPESP 2011/20186-6).

A 12-month follow-up study on *Toxoplasma gondii* infection in renal transplants recipients

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BACKGROUND: Solid-organ transplant recipients represent a vulnerable population for *T. gondii* infection.
METHODS: We conducted a cross-sectional study on seroprevalence of anti-*T. gondii* antibodies in donors and recipients of renal transplants, and performed a 12-month prospective study on recipients to evaluate anti-*T. gondii* seroconversion. IgG and IgM antibodies were detected by enzyme immunoassay and Western blot (WB), and a subset of 116 patients received nucleic acid testing (PCR) confirmation.
RESULTS: Among 230 cross-sectional participants, 198 (86%) were donor-receptor dyads surgically intervened; 77 of 230 (33.5%) had a positive IgG ELISA test, 59 (25.7%) had a positive WB test, and 57 (24.8%) had both tests positive (confirmed latent infection). Only 9 (3.9%) were IgM-positive by WB and 4 (44.4%) also IgG-positive (ELISA).The frequency of IgG anti-*T.gondii* antibodies was higher in donors than in receptors (38.4% vs 24.2%;P=0.03). The seroconversion rate was 18.8%, 20.8% and 22.4% at 3, 6 and 12 months of follow-up, respectively. From the 116 samples analyzed by PCR, one sample corresponding to a receptor has been positive. In a multivariate analysis, factors associated with receptors seroconversion during the 12-month follow-up were having a donor with latent infection (OR: 7.73, 95% CI: 1.06-13.13) and having an immunosuppression-associated infection (OR: 11.50, 95% CI: 1.37-96.24).
CONCLUSIONS: In this cohort we found evidence of an increased risk of seroconversion in renal transplant recipients over a 12-month follow-up. Donor serostatus and immunosuppression are the main determinants for the risk of acquiring *T. gondii* infection.

Parasitological and Ecological parameters associated to nematofauna of the Argentine goatfish, *Mullus argentinae* (Perciformes, Mullidae) and the Whitemouth croaker, *Micropogonias furnieri* (Perciformes: Sciaenidae) from coast of Rio de Janeiro, Brazil.

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BACKGROUND: The Argentine goatfish, *Mullus argentinae* and the Whitemouth croaker, *Micropogonias furnieri* are species of fish commonly commercialized and widely appreciated by consumers. *M. argentinae* is distributed from Brazil to northern Argentina. *M. furnieri* has a more extensive distribution from Costa Rica to Argentina, including Greater Antilles and Nicaragua. Studies on parasite helminthofauna of these fish species are scarce, despite its impact in both human and fish health, and mainly, in fishing industry. The aim of this study was to investigate ecological / parasitological parameters of the nematodes parasites of *M. argentinae* and *M. furnieri* during distinct seasons.
METHODS: Sixty specimens of each species, *M. argentinae* and *M. furnieri,* were collected in São Pedro Fish Market, municipality of Niteroi, Rio de Janeiro state (22° 52′ S, 43° 6′ O). Thirty of each were collected in the winter season (July-August) and the others in the summer station (January- February). Fish were necropsied and their nematode parasites investigated. The ecological / parasitological parameters were calculated.
RESULTS: Parameters defined for *M. argentinae* were: Winter: Prevalence 57%; Mean intensity 5; Mean abundance 2.8. Summer: Prevalence 50%; Mean intensity 4.3; Mean abundance 2.1. For *M. furniere* were: Winter: Prevalence 43%; Mean intensity 5.5; Mean abundance 2.4. Summer: Prevalence 27%; Mean intensity 6.9; Mean abundance 1.8. Regarding the site of infection, the distribution for *M. argentinae* and *M. furniere*, were, respectively: Intestine 59% and 27%; intestinal content 8% and 54%; stomach 11% and 5%; stomach content 1% and 4%; mesentery 14% and 9%; liver 5% and 0%; lungs 2% and 0%; swimming bladder 0% and 1%.
CONCLUSIONS: The parasitological parameters analysis showed no significant differences between seasons for *M. argentinae*, however, for *M. furniere*, a higher prevalence during the winter season was demonstrated. Intestine was the most important site of infection in *M. argentinae* and intestinal content in *M. furniere*.

Hospital dichages for *Toxoplasma gondii* infection in the Mexican public health system, 2010-2012

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BACKGROUND. In the context of the HIV/AIDS pandemics and the increasing use of immunosuppressants, a growing number of cases of toxoplasmosis can be expected. However, little is known about the health care burden and hospital mortality associated with toxoplasmosis in Latin America. To determine the epidemiological characteristics of hospital discharges associated with the diagnosis of toxoplasmosis.
METHODS. We analyzed the hospital discharges database of the years 2010 to 2012, of the National Health Information System (SINAIS, Ministry of Health: SSA, IMSS, IMSS oportunidades, ISSSTE, PEMEX, and the Secretary of Defense). Clinically defined toxoplasmosis records were identified by the code B58 (B58.0+, B58.1+, B58.2+, B58.3+, B58.8 and B58.9) of the International Classification of Diseases 10th revision. Only records of inpatients older than 18 years were considered for this analysis.
RESULTS. In the whole Mexican public health system, a total of 5.31, 5.45 and 5.64 millions of hospital discharges were registered during the years 2010, 2011 and 2012, respectively. In all, 315 adult patients (69.5% men, median age 37 years) were registered during the three years: 103 patients in 2010, 113 in 2011, and 99 patients in the year 2012. The clinical syndromes registered were *Toxoplasma* oculopathy (2.5%), hepatitis (1.3%), meningoencephalitis(14.3%), pulmonary toxoplasmosis (1.6%), and toxoplasmosis with other organ involvement or unspecified (80.3%). At a median hospital stay of 9 days (range: 1-70 days), the hospital case fatality rate was 20.7% (higher among meningoencephalitis and pulmonary toxoplasmosis). A non-significant trend for a higher mortality was observed in men, as compared with women (23.3% vs. 14.6%, respectively; P=0.08). Age ≥50 years was significantly associated with in-hospital mortality (OR: 2.06, 95% CI: 1.03-4.11).
CONCLUSIONS. In this adult population, the frequency of hospital discharges associated with toxoplasmosis was lower than expected, which suggest under diagnosis. Lack of awareness and limited resources may be involved in this phenomenon, an issue that needs further investigation.

Host specificity of an introduced parasite, *Centrocestus formosanus*, to a non-native fish in the Panama Canal

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BACKGROUND: To establish, persist, and spread, introduced parasites must be either "pre-adapted" to hosts in the new range and/or rapidly adapt to new hosts. Generalist parasites are predicted to be more successful at invading new geographic ranges because they can effectively colonize novel host species compared to specialist parasites. Yet patterns of host range are affected by both ecological and evolutionary factors that could drive a parasite to specialize on a new host species. Here, we evaluate to what extent does the introduced parasite, *Centrocestus formosanus*, specialize on particular hosts when it is introduced to a new environment?
METHODS: We used field comparisons to examine patterns of host use in nature and laboratory infection experiments to determine host specificity (compatibility) and host preference by holding encounter rates constant.
RESULTS: In nature, the invasive peacock bass, *Cichla monoculus*, exhibited higher prevalences and intensities of *C. formosanus* compared to 3 other cichlid species, the oscar (*Astronotus ocellatus*), the Nile tilapia (*Oreochromis niloticus*) and the native vieja (*Vieja maculicauda*). Laboratory infection experiments demonstrated that while all hosts are susceptible to infection, *C. monoculus* experiences significantly higher infection rates in both single species trials and mixed species trials compared to other cichlid fish.
CONCULUSIONS: In contrast to other research findings suggesting that *C. formosanus* is a generalist parasite that can infect a broad array of fish species, our results demonstrate specificity and perhaps specialization on a novel host species. We will discuss the implications of specializing on a novel invasive host species for the success of an introduced parasite and potential evolutionary drivers for this observed pattern. Finally, we will describe an experiment which will test if the observed pattern is the result of rapid adaptation of host-specialization in a human dominated landscape.

Selective Whole Genome Amplification Yields Near Full Length Genomes of Chimpanzee *Plasmodium* Parasites

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BACKGROUND: Wild living chimpanzees and gorillas are infected with at least six *P. falciparum*-like species (the *Laverania* subgenus), including the ancestor of *P. falciparum*. These parasites are widespread, highly prevalent in wild apes, and appear very host specific: three species are found only in gorillas, three only in chimpanzees, and none in nearby human populations. These species provide a unique opportunity to study the determinants of *Laverania* host specificity, and identify mechanisms by which *P. falciparum* adapted to humans.
METHODS: We obtained remnant screening blood samples from sanctuary chimpanzees in central Cameroon. Samples were screened for *Plasmodium* infection by bulk PCR, single genome sequencing (SGS), and qPCR. Two samples (one *P. gaboni* infected and one *P. reichenowi* infected) were chosen for whole genome sequencing. We enriched for *Plasmodium* DNA by methylation-dependent restriction digest and a novel selective whole genome amplification (SWGA) approach that uses phi29 polymerase and 10 *Laverania* specific primers. Enriched samples were sequenced (Illumina MiSeq) and assembled using a reference-guided pipeline.
RESULTS: Plasmodium DNA constituted 0.007-0.15% of total DNA in pre-enriched samples, by qPCR. After enrichment, >87% of reads mapped to the *Plasmodium* genome and <6% to the chimpanzee genome (600-12000x enrichment). Assembly of the *P. reichenowi* isolate yielded 19.7 Mb of sequence that could be aligned to the *Pr*CDC1 reference, and 650 unplaced contigs (1.45 Mb). Assembly of the *P. gaboni* isolate yielded 17.3 Mb of sequence that could be aligned to the *Pf*3D7 reference and 1100 unplaced contigs (3.1 Mb). SGS of select pre-enrichment sample genes identified no errors in the SWGA consensus.
CONCLUSIONS: Methylation-dependent digest and SWGA substantially enriches *Plasmodium* DNA from whole blood. This approach yielded sequences with sufficient quality and breadth of coverage to produce near full-length genomes of two chimpanzee *Plasmodium* species. Annotation and comparative evolutionary analyses of these genomes are ongoing.

Nitric oxide synthase dysfunction contributes to impaired cerebroarteriolar reactivity in experimental cerebral malaria

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BACKGROUND: In experimental cerebral malaria (ECM) by *Plasmodium berghei* ANKA, cerebrovascular dysfunction characterized by vascular constriction, occlusion and damage results in impaired perfusion and reduced cerebral blood flow and oxygenation, and has been linked to low nitric oxide (NO) bioavailability. METHODS: Cerebrovascular function was assessed in ECM using a novel cranial window method for intravital microscopy of the pial microcirculation and the role of NOS isoforms and phosphorylation patterns in the impaired vascular responses was probed.
RESULTS: Pial arteriolar responses to endothelial NOS (eNOS) and neuronal NOS (nNOS) stimulators (Acetylcholine (ACh) and N-Methyl-D-Aspartate (NMDA) were blunted in mice with ECM, and could be partially recovered by exogenous supplementation of tetrahydrobiopterin (BH4). Pial arterioles in non-ECM mice infected by Plasmodium berghei NK65 remained responsive to the stimulators. These findings, together with the observed blunting of NO production upon stimulation by the agonists, decrease in total NOS activity, augmentation of lipid peroxidation levels, upregulation of eNOS protein expression, and increase in eNOS and nNOS monomerization in the brain during ECM development strongly indicate a state of eNOS/nNOS uncoupling likely mediated by oxidative stress. Furthermore, the downregulation of Serine 1176 phosphorylation of eNOS, correlated with decreased cerebrovascular wall shear stress, implicates hemorheological disturbances in eNOS dysfunction in ECM. Finally, pial arterioles responded to superfusion with the NO donor, S-Nitroso-L-glutathione (GSNO), but with decreased intensity.
CONCLUSION: eNOS and nNOS dysfunction contributes to cerebrovascular dysfunction in ECM.

THE IMPACT OF INTERMITTENT PREVENTIVE TREATMENT (IPT) OF MALARIA WITH SULFADOXINE (SP) ON PLACENTAL MALARIA IN PARTS OF ABIA STATE SOUTH EASTERN NIGERIA.

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BACKGROUND**:** *Plasmodium falciparum* infection of the placenta (Placental malaria) remains a major medical challenge among pregnant women in Sub-Saharan Africa owing to the associated consequences. World Health Organization (WHO) Policy of Intermittent Preventive Treatment (IPT) in pregnancy with Sulfadoxine –Pyrimethamine has been adopted for control of malaria during pregnancy. The impact of this intervention policy on Placental Malaria (PM) was assessed in this study carried out in Abia State South Eastern Nigeria.
METHODS**:** PM prevalence was determined, the use of IPT with SP was established. Clinical and parasitological parameters were assessed among the pregnant women who enrolled for the study.
RESULTS**:** 74% prevalence rate of placental malaria was observed with 50% compliance to the IPT- SP policy. The use of IPT with SP was associated with prevalence and severity of placental malaria, maternal Hemoglobin level and birth weight of the babies. 67% of the enrolled women complied with the IPT-SP policy with 66% positive for placental malaria while of the 33% non IPT-SP group 89% were positive for placental Malaria. Severity was also higher in the Non IPT-SP group (66%) than the IPT-SP group (16%). Low birth weight of 62% was associated with Non IPT-SP group as compared with 37.9% for the IPT SP group. The non IPT SP group was predisposed to severe anemia (23%) more than the IPT-SP group (4%).
CONCLUSION: the implementation of the IPT-SP policy was significant in the control of placental malaria and its consequences however the prevalence rate observed showed that total eradication of placental malaria remains a challenge.

A widely spread, overlooked lymnaeid species, the first fossarine resistant to *Fasciola* infection: a new field for research

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BACKGROUND: Vector specificity of fasciolid parasites defines disease distribution and characteristics. Different lymnaeid species appear linked to different transmission and epidemiological patterns. When assessing disease characteristics in different endemic areas, unexpected results were obtained in studies on lymnaeid susceptibility to *Fasciola.*
METHODS: *Lymnaea schirazensis*, confused with the main vector *Galba truncatula* and/or other *Galba*/*Fossaria* vectors, were analyzed from geographical areas with human and/or animal fascioliasis endemicity of different continents. Morphometry, anatomy and egg cluster analyses allowed for phenotypic differentiation. Nuclear rDNA and mtDNA sequences and phylogenetic reconstruction were used. Experimental procedures with different geographical strains of *F. hepatica* and *F. gigantica* miracidia allow for susceptibility/resistance analyses.
RESULTS: A naturally-infected *L. schirazensis* specimen was never found, despite inhabiting localities where high fascioliasis prevalences are known. Molecular and phylogenetic studies highlighted an old evolutionary divergence from other *Galba*/*Fossaria*, and a low intraspecific variability suggesting a recent spread from one geographical source. Experimental assays showed that *L. schirazensis* is not a vector species: snail finding and penetration by *F. hepatica* miracidium occur but never lead to cercarial production.
CONCLUSIONS: *Lymnaea schirazensis* has been distorting fasciolid specificity/susceptibility and fascioliasis geographical distribution data. It is an efficient biomarker for the follow-up of livestock movements, a crucial aspect in fascioliasis emergence. It offers an outstanding laboratory model for genetic studies on susceptibility/resistance in *F. hepatica*/lymnaeid interaction, a field of applied research with disease control perspectives.

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Hybrids and mitochondrial introgression in Phyllosoma complex: analysis of new mtDNA *nad*1 gene and relationships with Chagas disease epidemiology

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BACKGROUND: The Triatomini species included in the Phyllosoma complex are of epidemiologic importance due to their parasitic infection and colonization indexes. Variable percentages of *Trypanosoma cruzi* infection have been reported. These vectors are found associated with human dwellings in central and southern Mexico.
METHODS: Multigenic combination of rDNA and mtDNA markers conforms the most appropriate genetic approach for the assessment of interpopulation and intrapopulation variability. Genetic differences, phylogenetic relationships and the resolution level of the complete *nad*1 gene of mtDNA *versus* nuclear rDNA (ITS-2) were obtained using specimens representing all members of the Phyllosoma complex.
RESULTS: Complete *nad*1 gene provided 17 new haplotypes for the Phyllosoma complex, showing 21.4% and 8.5% of nucleotide and amino acid variability, respectively, with an irregular distribution of variable positions along the entire gene. Five groups of *nad*1 sequences are described which do not correspond to a given morphological species, nor a geographical pattern of distribution. ITS-2 distinguishes three subspecific groups of sequences for this complex.
CONCLUSIONS: Because of the faster evolutionary rates of mtDNA genes, higher genetic distances between taxa of the Phyllosoma complex appear when analyzing the complete *nad*1 gene. The heterogeneity and discrepances of combined rDNA and mtDNA haplotypes in Phyllosoma highlight their classifications problems, corroborate the detection of hybridization and introgression between them and increase the genetic approaches to be considered when dealing with triatomines with mixed characteristics which may differ in behaviour, domestication, epidemiology or susceptibility to *T cruzi* infection.

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The discovery of a nuclear ribosomal DNA pseudogene in triatomine vectors opens a new research field of wide fundamental and applied implications in Chagas disease in North, Central and northern South America

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BACKGROUND: A pseudogene, paralogous to the 5.8S gene and ITS-2 spacer of the nuclear ribosomal DNA, is described in many triatomine species distributed throughout North America, Central America and northern South America.
METHODS: Such a nuclear rDNA pseudogene is very rare. In the 5.8S gene, criteria for pseudogene identification included length variability, lower GC content, mutations regarding the functional uniform sequence, and relatively high base substitutions in evolutionary conserved sites. At ITS-2 level, criteria were the shorter sequence and large proportion of insertions and deletions (indels). Pseudogenic 5.8S and ITS-2 secondary structures were different from the functional foldings, different one another, showing less negative values for minimum free energy (mfe) and centroid predictions, and lower fit between mfe, partition function, and centroid structures.
RESULTS: A complete characterization indicated a processed pseudogenic unit of the ghost type, escaping from rDNA concerted evolution and with functionality subject to constraints instead of evolving free by neutral drift. This pseudogene distinguishes different taxa and furnishes coherent phylogenetic topologies with resolution similar to the functional ITS-2. The discovery of a pseudogene in many phylogenetically related species is unique in animals and allowed for an estimation of its palaeobiogeographical origin, inheritance pathways, evolutionary rate and pattern, and geographical spread.
CONCLUSIONS: This relict pseudogene proves to be a valuable marker for specimen classification, phylogenetic analyses, and systematic/taxonomic studies. It opens a new research field, Chagas disease epidemiology and control included, given its potential relationships with triatomine fitness, behaviour and adaptability.

Studies funded by Red de Investigación Cooperativa en Enfermedades Tropicales - RICET (Project No. RD12/0018/0013 of RETICS), VI National Plan of I+D+I 2008-2011, ISCIII - Subdirección General de Redes y Centros de Investigación Cooperativa, Ministry of Health, Madrid, Spain; and by PROMETEO Project No. 2012/042, Programa of Ayudas para Grupos de Investigación de Excelencia, Generalitat Valenciana, Valencia, Spain.

Management of human fascioliasis endemic areas: from flukes and snails to environmental analyses, from transmission and epidemiology assessment to control intervention

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BACKGROUND: Human fascioliasis appears to be an extremely heterogeneous diseases, including two causal agent species *Fasciola hepatica* and *F. gigantica* presenting different geographical distribution, respectively diferent specific vector species of the *Galba*/*Fossaria* and *Radix* groups, and different environmental requirements and abiotic factor thresholds.
METHODS: Field and laboratory studies (both experimental and non-experimental) performed on human endemic areas distributed in Latin America, Europe, Africa and Asia during the last two decades have allowed for comparison analyses and the distinguishing of different transmission an epidemiological situations.
RESULTS: Different transmission patterns and epidemiological situations may be distinguished according to the different human endemic endemic areas. Different environments, among which abiotic factors linked to altitudinal characteristics appear to be crucial, and different human behaviours and traditions mainly related to livestock management and local diet may be considered in the front-line.
CONCLUSIONS: The significantly different situations indicate that made-to-measure control action, including from different diagnostic methods and techniques up to different human treatment and animal reservoir management strategies, is needed according to the different human fascioliasis endemic areas. This was verified, within the initiative of the World Health Organization, in a pilot intervention made in four countries of Latin America, Africa and Asia selected depending on their different disease characteristics.

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Determination of genotypes of *Toxoplasma gondii* in domestic animals and wildlife in captivity in Mexico

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BACKGROUND: Currently worldwide are described over 220 genotypes of *Toxoplasma gondii*. In Mexico, certain genotypes are mainly concentrated in two regions: on the north, with recombinant and atypical genotypes and central part of the country with genotypes I, III and I with extra alleles in some markers. The aim of this paper is to describe the *T. gondii* genotypes identified in domestic animals and wildlife in captivity in Mexico.
METHODS: Tissue samples of 92 animals, including cats, dogs, sheep, cattle, New World monkeys, Australian marsupials, lemurs and wild carnivores, with presumptive or confirmed diagnosis of toxoplasmosis were obtained. Extracted DNA was analyzed by PCR using B1, SAG2, SAG3, GRA6 and BTUB genes, genotyping was performed by PCR-RFLP using the same markers.
RESULTS: 35 samples for PCR amplification products was defined, determined partial genotypes for 19 animals. The determined genotypes corresponded to type I, I with extra alleles or atypical genotypes. The genotypes I (in domestic animals) and I with extra alleles, differ from those described in the literature in the case of New World monkeys and captive Australian marsupials, which died of acute toxoplasmosis. In the cases of the black bear and marsupials from the valley of Mexico, which both were asymptomatic, atypical genotypes were determined, similar to those described in these species in their home countries.
CONCLUSIONS: Our results allow us to infer that the genetic diversity of *T. gondii* in Mexico is higher than currently described, being of relevance to continue this type of studies to better understand the epidemiology and impact of *T. gondii* in México.

Identification of phosphatidylcholine transfer protein-like in the parasite *Entamoeba histolytica*

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BACKGROUND: Caveolin is the protein marker of caveolae-mediated endocytosis. Previously, we demonstrated by immunoblotting and immunofluorescence that an anti-chick embryo caveolin monoclonal antibody recognizes a protein in amoeba extracts. However, there is no caveolin gene in the *E. histolytica* genome database. The aim of this work was to isolate, identify and characterize the protein that cross-reacts with anti-chick embryo caveolin.
METHODS: We identified the protein using two-dimensional SDS–PAGE/LC-MS/MS. The complete gene was cloned, sequenced, expressed in *E. coli*; and polyclonal antibodies were raised against the recombinant protein.
RESULTS: The identified protein, *E. histolytica* phosphatidylcholine transfer protein-like (EhPCTP-L) is a member of the StAR-related lipid transfer (START) protein superfamily. The human homolog binds and transfers phosphatidylcholine and phosphatidylethanolamine between model membranes *in vitro*; currently, the physiological role of PCTP-L remains unknown. Studies *in silico* showed that EhPCTP-L has a central START domain that theoretically conserves its phosphatidylcholine binding function and also contains a C-terminal intrinsically disordered region. The anti-rEhPCTP-L antibody revealed that EhPCTP-L is found in the plasma membrane and cytosol; this is consistent with previous reports on the human counterpart. Additionally, assays using filipin to sequester cholesterol, showed that cholesterol levels regulate the expression of EhPCTP-L.
CONCLUSIONS: Localization results points to the plasma membrane as one possible target membrane for EhPCTP-L. Cholesterol regulation of EhPCTP-L expression contributes to the emergent evidence that phosphatidylcholine transport and cholesterol regulation are interrelated. The present study provides information towards understanding the possible function and regulation of PCTP-L expression in *E. histolytica*.

Evaluation of the capacity amebicide of resveratrol on trophozoites of *Entamoeba histolytica*

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BACKGROUND: Amebiasis is caused by the parasite protozoan *Entamoeba histolytica.* Every year there are 50 million people with amebiasis, of which 100 000 die. The current treatment against this parasitic disease is by metronidazole, nevertheless, it presents collaterals effects and the generation of resistant strains has been reported. For these reasons, it is necessary to find new and safer therapeutic agents against amoebiasis with fewer or no collaterals effects. Resveratrol, a compound found in grape seeds presents bactericidal, fungicide and parasiticide effects and it has been described as a compound with multiple benefits for the human health. In this work we studied the effects of resveratrol on *E. histolytica* trophozoites.
METHODS: We determined the resveratrol IC_{50}, on trophozoites of *E. histolytica* using different dose and incubation times with the drug. Evaluation of morphological changes was performed by optical and transmission electron microscopy. To determine the type of celular death we used immunofluorescence and western blot assays, using a polyclonal antibody against EhAtg8. DNA fragmentation was analyzed by TUNEL experiments while phosphatidylserine was studied using annexin V-FITC.
RESULTS: Resveratrol showed a dose dependent anti-amoebic effect on *E. histolytica* trophozoites, and the IC_{50} was 239 µM. After treatment with resveratrol, cells lost their pleomorphism, appearing rounded and with pyknotic nuclei. By immunofluorescence assays, EhAtg8 protein did not appeared relocates and western blot assay did not evidenced conversion of EhAtg8 to EhAtg8-PE indicating that autophagy was not the cause of dead. On the other hand, our experiment showed externalization of phosphatidylserine and DNA fragmentation, suggesting apoptosis as the cause of resveratrol treatment.
CONCLUSION: The treatment with resveratrol induces cellular death by apoptosis in trophozoites of *E. histolytica*.

IL-23 protection against *Plasmodium berghei* infection in mice is dependent on IL-17 from macrophages

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BACKGROUND: Malaria is still one of the most life-threatening infectious diseases. Effective vaccines are eagerly awaited for malaria control. A major obstacle for vaccine development is that the immune responses crucial for eradication of malaria parasites are not fully understood. Thus, to succeed in developing an effective vaccine, it is crucial that the mechanisms underlying protective immunity as well as that those that drive pathogenic responses are better understood. Although IL-12 is believed to contribute to protective immune responses, the role played by IL-23 (a member of the IL-12 family) in malaria is elusive.
METHODS: To evaluate the role of IL-23, mice deficient in IL-23 (p19KO) were infected with *Plasmodium berghei* NK65 and were analyzed for immune responses.
RESULTS: IL-23 is produced during infection, and p19KO mice had higher parasitemias and died earlier than wild-type (WT) controls. Interestingly, p19KO mice had lower numbers of IL-17-producing splenic cells than their WT counterparts. Furthermore, mice deficient in IL-17 (17KO) suffered higher parasitemias than the WT controls, indicating that IL-23-mediated protection is dependent on induction of IL-17 during infection. We found that macrophages were responsible for IL-17 production in response to IL-23. We observed a striking reduction in splenic macrophages in the p19KO and 17KO mice, both of which became highly susceptible to infection. Thus, IL-17 appears to be crucial for maintenance of splenic macrophages. Adoptive transfer of macrophages into macrophage-depleted mice confirmed that macrophage-derived IL-17 is required for macrophage accumulation and parasite eradication in the recipient mice. We also found that induction of CCL2/7 was responsible for IL-17 recruitment of macrophages to the spleen.
CONCLUSIONS: Our findings reveal a novel protective mechanism whereby IL-23, IL-17, and macrophages reduce the severity of infection with blood-stage malaria parasites.

Antileishmanial activity of methanolic extract of bark from *Bursera morelensis*

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BACKGROUND: Leishmaniasis represents a group of tropical diseases caused by infection with protozoan parasites of the genus *Leishmania*, it has an annual worldwide estimated prevalence of 12 million cases, approximately three-fourths are cases of cutaneous leishmaniasis (CL) caused mainly by *Leishmania mexicana*; pentavalent antimonial were considered the treatment mainstay in therapy for CL, however these drugs have been used for over 40 years. The WHO considered that investigation oplants used in medicine traditional to treat parasitic infections as an essential and high-priority field of study. Due to extensive use in mexican traditional medicine and its remarkable content of secondary metabolites, it is probably that *B. morelensis* has activity on *L. mexicana* promastigotes. In previous studies, we found that *B. morelensis* is anti-bacterial, anti-fungal and anti-protozoal, because of this we believe it is a good candidate to have anti-leishmanial properties. In this work the antileishmanial activity of *B. morelensis* on *L. mexicana* promastigotes was evaluated.
METHODS: The anti-leishmanial activity was determined in vitro by MTT; apoptosis was evaluated by analyzing phosphatidylserine externalization and mitochondrial membrane potential by flow cytometry; the cytotoxicity of the extract was determined in the cell line P388 by crystal violet technique and finally the chemical composition analysis was realized with gas chromatography–mass spectrometry (GC-MS).
RESULTS: We found that the extract of *B. morelensis* has in-vitro effect on *L. mexicana* promastigotes and the LC_{50} was 0.213 mg/ml, generated 83% of apoptosis in promastigotes; it is not cytotoxic according the CNI (IC_{50} of the extract was 0.238 mg/ml) and the GC-MS analysis recorded 6 major compounds.
CONCLUSIONS: Our results suggest that methanolic extract of *B. morelesis* has anti-leishmánial activity and induce apoptosis in *L. mexicana* promastigotes, is not cytotoxic and most abundant compounds are lanosterol, cycloartenol and terbutilbisfenol.
PAPIIT IN213713, IN211614, supported this work.

Thrombotic mechanisms involved in experimental cerebral malaria

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BACKGROUND: Cerebral malaria (CM), the most common clinical complication caused by *Plasmodium falciparum* infection, is a multifactorial syndrome that leads to local damages into brain tissue and blood flow blockage. Additionally to host immune response imbalance, coagulation disorders marked by endothelial activation, hemorrhagic spots and micro-thrombi formation are also observed in patients who succumb CM. C57BL/6 mice infected with *Plasmodium berghei* ANKA (PbA) has been successfully employed as an experimental model for cerebral malaria (ECM), however the relevance of this model is still not fully accepted.
METHODS: Carotid artery thrombosis was induced photochemically in PbA- (cerebral model) and PbNK65- infected mice (a non-cerebral line) and the blood flow was monitored with a specific probe. Coagulation pathways were evaluated by prothrombin time (PT) and activated partial thromboplastin time (APTT). Platelets number was evaluated using a hematology analyzer veterinary and platelet factor 4 was quantified by ELISA assay.
RESULTS: Carotid occlusion occurred significantly more rapidly in PbA-infected mice than mice infected by PbNK65, while PT and APTT were not altered in both groups. Treatment of PbA-infected mice with artesunate significantly reverted occlusion time despite no correlation between parasitemia and occlusion time was noticed. Moreover, the number of platelets is unlike to be directly related with this prothrombotic status. In contrast, the platelet factor 4 that has been associated with platelet activity is increased in PbA-infected mice plasma.
CONCLUSIONS: Our findings show that PbA infection leads to a pro-thrombotic condition, however this phenomenon is not associated to parasitemia levels and that functionality, rather than total number, of platelets plays a role in thrombi formation.

Amebicide effect of the methanolic extracts of three species of *Bursera* genus against *Naegleria fowleri*

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BACKGROUND: *Naegleria fowleri* is the causal agent of primary amoebic meningoencephalitis (PAM). *N. fowleri* has been isolated from waters where aquatic activities are realized and the infection is propitiated, because his route of dissemination is through the nasal passages, where they migrate to the brain and feed and destroy the tissues. PAM is almost always mortal.
The antifungic Amphotericin B is the only anti-*Naegleria* agent, which it is efficent against the amoeba but produce grave secondary effects, so the search of alternatives for the treatment of this disease is important. The methanolic extracts of *B. aptera, B. arida* and *B. morelensis*, can be effective against *N. fowleri* due to their biologic properties, specially their antifungical activity.Therefore, the objetive of this work was to determinate the amebicide effect of the methanolic extracts of *B. aptera*, *B. arida* and *B. morelensis.*
METHODS: The in vitro effect against *N. fowleri* of the extracts and their cytotoxicity were evaluated by crystal violet cell viability technique. The apoptotic effect of the extracts was evaluated by flow cytometry and their chemical composition was determinate by GC-MS.
RESULTS: The 3 species had in vitro effect against *N. fowleri*, being *B. arida* the one with lower LC_{50}. Of the three extracts, only *B. morelensis* had no cytotoxic effect. The 3 extracts showed apoptotic effect, being *B. morelensis* the specie that produces higher percentage of apoptotic cells. The chemical composition of the 3 extracts was different.
CONCLUSIONS: Some of the compounds of the extracts have been reported with biological activity, like medicinal properties, antibacterial, antimicotic, anti-parasitary, apoptotic and anti-cancerigen. It is probably that the activity of the extracts against *N. fowleri* is due to the presence of some of these compounds.
PAPIIT IN213713, IN211614, supported this work.

Anti-leishmanial activity of methanol extract of Bursera aptera Ramírez.

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BACKGROUND: Leishmaniasis is a parasitic disease that has three clinical forms. The treatments against this disease are becoming less effective because the parasite has generated resistance to these; it is therefore necessary to find alternatives to treat leishmaniasis. Traditional medicine is considered by WHO, like an alternative to combat parasitic infections. In this work the effect of the methanol extract of the bark of *Bursera aptera* on *Leishmania mexicana* promastigotes was evaluated.
METHODS: The in vitro effect of the extract was assessed by cell viability method with MTT; subsequently the apoptosis generation by the extract in *L. mexicana* promastigotes by flow cytometry with Annexin V kit-IP was evaluated. The anti-inflammatory effect of the extract in paw edema by carrageenan was measured. The cytotoxicity assay of the extract on P-388 macrophages was evaluated by Crystal Violet method and finally the chemical composition of the extract by GC-MS was determined.
RESULTS: *B. aptera* extract has anti-leishmanial effect with an LC_{50} of 0.408 mg/ml; a 20% of apoptosis on promastigotes was generated by the extract. 18 compounds from the methanolic extract were identified; inflammation was inhibited to 63.55% to a concentration of 500 mg / kg; finally we found that the extract is cytotoxic according to CNI criteria.
CONCLUSIONS: Our results suggest that the *B. aptera* extract shows antileishmanial activity generates apoptosis, besides inhibiting inflammation and is cytotoxic. These biological properties can be explained based on the chemical composition thereof.
PAPIIT IN213713, IN211614, supported this work.

In vivo characterization of the effect of the methanolic extract of the bark of _Bursera morelensis_ on the lesion caused by _Leishmania mexicana._

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BACKGROUND: Leishmaniasis is a parasitic disease prevalent in tropical areas of the world caused by different species of *Leishmania* protozoa, transmitted by hematophagous dipterous insects, creating a serious public health problem by high economic costs in addition to the treatments that are currently administered are the same as 40 years ago, for at that this parasite have acquired resistance to those treatment, besides its serious side effects, the OMS considered traditional medicine as an option for the development of new drugs. *B. morelensis* is used in traditional medicine to wound healing and in recent studies have shown that this plant have antimicrobial properties.
METHODS: We obtained the methanolic extract from *B. morelensis* that was applied topically over the lesion of mice infected with *L. mexicana*; the lesion was monitored for nine weeks, and samples from the lesions caused by the parasite were obtained and analysed by histology. Pro and anti-inflammatory Cytokines were measured by ELISA, and the anti-inflammatory effect of the extract with the carrageenan model was evaluated.
RESULTS: A decrease in size of the lesion was observed caused by *Leishmania mexicana,* the ELISA test revealed that the extract causes a decrease in pro-inflammatory cytokines and an increase in anti-inflammatory cytokines; finally it was found that it possesses anti-inflammatory activity approximately 60%.
CONCLUSIONS: Our results suggest that the *B. morelensis* extract, reduces the size of the lesion caused by *Leishmania Mexicana*, have an anti-inflammatory effect and increases the production of IL-4 and decreases TNF-α.
PAPIIT IN213713, IN211614, supported this work.

Genetic diversity of *Toxoplasma gondii* in Mexico

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Few studies on *Toxoplasma gondii* genotyping have been carried out in Mexico. The first report of isolation of *T. gondii* was done in free-ranging chickens from rural Estado de Mexico and D.F. in 2004. Genotyping of chicken isolates of this protozoan using the SAG2 locus indicated that five were type III and one was type I. Later, the DNA obtained from *T. gondii* isolates in dogs and cats from Durango, as well as four isolates from free-range chickens from Mexico, previously isolated, were genotyped by PCR-RFLP, using 11 markers. One was type III (strain #2), three recombinants (strains #73, #74 and other not previously described) and two atypical (strain #9). Two cases of lethal acute toxoplasmosis in squirrel monkeys of Mexico City were described in 2011. Digestion of the *SAG3* gene amplicon showed similar bands to type I reference strains. Four cases of perinatal toxoplasmosis diagnosed at the Instituto Nacional de Perinatología and Instituto Nacional de Pediatría were published in 2012. RFLP patterns of *BTUB* and *SAG2* presented unique alleles in two cases, which suggest that these parasites were different from, but related to type I strains. Genotype of the other two parasites was classic I as shown by *SAG3* and/or *SAG2.* Recently, a mouse virulent *T. gondii* strain was isolated from the heart of a wild puma. DNA isolated from culture-derived tachyzoites was characterized using 11 PCR-RFLP and a new genotype for Mexico was found (strain #222). Other works carried out by us in dogs, cats and ruminants from D.F., Hidalgo and Colima revealed types I, III and recombinants using four PCR-RFLP markers. In conclusion, classical, recombinant and atypical strains have been found in different parts of Mexico. Different patterns were observed among regions, with classical strains in the North of Mexico and more atypical strains in the center.

Anti-*Naegleria* and anti-*Acanthamoeba* activity of ethanol extract of two propolis of Mexican Republic.

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BACKGROUND: *Naegleria fowleri* and *Acanthamoeba castellanii* are free-living amoebae and are capable of causing primary amoebic meningoencephalitis and chronic granulomatous amoebic encephalitis respectively. So far there is no adequate treatment for infections caused by these amoebae since the drugs are very toxic. Considering the above, the search for new alternatives is necessary, the WHO have considered that the investigation of natural products used in traditional medicine to treat parasitic infections is an essential and high-priority field. Propolis is a natural product with antibacterial, antifungal, and antiprotozoal activities, among others. The antiamoebic activity of the ethanol extract of two propolis from Mexican Republic was evaluated.
METHODS: The antiamoebic activity was determined by the technique of crystal violet. The antiinflammatory activity was evaluated by carrageenan-induced paw edema. Furthermore was realized the chemical characterization of secondary metabolites by analyzing high performance liquid chromatography and gas chromatography coupled to mass spectrometry. Finally was realized the technique of cytotoxicity on macrophage to determine the IC50.
RESULTS: With respect to antiamoebic activity, the Guanajuato's propolis (GP) presented an IC50 of 0.009mg/mL and the Mexico State´s propolis (MSP) showed an IC50 of 0.039mg/mL on *N. fowleri*, to *A. castellanii* GP registred an IC50 of 0.617mg/mL and MSP of 0.677mg/mL. The antiinflammatory activity, showed that both propolis are anti-inflammatories. Regarding cytotoxicity was obtained an IC50 of 0.034mg/mL to GP and 0.068mg/mL for MSP. Pinocembrin, naringenin and others compounds were identified, these secondary metabolites may be responsible for the different biological activities of both propolis.

CONCLUSIONS: The two propolis have activity against *N. fowleri* and *A. castellani*. Both propolis have antiinflammatory activity. These two propolis have different chemical composition. The propolis aren't toxic.PAPIIT IN213713, IN211614, supported this work.

The pivotal host oncogene c-Myc is induced by a novel *Toxoplasma* effector, MYR1

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BACKGROUND: *Toxoplasma gondii* infection causes dramatic changes in the host transcriptome by manipulating key host regulators via secreted effectors. We have discovered that *Toxoplasma* tachyzoites mediate rapid and sustained induction of a pivotal host transcription factor, c-Myc. This induction is *Toxoplasma*-specific as the closely related Apicomplexan parasite, *Neospora caninum,* does not exhibit this phenotype and neither do nonviable intracellular parasites. The mechanism of c-Myc up-regulation and its effects on *Toxoplasma* infection will be discussed.
METHODS: To assess the effects of c-Myc induction during *Toxoplasma* infection we analyzed the transcript levels of c-Myc targets by microarrays. Using specific inhibitors the involvement of signaling pathways known to be involved in c-Myc regulation was tested. To further elucidate the mechanism of c-Myc induction, we employed a forward genetic screen to isolate *Toxoplasma* mutants defective in c-Myc up-regulation. The resulting mutants were sequenced and their phenotype was characterized *in vitro* and *in vivo*.
RESULTS: We show that the *Toxoplasma*-induced c-Myc is active and that transcripts dependent on its function are up-regulated. We further demonstrate that c-Myc induction by *Toxoplasma* is likely mediated through JNK pathway. Whole genome sequencing analysis of mutants revealed a novel *Toxoplasma* effector MYR1 (MYc Regulator 1) that appears to be responsible for this phenotype and necessary for full virulence during infection of mice.
CONCLUSIONS: Our results suggest that c-Myc regulation contributes to the dramatic host cell remodeling observed during *Toxoplasma* infection. Our study also uncovers a novel effector employed by *Toxoplasma* to modulate its host cell function.

The new vaccines

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BACKGROUND: Multi-epitope, multistage, fully protective, minimal subunit-based, chemically-synthesized vaccines are the answer to infectious diseases scourging humankind (malaria being one of them) due to the disease's high toll, accounting for 200-300 million cases and ~1 million deaths per year.
METHODS: 15-20 mer long, chemically-synthetized peptides, covering the complete length of the 40 most relevant merozoite (Mrz) and 20 sporozoite (Spz) proteins involved in the invasion of RBC and hepatocytes, respectively, were used for recognizing high activity binding peptides (HABPs). Conserved HABPs (cHABPs) were immunologically silent since they did not elicit any antibodies or protection against experimental challenge in *Aotus* monkeys and had to be specifically modified (mHABPs) to render them very high long-lasting antibody-inducing (VHLLAI) and protection-inducing (HIPI) mHABPs when used to immunize target *Aotus* monkeys.
RESULTS: Mrz-derived HIPI and Spz-derived VHLLAI mHABPs displayed polyproline-type II-like ($PPII_L$) structures when the 3D structure of ~200 of them was determined by ^{1}H-NMR, thereby allowing them to fit perfectly into the peptide binding region (PBR) of major histocompatibility complex class II (MHCII) molecules or HLA-DRβ1* in humans. Modifications determining a rotamer *gauche*$^+$ orientation in position 3 of the PBR sequence allowed perfect T-cell receptor (TCR) interaction to form a tri-molecule MHCII-peptide-TCR complex to induce an appropriate VHLLAI- and HIPI-based immune response.
CONCLUSIONS: Vaccine development follows the stereo-electron topological rules of chemistry; these are shown here for *P. falciparum* malaria, thereby paving the way forward for developing new vaccines against the diseases scourging humankind.

The methanolic extract of Bursera arida and its antileishmanial activity

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BACKGROUND: In Mexico, in recent years, has documented an increase in cases of cutaneous leishmaniasis caused by Leishmania mexicana. After 40 years of use, the drugs used are still expensive, with many side effects besides finding parasite resistance towards them. Because of this, the search for new alternative therapies to combat this disease is necessary. One of these alternatives are natural products derived from medicinal plants such as Bursera arida which presented high efficiency in treatment, respiratory, intestinal disorders of skin. In this paper antileishmanial activity of methanol extract of B. arida was evaluated.
METHODS: The in vitro effect of the extract was assessed by cell viability method with MTT; subsequently the apoptosis generation by the extract in *L. mexicana* promastigotes by flow cytometry with Annexin V kit-IP was evaluated. The anti-inflammatory effect of the extract in paw edema by carrageenan was measured. The cytotoxicity assay of the extract on P-388 macrophages was evaluated by Crystal Violet method and finally the chemical composition of the extract by GC-MS was determined.
RESULTS: *B. arida* extract has anti-leishmanial effect with an LC_{50} of 0.012 mg/ml; only a 3% of apoptosis on promastigotes was generated by the extract; it is noteworthy that the mortality of more than 70% of promastigotes by necrosis was recorded, indicating high activity antileishmanial. 6 compounds from the methanolic extract were identified and relationated with antileishmanial, apoptotic and anti-inflammatory activity; inflammation was inhibited to 60 % to a concentration of 500 mg / kg; finally we found that the extract is cytotoxic according to CNI criteria (IC_{50} <0.0048mg/ml).
CONCLUSIONS: Our results suggest that the *B. arida* extract shows antileishmanial activity generates more necrosis that apoptosis, besides inhibiting inflammation and is cytotoxic. These biological properties can be explained based on the chemical composition thereof.
PAPIIT IN213713, IN211614, supported this work.

Mapping cutaneous, mucocutaneous and visceral leishmaniasis in municipalities of one coastal department of Colombia (Sucre) using Geographic information system (GIS)

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BACKGROUND: Use of GIS for development of epidemiological maps in cutaneous (CL), mucocutaneous (MCL) and visceral leishmaniasis (VL) has not been extensively used in Colombia, particularly at the north coastal areas of Colombia, e.g. Sucre department, which have extensive endemic areas of disease. Then, we developed such maps.
METHODS: Surveillance cases data (2007-2011) were used to estimate annual incidence rates using reference population data, on CL, MCL and VCL (cases/100,000 pop) to develop the first maps in the municipalities of Sucre. GIS used was Kosmo® 3.1. We assessed also at the main hospital of the department (Hospital Universitario de Sincelejo, HUS, Sincelejo, Sucre) principal clinical and epidemiological aspects of VL cases. Fifteen thematic maps were developed according municipalities (5 for each form of leishmaniasis), years and disease incidence.
RESULTS: Between 2007-2011, 880 CL cases, 5 MCL cases and 40 VL cases were reported, for cumulated rates of 109.61, 0.62 and 4.98 cases/100,000pop, respectively. Highest CL and MCL incidences were reported at Ovejas municipality (382.87 cases/100,000pop, 2009; and 9.29 cases/100,000pop, 2007, respectively). Ovejas reported 32% of cases of the department. From 2009 its CL incidence rate decreased to 93.88 cases/100,000pop in 2011. From 2007 its MCL incidence rate decreased to 0.00 cases/100,000pop in 2011. Highest VL incidence was reported at Corozal municipality (13.28 cases/100,000pop, 2010). From 2010 VL incidence rate decreased to 1.65 cases/100,000pop in 2011. From those VL patients hospitalized at HUS (diagnosed with rK39 ELISA), 100% survived, being successfully treated with glucantime.
CONCLUSIONS: Burden of all forms of disease is concentrated in the north of the department. Although reduced, impact of VL is still important in this department. Colombia has 6 departments yet with this form of disease, then strategies to eradicate this vector-borne disease, as occurred with onchocerciasis, are necessary. Use of GIS-based epidemiological maps allow to integrate preventive and control strategies for joint control of all forms of disease in this area of the country.

Innate immune modulation by *Taenia crassiceps* and its antigens

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BACKGROUND. Helminth parasites have developed complex strategies of immunomodulation to remain in their host for long periods of time. A growing body of evidence has shown that immunomodulatory activities displayed by helminths can impact the outcome of dendritic cells (DCs) and macrophages' inflammatory responses. However, mechanisms associated with this type of immunomodulation are not completely understood.
METHODS. Female BALB/c and C57BL/6 mice were i.p. infected with 10-20 metacestodes of *T. crassiceps*. Peritoneal macrophages were harvested at early and late infections. Macrophages were stimulated with IFN-γ or IL-4 and phosphorylation of STAT1 and STAT6 was analyzed in 20 min, respectively. On the other hand, bone marrow derived dendritic cells or macrophages were exposed to different doses of *T. crassiceps* excreted/secreted antigens (TcES). Later these cells were stimulated with LPS or IFN-γ, respectively, and analyzed for different signaling pathways as well for inflammatory molecules such as IL-12, TNF-α, and nitric oxide.
RESULTS. We found that DCs exposed to TcES triggered cRAF phosphorylation and interfered with the LPS-induced NFκB p65 and p38 MAPK signaling pathways. Furthermore, TcES-induced cRAF signaling pathway was critical for down-regulation of the TLR-mediated DC maturation and secretion of IL-12 and TNF-α. Finally, we also showed that macrophages obtained from chronically *T. crassiceps*-infected mice displayed an impaired response to IFN-γ, but not to L-4, as measured based on the phosphorylation of STAT1 and STAT6, respectively. Such inhibition was phosphatase-dependent. Interestingly, macrophages exposed to TcES displayed increased expression of SOCS3 and SHP1.
CONCLUSIONS. These findings demonstrate new mechanisms by which helminths target intracellular pathways to block DCs and macrophages' activities.

Characterization of the effect *in vivo* of methanolic extract of bark of *Bursera arida* on *Leishmania mexicana.*

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BACKGROUND: The current treatment for all forms of leishmaniasis are the pentavalent antimonials, but an increasing number of resistant strains is higher and due to its high toxicity it is necessary to find new effective treatments against infection, and an alternative may be to use natural products such as plant extracts used in traditional medicine.
Bursera species are used as a traditional remedy for treating wounds; therefore it can be assumed that the extract of *B. arida* is an option for treating injuries caused by *Leishmania mexicana*.
METHODS: Topically the extract was applied to the lesion to mice and lesion size was measured during infection. Thereafter the effect of the extract on the production of cytokines (TNF-α and IL-4) by ELISA was determined. After this, the anti-inflammatory activity of the extract was determined using the model of carrageenan-induced edema. Finally histological sections were performed to analyse the effect on the lesion.
RESULTS: It was observed that the size of the lesion was gradually decreased during the infection. High low concentration of proinflammatory cytokines (TNF α) and antiinflammatory (IL-4) was obtained. An anti-inflammatory effect was obtained in the three extract concentrations tested. There was a reduction in inflammatory infiltrate in the epidermis and thickening.
CONCLUSIONS: Our results showed a favorable effect of the extract in the treatment of the lesion with decreasing size, have an anti-inflammatory effect and increases the production of IL-4 and decreases TNF-α; extract concentrations tested showed an anti-inflammatory effect, and was observed histologically positive effect on the injury.
PAPIIT IN213713, IN211614, supported this work.

Frequency and genotyping of *Toxoplasma gondii* infection in wild herbivores and carnivores from a private collection

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BACKGROUND**.** Prevalence of *Toxoplasma gondii* infection varies considerably worldwide, depending on the weather and cultural conditions in a given geographical region. Susceptibility to clinical problems development varies among host species. Some of them rarely develop toxoplasmosis, while others are particularly susceptible and in most cases, with fatal consequences like Pallas cat, Australian marsupials, canaries, meerkats, new world primates and lemurs. Currently, the genetic variability of *T. gondii* is higher than considered in past decades. Clustering methods have been used to organize the marked genetic diversity of 138 unique genotypes into 15 haplogroups. *Toxoplasma gondii* infections in captive animals are of particular importance because several species have chronic toxoplasmosis and they can be considered as indicators of the infective pressure of the interest area. The objective of this study was to determine the frequency and genotypes of *T. gondii* in wild herbivores and carnivores from a private collection. METHODS**.** Fifty-five European mouflons and 15 wild felids were bled to obtain serum samples and tested by indirect ELISA. Two highest seropositive mouflons as well as two senile lions were euthanized to collect target organs to extract DNA. PCR and RFLP assays were performed using 7 genetic markers (non-coding 529 pb fragment, *B1, GRA6, GRA7, SAG2*-alternative, *SAG3* and *BTUB*).
RESULTS**.** Four herbivores (7.3%) and 12 carnivores (80%) were seropositive. For most markers, expected products as well as additional amplicons were obtained by PCR. The PCR-RFLP analysis of the SAG2-alternative and SAG3 loci produced patterns completely different to those for the clonal lineages.
CONCLUSIONS**.** The results suggest that toxoplasmosis is of low prevalence within the collection, but can reach high rates after years of exposure. Also, the strains found in mouflons and lions may be atypical. To our knowledge, this is the first report of direct tissue genotyping from mouflons and lions in Mexico.

Efficacy of triclabendazole and prevalence of *Fasciola hepatica* in dairy cattle raised above 3200 meters the sea level in the provinces of Cajamarca, Hualgayoc and Celendín, Peru

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BACKGROUND: Bovine fasciolosis caused by *Fasciola hepatica* is one of the greatest health problems facing dairy farmers in the provinces of Cajamarca, Hualgayoc and Celendín in the northern highlands of Peru, above 3200 meters the sea level. Most are small producers with six animals on average. The operation performed by these producers is characterized by using fewer fasciolicides treatments per year, compared with producers in the valley, and the lack of technical assistance. Drug control is done mostly using triclabendazole.
METHODS: The efficacy of triclabendazole against *F. hepatica* was carried out by the fecal egg count reduction test (FECRT). Parasitological stool examination was performed by the technique of sedimentation in stool samples from 120 dairy cattle, three days before treatment and 28 days after treatment. Additionally, 1,231 stool samples were analyzed by the same technique.
RESULTS: The efficacy of triclabendazole was 82.90 % and the prevalence of *F. hepatica*, under these conditions, was 67.18 %.
CONCLUSIONS: Triclabendazole is ineffective in the treatment of *F. hepatica* in dairy cattle raised above 3200 meters the sea level in the provinces of Cajamarca, Hualgayoc and Celendín, Peru. It is also concluded that the prevalence of *F. hepatica* in this area is 67.18 %.

NUCLEIC ACIDS DISTRIBUTION OF THE *Giardia lamblia* TROPHOZOITES DURING THE NUCLEAR DIVISION.

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BACKGROUND: *Giardia lamblia (G. lamblia)* is a bi-nucleated unicellular eukaryotic protozoan. This parasite is a protist that causes the human diarrheal disease. It affects the duodenum and the proximal jejunum causing a symptomatic infection or chronic diarrhoea with malabsorption síndrome. The changes of the nucleic acids distribution of *Giardia lamblia* trophozoites during nuclear division are described.
METHODS: The acridine-orange stained nuclei of live and fixed *G. lamblia* parasite showed green DNA and orange RNA fluorescence, and the nucleic acid nature was confirmed by DNase and RNase treatment.
RESULTS: The RNA of both nuclei was organized in two forms: as an "eyebrows" and central oval corpuscles. These forms of RNA were fragmented in several condensations during nuclear division and distributed in the two new nuclei. The RNA in interphase did not disappear during mitosis. The DNA of both nuclei began to condense in several spherical bodies during prophase stage. In metaphase, was fully condensed as chromosomes-like structures. In early anaphase, the chromosome-like structures were very small and began to separate. In late anaphase, the chromosome-like structures were polarized. In telophase, the nuclei were elongated and the DNA began to decondense. Similar to other protozoa, karyokinesis was observed just before cytokinesis. In conclusion, the RNA of *G. lamblia* is fragmented during nuclear division and distributed in the two new daughter nuclei.
CONCLUSIONS: The DNA undergoes different degrees of condensation, from decondensed chromatin to chromosome-like structures and we were able to observe structures similar to chromosomes in the metaphase.

Presentation of Case

Acute Chagas is associated with oral transmission. Myocarditis

Report of 2 cases presented during March-April 2014.

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CASE 1

33 years male, coming from Centro Gaitan Casanare (Colombia). Presenting fever, headache, arthralgia, myalgia, bilateral palpebral edema with 20 days of evolution. The patient also had an outbreak. To the physical exam: cervical lymphadenopathy, splitting the first hart sound, pericardial rub, liver at 2 cm from the costal margin, distended abdomen, skin rash: pulmonary Cardiomegaly TAC, Pericarditis, EKG ,AV block first degree.

In the laboratory exams the results were: direct exam positive (parasites were observed ***Trypanosomes s.p***).At the health public laboratory of the department (LSP), the INS serologic tests were performed or were done to determine antibodies ***T. cruzi*** through enzymed-linked inmunosorbent test (ELISA) and it was confirmed through indirect inmunofluorence (IFI) , Echocardiography pericardial effusion 1000 cc, ejection fraction 60% .Etiologic treatment was given : benzonidasol 100 mg every 6 hours, Spironolactone, Enalopril, Carvedidol. Expectant management is defined by progressively decreasing pericardial effusion in the following checkups.

CASE 2

47 years male, coming from Centro Gaitan Casanare Colombia). Presenting fever, chills, and sweating, headaches, myalgia, arthralgia, palpebral edema, chest pain, dyspnea, edema in lower members , skin rash. To the physical exam: cervical lymphadenopathy, splitting the second heart sound, pericardial rub. Basel lung crackles, liver at 1 cm from RCD. Edema in lower members , Echocardiogram showed left ventricular dilatation with fraction ejection of 50%. Chest Rx. Cardiomegaly, keg PR prolongation or augmentation in V1 with negative tests direct from the laboratory. The INS serologic tests were done to determine antibodies ***T. cruzi*** through an enzyme-linked inmunosorbent test (ELISA) and it was confirmed through indirect immunofluorescence (IFI). Etiologic treatment was given: benzonidasol tablets of 100 mg, 3 tablets at 7.00 am and 2 and a half tablets at 7:00 p.m. Beta blockers, ace inhibitors and aldosterone inhibitors were given.

KEY WORDS: CHAGA DISEASE, MIOCARDITIS, ORAL TRANSMISION

Are *Rhodnius prolixus* and *R. robustus bona fide* species? If so, what are their roles in Chagas disease transmission to humans in Latin America? Lessons learned with the use of molecular markers.

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BACKGROUND: This presentation is intended to cover, in a historical perspective, the main findings my group (together with relevant results from other researchers), has generated on the molecular taxonomy, biogeography, and evolution of the species *Rhodnius prolixus* and *R. robustus*. Some discoveries are particularly important in terms of epidemiology and vector control strategies.

METHODS: Several molecular markers were employed. I will focus on recent data derived from mtDNA and rDNA sequences).

RESULTS: The diversity unveiled is far greater and complex than one could have anticipated. In certain instances, the genetic divergence observed between OTUs was so great that a new species was described. This is the case of *Rhodnius barretti* sp. n. I will present evidence that shows, beyond dispute, that *R. prolixus* is a valid species, separate from *R. robustus*. The latter, to our surprise, comprises a cryptic species complex.

CONCLUSIONS:
The sequence data generated was also used to develop molecular diagnostic essays to aid in the identification of “problem specimens” (i.e. the separation of *R. prolixus* from *R. robustus s.l.*). We have done so with the development of an mtDNA multiplex PCR assay, and with the identification and description of a *R. prolixus*-diagnostic nuclear SNP.

Flow cytometric analysis of various T cell subsets in BALB/c mouse spleen after *Plasmodium berghei* (NK-65) infection and immunization using parasite constituents.

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BACKGROUND: Infection with *Plasmodium* is characterized by both activation and suppression of the immune system during different phases of the disease. Lymphocytes play a crucial role in controlling malaria infection. Intracellular parasites manipulate host cells. The present data provides information on the cellular changes taking place in spleen during *P. berghei* infection and after immunizing mice with parasite constituents.
METHODS: Mice were injected with 100µg of total parasite antigen formulated in equal amount of Freund's complete adjuvant (FCA) on D0 and two booster doses in equal volume of Freund's Incomplete Adjuvant (FIA) and antigen at two weeks interval. Lymphocytes from infected and immunized spleen were separated using lysis buffer and centrifugation. For the immunophenotyping, antibodies viz. anti mouse CD3 (FITC conjugated), CD4 (PE conjugated) and CD8 (APC conjugated) were used. For the analysis of T-regulatory cells, antibodies viz. Anti mouse CD4 (FITC conjugated), CD25 (APC conjugated), $FoxP^3$ (PE conjugated) were used.
RESULTS: During *P. berghei* infection the percentages of CD3+, CD4+ and CD8+ cells were found to be high (45%, 17% and 18.3% respectively) which declined subsequently on day 5 post infection, whereas, in immunized mice the percentages of these cells were observed to increase constantly till clearance of infection. The percentage of CD4+ T-regulatory cells in infected group was found to be 38.2±0.8% during high infection 20.7±6.3%, whereas, it was 46.4±0.5% in immunized group before challenge.
CONCLUSIONS: The results of the study revealed that the frequencies of CD4+ and CD8+ cells changes in spleen during *P. berghei* infection which provides a switching over between Th1 and Th2 immune responses which can be useful for vaccine development.

Antioxidant defense systems against oxidative and nitrosative stress in *Giardia intestinalis*

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BACKGROUND: *Giardia intestinalis* is expected to face oxidative and nitrosative stress conditions *in vivo*. Elucidating the parasite antioxidant defense system may thus be a strategy to identify novel potential drug targets. Though lacking common antioxidant enzymes, such as catalase, superoxide dismutase and glutathione peroxidase, *G.intestinalis* was shown to encode, in addition to NADH oxidase, a flavodiiron protein (FDP), a flavohemoglobin (FlavoHb), a superoxide reductase (SOR) and two peroxiredoxins (Prx1a and Prx1b).
METHODS: These enzymes were functionally characterized both as isolated recombinant proteins and in living parasites by time-resolved spectroscopy, high-resolution respirometry and NO-amperometry. Expression in parasitic trophozoites and modulation in stress conditions were assayed by qPCR and immunoblotting.
RESULTS: We found that FDP detoxifies O_2 to H_2O, FlavoHb rapidly metabolizes NO to nitrate in aerobic conditions, and SOR quickly reduces superoxide anion to H_2O_2, whereas Prx1a and Prx1b are the first enzymatic system identified in *G.intestinalis* as being able to detoxify (hydro)-peroxides and peroxynitrite, a harmful reactive nitrogen species. The proteins were shown to be expressed in parasitic trophozoites as residential defense systems against oxidative and nitrosative stress.
CONCLUSIONS: Based on these results, it is suggested that the newly characterized enzymes may contribute to parasite survival in the human intestine and/or resistance to specific drugs. Consistently, overexpression of some of these enzymes was recently reported for *G.intestinalis* upon interaction with human intestinal cells and in drug resistant strains.

Comparison of secretome profile of *Entamoeba histolytica* pathogenic and non-pathogenic strain

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BACKGROUND: The enteric-invading protozoan *Entamoeba histolytica* is a causative agent of amebic dysentery and liver abscess in humans. In order to establish successful attachment and invasion of the ameba into the host, binding to the intestinal epithelium and destruction of the tissue are prerequisite for *E. histolytica*. Trophozoites released from cysts invade to colonic epithelial cells by a contact-dependent manner.
METHODS: In this study, we comparatively analyzed proteome profile of excretory-secretory products (ESP) of pathogenic (HM-1: IMSS) and non-pathogenic Rahman strains of *E. histolytica* through 2-dimensional electrophoresis followed by mass spectrometry.
RESLUTS: A total of 95 proteins was identified from the ESP of pathogenic strain by mass spectrometry. The identified proteins were largely classified into cellular (29%) and metabolic process (37%) in biological processes, binding (51%), transferase (12%) and oxidoreductase activity (11%) in molecular functions, and cell (54%) in cellular components in functional KEGG hierarchy. To order to address differential expression of proteins in ESP between pathogenic and non-pathogenic strains, we comparatively analyzed ESP proteomes of these strains through 2-DE followed by mass analyses. When we observed secretome profile of non-pathogenic strain, at least 27 protein spots revealed significant differences (over 2-fold); seven spots were shown to be increased in ESP of non-pathogenic strain, and 20 spots appeared to be decreased. Interestingly, we found that secretion of several enzymes involved in carbohydrate metabolism (transketolase, alcoholdehydrogenase, phosphoglucomutase, coronin, malic enzyme, pyruvate:ferredoxin oxidoreductase and enolase) and oxidative stress responsive superoxide dismutase were significantly decreased in ESP of non-pathogenic strain.
CONCLUSIONS: We established differential proteome profile of *E. histolytica* ESP of pathogenic and non-pathogenic strain by 2-DE and mass spectrometry for the first time. We were able to identify some enzymes involved in critical biological functions as cellular adhesion, antioxidant activity and local immune regulation, such as enolase, fructose 1,6-bisphosphate aldolase, and triosephosphate isomerase, which were found to be significantly reduced in the ESP. Our result suggested strongly that these enzymes might play functional roles in host-parasite interplay, thus exert their roles in pathogenesis of amoebiasis.

Alteration of immunoproteome profile of *Echinococcus granulosus* hydatid fluid with progression of cystic echinococcosis

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BACKGROUND: Cystic echinococcosis (CE), caused by *Echinococcus granulosus* metacestode, invokes a serious worldwide public health concern. Several antigen B-related molecules (EgAgB; EgAgB1-5) are immunopotent, but their individual properties are unclear because detection of EgAgB in many patients are variable and do not allow precise interpretation of its immunological relevance. Moreover, immunoproteome profile of hydatid fluid (HF) has not been addressed.
METHODS: We determined the proteome of HF of a single fertile cyst of CE1 and CE2 stages through two alternative approaches and analyzed immunoproteome profile employing patient sera of entire disease spectrum (CE1-CE5 stages).
RESULTS: Ninety-four *Echinococcus* proteins were identified, of which EgAgB and antigen 5 (EgAg5) were abundant. EgAgB isoforms, whose main functions included immune modulation and lipid transport, constituted mostly EgAgB1 followed by EgAgB2 and 4. EgAgB3 and 5 were detected in trace amounts. HF of CE1 and 2 cysts exhibited comparable spotting patterns, but CE2 HF harbored greater amounts of EgAgB complex. CE sera demonstrated complicated immune recognition patterns according to the disease progression; CE2 and 3 stages exhibited strong antibody responses against diverse EgAgB and EgAg5 while CE1, 4, and 5 stages mainly reacted to EgAg5 and cathepsin B. Alveolar echinococcosis (AE) sera showed cross-reactions against EgAgB isoforms (36%). EgAg5 and cathepsin B cross reacted with sera from neurocysticercosis, sparganosis, and AE. In conclusion, we demonstrated a global immunoproteome profile of CE for the first time.
CONCLUSIONS: Our data indicated that detection of a single defined molecule may not properly diagnose CE because specific immunopotent antigens are altered according to CE progression. Furthermore, serological cross-reactions between CE and AE may be inherent to some extent. Surveillance of immunoproteome profile combined with imaging scans may be essential to differentiate CE from AE and to clarify CE status. Our proteome data also highlights the plausible biological function of HF proteins, which might be intimately involved in homeostatic and pathophysiological adaptation of the parasite during long-standing infections, thus significantly deepening our understanding of this clinically important human pathogen.

Biochemical properties of two omega-type glutathione transferases of *Clonorchis sinensis*

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BACKGROUND: Glutathione transferases (GSTs) are a family of multifunctional enzymes ubiquitously present in living organisms. They are intimately involved in detoxifying class II enzymes of the helminth parasite. *Clonorchis sinensis* is a trematode parasite that thrives in the hepatobiliary tract of mammals including humans and is intimately associated with cholangiocarcinoma. *C. sinensis* excretory-secretory products (ESP) might constitute first-line effctor system affecting the host-parasite interrelationship by interacting with bile duct epithelium.
METHODS: We have cloned two isotypes of secretory omega-class GSTs (designated as CsGSTo1 and CsGSTo2) of *Clonorchis sinensis*. We subsequently expressed recombinant CsGSTo1 and CsGSTo2 proteins and characterized their biochemical properties.
RESULTS: The full length cDNA of CsGSTo1 was composed of 965 bp, whose open reading frame (ORF) of 741-bp encoded a 247-amino acid polypeptide. The full length cDNA of CsGSTo2 was composed of 1061-bp with an ORF of 753-bp that coded for a 251-amino acid polypeptide. These proteins showed high sequence similarities with *Schistosoma mansoni* omega-type GST. Two recombinant proteins showed enzyme activity toward all class GST substrate, 1-chloro-2,4-dinitrobenzene (CDNB). Interestingly, *C. sinensis* enzymes exhibited high activity toward 4-nitrobenzyle chloride, in contrast to other omega-type GSTs. They also showed enzyme activity against omega-class GST specific substrate, 4-nitrophenyl acetate, as well as dehyroascorbate and hydroethyl disulfide and suggested their roles as a thioredoxin. These enzymes demonstrated no activity against cumene hydroperoxide, 4-hydrozynonenal and ethacrynic acid. Secretion of these proteins was increased in response to bile stress. The proteins were localized in the tegument of the adult worm.
CONCLUSIONS: The two omega-type GSTs of *C. sinensis* showed multifunctional enzymatic properties of glutathione transferase and glutaredoxin features. Induction of the CsGSTs upon stimulation of exogenous stressors suggests that CsGSTo proteins exerted their principal roles in protection of the parasite against host oxidative stresses thus shaping the first-line defensive system.