Visceral Leishmaniasis (VL) or Kala-azar in the state of Assam India: Past, present and prospects of elimination

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Abstract

BACKGROUND: The history of Kala-azar in Assam dates back to 1875 where in Kamrup district alone more that 75,000 people died. Later in between 1875-1950 three major epidemics after a gap of 30, 45 and 20 yrs not only took thousands of human life but also sapped the state health and vitality. After 1950s the cases of Kala-azar were gradually declined due to extensive use of DDT in malaria eradication programme which eliminated sand fly population also from Assam and no new indigenous case of kala-azar was reported after 1960s. However, in 2008 outbreak of Kala-azar in Kamrup Metro district of Assam brought the state again into the limelight.

METHODS: Surveys for Kala-azar and PKDL cases using rK39 diagnostic test and clinical examination were done by house to house visit in 851 households having 4384 population. Inclusion criteria were case having fever more than two weeks old and tested negative for malaria (*Plasmodium falciparum & P. vivax*) or not responding to antimalarials. Kala-azar cases were treated with sodium stibogluconate (SSG) while PKDL cases were treated with Amphotericin b as per WHO guideline.

RESULTS: A total of 166 rK39 positive cases of VL and 6 PKDL cases were detected during 2008-14 (April). Kala-azar and PKDL cases treated with SSG and Amphotericin b respectively were cured. In pediatric age group, rK39 positivity was more than 27%.

CONCLUSIONS: Of the 166 cases of Kala-azar, many are below 10 years of age who have never visited Kala-azar endemic areas. The presence of *Phlebotomus argentipes* and infection in paediatric age group strongly suggest local transmission of infection. Unlike Bihar, Jharkhand and Bengal, Kala-azar is confined only to some of the areas of Assam and offers an opportunity to tackle this disease effectively and can be eliminated within the set target of Government of India.

Prevalence and Predictors of Intestinal Parasites among Food Handlers in Yebu Town, Southwest Ethiopia

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BACKGROUND: Most of the intestinal parasites of medical importance are transmitted by ingestion of food or water contaminated with the infective stages of these parasites. Hence food-handlers with poor personal hygiene working in food service establishments could be potential sources of infections with the intestinal parasites of public health importance.
METHODS: A cross-sectional study was conducted among a total of 118 food handlers in Yebu Town in January 2011. Fresh stool specimen was collected and processed using direct wet mount and Formol ether concentration technique.
RESULTS: The overall prevalence of intestinal parasites among the study subjects was determined to be 44.1% (52/118). *A.lumbricoides* and Hookworm spp were the predominant parasites identified from the stool of study participants. The age group [AOR: 4.8, 95% (1.1, 21.8)], hand washing practice before meal [AOR: 7.8, 95%CI (2.8, 24.8)], and finger nail status [AOR: 14.7, 95%CI (2.8, 75.4)] were the independent predictors of intestinal parasitic infection among food handlers in Yebu Town.
CONCLUSIONS: The present study showed high prevalence of intestinal parasites among the study subjects. The study also revealed poor personal hygiene like poor practice of hand washing and poor finge nail hygiene. Therefore pre-placement and periodic screening of food handlers for parasites, periodic deworming, health education on regular trimming or cleaning of fingernails would be the way forward for prevention of food borne diseases.

DNA- based molecular and in-silico characterization of paramphistomes (Trematoda: Digenea)

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BACKGROUND: Among digenetic trematodes, paramphistomes are the eitiological agent of “amphistomiasis”- the disease resulting in significant productivity losses and high death rates in domesticated ruminant mammals in subtropical and tropical areas. Molecular characterization of this morphologically-difficult-to-identify group of parasites is highly warranted.
METHODS: DNA was isolated from 14 species of paramphistomes collected from the ruminant livestock from various locations in Northeast India. Genetic markers viz., second internal transcribed spacer (ITS2), small and large ribosomal subunit DNA (18S and 28S rRNA) and mitochondrial cytochrome oxidase1 (mtCO1), were amplified and their sequences analyzed for phylogenetic studies. Molecular morphometrics were based on the secondary structure of ITS2 and D (D1+D2+D3) domains of 28S rRNAs. Restriction fragment profiles were generated using several endonucleases and DNA barcodes developed to ascertain the species identification utilizing relevant bioinformatics tools.
RESULTS: In sequence analyses, 18S rRNA emerged as the most conserved (showing 2.1% genetic variation) and mtCOI the most variable (22.3% variation).among the markers. The constructed phylogenetic trees showed an expected clustering of *Paramphistomum* spp, *Orthocoelium* spp and members of Gastrodiscinae, supporting morphology-based classification. All the 14 secondary structures differ in morphology and free energy values. Of the 28S rRNA D domains, only D2 comprised compensatory mutations in helices of its structural constraints. Restriction profiles revealed polymorphism among isolates of *Calicophoron* spp, *Olveria* spp and *Orthocoelium spp.* We also generated DNA Barcodes for all species.
CONCLUSIONS: Our results suggest that D2 domain is well suited for species distinction and may be considered a potential DNA barcode complementary to mitochondrial DNA. A molecular approach would expedite the estimation of this group of parasites of veterinary importance.

DNA-based molecular identification of Lytocestus spp from *Clarias batrachus* (L.) and *Heteropneustes fossilis* in Meghalaya, Northeast India

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BACKGROUND: Monozoic cestodes of the Order Caryophyllidea are the most predominant group of helminthes parasitizing freshwater siluroid catfishes in Northeast India and as many as seven species of *Lytocestus* are reported parasitizing Clarias batrachus alone. These frequently encountered species are distinguishable from each other on the basis of not too reliable phenotypic characters. Discrimination based on morphological characters alone may be ambiguous, more so when the bothria are inconspicuous. The present study was aimed to provide molecular characterization of the seven closely resembling species so as to supplement their phenotypic differences.
METHODS: For the purpose rDNA Internal Transcribed Spacer 2 region (ITS2), and mitochondrial DNA (mtCO1) were used as the parameter because of their proven usefulness in identification of taxa at the species level. The region was PCR-amplified using universal primers of platyhelminths and the amplicons were sequenced. The sequences obtained were analysed using BLAST and ClustalW bioinformatics tools. Evolutionary phylogenetic trees were constructed using Bayesian inference (BI) with MrBayes, version 3 and Neighbor-Joining (NJ) and Maximum Parsimony (MP) criterion with MEGA6.
RESULTS: Since, limited information on ITS2 and mtCO1 genes of any other caryophyllid is available in GeneBank, the query sequences were aligned with Pseudophyllidea the closely related group of cestodes. BLAST results showed that the sequences of the caryophyllid species closely matched with those of other members of the families Lytocestidae. The trees reconstructed reveal two major groups for ITS2 and mtCO1 genes - that of the family Caryophyllidae and Pseudophyllidae, well separated from each other.
CONCLUSIONS: Analysis of the results suggest that ITS2 and CO1 gene are effective interspecific markers for discrimination of caryophyllid species under study and does help in resolving taxonomic issues pertaining to species of Lytocestus.

In vitro efficacy of _Artemisia indica_ (Asteraceae) on helminth parasites, (Raillietina _echinobothrida_): Motility, mortality and electron microscopic observations on surface topography; histochemical and biochemical study

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BACKGROUND: Alcoholic extract of *Artemisia indica* (Asteraceae) was tested in vitro against helminth parasites, *Raillietina echinobothrida* from domestic fowl. The live adult parasites, collected from a freshly autopsied host, were exposed to different concentrations (5–50mg) of test plant extract in physiological phosphate-buffered saline (PBS) having 0.1% dimethyl sulphoxide (DMSO) at 37±1ºC. The treated parasites revealed complete inactivation and flaccid paralysis that was followed by death at varying periods of time. Motility and/or mortality of the worms were carried out through observations on the physical movement of the worms in the incubation media. Observation on morphological alterations was carried out with stereoscan, histological and ultrastructural observations on treated worms. Inhibition of important tegumental enzymes was carried out through histochemical and biochemical studies.
METHODS: Plant parts were grind and refluxed in ethanol (100 g/l) for 8 h at 60ºC. The cooled suspension was then filtered through Whatman filter paper No.1 to remove small particulates and then the volume of the solvent was reduced through distillation, to complete dryness at reduced temperature of 45ºC in an oven. The crude extract obtained as powder material was then refrigerated at 4ºC until further use. Control parasites were maintained in 0.9% PBS (pH 7.2) at 37±1ºC, having 0.1% DMSO only, whereas for treatment live worms were directly incubated in different concentrations of crude plant extract and its fractions dissolved with 0.1% DMSO in separate petri dishes.
RESULTS: *Raillietina echinobothrida* incubated in the control medium showed physical activity for a longer period of time, the controls survived for about 72.00±0.06 following which became immobilized and dead. On exposure to the test medium (plant extracts), the parasites proceeded from the state of vigorous movement to a relax state in which they remain till they attained paralysis leading to death at different time irrespective to dose concentration.
CONCLUSIONS: Analysis of the results sugges that *Artemisia indica* (Asteraceae) showed high efficacy against *Raillietina echinobothrida.* The present finding therefore, aims to provide clues on the likely targets on helminth parasites with anthelmintic components of the test plants.

Presence of Canine *Ancylostoma ceylanicum* in School Age Children of a Tribal Community in India

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BACKGROUND: Hookworm infections in humans are generally caused by *Necator americanus* and *Ancylostoma duodenale.* However, recent studies also revealed the presence of the canine hookworm *A. ceylanicum* in human stool. Hookworm infections were highly prevalent in school age children of a tribal community in India (29.8%), but it remains unknown which hookworm species are causing these infections. The main objective of this study was to characterize the hookworm infections in these children.
METHODS: Fifty samples from each individual infected with hookworm were randomly picked from a total of 185 individuals excreting hookworm eggs. These samples were collected during a cross sectional survey designed to assess the infection of soil-transmitted helminthes in 620 children of 2 to 5 years of age in 30 villages of Jawadhi hills (a tribal community). Molecular differentiation of the hookworms was based on a nested PCR targeting the ITS 1, 2 and 5.8s gene, which allowed differentiating between *N. americanus* (552 bp) and *Ancylostoma* spp. (404-408 bp). Species of *Ancylostoma* (*A. duodenale* and *A. ceylanicum*) were further characterized by means of RFLP assay.
RESULTS: The majority of the hookworm infections were caused by *N. americanus* (n = 33), and to a lesser extent by *Ancylostoma* spp. (n = 8). Co-infections between *N. americanus* and *Ancylostoma* spp. were seen in 3 of these samples. Of the 8 *Ancylostoma* infections, 6 were caused by *A. duodenale*, the remaining 2 by *A. ceylanicum*.
CONCLUSIONS: The results indicate that in this tribal community, human hookworm species account for the majority of the hookworm species. Although canine hookworm *A. ceylanicum* only accounts for a minority of the infections, it confirms that dogs may play a role in transmission of hookworm infections in humans, and hence highlighting the need to consider zoonotic transmission when developing strategies to control hookworm infections.

Effect of praziquantel treatment on *Clinostomum complanatum* infected gray heron (*Ardea cinerea*)

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BACKGROUND: *Clinostomum complanatum* is one of the fluke parasites of the piscivorous birds that rarely cause incidental infection in humans. In humans, they cause laryngopharyngitis called “halzoun” or “marrara” and the only known treatment is mechanical removal of the worms with anesthesia using lidocaine. However, other treatment methods that this were not even tried. In this study we report a case of chemotherapy to an avian host, gray heron (*Ardea* cinerea), infected with C*. complanatum*.
METHODS: A gray heron was rescued from Cheongju City, Chungbuk, Korea in May, 2013. The rescued bird was exhausted but no other clinical signs were detected in physical examinations except for finding of a lot of flukes inside of the buccal cavity. For treatment, we tried to use praziquantel solution 50mg/kg for oral ingestion; and we checked status of flukes every 15 minutes. At each time, we collected a worm for morphological examination.
RESULTS: Before drug ingestion, we found 21 flukes attached to mucous membrane of entire buccal cavity. After 1 minute of drug ingestion, the flukes loosed attachment and stayed hold by using oral sucker only, and the body was swollen. After 15 minutes, only 1 drooping fluke was found left, and during those minutes, the host kept swallowing behavior. At morphological examination, the fluke was damaged and lost their own shapes, especially genital organs. The body between 2 suckers was weakened and easily broken. At the first fecal examination after the treatment, we found a great number of yellowish fluke eggs from the bird. However, at the additional fecal examinations during the consecutive 3 days, we could not find any eggs of *C. complanatum;* and adult flukes were not found either from buccal cavity.
CONCLUSIONS: Our study shows praziquantel can be used for treatment of *C. complanatum* infection *in vivo.* This method may have some problems in removal of dead parasite bodies (spitting or swallowing), though it needs only a tablet with 15 minutes of cure time. Praziquantel was considered to be a way of effective treatment method for *C. complanatum* infection in humans.

Molecular diagnostics of Nematode species Infecting Ruminants in Egypt

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BACKGROUND: Molecular techniques are utilized for the diagnosis of parasitic diseases and identification of parasites, for the development of specific antigens for serological tests and studying immune response in the patients.
METHODS: In the present study, nematodes spp which were *Trichuris ovis* recoverd from goats *Trichuris ovis*, *Haemonchus contortus*, and *ovine strongyloides papillosus* rom sheep. *Toxocara vitullorum* from buffaloes and *Toxocara vitullorum* from cattle. All samples were checked with 3 different primers by the use of RAPD-PCR. The genetic profiles of all nematodes spp. from different ruminants hosts were compared. Pairwise comparisons were used to evaluate sequence homology and diversity of some variable regions was identified.
RESULTS: Interspecific variation in the regions exceeded that within species. Comparison between *Toxocara vitullorum* from cattle and *Toxocara vitullorum* from buffaloes revealed genetic polymorphism with genetic variability observed in DNA amplification with primer 3 and non genetic polymorphism with genetic variability in primer 1 and primer 2.
CONCLSIONS: The use of the Random Amplified Polymorphic DNA Polymerase chain reaction (RAPD-PCR) technique to amplify short regions of an organism's genome provide more specific method than conventionally employed in epidemiological studies.

Integrating parasite data and host genetic structure in the frame of an holistic approach for stock identification in Mediterranean Sea fish species

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BACKGROUND: The historical features of the Mediterranean Sea make fish stock assessment a challenge: up to date stock identification has been based on data from biology, morphometrics, artificial tags, otolith shape, and fish genetics, with less effort on the use of parasites as biomarkers. We review the state of the research on the stock structure of Mediterranean fish species in comparison with the Atlantic Ocean, within a multidisciplinary framework.
METHODS: A Procruster Rotation (PR) was carried out to assess the association between host population genetics and parasitological data sets concerning the distribution of *Anisakis* spp. larvae genetically identified in Mediterranean and Atlantic subpopulations of demersal (European hake) and pelagic (horse-mackerel, swordfish) fish species.
RESULTS: The larval distribution of *Anisakis spp.* recognized in hake from the different fishing grounds indicates distinct stocks of *M. merluccius* in Mediterranean and Atlantic waters. These data were concordant with population genetics of the fish species. Concerning horse mackerel, a structuring of subpopulations from the Mediterranean and Atlantic is highlighted by a PCA analysis inferred from Anisakis spp. larvae. The same analysis supports the existence of a "southern" and "northern" Atlantic stock. This finding was in accordance with the morphometric and biological data rather than with the fish population genetic. As for the swordfish, the PCA analysis based on the distribution pattern of *Anisakis* spp. showed the Mediterranean samples clustering separately from the Atlantic ones; in Atlantic waters, a cluster formed by the "northern" swordfish sample was distinct from a "southern" one. The use of *Anisakis* spp. as biomarkers agrees with genetic results for this fish.
CONCLUSIONS: Different resolution powers emerged from the use of different approaches to stock identification, due to the different time scales investigated: fish population genetics can detect changes over an evolutionary time scale, while parasites are more suitable biomarkers when considering fish stocks over smaller temporal and spatial scales.

New insights into the molecular systematics of anisakid nematodes of genus *Anisakis*: implications for their evolutionary ecology, epidemiology and relevance to human health

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BACKGROUND: The application, over the last decades, of the molecular systematics approach to the anisakid nematodes of the genus *Anisakis,* which is the main zoonotic agent of human anisakiasis, has advanced the understanding of their taxonomy, ecology and phylogeny. So far, molecular genetic markers as allozymes and sequences analysis of DNA genes, provided a rapid screen tool to identify the nine species of the genus *Anisakis*, so far genetically characterised.
RESULTS AND CONCLUSIONS: New insights concerning the use of several nuclear and mitochondrial genes in the correct identification of larval and adult anisakid nematodes of the genus *Anisakis*, and in revealing species boundaries within this group of parasites, have been recently obtained. We report recent results on the epidemiological data concerning parasitic infection levels by the zoonotic species of the genus *Anisakis* occurring in the fish hosts, including their differential distribution in different organs and tissues of the fish species. Also, we discuss their relevance to human health concerning the zoonotic disease (anisakiasis). Molecular genotyping of the zoonotic species of *Anisakis* and the human response to the infection by *Anisakis* spp., including allergic reactions, are presented. We show the occurrence of different *Anisakis* species in different definitive hosts, as an ecological consequence of host-parasite co-phylogenetic and host-switching phenomena, including host-parasite co-phylogeographic aspects. On the other hand, the link of these parasites to a "healthy marine ecosystem" is underlined, due to their life-cycle embedded in complex trophic webs. Based on these data, the population size and genetic variability of these parasites are finally suggested as environmental indicators.

Ecological data and genetic variability of two parasite species, *Contracaecum osculatum* sp. D and *C. osculatum* sp. E (Nematoda: Anisakidae) in fish from the Ross Sea (Antarctica): indicators of food webs stability of the Antarctic ecosystem?

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BACKGROUND: The Ross Sea, Eastern Antarctica, is considered a "pristine ecosystem" and a biodiversity "hotspot". This provides a rare chance to investigate a marine ecosystem scarcely impacted by human. The cryptic species *Contracaecum osculatum* sp. D and *C. osculatum* sp. E are parasites embedded in the natural Antarctic marine ecosystem, where they are in sympatry both in definitive host (Weddell seal) and in several fish paratenic hosts. Aim of this study was to: identify the larvae of *C. osculatum* s.l. recovered in fish hosts, during XXVII Italian Expedition to Antarctica (2012); perform a comparative analysis of the parasitic infection levels and genetic variability with respect to those reported in previous expedition (1994).
METHODS: 120 specimens belonging to four Antarctic fish species (*Chionodraco hamatus*, *Trematomus bernacchii*, *T. hansoni, T. newnesi*) were inspected for *Contracaecum* larvae, whose genetic identification was undertaken using multilocus allozyme electrophoresis and mtDNA *cox2* gene sequencing.
RESULTS**:** No significant differences were found in the infection levels by *C. osculatum* sp. D and sp. E genetically recognised on a temporal scale (2012 *versus* 1994). Statistical significant differences were found in the relative proportions of *C. osculatum* sp. D and sp. E: the latter resulted prevalent species in *T. bernacchii*; *C. osculatum* sp. D was prevalent in *T. hansoni* and *C. hamatus*. The two species showed differences in the host infection site: the relative proportion of *C. osculatum* sp. D was significantly higher in the fish liver. High genetic diversity at both nuclear (allozymes) and mitochondrial (mtDNA *cox2*) level were found in the two species.
CONCLUSIONS**:** The parasitic infection levels by *C. osculatum* sp. D and sp. E and their estimates of genetic variability showed no statistically significant variation between the two Antarctic campaigns, suggesting that the low habitat disturbance of the Antarctic region permits the maintenance of stable trophic webs.
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Saccharide epitopes and glycocalyx shedding in cercariae of avian schistosomes transforming to schistosomula

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BACKGROUND: Schistosomes of the genus *Trichobilharzia* are pathogens of aquatic birds as well as the causative agents of cercarial dermatitis in man. During skin penetration, cercariae transform to schistosomula, which is characterized by shedding of glycocalyx and formation of a double outer tegumental membrane. Schistosome glycocalyx has a specific composition of saccharides bound to lipids or proteins on the membrane. There is only limited information about the molecular mechanisms of its shedding.
METHODS: Changes in surface glycosylation and shedding of cercarial glycocalyx were studied in *Trichobilharzia regenti* and *T. szidati* by time-lapse photography, light and fluorescence microscopy, a set of dyes, fluorescently labelled lectin markers + appropriate sacharide inhibitors, and monoclonal antibodies against Lewis X antigen.
RESULTS: Lewis X antigen expression on *T. regenti* schistosomula started at the anterior end and gradually expanded over the entire body; *T. szidati* schistosomula did not react with the same antibodies. The pattern of lectin binding to transforming cercariae dramatically changed upon the contact with linoleic-acid-induced secretions of penetration glands; glycocalyx has been shed in a sleeve-like manner. Shedding was also induced by lectins alone, especially by those possessing specificity to fucose.
CONCLUSIONS: Cercariae transforming to schistosomula show dramatic changes in composition of surface saccharides. Secretions of penetration glands seem to be involved in the process of glycocalyx shedding. The process can be triggered by natural stimuli of cercarial penetration (linoleic acid), but also by various exogenous lectins. The involvement of mannose binding protein, a component of vertebrate innate immunity, as a possible trigger of glycocalyx shedding in penetrating schistosome cercariae will also be discussed.

Prevalence and Intensity of Soil-Transmitted Helminths among Pre-School Age Children in 12 Kindergartens in Jimma Town, Southwest Ethiopia

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BACKGROUND: Pre-school aged children (preSAC) remain difficult to reach in mass drug administration (MDA) programmes to control soil-transmitted helminth (STH, *Ascaris lumbricoides*, *T. trichiura* and hookworm) infections. Kindergartens provide a unique platform to increase the coverage of MDA in preSAC in Ethiopia, but surveys assessing STH among preSAC in kindergartens are scarce. Therefore, we assessed the prevalence and intensity of STH infections among preSAC in kindergartens of Jimma Town, southwest Ethiopia.
METHODS: Cross-sectional parasitological survey was conducted in 12 kindergartens of primary schools in Jimma Town from November to December 2013. Stool samples were processed by McMaster egg counting method.
RESULTS: Out of 622 children (1-5 years) screened across the 12 kindergartens, STH Infections were found in 290 (46.6%) children. *T. trichiura* was the most prevalent (38.9%), followed by *A. lumbricoides* (20.3%). Hookworm infections were observed in 5.5% of the preSAC. Both *T. trichiura* and *A. lumbricoides* infections, and hence any STH infection, were observed in all 12 kindergartens involved in the study. However, there was a large variation in the proportion of infected children across the kindergartens. This proportion ranged from 13.8% to 70.6% for any STH infection (in half of the schools more than 50% of the children excreted STH eggs), from 11.2% to 61.8% for *T. trichiura* infections, and from 1.9% to 39.3% for *A. lumbricoides* infections. The proportion of children excreting eggs of hookworm in the nine schools in which these STH eggs were observed ranged from 1.6% to 18.3%.
CONCLUSION: Our results indicate that STH are highly prevalent in preSACs. They also highlight the potency of kindergartens as an additional platform for MDA in Ethiopia, particularly when these kindergartens are often located within the same compound of the primary schools, allowing to reach both preSACs and school age children with a minimum of additional efforts.

Selfing in lymnaeid vectors of fascioliasis: A comparative study of American species of the *Galba*/*Fossaria* and *Pseudosuccinea* groups

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BACKGROUND: Fascioliasis is a snail-borne trematode zoonotic disease caused by liver flukes of the genus *Fasciola* and has a large veterinary and public health significance. Many freshwater lymnaeid snail species act as vectors in disease trasmission. In the Americas, several species of *Galba*/*Fossaria* and *Pseudosuccinea* transmit *F. hepatica*. Lymnaeid snail susceptibility to *Fasciola* infection depends on the genetic and bio-ecological requirements. The present study puts in evidence differences in reproductive behaviour by selfing and their importance in the transmission and spread of the disease.
METHODS: Specimens of *Fossaria* from Mexico and *Pseudosuccinea* from Colombia were isolatelly maintained under the same conditions in Heraeus climatic chambers: 12/12 hours light/darkness, 90% relative humidity, and 20 °C temperature. Monitoring of lifetime and cluster laying of each individual was made.
RESULTS: Among the many parameters analyzed, the following showed evident differences between the two groups: ife span in days (TV) = 75-125 (mean 94.80), laying period in days (PP) = 39-69 (48.50), number of clusters (NC) = 13-26 (16.20), and total non-laying days within the laying period (TSP) = 25-43 (33) for *Fossaria*; TV = 138-246 (187.63), PP = 66-193 (120.54), NC = 27-63 (39.08), and TSP = 36-149 (81.79) for *Pseudosuccinea*.
CONCLUSIONS: The marked differences between both lymnaeid groups highlight different reproduction and adaptation capacities. Their role in the transmission, emergence and spread of fascioliasis should be emphasized.

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Pooling faecal samples and Mini-FLOTAC for the assessment of gastro-intestinal nematode infection intensity and anthelmintic drug efficacy in sheep

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BACKGROUND: Faecal egg count (FEC) techniques are widely used to diagnose gastro-intestinal nematode (GIN) infections through the assessment of eggs per gram of faeces (EPG). Reduction in faecal egg count (FECR) is the method of choice to monitor anthelmintic drug efficacy and detect anthelmintic resistance in ruminants. However, there are some limitations that affect the use of FEC/FECR, as the time and cost to conduct FEC/FECR on a representative number of individual animals. An alternative is to examine pooled faecal samples.
METHODS: A field study was conducted to validate pooled faecal samples in sheep for FEC/FECR. Ten sheep farms located in southern Italy were selected. In each farm, individual faecal samples from 20 sheep were collected, before (D0) and after (D14) a treatment with albendazole. For each farm and at each time point (D0 and D14) the faecal samples were examined individually and as pools using Mini-FLOTAC and Specifically, three different pool sizes (5, 10 and 20 individual samples) and three different analytic sensitivities (10 using Mini-FLOTAC; 15 and 50 using the two variants of McMaster) were compared for FEC/FECR using individual and pooled samples.
RESULTS: GIN EPG of pooled samples correlated positively with mean EPG of individual samples, with very high correlation coefficients (ranging from 0.94 to 0.99) across the 3 different pool sizes and analytic sensitivities. Mini-FLOTAC was more sensitive compared to the two variants of McMaster and resulted in significant higher FEC ($p < 0.001$). FECR was higher than 98% at most farms independently of the pool size and analytic sensitivity.
CONCLUSIONS: Pooling ovine faecal samples was a rapid procedure that holds promise as a valid strategy for assessing GIN FEC and FECR in sheep.

The in vitro development of *Leishmania* parasite dissemination during late stage of macrophage infection

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BACKGROUND: Following sandfly inoculation, a low number of *Leishmania* promastigotes survives and differentiates into replicating amastigotes. Disease development is therefore strongly linked to the ability of amastigotes to spread to naïve macrophages. However, research in this area has been largely hindered by the lack of models for this stage of the parasite's life cycle. We have developed a human cells-based in vitro model representative of *Leishmania* dissemination at late infection stages.
METHODS: Terminally differentiated THP-1 monocytes were infected with two GFP expressing species, *Leishmania mexicana* (M5G) and *Leishmania aethiopica* (L8G) and treated with Camptothecin. Following 72 hours from infection, the infected cells were used to infect a newly differentiated population of THP-1 cells at different range of infection ratios. Flow cytometric analysis was carried out to detect the percentage of GFP positive parasite residing in the cell.
RESULTS: We found that 80% of cells were infected by M5G and L8G at 72 hours, under Camptothecin treatment. Re-infection of the healthy cells at 72 hours with both infected cells at a ratio of 10/1, 5/1 and 1/1 resulted in a significant increase ($p<0.05$) in the percentage of infection post 24 hours. This infection remained constant from 48 to 72 hours, eventually lowering at 96 hours. Importantly, there was no significant reduction in the total number of viable cell before and after re-infection. This indicated that the infection rise observed was related to the possible spread of the parasites and not caused by death of uninfected THP-1 monocytes.
CONCLUSIONS: Our development of an in vitro model of *Leishmania* infection of macrophage revealed a potential time frame for parasite dissemination during late infective stage. This will allow further investigation into the mechanism of parasites spreading.

Geospatial health - past, present and future

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The application of geospatial tools (e.g. geographical information systems - GIS, global positioning system, satellite-based remote sensing, virtual globes and spatial statistics) to spatial epidemiology in veterinary and medical parasitology have been nowadays firmly established for geo-positioning, collating, exploring, visualizing and analyzing health data in a spatially explicit manner. Geospatial tools can be very useful for mapping and spatio-temporal modeling of parasite distribution and abundance at local, regional and area-wide scales. The possibility of using geospatial tools in human and veterinary parasitology at different levels, from mapping to modelling, represents a very useful way to communicate with field researchers and decision-makers and to address targeting of parasite control treatments. Geospatial Health is an international, peer-reviewed scientific journal produced by the International Society of Geospatial Health (GnosisGIS, www.gnosisgis.org). This network (now society) was founded in 2000 and the inaugural issue of its official journal was published in November 2006 with the aim to cover all aspects of GIS applications, remote sensing and other spatial analytic tools focusing on human and veterinary health.

Molecular basis of matching phenotype theory in the interaction between *Biomphalaria glabrata* and *Schistosoma mansoni: a way to understand* compatibility polymorphism and co-evolutionnary processes.

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The co-evolution between hosts and parasites involves huge reciprocal selective pressures on both opponents. However, relatively few reports have evaluated the impact of these reciprocal pressures on the molecular determinants playing a key role at the core of the interaction, such as defense molecules of the host or antigens of the parasite. Here, we address this question in a host-parasite model in which coevolutionary processes were characterized: the interaction between the mollusk, *Biomphalaria glabrata*, and its trematode parasite, *Schistosoma mansoni*. This interaction is characterized by a compatibility polymorphism and a combination of data obtained from field and laboratory studies argues in favour of a matching phenotype model to explain this phenomenon. Investigations focusing on the molecular determinants of this compatibility polymorphism have revealed that the arms race plays at the level of the recognition processes as well as at the level of effectors/anti-effectors mechanism. Concerning immune recognition, we identified two repertoires of polymorphic and/or diversified molecules that have been shown to interact: the parasite antigens *S. mansoni* Polymorphic Mucins (SmPoMucs) and the *B. glabrata* Fibrinogen-related Proteins immune receptors (FREPs). Concerning immune effector molecules, we showed a clear link between the oxidant and antioxidant systems of the host and the parasite, respectively. The compatible/incompatible status of a specific snail/schistosome combination is the result of the integration of all these molecular pathways that are influenced by coevolutionary histories and probably the environment. The talk, we propose, will provide an update on recent progress on the issue of compatibility polymorphism in the interaction between *B. glabrata* and *S. mansoni*.

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

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

A Giardial ENTH Protein Regulates Vacuolar Homeostasis and Reveals a Bifunctional Role in Clathrin-Mediated Trafficking

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BACKGROUND: *Giardia* lacks a defined endosomal/lysosomal system but possesses peripheral vacuoles (PVs) that play roles in endocytosis, degradation, recycling, and secretion of proteins during growth and differentiation. Analysis of the role of heterotetrameric clathrin adaptor proteins in protein trafficking to the PVs, suggested that this organism preserved a highly reduced set of proteins involved in endosomal/lysosomal trafficking. In this work, we identified the protein GlENTHp (for *Giardia lamblia* ENTH protein) containing an ENTH domain present in the monomeric adaptors epsin or epsin-related (epsinR) proteins, which are implicated in the endocytosis and Golgi-to-endosomes protein trafficking, respectively.
METHODS: Immunofluorescence, immunoblotting, immunoprecipitation and yeast-two hybrid assays were carried out in stable clones expressing HA-tagged GlENTHp or GlENTHp mutants. Lipid binding was tested by performing protein-lipid blot overlay assays. The structure of the PVs in wild-type and transgenic parasites were examined by electron microscopy.
RESULTS: We found that GlENTHp binds clathrin, ubiquitin, and interacts with the alpha subunit of AP-2 and the gamma subunit of AP-1, with the events of endocytosis and lysosomal hydrolase trafficking being equally affected by GlENTHp downregulation. GlENTHp targeting to membrane required a conserved $lysine_{75}$, which allowed its binding to PI3,4,5P3 and PI4P, polyphosphoinositides linked in other cells to anchoring to the plasma membrane and Golgi, respectively. Either downregulation of GlENTHp or overexpression of a nonfunctional GlENTHp showed an unusual accumulation of dense material in the PVs and severely affected trophozoite growth.
CONCLUSIONS: Our findings suggest that the dual epsin-epsinR role of GlENTHp is implicated in the maintenance of the PV homeostasis providing new avenues for therapeutic intervention against *Giardia* and related parasites.

Comparative Evaluation of Egg, Metabolic, and Tegument Antigenic Extracts of *Fasciola gigantica* for the Serological Diagnosis of Bovine Fascioliasis

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BACKGROUND: Fascioliasis is a parasitic disease of ruminants and man. Diagnosis of bovine fascioliasis is based on clinical signs, post mortem examination coporological examination of faeces for eggs and the demonstration of the antigens or the antibodies by immunoassays. Various immunological reactions using crude antigenic extracts have been studied for fascioliasis. Results obtained have not always been uniform.
METHOD: Sera from 61 cattle were tested for the presence of *Fasciola* antibody by indirect Enzyme-linked Immunosorbent Assay (ELISA) using Egg, Metabolic, and Tegument extracts of *Fasciola gigantica.* This was done to compare the sensitivity and specificity of these extracts for routine immunological diagnosis of bovine fascioliasis on the field.
RESULTS: The metabolic and tegument extracts detected *Fasciola* antibody in 56/61 (90.30%) while the Egg extract detected antibody in 59/61 (96.7%) in the sera samples respectively. The mean Optical Density (OD) values in ELISA were 0.561±0.174 SD for metabolic extract, 0.619±0.219 SD for tegument extract and 0.650±0.199 SD for the egg extract. Western blotting using 5% non fat milk as blocking agents, the egg extract showed the presence of prominent specific low molecular weight (30 – 35 KDa) polypeptides which were not cross –reactive with sera from *Schistosoma bovis* and *Paramphistomum microbothrium* infections.
CONCLUSION: Egg extract of *F. gigantica* has potential as an immunodiagnostic antigen. It will complement the metabolic, and tegument antigens for detecting antibody in bovine fascioliasis

Comparison of Individual and Pooled Stool Samples for the Assessment of intensity of *Schistosoma mansoni* and Soil-Transmitted Helminth infections using Kato-Katz technique in South-west Ethiopia.

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BACKGROUND: Our group has recently shown that pooling of stool samples holds promise for a rapid assessment of intensity of soil-transmitted helminth infections (STH, *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm) using McMaster technique. In the present study we evaluated our pooling strategy for the assessment of intensity of *Schistosoma mansoni* and STH infections. We also assessed the time to screen individual and pooled samples.
METHODS: A cross-sectional survey was conducted in 360 children 5 to 18 years of age from six schools in Jimma Zone (southwest Ethiopia). In both individual and pooled samples (pools sizes of 5, 10 and 20) faecal egg counts (FEC) by means of eggs per gram of stool (EPG) were determined using the Kato-Katz technique.
RESULTS: Except for hookworms, there was a significant correlation (coefficient = 0.53-0.95) between the mean of individual FECs and the FECs of pooled samples for *S. mansoni, A. lumbricoides* and T*. trichiura*, regardless of the pool size. Mean FEC were 47 EPG, 2,596 EPG, 125 EPG, and 41 EPG for *S. mansoni A. lumbricoides*, *T. trichiura*, and hookworm, respectively. There was no significant difference in FECs between the examination of individual and pooled stool samples, except for hookworms. For this STH, pools of 10 resulted in a significant underestimation of infection intensity. The total time to obtain individual FECs was 65 h 5 min. For pooled FECs, this was 19 h 12 min for pools of 5, 14 h 39 min for pools of 10 and 12 h 42 min for pools of 20.
CONCLUSIONS: The results indicate that pooling of stool sample holds promise as a rapid assessment of infections intensity for Kato-Katz technique and *S. mansoni*. In this setting, time in the laboratory can be reduced with 70% when pools of 5 instead of individual stool samples are screened.

Genetic diversity of *Toxoplasma gondii* in animals and humans in Brazil

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Toxoplasma gondii population structure is complex, with geographic origin playing a central role. *T. gondii* isolates from South America have a high genetic diversity, particularly in contrast to North America and Europe. Parasite genetic characteristics can be one of the factors involved in the outcome of toxoplasmosis. In Brazil, the parasite is highly seroprevalent in domestic and wild animals and humans and the burden of toxoplasmosis is high in cases of AIDS or ocular disease. *T. gondii* genotypic profile of Brazilian strains has been largely investigated by PCR-RFLP of 10 or 11 markers. By now, complete genotyping has been achieved for 513 samples, corresponding to 144 genotypes and, of these, the genotypes #06 (Type BrI), #11 (Type BrII) and #08 (Type BrIII) have been the most frequent, with, respectively, 50, 35 and 26 samples; these called Brazilian clonal genotypes have also been already found in human samples. Only recently, complete PCR-RFLP genotyping was obtained from human samples originated from different cases of toxoplasmosis; these studies revealed high genetic variability in humans, but association between genotype and clinical presentation should be better investigated. Overlapping of genotypes circulating in animals and humans from the same region may reflect the environmental contamination with prevalent isolates. The majority of isolates genotyped until now are from domestic animals from Southeast region, which is the most populated and the most urbanized area in Brazil. Data are lacking from *T. gondii* diversity in the wild fauna of poorly anthropized regions of South America, such as the Amazonian rainforest. Recently, a set of 11 isolates from wild animals from Amazon region, North of Brazil, revealed eight new genotypes that clustered together, suggesting they could be a new haplogroup. Finally, an initial study of 138 samples based on microsatellites identified a high level of spatial structure occurring between Southwest and North/Northeast Brazil.

Assessment of the efficacy of Two Brands of Albendazole Available on the Local Market in Jimma Town, Southwest Ethiopia

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BACKGROUND: The Soil-transmitted helminths (STH) *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm cause the highest burden among all neglected tropical diseases. Up to date albendazole (ALB) and mebendazole (MEB) remain the most commonly administered drugs to control the morbidity caused by STH infections. There is a wide variety of ALB and MEB brands available on the local market, but the efficacy of these brands is often unknown. Therefore, we assessed the efficacy of two ALB brands available on the local market in Jimma Town (Ethiopia): OVIS® (400 mg, Korea, DAEHWA Pharmaceutical) and BENDEX® (400 mg, India, Cipla).
METHODS: A randomized efficacy trial was conducted to evaluate the efficacy of the two ALB brands against STH infections in 679 children (age group: 5 to 18 years). Stool samples were collected one day before and 14 days after drug administration. All samples were examined using the McMaster egg counting method. The efficacy of the two brands was evaluated by means of egg reduction rate (ERR) for the three STH species separately.
RESULTS: Both brands were highly efficacious against *A. lumbricoides* infections (BENDEX®: n = 106, ERR = 98.7%; OVIS®: n = 101, ERR = 97.8%), but showed poor efficacy against *T. trichiura* (BENDEX®: n = 137, ERR = 24.4%; OVIS®: n = 129, ERR = 20.4%). For hookworm infections, OVIS® (n = 56, ERR = 98.1%) was more efficacious compared to BENDEX® (n = 56, ERR = 88.7%, $p = 0.051$).
CONCLUSIONS: The results confirm that albendazole is highly efficacious against *A. lumbricoides* infections, but not against *T. trichiura* infections. They also point to possible discrepancies in efficacy between brands for hookworms, which underscores that poor efficacy of anthelminthic drugs may not automatically be due to anthelminthic resistance.

Canine leishmaniosis in Paraguay, management by the National Program of Zoonoses Control, year 2013

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BACKGROUND: Dogs are considered to be the main domestic reservoirs of *Leishmania infantum*, they play and important role in the epidemiological cycle of visceral leishmaniosis transmission to human host. The purpose of this work was to determine the seroprevalence of canine leishmaniosis (CL) by immunochromatographic rK39 dispstick test in serum samples obtained for routine exam request by dog`s owners and veterinarians, active surveillance in areas of silence transmission and control of human visceral leishmaniosis cases notified for the National program of vector-borne diseases control (SENEPA) to the (PNCZyCAN) in 2013.
METHODS: A total of 12115 canine blood samples were analyzed by rK39 dispstick test (BIOCAN, Canada), in the laboratory of leishmaniosis of the PNCZyCAN coming from 10/17 departments of Paraguay.
RESULTS: The 6537 blood samples coming from routine exam from Asunción (capital city) and the departments: San Pedro, Cordillera, Central, Caaguazú, Paraguarí, Misiones, Alto Paraná, Presidente Hayes, Boquerón and Alto Paraguay, showed 3182 positive serum with a prevalence of (48.6%) of canine leishmaniosis. The active surveillance coming from Asuncion and the departments: Central, Cordillera, Itapúa and Misiones showed that 301 out of 1505 dog`s blood samples were identified as positive with a prevalence of (20%) of CL. From the 50 focus of human visceral leishmaniosis cases, coming from Asunción and the departments: Central, Cordillera, Caazapá, Misiones, Paraguarí; it was observed that 811 out of 4073 dog`s blood samples showed a prevalence of 19.9%. It was observed a global prevalence of (35.4%). Euthanasia procedures was performed in 165 positive dogs (54.8%) coming from active surveillance and 366 positive dogs (45.1%) coming from focus of human visceral leishmaniosis.
CONCLUSIONS: The high canine visceral leishmaniosis shows the urgent need to continue a strict epidemiological surveillance, sanitary education and community participation by the MSPyBS in the control of this disease in Paraguay.

Eosinophilia and anemia in an endemic area of parasitic infections in Bahia, Brazil

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BACKGROUND: Intestinal parasitosis still constitutes one of the major causes of public health problems in the world, especially in tropical and subtropical areas. Enteroparasites are able to develop on their hosts a number of pathophysiological changes, including allergenic reaction and blood loss, responsible for development of eosinophilia and anemia in parasitized individuals, respectively. The aim of this study was to verify the prevalence of anemia and eosinophilia in infected individuals of an endemic area in the northeast of Brazil.
METHODS: Blood and feces samples were collected for carrying out hematological (automation and observation of blood smears) and parasitological (spontaneous sedimentation) analyses, respectively. The hemoglobin value and the eosinophils count were used as parameter to define the presence of anemia and eosinophilia, according the limits established by local studies.
RESULTS: Among 137 subjects, 83.2% (114) were positive in the coproparasitological examination. The enteroparasite with the highest prevalence (64%) was hookworm, followed by *A. lumbricoides* (48%), *E. coli* (42%), *T. trichiura* (36%), *E. nana* (24%) and *E. histolytica* (14%). Among the infected individuals (114), 11.8% (12/114) had anemia and 62.7% (64/114) presented eosinophilia. However, despite the association between enteroparasitosis and the presence of anemia and eosinophilia in some individuals, it was not significant in statistical tests (p=1 and p=0.29 respectively, Fisher's exact test).
CONCLUSIONS: High prevalence of enteroparasitosis in the studied community demonstrates the difficulty to control these diseases and emphasize the need for adequate preventive and educative measures adapted to the reality of each region.The growth of circulating eosinophils and the anemia was not significantly associated to the prevalence of intestinal parasites.

Identification and characterization of microRNAs in *Echinococcus granulosus sensu stricto* G1 and *Echinococcus canadensis* G7 using a high throughput approach

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BACKGROUND: *Echinococcus granulosus sensu stricto* G1 and *Echinococcus canadensis* G7 are etiological agents of cystic echinococcosis in animals and humans worldwide. These cestode parasites have complex life cycles and differ in biological features such as host specificity. MicroRNAs are key regulators of gene expression and are likely to have a role in development. In this study, we have used a high throughput approach to identify and characterize microRNAs in *Echinococcus* spp.
METHODS: Small RNA libraries from protoscoleces of *E. granulosus s.s.* G1 and from protoscoleces and cyst walls of *E. canadensis* G7 were sequenced using Illumina technology. For microRNA prediction, the processed reads were mapped to *E. multilocularis* reference genome and then, miRDeep2 core algorithm was used. The output list of candidate precursors was manually curated to generate a high confidence set of microRNAs. Differential expression analysis of microRNAs between species or stages was estimated with DESeq.
RESULTS: In this study we identified 36 microRNAs including 13 novel ones. We found that two microRNAs were highly expressed accounting for about 50% of the total microRNA expression in each sample. Differential expression analysis showed 16 microRNAs with stage biased expression in *E. canadensis* G7. No significant differences in the expression of miRNAs were found between protoscoleces of *E. granulosus s.s.* G1 and *E. canadensis* G7.
CONCLUSIONS: Our results show the existence and expression of a broader repertoire of microRNAs in *Echinococcus* spp. than previously reported, including some microRNAs considered as evolutionarily lost. Also, our results suggest that microRNAs could play important functional roles in development and could be evaluated as potential targets for therapy.

Co infection of *Leishmania infantum*, *Dirofilaria immitis, Anaplasma platys, Ehrlichia canis* and *Borrelia burgdorferi*, in dogs in the metropolitan area of Asunción, Paraguay

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BACKGROUND: Vector-borne diseases of dogs are widely distributed throughout areas with climatic conditions that allow the development of arthropods (e.g., mosquitoes, sand flies and ticks). The objective of this study was to determine the seroprevalence of *Leishmania infantum*, *Dirofilaria immitis, Anaplasma platys, Ehrlichia canis* and *Borrelia burgdorferi*, in dogs treated in the Veterinary Hospital of the Faculty of Veterinary Sciences.

METHODS: All the 195 collected blood samples includes in the study were tested using an in-clinic enzyme-linked immunosorbent assay (ELISA) SNAP ®4Dx® Plus that detects *A. phagocytophilum/platys*, *B. burgdorferi*, and *E. canis/ewingii* antibodies, and *D. immitis antigens*, and the SNAP® *Leishmania*, (both of them from IDEXX Laboratories, Inc, Westbrook, ME, USA) according to manufacturer´s indications.

RESULTS: The results showed that 92/195 (47.1%) of dogs were seropositive for *L. infantum*; 26/195 (13.3%) of dogs were seropositive for *A. platys*; 74/195 (37.9%) of dogs were seropositive for *E. canis*. A co infection seroprevalence was observed for *Leishmania*/*Anaplasma*/*Ehrlichia* in 11/195 (5.6%) of the dogs and a *Leishmania*/*Ehrlichia* co infection in 27/195 (13.8%) of the dogs. There were no found canines with infection by *D. immitis* or *B. burgdorferi*.

CONCLUSIONS: This study reports evidence of exposure to specific arthropod-borne pathogens in dogs in the metropolitan area of Asunción, Paraguay therefore it is recommended that routine screening for canine vector-borne diseases be done for the adequate diagnostic, treatment and prevention of these pathogens.

Identification of *Blastocystis* subtypes in children carriers from Michoacan, Mexico.

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BACKGROUND*:* In Mexico, the knowledge of genetic subtypes (STs) of *Blastocystis* is scarce. The objective of present study was to identify the main STs, in children carriers of suburban and urban areas from Michoacan, Mexico.
METHODS: Fecal samples of symptomatic and asymptomatic children (from 1 to 17 years old) from Morelia City (urban area) and other closer small towns (suburban areas), positives for *Blastocystis spp* by coprological analysis, were processed for to obtain DNA, after PCRs were performed using primers for a fragment of small unit of rDNA; amplicons obtained were purified and sequenced. Sequences were aligned and a phylogenetic inference was built. Besides, a questionnaire was applied in order to identify some risk factors as well as main signs and symptoms, associate to STs.
RESULTS: From 113 samples, 92 amplicons were purified, but only 74 sequences were obtained. Alignments and the phylogenetic inference showed that samples were 32 (43%), 19 (26%), 22 (30%) and 1 (1%) for STs1, 2, 3 and 7, respectively. Interestingly, the main STs for urban carriers was ST1, in contrast, ST3 was most frequent in suburban carriers. Constipation and abdominal pain were associated to ST2 ($P<0.05$), while ST1 was associated with animals concurrence.
CONCLUSIONS: We identified for first time in Mexico, one case of ST7 in a symptomatic carrier, whilst that ST1, 2 and 3 were the main *Blastocystis* STs identified in our population. Interestingly, differences within frequencies of STs from urban and suburban carriers, are agree with the cryptic host specificity that it has been argued in *Blastocystis* about that human strains grouped into other clades different of ST3 might be the result of a zoonotic transmission, but our results could be in opposition with the statement that the human ST3 infections are caused predominantly by human-to-human transmission.

Early responses to *Trypanosoma cruzi* infection: Implications for vaccine development

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Prevention of *Trypanosoma cruzi* infection in mammals likely depends on either preclusion of cell infection by infective trypomastigotes or the rapid recognition of infected cells by the host immune response. We have previously shown that immune recognition of *T. cruzi* infection is significantly delayed both at the systemic level and at the level of the infected host cell. The systemic delay appears to be the result of a stealth infection process that fails to trigger substantial innate recognition mechanisms while the delay at the cellular level is related to the immunodominance of highly variable gene family proteins, in particular those of the trans-sialidase family, that are presented on infected host cells late in the infection process. The implications for these factors in the development and use vaccines to prevent *T. cruzi* infection and possible work-arounds to these potential roadblocks will be discussed.

Molecular identification of *Leishmania infantum* isolated from domestic dogs of the National Program of Zoonoses Control and National Rabies Center of Paraguay

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BACKGROUND: Visceral leishmaniosis (VL) is a zoonotic disease caused by *Leishmania infantum* in America and transmitted by female sand flies, dogs being the main urban reservoir. Endemic condition and increasing urbanization have increased the risk of human infections in Paraguay. At present, the standard diagnostic method is Dipstick rK39, which although a genus-specific assay, doesn´t allow species characterization. In this study the 70-kDa heat shock protein-encoding gene was analyzed to determine taxonomic identity and evaluate the genetic diversity of parasites isolated from dogs diagnosed with LV.

METHODS: Forty samples from symptomatic dogs with a positive diagnosis for rK39 were collected and cultured on NNN medium for DNA isolation. The hsp70 gene was amplified by PCR for *Leishmania* detection and the product was digested with HaeIII, for species differentiation.

RESULTS: A region of 1300-bp in the hsp70 gene was amplified a as a valid and highly sensitive target for detection of *Leishmania*. VL parasites were genotyped as *L. infantum* through PCR-RFLP which revealed different profiles between *L. infantum*, *L. braziliensis* and *T. cruzi.*

CONCLUSIONS: With the enzymatic restriction product all the analyzed isolates showed profiles corresponding to *L. infantum*. The PCR-RFLP technique appears to be a sensitive assay for the detection and differentiation of *L. infantum* and might be an interesting alternative for the identification of *Leishmania* strains that are responsible of VL in Paraguay.

A Comparative Analysis Of Intestinal Parasitic Infections Among HIV Infected Persons With And Without Antiretroviral Treatment In Ejisu-Juaben Municipality, Ghana

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BACKGROUND: There is little information on the distribution of intestinal parasites among HIV infected individuals on ART and HIV infected individuals not on ART in this semi urban town located in the forest region of Ghana. The study sought to determine the prevalence and distribution of intestinal parasites in non-HIV infected individuals and HIV individuals with and without antiretroviral treatment in 3 hospitals in Ejisu-Juaben Municipality in the Ashanti region of Ghana. The study also sought to obtain information on the knowledge and attitudes of patients about these parasites and assess their personal hygiene habits.
METHODOLOGY: A total of 154 stool samples were obtained from 99 HIV patients on ART, 26 HIV patients not on ART and 29 non-HIV patients at the above mentioned hospitals. Samples were examined macroscopically and microscopically using the wet preparation and formol saline techniques. Questionnaires were also issued to study subjects.
RESULTS: Out of the 154 subjects, 23(14.9%) were infected with intestinal parasites. There were a total of 102 females of which 13(12.7%) were infected and a total of 52 males of which 10(19.2%) were infected. From this study, 12 (12.1%) of the 99 HIV patients on ART, 9(34.5%) of the 26 HIV patients not on ART and 2(6.9%) of the 29 non-HIV patients were infected. Of the 21 HIV and intestinal parasite co-infested study participants, 1(4.8) had CD4+ T-cell count >500/µL, 15(71.4%) had CD4+ T-cell count between 200-500/µL and 5(23.8%) had CD4+ T-cell count <200/µL.
CONCLUSIONS: Intestinal helminthes infections among the study subjects were high. The study showed that the prevalence of intestinal parasites is higher in males than in females independent of age. HIV patients on ART had lower prevalence than those not on ART.

Ecological aspects of parasitic helminths of Lorna Drum *Sciaena deliciosa* (Tschudi, 1846) (Perciformes: Sciaenidae) acquired at the fishing terminal of Ventanilla, Callao, Peru

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BACKGROUND: The aim of this study was to assess the ecology of helminth parasites from 35 *Sciaena deliciosa* of the Terminal of Fish from Ventanilla, Peru during the months of January to May 2013.
METHODS: The parasites were collected and evaluated using standard protocols.
RESULTS: The four most prevalent parasites and mean abundance were *Cynoscionicola sciaenae* (Tantaleán, 1974) (Monogenea); *Helicometra fasciata* (Rudolphi, 1819) (Trematoda), *Dycheline (Cucullanellus) amaruincai* (Freitas, Vicente & Ibañez, 1969) (Nematoda) and *Corynosoma obtuscens* (Lincicome, 1943) (Acanthocephala). Endoparasites predominance on ectoparasites was observed. All five helminths showed with aggregation indices a clumped distribution and only *Hargicotyle louisianensis* (Hargis, 1955) Mamaev, 1972 (Monogenea) was evenly distributed. The prevalence and abundance of *D. amaruincai* and *Diphyllobothrium pacificum* (Nybelin, 1931) (Cestoda) were correlated with the total length of the host. The sex of *S. deliciosa* was observed to be related to the prevalence and abundance of *D. amaruincai*. Regarding the relative condition factor (k_n) only fish parasitized by *C. sciaenae* and ectoparasites had higher values compared with non-parasitized. Infective stages of *Anisakis* sp, *C. obtuscens* and *D. pacificum* were also found with zoonotic importance on the Peruvian coast.
CONCLUSIONS: The monogeneos *H.* louisianensis and *Macrovalvitrema* sp. are considered new records for the Peruvian coast.

The interleucin-like growth factors in the human pathogen *Trichomonas vaginalis*.

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BACKGROUND: *Trichomonas vaginalis* is the causative agent of human trichomoniasis, a sexually transmitted human infection, with ~276 million cases occurring annually worldwide. Cellular proliferation is a critical step in parasite colonization of the urogenital epithelium. However, the molecular mechanisms involved on *T.vaginalis* proliferation are not well understood.
METHODS: Three *T. vaginalis* growth factors were cloned and expressed (TvGf1, TvGf2 and TvGf3). Proliferation assays as well as dose responde assays were performed in presence or abscense of *Trichomonas* recombinant growth factors (TvGfr). Cell localization was determined by confocal immunofluorescence and electron microscopy whereas ^{35}S methionine labelling and immunoprecipitation were used to identify secreted growth factors.
RESULTS: TvGfr1 displayed a single domain of the DUF1764 super family. TvGfr2 displayed a single domain of the DUF1764 super family and a IL 7 motif. TvGfr3 did not display a known functional domain but had a IL 2 motif. A dose-dependent increase of the parasite number (40-60%) was observed in *T.vaginalis* cultures incubated with the TvGfr respect to controls incubated without them. Confocal microscopy reveals that growth factors were present mainly in the first three hours of culture. Electron microscopy reveals that TvGf1 and TvGf3 were localized inside of secretory or endocytic vesicles whereas TvGf2 was observed inside of lysosomes. Finally, the secretome of methionine ^{35}S labelled parasites followed by immunoprecipitation with polyclonal antibody raised against TvGf3 shown a single band confirming that proteins are secreted by parasite.
CONCLUSION: These body of evidences strongly suggest that *in vitro T.vaginalis* proliferation is induced by protozoan secreted growth factors.

Comparison of Western blot using the recombinant antigen rTES-30 versus TES-ELISA to detect antibodies in Toxocarosis patient

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BACKGROUND: Diagnosis of human toxocarosis currently relies on serologic test that use *Toxocara* excretory- secretory (TES) antigen to detect immune globulin G (IgG) antibodies. The use of recombinant antigens offers significant benefits for detection; assays using recombinant antigens have increased specificity compared to those of assays using native TES antigens.
METHODS: To produce the recombinant antigen, *tes30* gene was synthetized via assembly PCR, subcloned into an *E. coli* expression vector with the 6xHis system. Bacterial culture conditions were adjusted in order to obtain optimal expression and purification conditions using FPLC under denaturing conditions with urea. The performance of the Western blot based on the recombinant antigen, rTES30, was then compared with that an IgG- ELISA based on the *Toxocara* excretory- secretory (TES) antigen. Protein purity was assesed with capillary electropherisis system, Bioanalyzer 2100.
RESULTS: We obtained a recombinant protein from *T.canis*, which was purified efficiently on a small scale (5-10 mg) with high reproducibility and 95% purity. Sera from patients with probable cases of ocular toxocarosis recognized this recombinant protein. The assay based on rTES30 demonstrated a sensitivity of 73% (46/63) and a specificity of 100% (21/21), compared to TES-ELISA. The results showed that 46 of the positive sera by ELISA (TES) were also reactive by Western blot rTES30. Similarly, all sera of the negative control group, which were non-reactive by TES-ELISA, were also negative by the Western Blot. Out of the 21 sera with diagnosis of other parasitic diseases, 19 were reactive by ELISA (TES), whereas only 7 of these were positive with the immunoblot.
CONCLUSIONS: We describe the production of a recombinant antigen from *T. canis,* rTES30, and its use in Western blot assays, which is promising for the toxocarosis serodiagnosis tool for epidemiological studies in Colombia.

Parasitological indices of the Peruvian Hake *Merluccius gayi peruanus* Ginsburg, 1954 (Perciformes: Merlucciidae) acquired at the fishing terminal of Ventanilla, Callao, Peru

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BACKGROUND: The "Peruvian hake" *Merluccius gayi peruanus* (Ginsburg, 1954), is the most abundant and economically important fisf in the trawl fishery in Peru. The aim of this study was to assess the parasitological indices of Peruvian hake *M. gayi peruanus* from the Fishing Terminal of Chorrillos during the months of January to February 2014.
METHODS: Parasites were collected, fixed, preserved and quantified using standard procedures.
RESULTS: A total of fifteen parasite species, with a mean total abundance and species richness of 1.55 and 0.90 was collected. Ectoparasites were represented by the monogenean *Anthocotyle americana* and copepod *Caligus debueni* and *Neobrachiella insidious pacifica*. Endoparasitic digeneans were *Aporocotyle wilhelmi*, *Derogenes varicus* and *Lecithochirium genypteri*. The tapeworms were *Diphyllobothrium pacificum*, *Diphyllobothrium arctocephalinum*, Callitetrarhynchus gracilis and *Clestobothrium crassiceps*. Identified nematodes were *Anisakis simplex* and *Contracaecum* sp. The acanthocephalans were *Corynosoma obtuscens* and *Bolbosoma* sp. *Clestobothrium crassiceps*, *A. wilhelmi*, *A. americana* and *A. simplex* showed the highest frequency of dominance. The prevalence and mean abundance of *C. crassiceps* was associated with the total length of the host. Only A. *wilhelmi* presented higher condition factor values of Peruvian hake in parasitized fish. Rates of interactivity (CC_{50}) of ectoparasites and endoparasites showed that parasite communities were largely non-interactive or isolated. The four nonparametric estimators Chao- 2, Jacknife-1, Bootstrap and Jacknife -2 to determine the parasite species richness indicated that an increased sampling effort of Peruvian hake is needed.
CONCLUSIONS: Parasites *D. varicus*, *L. genypteri*, *D. arctocephalinum*, *G. dollfusi*, *Contracaecum* sp., and *Bolbosoma* sp. are new records for *M. gayi*. These digeneans, tapeworms and acanthocephalan are new additions to the parasitological fauna of Peru.

Impact Of Molecular Detection Of *Comamonas Sp.* Symbionts For Diagnosis Of *Spirocerca Lupi* In Dogs

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BACKGROUND: The presence of a novel bacterial symbiont in *Spirocerca lupi* that is closely related to *Comamonas* species (Brukholderiales: Comamonadaceae) of the beta-proteobacteria has been shown using PCR base application of 16S rDNA-gene. Spirocercosis is one of the nematode infections transmitted by coprohagic beetles and adult worms form nodules in the thoracic part of the esophagus. Most of the clinical cases are fatal and diagnosed only in the later stages based on the presence of *Spirocerca* eggs in the feces or detection *Spirocerca* nodules in the thoracic part of the esophagus mainly at post mortem examination. Therefore, present study was carried out to assess the possibility of detecting the presence of the bacterial symbiont *Comamonas* to diagnose *S. lupi*.
METHODS: We have carried out PCR based application of 16S rDNA of *Comamonas sp.* for this purpose. Twenty blood samples from spirocercosis suspected dogs presented to Veterinary Teaching Hospital were used in this study. Serum sample of all animals were subjected to western blotting against excretory secretory products of *Spirocerca lupi* and only four samples gave specific band pattern. Thereafter, DNA was extracted from all 20 serum samples and subjected to PCR using *Comamonas sp.* specific primer sets targeting part of the 16S rDNA.
RESULTS: The results revealed that all western blotting positive samples gave bright PCR band in 580bp in ethidium bromide incorporate gel electrophoresis. PCR was also performed using DNA extraction of *Ancylostoma caninum, Toxocara canis, Dirofilaria repens* and *Dipylidium caninum* simultaneously with *S.lupi* in order to check if this symbiont is present in other common helminths in dogs in Sri Lanka. However, only *D. repens* was shown PCR reaction with *Comamonas sp.* Specific primers.
CONCLUSIONS: Presence of *Comamonas sp*. symbiont could be used as a diagnostic tool to detect Spirocercosis, and early detection of the disease is possible with this test, which will enable the practitioners to institute appropriate treatment early to save lives of affected dogs.

Gastrointestinal parasites in amphibians of genus *Telmatobius* (Telmatobiidae) of an area of high Andes, Puno, Peru

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BACKGROUND: Aquatic frogs of the genus *Telmatobius*, are endemic, diverse and taxonomically problematic group of South American frogs that inhabit aquatic environments of the Andean moorlands and submoorlands, presenting a distribution range from 1500-5000 meters. The aim of this study was to characterize the gastrointestinal parasites of three species of frog of the genus *Telmatobius* of the Andean region of Puno, Peru.
METHODS: 80 specimens of the amphibians genus *Telmatobius* (Telmatobiidae) were collected in the high Andes of Puno: *T. jelskii* (n=56), *T. marmoratus* (n=14), and *T. parkeri* (n=10). Platyhelminths and acanthocephalans were collected, fixed and preserved in 70% alcohol, stained with acetic carmine of Semichon, or Gomori Trichrome Delafield hematoxylin and mounted in Canada balsam or Entellan. Nematodes were fixed in hot alcohol (70%) and rinsed in a mixture of alcohol-phenol.
RESULTS: The following 14 parasites were identified: Digenea: *Gorgoderina parvicava*, *Gorgoderina chilensis* and *Haematoloechus arequipensis*. Cestoda: *Cylindrotaenia americana* and *Ophiotaenia* sp. Nematoda: *Aplectana hylambatis*, *Aplectana vellardi*, *Rhabdias sphareocephala*, *Hedruris moniesi*, *Hedruris* sp., *Falcaustra* sp. and *Physaloptera huascari.* Acantocephala: *Anuracanthorhynchus lutzi* and *Centrorhynchus* sp.
CONCLUSIONS: This work constitutes the first report of the genus *Ophiotaenia* parasitizing *Telmatobius* amphibians in Peru and in South America.

Restrospective distribution of *Trypanosoma cruzi* I genotypes (TcI_{Dom} and Sylvatic TcI) in Colombia

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BACKGROUND: *Trypanosoma cruzi* displays a remarkable genetic diversity evinced in six Discrete Typing Units (DTUs). Recently, TcI the genotype with the broadest geographical distribution has been divided into an enigmatic genotype named TcIDom and sylvatic isolates. The aim of this work was to conduct a retrospective detection of TcIDom and sylvatic-like isolates across a set of 105 isolates from Colombia, ranging from 1984 to 2012.

METHODS: We typed 105 strains isolated from humans, *Didelphis marsupialis, Canis familiaris, Oryzomis*, *Rattus rattus, Rhodnius robustus, R. colombiensis, R. pictipes, R. prolixus, Triatoma venosa, T. dimidiata* and *Panstrongylus geniculatus*. The TcI strains were subjected to TcI genotypes discrimination using specific primers based on SL-IR region. The samples were typed in blind using as reference controls the strains DA (TcIDom) and GC (Sylvatic TcI).

RESULTS: After analyzing the PCR profiles of the 105 strains studied, we observe that 30% were TcIDom and 70% were Sylvatic TcI. Regarding humans, 40% were typed as TcIDom and 60% as Sylvatic TcI. In mammal reservoirs, 99.7% were typed as Sylvatic TcI and 0.3% as TcIDom. Finally, among the insect vectors we observed 52% typed as TcIDom and 48% as Sylvatic TcI.

CONCLUSIONS: These findings suggest that despite of the recent description of TcIDom genotype. This enigmatic near-clade has been circulating across insects and humans in Colombia since 1984. Also, the strict association with vectors and humans imply a host-selection or biological adaptation of this genotype that needs to be further unraveled.

Infection and immune response patterns in dams experimentally infected with *Neospora caninum* in the second trimester of pregnancy.

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BACKGROUND: *Neospora caninum* is a leading cause of abortion in cattle worldwide. The pathogenesis of bovine neosporosis is only partially understood, and the reasons why only some animals abort remain unclear.
METHODS: Fetal and maternal infection and immune responses 3, 6 and 9 weeks post infection (wpi) were investigated in cows experimentally infected with *N. caninum* tachyzoites on day 110 of gestation. Presence of parasite, antibody dynamics, lymphocyte subpopulations and cytokine gene expression in dams and fetuses were examined.
RESULTS: Transplacental infection took place on all analyzed fetuses. One dam from the group euthanized at 6 wpi had a dead fetus at necropsy and showed extensive lesions in the placenta. Fetuses had lower percentages of spleen T cell subpopulationsat 6 wpi, with the lowest percentages observed in the dead fetus. Fetuses alive at 6 wpi showed increased expression of most cytokines. Up-regulated Th1, Th2 and Treg expression was also observed at 6 wpi in the dams. At the placental level, while most cytokines were down-regulated from 6 wpi, up-regulation of IL-4 expression was observed at 6 wpi in the caruncle.
CONCLUSIONS: Our results suggest that parasitic replication and the immune response taken place at 6 wpi were crucial for fetal survival in this model of infection.

Community of metazoan parasite of Corvina Drum *Cilus gilberti* (Abbott, 1899) (Perciformes: Sciaenidae) in the coastal zone of Chorrillos, Lima, Peru

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BACKGROUND: The Corvina Drum *Cilus gilberti* (Abbott, 1899) (Sciaenidae), is a demersal and carnivorous species distributed from Sechura Bay (Peru) to Lote (Chile). To date in Peru, there is no work to analyze community wildlife parasites from *C. gilberti* from the marine Peruvian central coast. This work represents a qualitative and quantitative analysis of *C. gilberti* parasitic communities, with the aim of assessing their metazoan parasite community in the coastal area of Chorrillos, Lima, Peru.
METHODS: 103 specimens of *C. gilberti* from August to September 2011 were acquired in Fishing Terminal of Chorrillos, Lima, Peru. Parasites were collected, fixed, preserved and quantified using standard procedures.
RESULTS: During sampling a total of 257 parasites were collected, with a total mean abundance of 0.28. Ectoparasites are represented by monogeneans *Hargicotyle louisianensis* (Hargis, 1955) Mamaev, 1972 (Diclidophoridae), *Choricotyle* sp. (Diclidophoridae) and *Cynoscionicola americana* Tantalean, Martínez & Escalante, 1987 (Microcotylidae) and copepods *Lernanthropus pacificus* Olivia & Duran, 1982 (Lernanthropidae) and *Neobrachiella sp.* (Lernaeopodidae). Endoparasites, by the flukes, *Villarrealina peruana* Bolaños & Salas, 1982 (Opecoelidae); *Helicometra fasciata* (Rudolphi, 1819) Odhner, 1902 (Opecoelidae), *Plagioporus* sp. (Opecoelidae) and *Didimozoidae*. Cestodes correspond to larval forms of *Diphyllobothrium pacificum* (Nybelin, 1931) (Diphyllobothriidae). Nematodes were identified as *Dycheline elongatus* (Tornquist, 1931), *Ascarophis* sp. (Spiruroidea), *Heterotyphlum* sp. and *Proleptus carvajali* Fernández & Villalba, 1985 (Spiruroidea). The acanthocephalans correspond to cystacanth of *Corynosoma obtuscens* Lincicome, 1943 (Polymorphidae). The hirudinea was identified as Piscicolidae. Nonparametric estimators indicated that requires no effort to increase the sampling of fish hosts.
CONCLUSIONS: The following parasites: *Plagioporus* sp. and *Ascarophis* sp. are recorded for the first time on the Peruvian coast. All parasites recorded in this research, except: *Lernanthropus pacificus*; *Neobrachiella* sp.; *Helicometra fasciata*; *Villarrealina peruviana* and *Diphillobothrium pacificum* are considered new records for *C. gilberti* in Peru. The discovery of the plerocercoid of *D. pacificum* in Corvina Drum studied indicates that this host species has zoonotic potential in the central Peruvian coast.

Frequency of intestinal parasites in patients treated in hospitals and their correlation with climate, over the period 2008-2012.

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BACKGROUND: Some authors have suggested that seasons can influence the transmission of protozoan parasites. The aim of this study was to identify the frequency of major gastrointestinal parasites and its correlation with rainfall periods.
METHODS: A comparative and retrospective study of medical records of all patients with gastroenterological disorders that were attended during 2008-2011 at Children's Hospital of Morelia, Michoacan; Children's Hospital of Mexico (HFG) and a Hospital General (HGG), both placed in Mexico City of Mexico, was conducted. Coproparasitoscopic studies (CPS) and other demographic data were compiled. Furthermore, monthly rainfall data for Morelia and Mexico City were proportioned by National Weather System of Mexico. For statistical analysis, chi-square test and Pearson correlation coefficient were performed.
RESULTS: The results of 16,235 patients (3,474 adults and 12,761 children) were analyzed. Frequencies of positive samples were 35%, 29% and 30%, for HIM, HFG, and HGG, respectively. In all institutions, the most common protozoa were *Blastocystis* spp. (45% for HIM and ~20% for HFG and HGG); *Endolimax nana* (16% for HIM and ~10% for HFG and HGG); *Entamoeba coli* (15% for HIM and ~3% for HFG and HGG); helminthes ova were detected on only few cases. Interestingly, adult male of HGG showed a condition of protection for *Blastocystis* infection (odds ratio=0.62, 95% confidence interval =0.26-0.80, *P*=0.014). Although, during the rainy months (>10 mmH_2O rain average), an increase in the frequency of parasites was observed, statistical significance was not found; however, Pearson indexes for the correlation between the number of positive CPS and rainfall data, were 0.56, 0.59 and 0.76 for HFG, HGG and HIM, respectively, showing from a moderate to a high correlation.
CONCLUSIONS: Our results suggest that the rain could disperse parasitic infective structures, because we found a correlation between the rainy months and those parasitized cases.

Endoplasmic reticulum stress during *Plasmodium berghei* sexual development

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BACKGROUND. Sexual development of *P. berghei* is regulated by transcription mechanisms that allow establishment of infection in the mosquito vector. The parasites undergo morphological and physiological changes that increase their metabolism and temporal protein expression. Those processes induce a state of oxidative stress (OxS) and *Plasmodium* parasites are highly susceptible to OxS, so redox homeostasis is vital to continue their development. In addition, trafficking of proteins provokes overload in the endoplasmic reticulum (ER) that triggers an adaptive response. In mammals, the activation of the PERK pathway, induces an antioxidant response and phosphorylation of the translation initiation factor eIF2α (P-eIF2α). P-eIF2α has been reported in the differentiation of other Apicomplexa.
METHODS. In this work, we evaluated *P. berghei* response to protein overload at different time points during its differentiation from gametocytes to ookinetes. Also, we evaluated P-eIF2α in ookinetes with ER stress induced with Tunicamicin.
RESULTS. During its differentiation, we observed an oscillating pattern in the activation of the system redox and P-eIF2α only in mature ookinetes. Also, administration of Tunicamycin induced P-eIF2α in ookinetes.
CONCLUSIONS. These results suggest that *P. berghei* has mechanisms of adaptive response induced by the accumulation of proteins during sexual differentiation and by the stimulation with drugs that induce ER stress. The study of molecular processes during invasion of the mosquito midgut could provide information to identify potential therapeutic targets.

Evaluation of morphometric features and molecular characterization of Its2 and 28s genes of *Anoplocephala* sp from a Sri Lanka elephant

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BACKGROUND: Cestodes of Anoplocephalidae family are parasites infesting a range of vertebrates that include ruminants, horses, primates, and elephants. A few studies were reported on *Anoplocephala* sp found in elephants. The present work is the first detailed morphological and molecular description of *Anoplocephala* sp in elephants.
METHODS: The adult worms were recovered from a wild elephant in Udawalawe, Sri Lanka. Necropsy findings revealed severe cestode infestation in the small intestine.
RESULTS: These tapeworms were tightly attached to the intestinal mucosa, resulted in hyperemia, mild ulceration, mucosal thickening and variable size with irregular well-demarcated multifocal nodules. The length of the strobila was 5.94±0.24 cm and width ranged from 0.7- 1.8 cm. The mean circumference and the diameter of the scolex are 1.75±0.07 cm and 0.69±0.01 cm respectively. It comprises of four anteriorly directed oral suckers with the diameter of 0.1 cm. Other features are: oval shaped ovary, longitudinal sacculated uterus, numerous transversely arranged testes, osmoregulatory canal, unilateral genital pore, genital atrium, genital papillae, cirrus pouch, internal and external seminal vesicles and ventral longitudinal canal. No lappets were observed beneath the suckers as in *Anoplocephala perforliata*. Nuclear ribosomal (ITS-2 and a portion of the 28S region) genes were amplified; PCR products were approximately 750 bp and 1200 bp respectively. In addition, 598 bp and 404 bp in length DNA sequences were obtained for ITS and 28S regions. However, nucleotide BLAST search revealed that the identity of the ITS2 region characterized (598 bp) is 99% (only a portion, 132/134 bp) among *Anoplocephala* species in elephants and *A. perfoliata* in a horse. The identity between *A. mamilana* and elephant *Anoplocephala* sp is 98% (only a portion, 129/131 bp). Analysis of the large ribosomal subunit 28S gene indicates 95% similarity between *Anoplocephala* species and *Anoplocephaloides dentate, Paranoplocephala kalelai* and *Paranoplocephala blanchardi*.
CONCLUSIONS: Further studies are needed to determine the species of elephant tapeworms that occur in Sri Lanka.

Description of *Trypanosoma cruzi* DTUs in *Triatoma maculata, Panstrongylus geniculatus* and *Rhodnius pictipes* from Colombia

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BACKGROUND: In Colombia, the vectorial control programs have been directed to the control of domiciliated species as *Rhodnius prolixus*. However, there are known vector species, with sylvatic as *Triatoma maculata, Triatoma venosa, Rhodnius pallescens, Panstrongylus geniculatus and Rhodnius pictipes*. These species can be involved in the oral transmission of *Trypanosoma cruzi,* especially acute cases of the disease. Therefore, it is necessary to evaluate the infection rate in these vectors and their infective DTU.
METHODS: We collected 37 bugs of two different regions from Colombia (Guajira and Meta). Twenty-five corresponded to *T. maculata*, nine to *P. geniculatus* and three to *R. pictipes*. For *T. cruzi* detection, we performed direct examination of stool, conventional PCR (kDNA) and *T. cruzi* typing was conducted by means of SL-IR and 24Sa.
RESULTS: We applied kDNA, SL-IR and 24Sa to the 37 bugs observing that *T. maculata* presented a rate of infection of 76%, which corresponded to 84% infected with TcI and 16% infected with TcIII. Within TcI, 12% were typed as TcIDom and 88% as sylvatic TcI. All *P. geniculatus* and *R. pictipes* were infected with TcI (Sylvatic type).
CONCLUSIONS: These results suggest that most of the sylvatic triatomines in Colombia harbor *T. cruzi* I infection. Also, the evidence of *T. maculata* as an important host of TcIII. This finally suggests the importance of sylvatic vectors in terms of epidemiological relevance in those areas where *R. prolixus* has been controlled.

Deployment of an accurate High Resolution Melting (HRM) assay for the discrimination of New World Leishmania species

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Background: Leishmaniases are tropical zoonotic diseases, caused by kinetoplastid parasites from the genus Leishmania. New World (NW) species are related to sylvatic cycles although urbanization processes have been reported in some South American Countries. This eco-epidemiological complexity imposes a challenge to the detection of circulating parasite species, not only related to human cases but also infecting vectors and reservoirs.
Methods: Herein, we conducted a systematic High-Resolution Melting (HRM) assay targeted to HSP70 and ITS2 genes in order to generate an algorithm that will allow the identification of at least six NW Leishmania species. In order to validate the herein described algorithm, we included 25 natural isolates obtained from human cases with cutaneous leishmaniasis, insect vectors and mammals.
Results: Our genotyping assay allowed the correct assignment of six NW Leishmania species (L. mexicana, L. infantum (chagasi), L. amazonensis, L. panamensis, L. guyanensis and L. braziliensis) based on reference strains. When the algorithm was applied to a set of well-characterized strains by means of MLEE and monoclonal antibodies (MA) we observed a tailored concordance between the HRM and MLEE/MA (KI= 1.0). Additionally, we tested the limit of detection for the HRM method showing that this is able to detect at least 10 equivalent-parasites per mL.
Conclusions: This is a rapid, accurate and reliable method to conduct molecular epidemiology and host-parasite association studies in endemic areas. We hope that findings will support further analysis on the detection of Leishmania parasites from human, vector and mammal samples.

Population genetic analysis of G*iardia duodenalis*: high levels of genetic diversity and low genetic structure in a metropolitan region

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BACKGROUND: *Giardia duodenalis* is a flagellate protozoan that causes giardiasis, one of the most common waterborne diseases in the world. This parasite has a great genetic diversity and consists of eight distinct genetic assemblages (A-H). The aim of this study was to determine the genetic diversity in a set of clinical and environmental samples and the genetic structure among distinct populations of *Giardia duodenalis*.
METHODS: The isolates were obtained in several strategic places of a metropolitan region. Genotypes were identified by multilocus sequence-based genotyping using three unlinked gene loci (*tpi*, β-giardin and *gdh*). Genealogical relationships among sequences were estimated and haplotype diversity was calculated at the intra-population level. At the inter-population level, the genetic structure of the populations was inferred by an analysis of molecular variance and the population pairwise F_{ST}.
RESULTS: Most of samples were assigned to assemblages AII and BIV. Isolates with zoonotic potential and mixed assemblages have also been recognized. Genealogical relationships revealed shared haplotypes and pairwise FST indicated low genetic structure among groups. AMOVA among groups indicated that most of the variance was found within the populations.
CONCLUSIONS: This study shows that important environmental sites are contaminated by isolates with zoonotic potential. The low genetic structure detected among populations reveals that multiple sources of contamination can be related which represents a question of great concern about public health.

Effect of immunization with *Schistosoma mansoni*-adenylate kinase (ADK) and -adenosine kinase (AK) during inflammation in mansoni schistosomiasis experimental model

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BACKGROUND: Mansoni schistosomiasis is considered one of the most important human helminthiasis. Several studies on finding new vaccine targets for the control of this disease have been proposed. Having this in mind we have been investigating putative vaccine candidate antigens coding different enzymes in metabolic pathways of *S. mansoni*. In this study, the effect of immunization with *S. mansoni* ADK/AK enzymes during the inflammatory process was analysed through liver histopathological analysis.
METHODS: Mice (BALBc) were divided into 5 groups: control without infection (n=7); infected without immunization (n=7); ADK-immunized and challenged (n=6); AK-immunized and challenged (n=5) and ADK/AK-immunized and challenged (n=6). Experimental groups were immunized with 3 doses with 15 days intervals between each other. 15 days after the last immunization animals were infected (challenged) with 80 cercariae of *S. mansoni*. 48 days post-parasite infection mice were euthanized and their livers were removed and included in paraffin for posterior histopathological analysis.
RESULTS: We were able to observe that liver from mice immunized with ADK/AK enzymes and challenged with cercariae from *S. mansoni* presented a more conversed structure of liver cells and also demonstrated a partial reduction control on the inflammatory process when compared to the group infected without immunization. In the group infected without immunization granuloma formation with rich inflammatory infiltrate dispersed throughout the tissue parenchyma and preservation of parasite egg structure was observed.
CONCLUSIONS: The results suggest that immunization in association with ADK/AK enzymes has a protective effect on the liver, with the reduction on the inflammatory process and the formation of granuloma, thus it is suggested that these enzymes able to modulate the inflammatory response by *S. mansoni* infection.

Programs of qualification and quality control at slaughterhouse to control trichinellosis

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In the next two decades the meat consumption will double in the world with a necessity to the rise of pork and poultry production in emerging countries. Beside environmental consideration, harmful effects on public health can occur. Particularly meat borne-parasites like *Trichinella* can emerge or re emerge in countries consuming more meat without efficient control system. So, in 2012 FAO with Codex Alimentarius, under the auspice of OIE and WHO, performed an expertise on neglected food borne parasites to develop a ranked list of parasites of global importance. Amongst these parasites *Trichinella* have a unique position. It is obviously one of the two main meat borne parasites with *Cysticercus* at the world level with a strong economic impact. Even if in developed countries *Trichinella* is no more an important public health, this nematode parasite exact an economic burden particularly in the control cost for meat exportation. At the opposite several endemic countries (numerous countries in Asia, Argentina, some Eastern European countries, Russia.) cannot avoid human contamination due to failure in quality insurance control. We will develop the role of national and international organizations to control *Trichinella* with an emphasis on the national and international standards that was edited recently. it is the combined efforts of all described organisms that can insure the challenge to control Trichinella. Some critics emerge with some local organizational complexity which interferes with an efficient surveillance system. In any case when all these defined structures are well fixed and coordinated, it is possible to get food free of parasites.

Evaluation of LIAISON® XL MUREX Chagas assay for diagnosis of the *Trypanosoma cruzi* infection in non-endemic area (Spain)

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Background: *Trypanosoma cruzi* infection, or Chagas disease (CHD), is a public health problem in Latin America and even, due to immigration, in non-endemic countries. The majority of CHD patients are in the chronic phase of this illness and the anti-*T. cruzi* IgG detection is the main tool to confirm a suspicion case. Therefore, our aim was the evaluation of both sensitivity and specificity of the LIAISON® XL MUREX Chagas assay (DiaSorin S.p.A.), a new automated test based on chemiluminescence.
Methods: Samples were selected considering individual clinical-epidemiological background and results by conventional tests. This panel consisted of sera from individuals with CHD (n=400), visceral leishmaniasis (VL, n=50), malaria (n=56), other parasitic infections (OPI, n=56) and from seronegative individuals (n=500). All samples were analyzed by LIAISON® XL Murex Chagas assay (LIAISON), Chagatest ELISA recombinante v.4.0, and in-house tests (ELISA and IFAT). Data were analyzed using Epidat 3.1, Bayes Latent Class Models 1.4, and Binomial/Poisson distributions.
Results: 399 sera from CHD patients, 11 from non-chagasic individuals, 1 from VL, 2 from malaria and none from OPI were reactive by LIAISON test. Taking into account these results, the sensitivity and specificity of the LIAISON test were 99.8% (CI 95%: 98.6–100%) and 99.2% (CI 95%: 97.9–99.8%), respectively.
Conclusion: The LIAISON® XL MUREX Chagas assay is a recombinant test that showed a high sensitivity and specificity for the diagnosis of *T. cruzi* infection. On the other hand, it is noteworthy the few cross-reactions with VL and malaria. The LIAISON test is a new assay to detect antibodies against *T. cruzi*.

Cytotoxicity evaluation of endofitic compounds as therapeutic targets against *Leishmania infantum/chagasi*

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BACKGROUND: Visceral leishmaniasis (VL) has a very high incidence and lethality, primarily in untreated individuals and malnourished children, and is also considered to be emerging in individuals carrying HIV infection. In Brazil the causative agent of this disease is *Leishmania infantum chagasi*. Treatment of individuals with LV is usually problematic, since the drugs used in clinical practice are toxic and not always promote effective cure. Currently, leishmaniasis control consists mainly on chemotherapeutic treatments since no effective vaccine is yet available. In recent years science in general have been searching for new potential therapeutical agents as by-products of various microorganisms metabolism. Thus, having all this in mind the search for new compounds that exhibit therapeutical activity against *Leishmania infantum chagasi* is a promising path for control of this disease.
METHODS: A pre-selection of compounds produced by bacteria isolated from the Brazilian Cerrado Biome was made. Before the trials and to test whether these compounds have potential leishmanicidal activity a cytotoxic evaluation of these compounds was conducted using human gum fibroblasts (HP024). This test was conducted using MTT method, to determine cell viability after exposure to these compounds. The compounds were tested for concentrations of 400; 200; 100; 50 and 25 µg. Moreover, the production of nitric oxide (NO) was determined, by the accumulation of nitrite measured spectrophotometrically (260nm) using the Griess reagent.
RESULTS: Cell viability was determined. A low level of cellular death was observed for the interval of concentrations tested. Compounds named 1102 and 0603 did not present cytotoxic effect on HP024 cell line. In relation to NO no significant result was obtained.
CONCLUSIONS**:** Based on these initial results compound doses will be increased until IC50 dose calculated and for these further tests to assess the effect as leishmanicidal agent against *Leishmania infantum chagasi* promastigote and amastigote.

Analysis of *Trypanosoma cruzi* screening reported to the External Quality Control Program (EQCP) in Mexican blood banks

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BACKGROUND: The transmission of *T. cruzi* by blood transfusion has received increased attention in the last years. No test has been found to be sufficiently sensitive and specific to be designated for only screening assay. The Pan American Health organization suggested that blood donors should be tested by at least two different methods (HAI,IFI, ELISA) to detect true seroreactive donors. The present study was designed to compare the commercially available test kits for *Trypanosoma cruzi* reported in the EQCP applied by the NCBT twice a year to the 559 public and private banks of the country.

METHODS: Rate of false negative (FNR) and false positive results (FPR) for the serologic Chagas test reported from the blood banks of the country were evaluated from 2010 to 2012.

RESOULTS: An average or 347 banks participated in the EQCP during the period 2010-2012. The participation of banks in the Chagas screening was 57% in 2010, 68%in 2011 and 87% in 2012. 65% FNR were from public banks. 18% of FNR is related to the use of fast test, 15% with ELISA, and 4% with chemiluminiscence assay. 33% of FPR is related to the use of fast test, 8% with ELISA and 23% with chemiluminiscence assay. 50% of the bank used the ELISA, 31.7% chemiluminiscence assay and 16% rapid test. The state with more erroneous results was Michoacán (one of the states with highest number of cases) and Mexico City (where blood banks are mostly concentrated).

CONCLUSIONS: An increase on Chagas tests and FPR were observed. The results are attributed to the use of diverse methods or commercial kits and because the screening was not mandatory on 2011 and was usually not carried out. The NCBT informs the results to the blood banks with recommendations. The upgrade of regulatory policies in Mexico in December 2012, establishes that participation in the EQCP and the *T. cruzi* screening as mandatory. For the year 2014 a 100% of part6icipation is expected. A test using native antigens of strains of *T. cruzi* is needed due to the different lineages of *T. cruzi* present in the country in order to diminish FNR and FPR

Finding of *Trichinella pseudospiralis* in South America

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BACKGROUND: The genus *Trichinella* is composed by nine encapsulated taxa, (i. e. *T .spiralis, T. nativa, T. britovi, T. murrelli, Trichinella* T6*, T. nelsoni, Trichinella* T8*, Trichinella* T9 and *T. patagoniensis*), and three species that do not encapsulate (i. e. *T. pseudospiralis, T. papuae* and *T. zimbabwensis*). The encapsulated species and genotypes only infect mammals, whereas the non-encapsulated species infect mammals and birds (*T. pseudospiralis*) and mammals and reptiles (*T. papuae* and *T. zimbabwensis*). The species *T. pseudospiralis* is widely distributed but has never been reported in South America.
METHODS: We analysed a non-encapsulated isolate of *Trichinella* obtained from a domestic pig in Rio Gallegos, Santa Cruz, Argentina. The molecular identification at the species level was done by multiplex PCR. Furthermore, PCR amplification products from the expansion segment V (ESV), the D3 domain of nuclear ribosomal DNA (D3 rDNA) and the mitochondrial cytochrome C oxidase subunit I (COI) were sequenced and compared to known sequences from Palearctic, Nearctic and Australian populations of *T. pseudospiralis*
RESULTS: The multiplex PCR from the *Trichinella* isolate in study displayed an amplification product corresponding to *T. pseudospiralis.* The D3 rDNA and COI DNA sequences from this isolate were identical to the respective sequences of the reference isolate of *T. pseudospiralis* from the Palearctic region, but exhibited some variation with Nearctic and Australian populations. Likewise, the DNA sequences from the ESV fragments show more similarity to Palearctic sequences.
CONCLUSIONS: The study reveals the finding of an isolate of *T. pseudospiralis* from the Neotropical region, closely related to those from the Palearctic region, that probably were introduced to Argentina after European colonization. It is the first report of a non-encapsulated species of *Trichinella* in South America and represents the third species of this genus in the region, besides *T. spiralis and T. patagoniensis*.

Ecology and Diversity of monogenean parasites of marine catfishes (Ariidae), off Visakhapatnam Coast, Bay of Bengal

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BACKGROUND: Parasitology today represents an essential part of ecology and evolutionary biology for which it offers the best models. The present work has been carried out on the diversity and ecology of monogenoidean parasites infecting two species of marine catfishes *Arius jella* and *Arius dussumieri* belonging to the family Ariidae. Data collected on various parameters like prevalence, abundance, species richness, diversity etc. was used to study the community structure of these monogenean parasites.
METHODS: A total of 973 individuals of *Ariid* fishes belonging to 2 species were examined for monogeneans parasites. All the monogeneans found were identified up to species level. The molecular and morphometric methods were employed as supplementary to traditional morphological methods for identification of the species.
RESULTS: Out of 973 fish examined 815 were found to be infected showing a prevalence of 83.76%, mean intensity of 20.08 and a mean abundance of 16.82. The present study on the parasite fauna of the *A. jella* and *A. dussumerei* reveals the presence of fourteen species of Monogenoidean parasites distributed under four genera viz. *Hamatopeduncularia* Yamaguti, 1953 with 6 species, *Chauhanellu* Bychowsky and Nagibina, 1969, with four species *Neocalceostomoides* Kristsky et.al,1978 with two species and a New genus identified and named as *Thysanotohaptorrex* n.gen.,n.sp (Monogenoidea: Neocalceostomatidae).The mean number of species per fish was found to be 2.17, showing a Richness index value of 0.48(±0.14).A moderate diversity index value of 0.72(±0.20) was noted in the distribution of the fauna, whereas the Evenness' index value was found to be 0.78(±0.14).Core and Satellite species were also distinguished.
CONCLUSIONS: The present study reveals that compared to many other marine fish, the diversity of monogenoidean parasites in *A. jella* and *A. dussumieri* were found to be very high and these Ariid fishes are considered as most suitable hosts for monogenoidean parasites.

The entomological indices of indoor resting *Anopheles* populations in a rural community in the northern guinea savannah of Nigeria

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BACKGROUND: - The entomological indices of indoor resting adult *Anopheles* were investigated in a rural community in the northern guinea savannah of Nigeria, to determine species composition, sporozoite, entomological inoculation and human biting rates.
METHODS: - Mosquitoes were caught by the pyrethrium spray technique in three hamlets of the community where inhabitants had slept the previous night. *Anopheles* collected were morphologically identified to separate the main species of *Anopheles gambiae* senso lato (sl) and *Anopheles funestus* sl. Using the polymerase chain reaction technique (pcr), *Anopheles gambiae sl* caught were further delineated to *Anopheles gambiae* senso stricto (ss) and *Anopheles arabiensis*. The four (4) *An. funestus* sl, caught during the study, were not identified by pcr to species level because of small sample size. All the adults of *An. gambiae* ss*, An. Arabiensis* and *An. funestus* sl were assayed by the Enzyme Linked Immuno-Sorbent Assay (ELISA) to determine the *Plasmodium* sporozoites infection rates. The entomological inoculation and human biting rates, respectively, were estimated from the sporozoite rates of the mosquitoes by calculations in relation to the number of mosquitoes caught with blood in the abdomen in relation to the number humans who slept in the rooms where the mosquitoes were collected.
RESULTS: - The results showed that of the one hundred and thirty four (134) anopheline caught, *An. gambiae ss* was more abundant with 85.07% (114/134) than *An. rhodesiensis* 8.21% (11/134), *An. Arabiensis* 3.73% (5/134) and *An. funestus* 2.96% (4/134), respectively. The sporozoites rates were 4.48% (3/63), 20.00% (1/5), 0.00%and 0.00% for *An. gambiae* ss, *An. arabiensis*, *An. funestus* and *An. rhodesiensis,* respectively. The mean human biting rates was 0.79 while the entomological inoculation rate is 104.4.
CONCLUSION: - The studies suggest that *An. gambiae* ss and *An. arabiensis* are main vectors involved in the transmission of *Plasmodium* malaria in the rural community in the northern guinea savannah of Nigeria.

Identification of *Trypanosoma cruzi* lineage and blood meal sources by Q-PCR in triatomine gut samples in México

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BACKGROUND: The use of molecular techniques such as PCR facilitates the specific detection of *T. cruzi* in treatomine vectors. In Mexico, there are scant studies about the lineages of *T. cruzi* present in the country. In addition, there are no studies describing the distribution of *T. cruzi* lineages in triatomines collected in the state of Michoacán.

METHODS: 109 triatomine vectors were collected from human dwellings in Michoacán México. Blood meal sources were identified by a real time polymerase chain reaction (Q-PCR) using DNA extracted from triatomine guts. The assay was performed with only one specific primer set, to amplify a fragment of the mitochondrial 12S ribosomal gene from vertebrate species. *T. cruzi* discrete taxonomic units (DTUs) were identified by Q-PCR with two sets of primers that amplify the mini-circle region (miniexon) and 18S ribosomal mitochondrial gene.

RESULTS: We identified the blood meal sources by Q-PCR using DNA extracted from triatomine guts. Blood meal origins were identified in 88 of the 109 PCR products sequenced. The most frequent sample corresponded to *O. cuniculus* (17%), followed by *M. musculus* (16%), *Canis familiaris* (15%) *H. sapiens*(13%). A total of 30 triatomines were positive to T. cruzi. Furthermore, 20% of the isolates were typed as TcIV, 46.66% as TcI, 13.33% as TcII and 6.66% as Tc III.

CONCLUSION: The sequences obtained from the 18S ribosomal gene amplifications confirmed the presence of T. cruzi I and II lineages, and provide evidence of the presence of lineage TcIII and TcIV. Further studies are required to determine the actual lineage distribution of *T. cruzi* across the country, to identify which of the lineages is most abundant and to determine the relationship *between T. cruzi* distribution and the development of Chagas disease in patients living in the endemic área of Michoacán.

Occupational risk for spotted fever: an evaluation of knowledge, attitudes and prevention practices among veterinary medicine students

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BACKGROUND: Spotted fever (SF) is a tickborne rickettsial disease, which in Brazil affects the economically active population and has a high coefficient of lethality. The occupational risk attributed to veterinarians and animal handlers is due to exposure to vectors. This study assesses the knowledge, attitudes and preventive practices related to SF in a group of veterinary medicine students.
METHODS: It was performed a descriptive study with 173 students from Education Institution in the Brazilian Federal District, in August 2013. The knowledge (K), attitudes (A) and practices (P) were evaluated through interviews. For (K) it was evaluated: Have you ever heard about SF? Where? What is SF? How one acquires? Which environment? Do you known how to prevent yourself? For (A): Have you found ticks on the body? Which environment and what did you do? And for (P): What kind of clothes is used in practical classes?
RESULTS: Show that 84.39 % of the respondents have heard about SF, 62.32 % heard during classes, 47.94 % answered that SF is a tickborne disease, 65.75 % stated that it is acquired through tick bites, 52.73 % reported that the infection environment are places with the presence of ticks, 57.53 % said they know the methods of prevention, but only 39.28 % said the main form of prevention is avoiding contact with ticks. As for (A) it was observed that 70.52 % have already found ticks on the body, 79.10 % had contact with the vector in rural areas and 22.2 % self-medicated after contact. Regarding to (P) none of the respondents use appropriate protective equipment when exposed to vector.
CONCLUSIONS: Although this population is well informed about the SF and its preventive measures, such knowledge was not reflected when implementing prevention practices.

Increased intensity of *Ascaris lumbricoides* and hookworm from agricultural activities diminishes benefits of intensifying subsistence agriculture for preschool child growth in rural Panama

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BACKGROUND: Intensifying subsistence agriculture in rural communities of developing countries can benefit preschool child health through increasing food production, leading to greater household food security and diet quality. However, intensified agricultural practices may also increase exposure of children to environmentally transmitted parasites, notably the soil-transmitted helminths (STH).
METHODS: Our longitudinal study examined the effects of subsistence agriculture on preschool child growth (height-for-age (HAZ) z-score) in poor, rural communities in Panama. Through questionnaires, we measured intensity of agriculture (quantities of crops planted, number of animals kept, methods used, area planted) and extent of family participation in, and contact with, agricultural activities. Using multivariate regression models, we related these to prevalence and eggs/gram (epg) feces of *Ascaris lumbricoides* and hookworm infections of preschool children and to household food insecurity. We then examined the effect of household food insecurity, STH infections and agricultural practices on child HAZ.
RESULTS: *Ascaris* and hookworm epg in preschool children were higher if children accompanied caregivers to the agricultural plots but lower if plots were larger. *Ascaris* epg was also higher in households with more pigs. Households were more food insecure if they grew less staples. Low child HAZ was associated with food insecurity, less education of the caregiver, accompaniment of the child to the agricultural plot, and hookworm infection.
CONCLUSIONS: Preliminary analyses confirm that intensifying subsistence agriculture has benefits for child growth through improving household food security, but also creates risks for stunting through increased risk of transmission of STHs, which is detrimental for child growth.

Intestinal parasitic infections in Cuban schoolchildren and control measures

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BACKGROUND: Intestinal parasitic infections are one of the most prevalent and persistent infections among childhood worldwide. These infections may have important health consequences, but morbidity – especially for school-aged children – is often underestimated. In this study we determined the prevalence of intestinal parasitic infections in schoolchildren in urban and rural settings from San Juan y Martínez Cuban municipality and applied control measures.
METHODS: A cross-sectional study was carried out in 392 schoolchildren during 2003 and 1 377 schoolchildren during 2009, they were analysed by stool examinations for intestinal parasitic infections. Control measures such as the treatment for all the positive cases, training the healthcare personnel and an educative intervention to the community were applied.
RESULTS: General prevalence rates for intestinal parasite infections were 45.0 % in 2003 and 21.8 % in 2009 ($p < 0.001$); for helminthic infections, were 24.0 % and 10.5 % respectively for the same years ($p < 0.001$), and for protozoa infections were 29.0 % and 13.3 %, correspondingly ($p < 0.001$). Infected children with soil - transmitted helminths were treated with periodic selective treatment with a single dose of 500 mg mebendazole. Children infected with pathogenic intestinal protozoa were referred to their family doctors. Doctors, nurses and laboratory technicians were trained for active screening of cases and diagnosis. The educative intervention included the design of educational materials to be distributed in gathering places of the municipality and the formation of children as health promoters.
CONCLUSIONS: Paediatric intestinal parasite infections are still prevalent in certain areas from Cuba and the applied control measures decreased the prevalence of pathogenic intestinal parasitic infections.

Stage specific antigens in *Trichinella* genus

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BACKGROUND: The life cycle of *T. spiralis* is completed within a single host species and infection starts with the consumption of infective muscle larvae (ML). Larvae undergo four fast molts in intestinal epithelial cells and eventually develop into sexually mature adults (Ad) approximately 2-3 days post infection (pi). Freshly released newborn larvae (NBL) are carried to host tissues by blood flow and invade new host cells. The NBL penetrate striated muscle cells and undergo developmental changes. To date, little is known about the molecular mechanisms that are involved in parasite development and survival within the cytoplasm of host cell. Identification of stage-specific genes/proteins will be important for elucidation of these mechanisms.
METHODS: In an attempt to identify stage-specific genes of *T. spiralis*, subtracted cDNA libraries of NBL, Ad3 and Ad5 were constructed respectively, using a suppression subtractive hybridization (SSH) technique and differential screening with serum of experimentally infected pigs of various cDNA libraries.
RESULTS: Six genes were identified as NBL stage-specific, including one member of the *T. spiralis* gene family encoding glutamic acid rich proteins, two genes encoding novel serine proteases, two closely related genes encoding proteins that are members of a deoxyribonuclease II (DNase II)-like family and one nucleotidic sequence with no similarity to known genes. Their role in the development of *Trichinella* will be discussed. Another selected gene encodes an adult specific protein with strong similarities with a molting marker of *C. elegans*. Additional immunodominant stage specific antigens were finally identified at the very early development of *Trichinella* in the intestinal tract between 14hours pi and 48hours pi.

Dissecting the role of rosetting in *Plasmodium vivax* malaria

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BACKGROUND*: Plasmodium vivax* is the most prevalent parasite that causes malaria outside of Africa, however its pathogenesis is still poorly understood. We have previously demonstrated that *Plasmodium vivax*-infected erythrocytes (Pv-iEs) cytoadhere to endothelial cell receptors and form rosettes, a typical feature observed for *Plasmodium falciparum*. Rosette is a cytoadhesion phenotype where infected red blood cells can adhere to non-infected red blood cells and in falciparum malaria rosettes are normally associate to poor clinical outcomes and severity, and could play a role in improving parasite infectivity. Nevertheless, we have recently shown that although Pv-iEs harvested from Thai-Myanmar border patients form rosettes they are unlikely involved in severity as well in facilitating infection. These observations prompt us to investigate the biological role of rosetting formation of Pv-iEs.
METHODS: Here, we used different approaches to dissect the role of rosetting in vivax malaria. Phagocytosis assays were performed. Cytokines and immunoglobulins were quantified. The role of anti-VIR antibodies was analyzed in a rosetting disruption assay.
RESULTS: We noticed that autologous plasma improved significantly rosette formation, suggesting a key role of plasma components in rosetting formation. Indeed, we found positive correlation of IgM levels and an inverse association of IL6 and IL10 with rosetting rates of Pv-iEs, whereas antibodies towards to VIR proteins disrupt rosettes significantly. Importantly, rosetting parasites were less likely to be phagocyted *in vitro*. Thus, we hypothesize that rosetting acts as a mechanism of immune evasion by avoiding parasites to be phagocyted, in which high levels of IL10 counterbalancing the macrophage activity.
CONCLUSION: Finally, although rosettes formation is a common feature between *P. falciparum* and *P. vivax*, we have shown that the mechanisms involved for both parasite species markedly differ, implicating in distinct strategies adopted by these species.

Relationship between host size and morphological variation of *Sparicotyle chrysophrii* (Monogenea: Microcotylidae), a pathogenic parasite of gilthead sea bream (*Sparus aurata*)

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BACKGROUND: The host effect on monogeneans size has been widely studied from an interespecific and phylogenetic viewpoint, but very few studies have explored in depth the intraspecific variability in monogeneans size due to this factor. In this study, the relationship between size variability of a polyopisthocotylean species, *Sparicotyle chrysophrii*, and the size of its host, the gilthead sea bream (*Sparus aurata*), is analysed.
METHODS: Two hundred and sixty-nine adult specimens with similar number of clamps were removed from fish of different sizes, stained and mounted on permanent slides. Total body length, body width, haptor length, largest clamp width and smallest clamp width, total clamp pair number and number of clamps per 250 µm of haptor were measured.
RESULTS: Parasite growth rate was similar for hosts of different lengths. However, significant differences were found on total body length and the largest clamp width among parasites collected from hosts different in length. The smallest clamp width and the clamp number per 250 µm of haptor were independent of host length.
CONCLUSIONS: The results of this analysis suggest that those factors affecting fish growth, which is promoted in aquaculture, could also have an influence on parasite size. Therefore, parasite load and the damaged area of gill should be proportionally considered in order to evaluate parasite effects. Present study has been funded by AGL 2010 – 20892 of the Spanish government and ISIC/2012/003 of the Valencian local government.

Larvicidal activity of *Eugenia candolleana* DC. (Myrtaceae) oil against *Aedes aegypti*

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BACKGROUND: *Aedes aegypti* is the major vector of dengue fever, an endemic disease in Brazil. In the search for alternative control against *A. aegypti*, many researches are developed and encouraged in order to find new natural products. The most widely adopted strategy to decrease the incidence of these diseases is to control the population of mosquito larvae. The objective of this study was to evaluate the larvicidal activity of *Eugenia candolleana* DC oil in larvae of *A. aegypti* in laboratory.
METHODS: Eggs of *A. aegypti* Rockefeller strain, were set to hatch in pots containing 1 L of water and kept at 36 ± 1 ºC. The larvae were fed daily with food for fish and tests were conducted in quadruplicate in plastic beakers containing 10 mL of dechlorinated water for each 10 stage larvae L3. The oil was tested at concentrations 0.08 µg/µl, 0.15 µg/µl, 0.25 µg/µl, 0.35 µg/µl and 0.50 µg/µl. The oil was soluble in DMSO and larvicidal activity was determined in percentage of larval mortality after 24h and 48 h of treatment. Data from toxicity tests was used to determine the median lethal concentration (LC50 and LC90).
RESULTS: In these studies, the *Eugenia candolleana* DC oil showed larvicidal activity reaching a rate of 100% mortality at the concentration of 0.50 µg/µl in 24 hours. The LC50 was found to be 0.30 µg/µl and LC90 was 0.45 µg/µl. The control with DMSO showed no larvicidal mortality which proves the effectiveness of the oil.
CONCLUSION: Our results has shown an effec of *Eugenia candolleana* oil on larvae of *Aedes aegypti* indicating that this oil may be a potential alternative to the use of chemical insecticides in control of mosquito.

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Signaling pathways identified by systems analysis of *Leishmania* differentiation

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BACKGROUND: Protists of the genus *Leishmania* are obligatory intracellular parasites that cause a wide range of cutaneous, mucocutaneous and visceral diseases in humans. They cycle between phagolysosomes of mammalian macrophages and the sand fly mid-gut, proliferating as intracellular amastigotes and extracellular promastigotes, respectively. While much of the molecular mechanism of development inside macrophages remains a mystery, development of a host-free system that simulates phagolysosome conditions (37ºC and pH 5.5, 5% CO_2) has provided new insight into these processes. Transcriptomic and proteomic analyses indicated that differentiation is a coordinated process that results in adaptation to life inside phagolysosomes. Quantitative phosphoproteomics revealed extensive differences in phosphorylation between promastigotes and amastigotes, and identified stage-specific phosphorylation motifs.

MRTHODS: We used Isobaric Tag for Relative and Absolute Quantitation (iTRAQ) to investigate the dynamics of changes in phosphorylation profile during *L. donovani* promastigote-to-amastigote differentiation.

RESULTS: These experiments revealed protein kinases that phosphorylate specifically by the differentiation signal at the beginning of differentiation, but not by either high temperature or acidic pH alone. The results of these analyses have begun to reveal the molecular basis of differentiation, including a role for protein kinase A (PKA) in its regulation.

CONCLUSIONS: This work constitutes the first genome-scale interrogation of phosphorylation dynamics in a parasitic protozoa; revealing the outline of a signaling pathway during *Leishmania* differentiation.

Structure of the helminthofauna of *Rana macrocnemis* Boulenger, 1885 on Gomarety plateau (Lesser Caucasus)

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BACKGROUND: Regions of the Lesser Caucasus differ according to their climatic and hydrological conditions, soil and vegetation. All these contribute to the diversity of free living and parasitic fauna. Within provided investigation helminthofauna of *Rana macrocnemis* is studied on the Gomarety plateau of the Lesser Caucasus (1450m a.s.l).
METHODS: Sampling was performed in summer and autumn 2013. Complete parasitilogical dissection method was used. Temporary and permanent slides were made. Species identification was performed using morphometric data.
RESULTS: 100% invasion of *R. macrocnemis* by helminthes was registered. Eight species of helminthes are identified: Monogenoidea- *Polystoma integerrirum* (Frohlich, 1798); Trematoda - *Dolichosaccus rastellus* (Olsson, 1876); *Haplometra brevicaeca* Timon-David, 1962; Cestoda - *Nematotaenia dispar* (Goeze, 1782); Nematoda - *Rhabdias bufonis* (Schrank, 1788); *Oswaldocruzia filiformis* (Goeze, 1782); *Aplectana sp.; Cosmocerca ornata* (Dujardin, 1845). Among the fauna predominated intestine helminthes with high invasion intensity: trematode - *D. rastellus* (intensity 14-17), cestoda – *N. dispar* (intensity 9-12) and nematode – *O. filiformis* (intensity 22-25). Specific helminth for *R. macrocnemis* - *H. brevicaeca* is found for the first time for Georgian fauna. In all investigated hosts simultaneous invasion by different species of helminths is revealed.
CONCLUSIONS: This is the first publication of parasitological investigation of *Rana macrocnemis* from the Gomarety plateau. High percentage of invasion by lung and intestine helminthes and high species diversity is registered. Co-occurrence of 4-5 different species of helminthes in the host has well developed pattern. In the same organ of the host, species of the different the high taxonomic groups can co-exist, but systematically close species parasite in different organs that should be provided by interspecific concurrence.

Malnutrition and fate of *Strongyloides stercoralis* ontogeny under heterogonic conditions

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BACKGROUND: *Strongyloides stercoralis* (*Ss*) is a common worldwide nematode whose pathogenicity may be potentiated by host immunosuppressive factors, such as malnutrition, corticotherapy, oncotherapy or HTLV-1 co-infection. Few are known about clinical background and its influence on worm fate and subsistence under environmental stress. The present study aimed to determine the predominant worm stages developed through in vitro induction of heterogonic life cycle for establishing a potential relationship between clinical severity and worm diversity.
METHODS: From June 2012 to May 2013, adult inpatients and those presenting at emergency service from Hospital Nacional Cayetano Heredia with diarrhea were enrolled. Concentration methods (Lumbreras' cup-modified Baermann, Tello's spontaneous sedimentation, Lumbreras' rapid sedimentation) were performed to detect stool multiparasitism. For ontogenic study, agar plate culture was used. Morphology and specific feature measurements (body, tail, esophagus-gut junction and buccal capsule) were considered.
RESULTS: Parasites were detected in 42% (8/19), *Ss* was the most prevalent (31.5%), followed by *Blastocystis hominis* (26.3%), *Giardia lamblia* (10.5%), *Cystoisospora belli* (5.3%) and hookworm (5.3%). Weight loss and nausea were the most frequent clinical features associated to diarrhea among strongyloidiasis patients but 50% was reported with malnutrition. Females were directly related to malnourished and anaemic patients, and filariform larvae prevailed on patients with malnutrition. However, males were only associated with weight loss, edema and fever.
CONCLUSIONS: Our results suggest that females and filariform larvae more proliferate when host nutrition status is so impaired that may be life-threatening. Male absence could be explained by female protandrogonous behaviour. Worm burden and stage predominance may be helpful for treatment scheme choice.

Prevalence of Myxoboliasis in cyprinid fish (*Schizothorax esocinus*) in Kashmir, India

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BACKGROUND: The valley of Kashmir has rich water resources that harbor tremendous fisheries especially in cold-water sector.
The present study was designed to study prevalence of Myxozoan infections in local fish *Schizothorax esocinus* from different localities of Kashmir. Myxosporeans are common parasites of fish worldwide which cause serious damage to economically important fresh water and marine fish species. The present study was aimed to isolate Myxozoan parasites in naturally infested *Schizothorax esocinus* from Kashmir.
METHODS: For this study, 200 live *S. esocinus* were randomly collected from different water ecosystems in Kashmir (India).
RESULTS: Parasitological examination revealed great number of spores inside the cysts. Clinical signs revealed mucous laden glls. Gills were found infected with Myxozoan parasites which belong to genus myxobolus. Results revealed that 23% of the examined fish were infested with myxoboliasis and the highest rate of infection was found in female specimens than males. Infection was higher in fishes collected from Mansbal Lake as compared to Dal Lake and River Jhelum.
CONCLUSIONS: This is the first ever study of Myxozoan parasites of fishes of Kashmir valley.

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Analysis of the ultrastructure and the secretome of *Leishmania Viannia braziliensis* during interaction with collagen matrices

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BACKGROUND: *Leishmania (Viannia) braziliensis* is the main etiological agent of American Tegumentary Leishmaniasis (ATL) in Brazil. This species has been associated to distinct clinical ATL manifestations ranging from cutaneous to mucosal and disseminated lesions. During the blood meal of the sandflies, the metacyclic promastigotes of *Leishmania* are inoculated into the skin of the vertebrate host and need to cross the components of the extracellular matrix (ECM) to establish the infection process. Parasites migration, inactivation of macrophages and intracellular multiplication depend on the interaction of *Leishmania* with the components of the ECM. METHODS: In the present study, isolates associated with cutaneous and disseminated clinical manifestations of ATL were analysed, using Scanning electron microscopy (SEM), regarding their ability to invade and migrate through synthetic 3D Collagen I (COLI) matrices. Proteins secreted during parasite-COLI matrix interaction were identified by mass spectrometry (MS). In addition, the ability to degrade collagen substrate was evaluated in the presence or absence of peptidase inhibitors. Peptidase expression was analysed by zymography. RESULTS: SEM showed that promastigotes from both isolates adhered and altered the organization of COLI matrix. However, they exhibited different mechanical features during the interaction with the collagen fibers. The isolate related to the disseminated form of ATL showed a higher percentage of degradation of the collagen when compared to the isolate associated with the cutaneous form of the disease. Zymography assays revealed the presence of parasite metallopeptidases in the supernatant of the *Leishmania*-matrix interaction. Both cysteine and metallopeptidases were identified by MS. Incubation of parasites with cysteine and metallopeptidases inhibitors decreased the percentage of matrix degradation. CONCLUSIONS: cysteine and metallo-peptidases actively secreted by *L.(V.)braziliensis* play an important role in the invasion, migration and remodeling of 3D COLI matrix. In addition, *L.(V.)braziliensis* strains associated to different clinical manifestation exhibit distinct capabilities of 3D matrix degradation.

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Echinococcus multilocularis in foxes in western Europe

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BACKGROUND: Increase in red fox populations in many parts of Europe due to the successful rabies control campaigns might have extended the parasite distribution. Hence, it cannot be decided whether *E. multilocularis* recently has extended its range or whether the parasite has simply remained undetected until now. In The Netherlands, *E. multilocularis* was detected for the first time from a population of foxes in1997 in the northern region of Groningen adjacent to Germany and the southern region of Limburg adjacent to Belgium. These areas are considered the geographical westernmost border area of the parasite distribution in Europe.
METHODS: Studies in foxes in The Netherlands and Belgium were used to study the spatial distribution in both countries. Moreover, in the Netherlands various studies in foxes were used to determine the spread of the parasite and the risk for public health. In 2013, the latest study was performed to assess the prevalence of *E. multilocularis* in red foxes in the area east of Maastricht.
RESULTS: In Western Europe, the fox population density appeared indeed to be increasing. The number of human cases of alveolar echinococcosis is small, but still underreported, and is likely to increase in the near future. Results of the fox study in 2013 showed that there is a steep increase compared with the 2006 from 11% (95% confidence interval 6.7-18.4%) to 59% (95% confidence interval 43-74%) in the same area.
CONCLUSIONS: Studies in the borderline areas in Europe might be very interesting to elucidate the dynamics of the infection. We identified a steep increase in the prevalence of *E. multilocularis* in the southern part of the Netherlands. It remains to be determined how the increased prevalence influences the infection pressure, and how this translates to the estimated number of human cases of alveolar echinococcosis.

Biological characterization of *Trypanosoma cruzi* isolated from triatomines in the state of Morelos, Mexico.

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Introduction. Chagas disease (CD) is caused by *Trypanosoma cruzi (T. cruzi) and* transmitted to humans and animals by exposure to feces of infected triatomine. Morelos is an endemic area for CD. *T. cruzi* is a population with a broad genetic heterogeneity that could explain its tissue tropism and response to tripanocidal drugs. This work analyzed some bological aspects of *T. cruzi* isolated from triatomines of Morelos.

Material and Methods. Parasites were isolated from feces of triatomines from different localities of Morelos. Isolates were cultured in LIT medium and 1 x 10^5 trypomastigotes were inoculated i.p to Balb/c mice (6 mice by each isolate); a non-infected control group was included. Parasitemia, survival and heart tropism were evaluated according to standardized procedures.

Results. Isolated parasites were designated as: Jp/Mor/Mex2013, Tl/Mor/Mex/2013, Yt/Mor/Mex/2013, and Pi/Mor/Mex/2013. Parasites were not observed by microscopy during the infection process. One hundred percent of inoculated mice with the isolate Jp/Mor/Mex/2013 survived for 90 days of observation, and 20% of those inoculated with Tl/Mor/Mex/2013. However, those inoculated with both the Yt/Mor/Mex/2013 and Pi/Mor/Mex/203, showed signs of infection such as paralysis of hind limbs, urinary incontinence, bristling hair and death at 25 and 35 days post infection, respectively. Also, amastigote nests were detected in heart of infected mice with these isolates.

Conclusion. This study confirm the heterogeneity of *T. cruzi* isolates and their biological relevance in the host-parasite relationship.

Integrated surveillance of ticks and tick-borne diseases

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BACKGROUND: Tick-borne disease emergence is a complex and dynamic process. Interactions between multiple disciplines and responsible health and environmental authorities are often needed for an effective early warning, surveillance and control of vectors and the diseases they transmit. To fully appreciate this complexity, integrated knowledge about the human and the vector population is desirable.
METHODS: Important parameters and terms of both public health and medical entomology are defined in order to establish a common language that facilitates collaboration between different disciplines. Special focus is put on different contexts with respect to the current presence or absence of the disease, the pathogen and the vector in a given location. Depending on the context, whether a VBD is endemic or not, surveillance activities are required to assess disease burden or threat, respectively. Following a decision for action, surveillance activities continue to assess trends.
RESULTS: The above methodology is exemplified with the emergence of Lyme borreliosis in The Netherlands. Nationwide cross-sectional retrospective studies have shown a strong increase in general practitioner (GP) consultations for tick bites and diagnoses of erythema migrans between 1994 and 2009 in the Netherlands. Analyses of (historical) monitoring data from different sources provided strong indications for an overall increase in the total number and activity of Borrelia-infected ticks. As vaccines for the prevention of Lyme borreliosis are currently unavailable, control measures to decrease the risk of acquiring Lyme borreliosis are being developed and implemented at different levels in the surveillance pyramid.
CONCLUSIONS: Improved surveillance of ticks and tick-borne diseases with harmonized approaches for comparison of data enabling the follow-up of trends at the international level will improve the messages on risk related to tick-borne diseases to policy makers, other stake holders and to the general public.

Do cattle belong to the main livestock species attributing to human toxoplasmosis?

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BACKGROUND: Cattle seem not to readily acquire infection however, reported seroprevalence is high although the validation might be difficult since tissue cysts are only infrequently recovered from (experimentally inoculated) cattle and also rarely found in natural infected cattle or beef samples. Therefore, beef is thus often considered of low risk for human infection.
METHODS: PCR based detection of *T. gondii* in bovine samples and bioassay results originating from a Dutch and French study were used as input in a quantitative microbiological risk assessment approach (QMRA).
RESULTS: PCR results showed that positive cattle could be identified in the Netherlands and using the QMRA beef could contribute importantly and up to 67% of the predicted human infections. One popular beef product in the Netherlands eaten raw, filet-americain contributed to 38% of the predicted Dutch human infections. However, PCR based detection does not necessarily reflects the presence of infectious parasites. Results from a French study using bioassay showed a prevalence of viable cysts (0.96%) in cattle. If these data were used in the QMRA, the relative attribution of beef remained high at 52.5%.
CONCLUSIONS: PCR and bioassay studies in cattle combined with QMRA based methods indicate that cattle play a more important role in the transmission route to humans. Gaining information and knowledge on the presence and infectivity of *T. gondii* cysts in meat and other edible tissues in main meat-producing animals and its relationship with *T. gondii* seroprevalence in animals is the aim of an European project that started at the end of 2013 and will be financed by the European Food Safety Authority (EFSA). Results of this project will be used to advise for more adequate prevention measures to prevent public health.

HLA-DRB1*01 allele group increases the risk for relapsing ocular toxoplasmosis

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BACKGROUND: *Toxoplasma gondii* is an obligate intracellular parasite and cosmopolitan, whose infection congenital or acquired nature, results in different forms of toxoplasmosis. The aim of this study was to investigate the allele frequencies HLA-DRB1 as a risk factor for ocular form disease, in a population northwest of São Paulo State, Brazil.
METHODS: Peripheral blood was collected from 140 unrelated patients with serologically-diagnosed for toxoplasmosis. The patients were divided into two distinct groups according to the ocular manifestation of the disease (G1- ocular form, 46; G2- without ocular form, 48). Still, G1 group was divided into two subgroups according to the relapsing event of ocular toxoplasmosis (G3-lesion/scar, 37; G4-relapse, 9). The genomic DNA was extracted from leukocytes using the Pure Link kit® (Invitrogen). HLA typing was carried out according to the manufacturer's specification for LABType® SSO Typing, testing for each locus using Luminex® Technology (One Lambda, INC, USA) and the retrieved output was analyzed by HLA Fusion™ software (One Lambda, INC, USA) for allele identification. The frequencies were determined by Arlequin 3.1. software and the comparison of allele frequencies was analyzed using the chi-square test with Yates' correction or Fisher's Test. To estimate the disease risk, odds ratio and 95% confidence interval were calculated. Statistical analyses were performed using the *Open Epi* statistics software. P values ≤ 0.05 were considered statistically significant and Bonferroni correction was used to correct the p value (pc).The Hardy-Weinberg equilibrium was achieved by calculating the expected genotype frequencies and comparing them to the observed values.
RESULTS: Statistically significant susceptibility to relapse ocular toxoplasmosis was found for HLA-DRB1*01 allelic group (G4 *vs* G2 pc=0.0006; OR=9.831; IC=2.541-40.06).
CONCLUSIONS: These result suggest that the HLA-DRB1*01 conferred susceptibility for relapse form of ocular toxoplasmosis.

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EhDNALigI from *Entamoeba histolytica* is a high fidelity ligase involved in oxidative stress and UV irradiation response

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Background The protozoan parasite *E. histolytica* is exposed to oxidative stress during colonization and tissue invasion and metronidazole drug treatment. Although the mechanisms of DNA repair in this parasite are largely unknown, the genome of *E. histolytica* contains enzymes involved in several DNA repair pathways such as Base and Nucleotide Excision repair (BER and NER). The majority of these pathways use a DNA ligase to seal DNA nicks. In contrast to higher eukaryotes, the genome of this deep branching organism encodes only one DNA ligase dubbed EhligI. Our data suggests that EhLigI could be involved in both DNA replication and DNA repair processes. In this work we initiate the study of EhligI from *E. histolytica*.
Methods We characterized the ligation fidelity and the ability of EhDNAlig I to ligate opposite DNA mismatches, oxidative DNA lesions and UV irradiation damages. We also analyzed EhDNAligI expression during oxidative stress and UV irradiation using RT-PCR and finally we analyzed its localization using confocal microscopy.
Results We found that EhDNAlig I is a high fidelity DNA ligase in comparison to T4 DNA ligase. EhDNAligI also discriminates erroneous base pairing opposite DNA lesions. We found that EhDNAlig I locates and colocalize with EhPCNA.
Conclusions Our data indicates that EhDNAlig I is involved in DNA replication and repair pathways.

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Immunity to Ascaris sp. Infection: insights from the experimental infection in humans and animal models

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Ascariasis is a worldwide parasitic infection and the most prevalent neglected tropical disease, affecting 1.2 billion people and an important cause of economic losses in the animal production. To date, studies related to the biological aspects of *Ascaris* spp. infection are still scarce and further studies are necessary, mainly to elucidate the early events in the immune response and focusing on new immunoprophylatic strategies. In the current presentation, focus will be made on the characterization of the parasitological and immunological aspects of experimental infection with *Ascaris suum* in humans and *A. suum and A. lumbricoides* in the murine model. The pattern of immunity during primoinfection and multiple exposures to the parasite, mainly in the early phase of the infection, will be explored. Moreover, the contribution of eosinophil response, nitric oxide production and coinfections during *Ascaris* spp. infection will be discussed.

Using Next Generation Sequencing to Study *Entamoeba histolytica* Diversity

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Entamoeba histolytica is the causal agent of amebic diarrhea and dysentery in young infants in developing countries. During prospective longitudinal studies of enteric diseases in disadvantaged Bangladeshi children 80% were infected with *E. histolytica* by their second birthday. Not all new infections however resulted in symptomatic disease, which occurred in only 20% of cases. We hypothesize that while host and environmental factors play a role, amoeba parasites also vary in their potential to cause disease. This theory is supported by studies using tRNA associated short tandem repeats and our earlier work using the common *E. histolytica* SNPs $2725^{C/T}$ & $2730^{A/G}$ to genotype parasites (the Reference allele occurred in 50% in asymptomatic cases but in 84% in diarrhea isolates: n=63, p=0.02).

Molecular tools, such as whole genome sequencing (WGS), offer greater potential to genetically characterize phenotypically different parasites. *E. histolytica* parasites however are typically present at only low copy numbers in the clinical fecal samples commonly available for analysis. Sample sequencing at the depth required to reliably detect and obtain the pathogen WGS by a metagenomic approach is difficult and incompatible with efficient multiplex sample sequencing. Our studies have focused on the development of a pipeline to genetically sequence the *E. histolytica* parasite genome in complex sample mixtures. Short-term, mixed cultures established from *E. histolytica* positive fecal samples typically contain $1.4 \pm 1\ 10^8$ bacteria per ml and $0.57 \pm 2\ 10^7$ *E. histolytica* trophozoites. Using a phi29 based selective whole genome amplification protocol we have selectively enriched *E. histolytica* DNA over 100 fold. Given the smaller genome size of bacteria this results in the majority of DNA in the sample being derived from the protozoan parasite. Our future plans are to sequence sufficient *E. histolytica* genomes to detect virulence-associated parasite SNPs and build a tool for multi-virulence-locus sequence typing (MVLST) of *Entamoeba histolytica*.

Word count =300

Subtle Morbidities Associated with Malaria Co-infection with Schistosomiasis Among Children In South-West Nigeria.

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BACKGROUND: Malaria co-infection with schistosomiasis is known to modulate the immune response and thereby potentially altering the pathophysiological and immunological profile of the diseases. The aim of the study was to determine the relationship between subtle morbidities and co-infection with malaria and schistosomiasis, and the immunological responses to the two diseases, among children in rural southwest Nigeria.
METHODS: A cross-sectional survey was conducted between April and July 2012 among primary and secondary school children in Eggua, Yewa North LGA, Ogun State and Omi-Adio, Iddo LGA, Oyo State. A total of 240 children (Yewa 91, Iddo 149) participated in the study. Blood and urine samples were collected from the children and analysed by microscopy for *Plasmodium falciparum* and *Schistosoma haematobium.* all the samples were analysed for IL-10, IFN-γ, and some for antibodies to *Plasmodium falciparum* $MSP1_{19}$. Packed cell volume (PCV) and some anthropometric indices (height, weight) were measured as indicator of subtle morbidities of infection with the two parasites.
RESULTS: The prevalence of co-infection with the two parasites in the study was 16%. Malaria prevalence was 35.6% in Eggua, 20.13% in Iddo, and highest in the 11-15yr age group. Average malaria parasite density was 195.67parasites/µl blood. Schistosomiasis prevalence was 20.8% in Iddo, 30.8% in Eggua, with highest intensity of infection in age group 11-15 years in both areas, anaemia was not prevalent among co-infected people (16%). Antibodies to $MSP1_{19}$ were found in 36.7%. Peripheral IL-10 levels did not differ significantly among malaria, schistosomiasis, or co-infected individuals, but IFN-γ was higher among older children with schistosomiasis.
CONCLUSIONS: Anaemia was not a very discriminating index to indicate morbidity from the diseases in this study area.

Identification of *Giardia lamblia* proteins that contribute to direct mast cell activation

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Mast cells, IL-6 and TNF-alpha play a central role in the early clearance of the intestinal parasite *Giardia lamblia*. In a previous study we reported the direct activation (IgE-independent) of mast cells by *G. lamblia* live trophozoites or trophozoite-derived total soluble proteins, followed by an increase in mast cell tryptase expression and the significant release of histamine, IL-6 and TNF-alpha. To identify the molecules that contribute to mast cell activation, trophozoite-derived total soluble proteins were separated in three fractions (F1-F3), and their ability to induce mast cell activation was tested. F2 fraction stimulates mast cell activity in a greater extent than F1 and F3 fractions. Electrophoretic analysis of the F2, using the 1-D analysis software Quantity One (Bio-Rad), showed at least 25 protein bands of moderate to high intensity, five of them were unique to this fraction and seven bands with high intensity were shared with F1 and F3 fractions. Sixteen different *Giardia* proteins were identified in unique and high intensity bands by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Six of them, were uncharacterized proteins of unknown function, such as hypothetical proteins, proteins 21.1, HCP lateral transfer candidate, FixW and GTA-1. Other proteins, such as the enzymes PDI5, NEK-unclassified and Peroxiredoxin, which are involved in protein transport and oxidative stress resistance, respectively; and proteins 14-3-3, BIP, DPP IV, with an important role during different cellular processes, including cell cycle and encystment, were also identified. Interestingly, the well characterized parasite proteins, enolase and alpha giardins, were identified in some of the unique bands of the F2 fraction. These two parasite molecules have been previously considered as potential virulence factors and vaccine targets in giardiasis and our data suggest they have potential capacity to induce mast cell activation.

_CCR5_Δ32 in the evolutionary forms of the chronic chagas disease

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BACKGROUND: Chagas disease is an infectious disease caused by the protozoan *Trypanosoma cruzi*. It is endemic in the Americas, and represents an important public health problem in Latin America. There are estimates that 10 million people infected with *T. cruzi* are at risk of developing chronic forms of the disease: cardiac, digestive or mixed. Chemokines are small proteins, which act through specific receptors guiding the migration of leukocytes to sites of inflammation. The CC chemokine receptor 5 (CCR5), receptor for chemokines CCL3, CCL4 and CCL5, has been investigated in chronic Chagas disease. Genetic polymorphisms that alter the expression of CCR5 were associated with differential susceptibility to Chagas cardiomyopathy. The deletion of 32 base pairs in the gene *CCR5* (*CCR5*Δ32) results in a non-functional receptor, and CCR5 expression in heterozygous individuals is reduced. Accordingly, the objectives of this study were to investigate *CCR5*Δ32 (rs333) polymorphism in patients with the cardiac and digestive forms of Chagas disease, comparing the patients genotypes and alleles with the evolutionary forms of the disease.
METHODS: A total of 333 patients with reagent serology for *T. cruzi* were evaluated: (165 patients with the cardiac form of Chagas disease, and 168 patients with the digestive form). PCR was used to identify the *CCR5*Δ32 deletion. The Fisher's exact test was used in the comparison of both groups.
RESULTS: Differences in the distribution of genotypes and alleles between patients were not statistically significant.
CONCLUSIONS: The *CCR5*Δ32 polymorphism seems not to be involved in the differential susceptibility of the evolutionary forms of the chronic Chagas disease. Future investigations are necessary to clarify this matter further.

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Feline sporotrichosis: Histopathological profile of cutaneous lesions and their correlation with clinical presentation

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BACKGROUND: Cutaneous lesions of feline sporotrichosis show high fungal load and are associated with severe disease and elevated zoonotic potential. The present study describes the histopathology and fungal load of the lesions in different clinical presentations of feline sporotrichosis.
METHODOLOGY: Cats with sporotrichosis were separated into groups L1, L2 and L3 (lesions in one, two and three or more locations, respectively) and subjected to skin biopsies for histopathology.
RESULTS: Eighty-six cats were included in the study. Lesions were suppurative granulomatous in 84 cases and poorly formed granulomas were predominant. The well-formed granulomas were associated with group L1. The high fungal load was predominant in group L3 and in poorly formed granuloma cases and did not occur in well-formed granulomas cases. The good general condition was associated with low fungal load.
CONCLUSIONS: The results suggest a relationship between a well organized immune response (well-formed granulomas and presence of epithelioid cells) and control of fungal load, as well as its association with the general condition of the animal and clinical presentation of the disease. These findings suggest that the fungal load control in animals with more localized lesions and well-organized response is linked with the improvement in the outcome of infected cats.

Blood flukes (Trematoda, Aporocotylidae) infecting the Mediterranean Bluefin tuna (*Thunnus thynnus*): Three species of *Cardicola* parasitize the same host species

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BACKGROUND: Fish blood flukes (Trematoda, Aporocotylidae) probably are the most pathogenic parasites infecting pelagic fish in aquaculture. Aporocotylid eggs get trapped in gill vessels, where they hamper circulation and produce asphyxia as the eggs hatch and break gill epithelium. Pathologies associated to these aporocotylids have been reported in tunas (*Thunnus* spp.) are considered the most potentially dangerous pathogens in Australia and japan. At the Mediterranean Sea no severe pathologies have been described in *T. thynnus*. During the present study, high aporocotylid egg prevalences have been detected. Our aim has been identifying the species from the genus *Cardicola* which infect the *T. thynnus* to define useful detection specific protocols for the diseases control in this area.
METHODS: Gills from 102 tunas from farms in the Spanish Mediterranean coast have been analysed for parasites. DNA from eggs and adults fixed in 100% ethanol was extracted. ITS2 rDNA was amplified with different primer pairs.
RESULTS: The three *Cardicola* species described to date in bluefin tunas were found in the Mediterranean tunas (prevalence, 92%). Molecular data corroborated the morphological findings.
CONCLUSIONS: This is the first time that three species of *Cardicola* are cited together in the same species and area and the first record of *C. orientalis* in *T. thynnus*. Present study indicates that all potentially pathogenic parasites are also present in the Mediterranean cultures.
Study funded by project AGL-2010/20892 of the Spanish Government and ISIC/2012/003 of the Valencian Government.

Evaluation of partial sequence regions of its1 and 2 as barcoding markers in *Toxoplasma gondii*

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BACKGROUND: *Toxoplasma gondii* is a zoonotic parasite of global distribution. Studies to characterize the species have used RFLP but this method does not attain enough resolution. Progress in the species genotype characterization requires additional molecular markers such the non-coding ribosomal internal spacer (ITS) regions that have the advantage of the absence of selection pressure. The purpose of this study was to evaluate the ITS1 and 2 rRNA as a molecular marker of high resolution in representative strains of *Toxoplasma*: ME49, GT1 and VEG.
METHODS: Partial sequences of ribosomal ITS were downloaded from NCBI and ToxoDB web sites. The sequences were aligned by ClustalW and then imported into MEGA program 6v and TNT. ITS phylogenetic trees were constructed by the method of maximun likelihood, calculating distances paired by PWD (p- distance). Ribosomal distance matrix was used by Neighbor -Joining with Jukes and Cantor model and bootstrap of 1.000. ITS 1 and 2 were amplified by PCR from DNA of reference strains with specific primers.
RESULTS: Phylogenetic trees in the ITS showed that separation between GT1 and VEG and ME49 was well defined, but GT1 and VEG were grouped together. The most parsimonious topologies showed a pattern related GT1 with VEG, sustaining this relationship with a node bootstrap of 100 for the ITS1 and ITS2. PCR successfully amplified ITS1 and 2 from the three major clonal lineages.
CONCLUSIONS: As complete sequencing of ITS from reference strains are not available on web databases and present analysis was done with partial sequences, we need now to obtain complete sequencing from isolates *in vitro* to obtain more informative information.

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Leishmania species causing cutaneous leishmaniasis in an endemic area of the Peruvian Northern Highlands: a field study

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BACKGROUND. Identification of *Leishmania* species is critical for appropriate therapeutic management. The World Health Organization recommends surveillance activities in endemic areas to know the *Leishmania* species distribution and to improve its control (prevention, case management and vector control). We performed a cross-sectional study to determine which *Leishmania* species are causing cutaneous leishmaniasis in the northern highlands of Peru.
METHODS. Patients with suspected cutaneous leishmaniasis were recruited in five communities of the districts Luya and Chachapoyas (department of Amazonas). Clinical and epidemiological data were recorded, and filter paper imprint and skin scraping samples were taken and submitted to our reference laboratories, where Giemsa-stained direct smear and PCR tests were done to diagnose leishmaniasis and to identify the *Leishmania* species.
RESULTS. Forty-three patients were included in this study. Their median age was 26 years (range:1–86 years). Twenty-four (56%) were female, thirty-eight (88%) were permanent residents, the median of disease duration was 5 months (range:0.1-48 months); and 37 (86%) had only one lesion. Cutaneous leishmaniasis was confirmed in 42 out of 43 cases (98%) by kDNA-PCR and in 32 out of 43 cases (74%) by positive direct smear. Only one case was not confirmed by any of the performed tests. *Leishmania* species identification by nested RT-PCR was possible in 19 (44%) samples and *Leishmania (Viannia*) *peruviana* was detected in all of them.
CONCLUSION. PCR of non-invasive sampling (filter paper and scraping samples) was useful for diagnostic confirmation of cutaneous leishmaniasis. In about half of the samples, the identification of the species was not possible. This may be due to the quality of the sample under field conditions or to a small quantity of DNA in the sample. L. *(Viannia) peruviana* is an important cause of cutaneous leishmaniasis in the Peruvian northern highlands.

Ribosomal internal transcriber spacer (ITS)-1 and -2 and mitochondrial cytochrome "c" oxidase subunit 1 (mCOX1) genomic sequences of *Hymenolepis nana* isolated from human and rodents

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BACKGROUND: *Hymenolepis nana* is a hookworm that parasitizes the small gut of humans and it is associated with intestinal disorders especially in children. Egss and adult worms recovered from human and rodents show same morphology and zoonotic potential of the parasite has been suggested. Nuclear ribosomal internal transcriber spacer (ITS) and mitochondrial cytochrome "c" oxidase subunit 1 (mxCOX1) genomic sequences have extensively used for identification of several cryptic species including helminthes parasites.
METHODS: Eggs of *H. nana* were isolated from feces of infected humans and adult worms of *Hymenolepis* sp were recovered from captured peridomestic mice (*Mus musculus*) and rats (*Rattus norvegicus*). ADN from the parasites was isolated and the ITS1, ITS2 regions and partial mCOX1 gene were amplified by PCR. PCR products were sequenced and analyzed.
RESULTS: The analysis of the mCOX1 sequence of *H. nana* showed 98-100% of similitude among isolates from humans in accordance to *GenBank* reference sequences. After the phylogenetic analysis only one cluster was observed in isolates of different host and from others species of *Hymenolepis*. This cluster is subdivided in different sub-cluster in accordance to host origin. In this way a monophyletic group of *H. nana* was divided in isolates from humans, mice and rats. ITS1 sequences were 100% homologous between both human and mouse isolates, but they showed 0.5% difference with at least one sequence of the *GenBank* database. Respect to ITS2 analysis sequence, high homology (99.9-100%) was observed among human isolates.
CONCLUSIONS: Isolates identified as *H. nana* from human and mouse showed intraspecific differences using a partial sequence of mxCOX1. Besides, region ITS1 and ITS2 kept high homology between isolates of human and *Mus musculus* but showed intraspecific differences with the isolates of *Rattus norvegicus* at level of nucleotide and sequence side.

Plasmodium falciparum suppresses the host immune response by inducing the synthesis of insulin-like peptides (ILPs) in the mosquito _Anopheles stephensi_

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BACKGROUND: The insulin-like peptides (ILPs) and their respective signaling and regulatory pathways are highly conserved across diverse phyla. In invertebrates, ILPs are known to regulate a multitude of physiological processes, including anti-bacterial immunity. However, their role in the mosquito immune response to malaria parasite infection has remained unknown. Previously, we reported that infection with the human malaria parasite, *Plasmodium falciparum*, induces *ILP* gene expression in a mosquito host, *Anopheles stephensi*, suggesting that ILPs are produced in response to infection-specific signals and may modulate sporogonic development.

METHODS: In this study, we examine the effects of soluble *P. falciparum* products, as well as bacterial and fungal products, on ILP expression and secretion in cultured *A. stephensi* cells. Additionally, we examine the significance of *A.stephensi*ILP production in the context of immunity using antisense morpholino technology to knockdown ILP levels *in vivo* during *P. falciparum* infection.

RESULTS: Our data revealed that soluble *P. falciparum* products, but not bacterial or fungal products, induced both ILP expression and secretion in cultured *A. stephensi* cells. This induction was controlled through the MEK-ERK branch of the mosquito insulin/insulin-like growth factor signaling pathway. Further, we show that knockdown of *P. falciparum*-induced ILP3 and ILP4 *in vivo* decreases parasite survival through distinct effects on mosquito innate immunity.

CONCLUSIONS: The observation that *P. falciparum*-mediated signaling events induce ILPs to dampen the expression of immune effectors in *A. stephensi* is a novel example of immunosuppression by a malaria parasite in its mosquito vector. This work enhances current understanding of both immune evasion by *P. falciparum* and the diversification of ILP function in mosquitoes.

Anaerobic fermentation of pig manure with different levels of molasses and its effect in eliminating *Ascaris* eggs.

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BACKGROUND: Approximately 25% of the world population may be infected with *Ascaris spp*. It is a serious environmental problem related to the use of pig manure as fertilizer. Anaerobic bio-digestion has been raised as an option for the stabilization of sewage. Compared to other processes, anaerobic bio-digestion lowers the survival of pathogen when the proportion carbon/nitrogen (C: N) is increased. For monitoring parasites in general it is common to use *Ascaris* eggs. The purpose of this research was to determine the levels of molasses needed in anaerobic bio-digestion for the elimination of *Ascaris* eggs.
METHODS: Manure was diluted to achieve a 2% dry fecal matter. Treatments were control (T0) without molasses, 3% (T1), 6% (T2) and 9% (T3) of molasses, pH 7, at 35° C for 90 days, 5 replicates, in flasks 2 L sealed with screw cap containing 1 L of mixture. The initial number of eggs was 2767.
RESULTS: The results showed a reduction in the number of eggs of *Ascaris* in T0, T1, T2 and T3 (1100, 400, 0 and 250 eggs respectively). The pH decreased according to the concentration of molasses (6.4, 6.3, 5.6 and 5 respectively). Increasing the ratio C:N by the addition of molasses balanced the nutrients availability for anaerobic bacteria which increased the substrate bio-digestion. Furthermore the pH is decreased in proportion to the addition of molasses, indicating the accumulation of metabolites and their effect on the destruction of *Ascaris* eggs.
CONCLUSIONS: We concluded that the balance of C:N increases fermentation of excreta and is associated with the elimination of *Ascaris* eggs. More research is needed on the effect of anaerobic fermentation and viability of *Ascaris* eggs.

Fermentación anaeróbica de excretas de cerdo con distintos niveles de melaza y su efecto en la eliminación de huevos de *Ascaris*.

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INTRODUCCIÓN: Aproximadamente el 25% de la población mundial puede estar infectado con *Ascaris spp*. Es un grave problema ambiental relacionado con el uso de excretas de cerdo como fertilizante. Se ha planteado la bio-digestión anaerobia como opción para la estabilización de excretas. En comparación de otros procesos, la bio-digestión anaeróbica abate la supervivencia de patógenos, cuando se aumenta la proporción de Carbono/Nitrógeno (C:N). Para el monitoreo de parásitos en general es común el uso de huevos de *Ascaris suum*. El propósito de esta investigación fue determinar los niveles de melaza en la bio-digestión anaeróbica para la eliminación de huevos de *Ascaris*.
MATERIAL Y METODOS: Las excretas se diluyeron hasta alcanzar un 2% de materia seca fecal. Los tratamientos fueron testigo (T0) sin melaza, 3% (T1), 6% (T2) y 9% (T3) de melaza, pH a 7, a 35º C por 90 días, 5 repeticiones, en frascos de 2 L sellados con 1 L de la mezcla. El conteo inicial de los huevos fue 2767.
RESULTADOS: Los resultados muestran una reducción en el número de huevos de *Ascaris* en T0, T1, T2 y T3 (1100, 400, 0 y 250 huevos respectivamente). El pH disminuyo de acuerdo con la concentración de melaza (6.4, 6.3, 5.6 y 5 respectivamente). El incremento de la proporción C:N por la adición de melaza equilibró la disposición de nutrientes para las bacterias anaeróbicas lo que aumenta la bio-digestión del sustrato. Además el pH disminuye en proporción a la adición de melaza, lo que indica la acumulación de metabolitos y su efecto sobre la destrucción de los huevos de *Ascaris*.
CONCLUSIONES: Se concluye que el equilibrio de C:N aumenta la fermentación de las excretas y está relacionado con la eliminación de huevos de *Ascaris*. Se requiere de más investigación sobre el efecto de la fermentación anaeróbica y la viabilidad de los huevos de *Ascaris*.

Experimental development of the life cycle of *Gnathostoma turgidum* in Sinaloa

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BACKGROUND: Human gnathostomosis is caused by third advanced stage larvae (L3A) of nematodes of the genus *Gnathostoma* which is endemic in Southeast Asia and an emergent zoonosis in the Americas. This parasite has a heteroxenicus life cycle. In Mexico *G. binucleatum* has associated with human infection; however, our group has reported the discovery of *G turgidum* in an endemic area of human gnathostomosis and *Didelphis virginiana* has been identified as its definitive host. Even some hosts of *G. turgidum* in the wildlife are unknown, this study aimed to recreate the life cycle of *G. turgidum in vitro*.
METHODS: *G. turgidum* eggs were obtained from feces of opossums infected in wildlife and captured from an endemic area of gnathostomosis. Eggs were incubated at room temperature in water until eclosion of larvae L1. Once the larvae were obtained, they were provided as food for several species of copepods growing *in vitro*. The copepods were monitored by direct observation until development of the third early stage larval (L3E) and were co-cultured with a group of tadpoles of *Rana pipiens* for the development of L3A. Finally, the infected tadpoles were used for adult opossum experimental infection.
RESULTS: Viable larvae of *G. turgidum* were observed into copepods after ten days of eggs incubation. The copepods *Mesocyclos edax* was infected and after several days post infection L3E were observed. The behavior observed in tadpoles included facets of cannibalism causing that a single specimen was infected with eight larvae. All the Virginian opossum experimentally infected with *G. turgidum*-infected copepodes developed the infection and eggs of the parasite were observed after 7 months post infection. CONCLUSIONS: These findings demonstrate that *Mesocyclops edax* and *Rana pipiens* could participate as first and second host in the life cycle of *G. turgidum*, at least under our experimental conditions.

Environmental change impacts on hatching of ovine gastrointestinal nematodes

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BACKGROUND: Projections for climate change forecast up to ~5°C higher temperature for temperate zones, and more extreme and frequent warmer winters and summers. This environmental change is expected to impact on disease epidemiology, including parasitic gastroenteritis. The free living stages of ovine gastrointestinal nematodes, the main cause of parasitic gastroenteritis, may be sensitive to this environmental change. Our hypothesis is that an increase in environmental temperature will accelerate hatching of gastrointestinal nematode eggs.
METHODS: For this study, *Teladorsagia circumcincta* eggs, extracted from faecal samples of monospecifically infected sheep donors, were incubated *in vitro* at eight different temperatures, i.e. from 5°C to 40°C with increments of 5°C. Incubation was stopped at specific time points and total number of unhatched eggs and hatched 1st stage larvae (L_1) were counted in triplicated samples. Each incubation temperature was replicated three times. The percentage of hatched L_1 was calculated from the total number of L_1 and unhatched eggs counted.
RESULTS: Larval hatching occurred at all temperature levels within 5°C – 40°C. By increasing temperature from 5°C to 25°C, hatching of gastrointestinal nematode eggs was accelerated. Every increment of 5°C between 5°C and 25°C halved the time required for the same percentage of eggs to hatch. The biological variation within the trials at the same temperature was relatively small.
CONCLUSIONS: Our data support the view that a rise in environmental temperature increases the rate of larval hatching, which has implications for pasture infectivity. Thus, under the expected climate change scenarios, it might be predicted that hosts are infected earlier and more severely in the grazing season, resulting in earlier outbreaks of parasitic gastroenteritis.

Phytomedicines from *Warbugia ugandensis* and *Zanthoxylum usambarense* against *Plasmodium knowlesi*

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BACKGROUND: Malaria affects about 500 million people annually with a mortality of up to 2.7 million. The high cost of effective treatment hampers malaria control. Herbal medicine is used by local communities in the developing world to treat various infectious diseases (Bidla *et al*., 2004). In Africa, about 75% of the population either does not have access to or cannot afford conventional medicine and therefore traditional medicine for malaria treatment is an alternative (Azas *et al*., 2001; Bourdy *et al*., 2004). *Warburgia ugandensis* Sprague (Canellaceae) and *Zanthoxylum usambarense* (Engl.) Kokwaro (Rutaceae) are commonly used as traditional medicine by many communities in Kenya (Njoroge and Bussmann, 2006). Both plants grow in both highland and lowland areas especially in forests around Nairobi, Masaai Mara, Samburu, Southwest of Mt. Kenya and Kakamega (Nanyingi *et al*., 2008). Hot and cold decoctions from leaves and stem barks of *Warburgia ugandensis* are culturally used to treat tooth decay, asthma and bronchitis while *Zanthoxylum usambarense* is used to treat malaria, upper respiratory tract infections, tooth decay and sore gums (Nanyingi *et al*., 2008; Njoroge and Bussmann, 2006). Phytochemical studies on *Zanthoxylum usambarense* revealed that it contains alkaloids of tetrahydroprotoberberine type (Kato *et al*., 2007). Moreover, its *in vitro* antimalarial activity against *Plasmodium falciparum* has been reported (Kirira *et al*., 2006). *Plasmodium knowlesi* is widely distributed in parts of Kenya, Malaysia, the Philippines, Myanmar and Thailand, and can also be fatal, presenting an urgent need for more focused investigations on its control (Tek *et al*., 2008; Luchavez *et al*., 2008).
OBJECTIVE: The aim of this study was to determine the anti-plasmodial activities of extracts from *Warburgia ugandensis* and *Zanthoxylum usambarense* against the fifth human malaria parasite (White, 2008), *Plasmodium knowlesi*.
METHODS: Eight plant extracts were screened for *in vitro* anti-plasmodial activity *against Plasmodium knowlesi*, in a 96-well plate incubated at 37 ◦C on a RPMI culture medium supplemented with Baboon serum.
RESULTS: Inhibitory concentrations (IC50) values of between 3.14 and 75_g/ml, up to 69% chemosuppression of parasites growth and over 80% survivorship of treated mice were observed. CONCLUSIONS: The two medicinal plants, *Warburgia ugandensis* and *Zanthoxylum usambarense* possess bioactive compounds against malaria parasites and could be exploited for further development into malaria therapy.

Metazoan parasites of the channel catfish (*Ictalurus punctatus*) from three dams in Nuevo Leon, Mexico

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BACKGROUND: The freshwater channel catfish (*Ictalurus punctatus,* Rafinesque, 1818) is one of the world-wide highlight species in aquaculture. Despite the growing presence of this fish in the farming industry, its parasitic fauna is rarely studied in Mexico. This survey was designed to provide insight into the occurrence and abundance of parasitic fauna of *I. punctatus* and to contribute to the national inventory of parasites of this fish.
METHODS: This study was performed at the following three dams in the state of Nuevo Leon, Mexico: Cerro Prieto, Rodrigo Gomez, and El Cuchillo-Solidaridad. Freshwater channel catfish (9 from Cerro Prieto, 8 from Rodrigo Gomez, and 7 from El Cuchillo-Solidaridad) were obtained from local fishermen and transported to the laboratory. External surfaces were examined for ectoparasites. Then, the gills were examined to detect copepods and monogeneans using a stereo dissecting microscope. The internal organs, including the heart, liver, intestines, and stomach, were separated and examined for metazoan parasites.
RESULTS: Seven helminths and 1 copepod species were recorded from the 24 *I. punctatus* collected. This record consists of a total of 4,687 parasites, of which 108 were endoparasites and 4,579 ectoparasites.
CONCLUSIONS: Our findings demonstrate a great diversity of helminth parasites, including *Ligictaluridus floridanus*, and *Corallobothrium fimbriatum*, and new locality records for *Megalogonia ictaluri*, *Centrocestus formosanus*, *Diplostomum* (*Austrodiplostomum*) *compactum*, *Spiroxys* sp., the copepod *Ergasilus cerastes,* and a new host and distribution record was reported for *Spinitectus tabascoensis*, originally described from *I. furcatus* from Tabasco, southern Mexico.

Serodiagnosis of Chagas Disease in cohabitants of blood donors seropositive for *Trypanosoma cruzi*.

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BACKGROUND: Infection with *T. cruzi*, the causative agent of Chagas disease, has been reported in different blood banks in Mexico. However, only regular blood donors are diagnosed, but not their cohabitants who share risk conditions (housing, proximity to vector, etc). Therefore, in this work the serodiagnosis of Chagas was performed to cohabitants of thirty-seven blood donors seropositive for Chagas, who currently live in the north of Mexico City and its metropolitan area.
METHODS: Confirmatory tests for the infection with *T. cruzi* for blood donors were performed. Their cohabitants were invited to the same diagnosis performed by ELISA and Western blot. Epidemiological data were collected by an *ad-hoc* questionnaire.
RESULTS: Thirty-seven blood donors (index cases) and their cohabitants agreed to participate in this work. A total of 77 cohabitants were assessed by the serological techniques described above. We found that 14 (18%) of them were seropositive for *T. cruzi*. The age range of seropositive cohabitants was 13 to 41 years. Most of the positive cases were sons and daughter (8 cases, 57%) and siblings (4 cases, 28%) of the index cases. Nine of fourteen (64%) were women and six men (36%). Three cases (31%) were under 15 years. Ninety percent of cohabiting cases shared at least one risk factor with the index case, as rustic house, have lived or have frequent stays in endemic areas, and had been in contact with the transmitter vector during childhood.
CONCLUSIONS: The results show that it is important to diagnose *T. cruzi* in the cohabitants of the seropositive individuals, because unnoticed cases can be diagnosed and in this way to contribute to the unknown aspects of epidemiology of Chagas Disease in Mexico.
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Characterization of profilin, an Actin-Binding Protein of *Trypanosoma cruzi*.

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BACKGROUD: Actin is a major component of the eukaryotic cytoskeleton that is involved in a wide range of cellular processes. One central property of actin is the capability to form polymers, known as microfilaments. The dynamics of formation, structure, and localization of the microfilaments is controlled by a diverse set of actin-binding proteins (ABPs). Typical microfilaments have not been identified in trypanosomatids, but there is a set of genes encoding for putative ABPs. Profilin is a conserved eukaryotic ABP that regulate actin polymerization dynamics. This activity is performed by its simultaneous binding to actin and to poly-proline motifs present in actin-regulatory proteins. In this work, we present preliminary data on the characterization of profilin in *T. cruzi*.

METHODS: Profilin of *T. cruzi* fused with the GST tag was expressed in *E. coli* and purified by affinity chromatography. Mice were immunized with the recombinant profilin for obtaining poly-clonal anti-profilin sera. Immunocytochemical studies were performed to determine profilin expression and subcellular localization for three stages of *T. cruzi* (CL Brener): epimastigotes and cell culture derived bloodstream trypomastigotes and amastigotes. Metacyclic trypanomastigotes were not included.

RESULTS: Poly-clonal sera raised against the recombinant profilin identify a protein of the expected molecular weight in *T. cruzi* protein extracts by immunoblot assays. Similar levels of expression were found in the three stages of the life cycle analyzed. Immunolocalization shows that profilin is a predominantly cytoplasmic protein.

CONCLUSIONS: Profilin is a cytoplasmic protein that is constitutively expressed along the life cycle of *T. cruzi*. We are currently performing assays to demonstrate the interaction of profilin with *T. cruzi* actins and to identify potential novel actin-regulatory proteins. Functional studies to analyze the role of profilin in the actin dynamics are also in process. To our knowledge, this is the first study of an ABP in *T. cruz*i.

Relative expression analysis of *IL-5* and *IL-6* interleukines from leucocytes stimulated by *Haemonchus contortus* excresion/secresion products

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BACKGROUND: Gastrointestinal nematodes (GIN) affect sheep industry. *Haemonchus contortus* is considered as one of the most important GIN worldwide, provoking anemia, malnutrition, weight lost and the dead of young sheep. Chemotherapy is the common practice of control; however, it has lead to anthelmintic resistance. Using immunizing agents from parasites in being considered as a measure to induce a protective immune response. GIN excretory/secretory products (ESPs) possess activity on interleukin 5 and 6 (IL-5–IL-6), involved in specific immune response. They contribute to the activation and differentiation of T cells, eosinophils and to the specific antibody production.
METHODS: The expression of two interleukin (*IL-5* and *IL-6*) by RT-qPCR and (lymfoproliferation) was assessed. Leucocyte activation was expressed by two *H. contortus* L_4 (HcL4) ESP (70 and 13 kDa). ESP`s HcL4 were obtained from HcL4 *in vitro* cultures. The lymfoproliferation technique was standardized using phytohaemagglutinin (PHA) and lipopolysaccharides (LPS) as positive controls. Each lymfoproliferation assay was standardized using $2.5x10^5$ and $5x10^5$ cells. PHA and LPS were tittered (5, 10 and 25 µg/mL) and LPS (5 ng/mL, 50 pg/mL and 5 pg/mL) for 72 h incubation.
RESULTS: ESP´s were assessed using 5, 10 and 20 ng/mL for 48 and 72 h incubation times. Higher cell stimulation in positive controls was: for PHA=5 µg/mL and for LPS=5 ng/mL. The maximum ESP stimulation was recorded with 5 ng/mL at 72 h for incubation. White cells were scaled at $4X10^6$ and activated with ESP (5 ng/mL). Total RNA and the relative expression of *IL-5* and *IL-6* genes were obtained through RT-qPCR. Both protein bands from HcL4 ESP showed immunosuppressive activity, decreasing the expression to *IL-5* and *IL-6* genes when compared with the constitutive *gapdh* gene.
CONCLUSIONS: An analysis of the biological function of *H. contortus* (L4) ESP´s using other interleukInes will be required to confirm the present results.

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

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

Trypanosomes in chimpanzees and their insect vectors

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BACKGROUND: African trypanosomes of the *Trypanosoma brucei* group are the most famous parasitic flagellates, as they are the causative agents of the neglected yet often fatal African sleeping sickness of humans and nagana of cattle. Members of this clade differ in mechanisms of escaping lysis by the host serum. Sera of some primates such as baboon, mangabey and mandrill efficiently kill *T. brucei brucei*, *T. b. rhodesiense* and *T. b. gambiense* via the apolipoprotein L1 (ApoL1) protein, while human sera lyze the latter two only. Although chimpanzees live in endemic regions and are known being susceptible to the infection in captivity, they lack the respective gene and, hence, do not lyze any of mentioned trypanosomes.
METHODS and RESULTS: We amplified trypanosome intergenic region 1 (ITS1) from about 10% of tissue samples of dead chimpanzees, colobus monkeys and mangabeys collected through several African countries. For first time, we succeeded to amplify trypanosome DNA from fecal samples of wild chimpanzees in the Täi National Park (Côte d'Ivoire), which will allow monitoring of trypanosome infections in highly protected mammals. With one sequence falling into *T. theileri* clade, all other ITS1 sequences belong to the *T. brucei* clade. Next, 564 individuals of tsetse flies (*Glossina* spp.) and a total of 81 specimens of tabanids were molecularly screened for trypanosomes. While 33% of tsetse flies were found to transmit either *Trypanosoma congolense*, *T. simiae*, *T. theileri* or at least four new members of the genus *Trypanosoma*, the other blood-sucking vectors were 59% positive exclusively for *T. theileri*. Finally, DNA was individually isolated from 1003 specimens of *Glossina* spp. and subjected to library-based screening of animals from which they sucked the blood. While most of them engorged on buffaloes, humans, sitatunga/bongo and suids, we provide evidence that in both studied locations, these tsetse flies also occasionally take blood from chimpanzees.
CONCLUSIONS: Since all attempts to amplify single-copy trypanosome genes failed, based on the ITS sequences, not allowing the distinguishing among the *T. brucei* subspecies, we speculate that chimpanzees are infected with these flagellates and control the infection by an ApoL1-independent mechanism. In addition to this, we provide the evidence that trypanosomes can be transmitted to great apes via testse fly vectors.

A randomized-controlled trial to determine the benefit of deworming on growth in early preschool-age children in Iquitos, Peru

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BACKGROUND: WHO recommends deworming of young children as of 12 months of age in soil-transmitted helminth (STH) endemic areas; however, the optimal timing and frequency have been understudied in early preschool-age children. These children may be particularly vulnerable to adverse effects from STH infection as they are in the most critical window for growth.
METHODS: We conducted a randomized controlled trial of deworming (500mg single-dose mebendazole) in 12 month-old children in Iquitos, an STH-endemic area of the Peruvian Amazon. A total of 1760 children were enrolled between September 2011 and July 2013 and followed for one year. Children were randomly allocated to one of four groups: 1) deworming at 12 months of age and placebo at 18 months of age; 2) placebo at 12 months of age and deworming at 18 months of age; 3) deworming at 12 and 18 months of age; or 4) placebo at 12 and 18 months of age. Height, weight and STH infection were assessed at each visit.
RESULTS: A total of 1563 children (88.8%) attended their 24-month visit. At baseline, the prevalence of STH infection was 12.4%. The species distribution was 11.5% for *Ascaris*, 4.5% for *Trichuris* and 0.6% for hookworm. STH prevalence rose to over 40% at 24 months. There was a statistically significant improvement in weight gain in those receiving deworming once at 12 months, compared to those receiving deworming once at 18 months (p=0.028). No additional benefit was detected for twice-yearly deworming.
CONCLUSIONS: Our results indicate that once-yearly deworming at 12 months of age has important benefits on growth. These results contribute to the evidence-base on deworming policy in over 100 STH-endemic countries worldwide. Emphasis should be placed on providing children in this vulnerable age group with cost-effective, integrated interventions to reduce health and nutritional burdens.

Distribution of *KIR* genes and their *HLA* ligands in a malaria-endemic population of Porto Velho, Brazilian Amazon

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BACKGROUND: Killer immunoglobulin-like receptors (KIR) regulate the activity of natural killer and T cells through interactions with human leukocyte antigen (HLA) class I ligands. Some studies have been associated KIR genes and genotypes KIR/HLA ligands with incidence and progression of several diseases, including malaria. In this context, the aims of this study were to describe the diversity of KIR genes, KIR genotypes and KIR-HLA pairs in a malaria-endemic population of Porto Velho, Brazilian Amazon.
METHODS: Here we analyzed, by PCR-SSP**,** 377 unrelated and randomly selected individuals from Porto Velho communities. Prism version 5 and R statistical software were used to analyze data according the statistical test necessary (Principal Component Analysis_PCA, Chi-square test, Student's t test and Mann-Whitney).
RESULTS: All 16 *KIR* genes investigated were present in the studied population. The PCA was performed on KIR gene frequencies for 52 worldwide populations including our study population (Brazil Porto Velho communities). The biplot representation showed Porto Velho population close to Venezuela Mestizos and California Asia Americans. Forty-eight KIR genotypes were defined. The most frequents (numbers 1 and 2) were present at a similar frequencies in the Americas. Seventy-five KIR-*HLA* genotypes were identified. The KIR-2DL2/3_*HLA*-C1, KIR3DL1_*HLA*-Bw4, and KIR2DL1_*HLA*-C2 pairs were the most commons. Forty-nine percent of the individuals carried KIR2DL2 and/or KIR2DS2 genes. Of them, 78% displayed their respective ligand *HLA*-C1. The number of previous malaria was highest in individuals who carried KIR2DL2 and/or KIR2DS2, regardless of the presence of *HLA*-C1.
CONCLUSIONS: The data obtained in this work suggest a possible association of *KIR/HLA* genes and malaria susceptibility. Moreover, such results may contribute to future studies on the functional impact of these genes in regulating the immune response in relation to the incidence and clinical course of the disease not only in malaria as well as in other infectious diseases.

Bone marrow-derived dendritic cell stimulation with recombinant *Taenia solium* Calreticulin

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Background: *Taenia solium* is a helminth that causes neurocysticercosis and taeniosis in humans. In general helminth infections are associated with a Th2-type immune response and although the induction mechanisms have not been well defined, dendritic cells play a critical role in the regulation of such responses. Calreticulin is a multifunctional and highly conserved protein involved in many physiological processes and has immune regulatory properties in other parasites. Our group demonstrated that oral immunization of BALB/c mice with functional recombinant *Taenia solium* calreticulin (rTsCRT) induced a Th2-biased immune response. The aim of this study was to analyze the cytokine response of murine bone marrow derived dendritic cells (BMDC) to TsCRT stimulation.
Methods: rTsCRT was purified by differential centrifugation, electrophoresis and electroelution. Monocytes were obtained from bone marrow of Balb/c mice, differentiated with GM-CSF, harvested and stimulated with 10µg of rTsCRT or 10µg rTSCRT + PmB on the 8th day. TNF-alfa and IL-10 cytokines were measured by ELISA 24h after stimulation. Flow cytometry was employed to confirm the dendritic cell phenotype using specific antibodies: anti-CD11c and anti-MHCII.
Results: Purification of rTsCRT was not easily achievable since the desalting process and detoxification resulted in a recovery rate of 25%. Flow cytometry analysis showed that about 50% of the BMDCs expressed MHCII and 70% were $CD11c^{+}$. Stimulation with rTsCRT did not induce a significant amount of TNF-alfa or IL-10, while stimulation with rTSCRT + PmB led to significant cytokine production compared to the unstimulated control.
Conclusions: The presence of PmB might induce physicochemical changes to rTsCRT that results in cytokine induction and differential recognition by BMDC. The molecular mechanisms involved need further study. In addition different rTSCRT concentrations and stimulation periods should be assayed.
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