**Towards an Understanding of the Epidemiological of Amoebiasis: An Evolutionary Standpoint**

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BACKGROUND: Amoebiasis is still a public health problem in the American Asian and African continents. The last global prevalence (1986) was 10% of the worldwide population. Current data after the establishment of the existence of two different species of *Entamoeba* are those obtained in different geographic locations or specific groups of communities or institution for handicapped individuals or individuals with high risk sexual practices.
Our major scientific interest is the estimation of the actual burden of *Entamoeba histolytica* and *Entamoeba dispar* infection and morbidity of disease. Genotyping of most prevalent strains the geographic distribution of theme and their phylogenetic relationship.
METHODS: Estimation of the molecular frequency of infection due to *E. histolytica* and *E. dispar* in Sonora and Morelos were performed, using as target, the gene for the small subunit of rRNA. The genotyping of *E. histolytica* and *E*. *dispar* were done using as targets Chitinase, SRP gene, and STR of intergenic regions of tRNA genes. Phylogenetic assay and genealogy of *E. histolytica* isolates from human amoebic infection were performed.
RESULTS: Comparison of incidence official data of intestinal amoebiasis and amoebic liver abscess will be presented. Data on molecular frequency of infection in Sonora and Morelos states will also be shown.
Genotyping of isolates of *E. histolytica* and *E. dispar* from intestinal infected individuals also show a high genetic variability both inter-species and intra species. The molecular targets can be coding DNA regions (Chitinase and Serine rich protein genes) or intergenic non codifying DNA sequences (inter-genes STRs related with tRNA genes).
CONCLUSIONS: In our opinion the studies on population genetics of pathogenic parasites allow us to know the population structure of prevalent genotypes, the phylo-geography, genealogy and the evolutive history, in this case of *Entamoeba histolytica* and *E. dispar*.

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

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

# Morphological and molecular characters evidencing the presence of new species of *Gyrodactylus* von Nordmann, 1832, (Platyhelminthes: Monogenea) from Poeciliid fish in Mexico

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Background: Livebearer fish from the Poeciliidae family are one of the most dominant fish groups in the lowland fresh and brackish waters of Middle America and the Western Indies. There are approximately 80 poeciliid species in Mexico. Eleven species of *Gyrodactylus* have been recorded from poeciliids in America and Africa. From these, only 3 have been recorded in Mexico, *G. bullatarudis* from *Poecilia mexicana*, *G. jarocho* from *Xiphophorus hellerii* and *G. xalapensis* from *Heterandria bimaculata.* These 3 species were described using morphological characters only.
Methods: Specimens of *gyrodactylid* were collected from *Heterandria bimaculata*, *Poeciliopsis gracilis*, *Poecilia mexicana* and *Xiphophorus hellerii* from Nautla, Antigua and Tecolutla river basins in Mexico. Morphology of haptoral structures was studied. Sequences of ITS were obtained and compared with those of gyrodactylids infecting poeciliids, available in GenBank. Nucleotidic differences were obtained and phylogenetic analysis under Bayesian inference was performed.
Results: two new *Gyrodactylus* species were identified by the morphology of the marginal hooks and hamuli, one from *Poecilia Mexicana,* and one from *Poeciliopsis gracilis*. Sequence difference of these two new species with the rest of Gyrodactylus species, ranged from 13.3% to 31.1% and from 25.4% to 30.1% respectively; between these two new species genetic variation was 31%. Bayesian analyses showed these are 2 new species.
Conclusions: Monogeneans are the least studied helminth group in Mexico, and only 3 species of *Gyrodactylus* have been recorded in poeciliid fish in the country. Morphological, molecular and phylogenetic studies demonstrated the presence of 2 new *Gyrodactylus* from poecillids from Mexico.

**Parasitic diseases in Venezuela: changes and challenges**

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BACKGROUND: In Venezuela, the three most important parasitic diseases: malaria, schistosomiasis and Chagas disease (ChD), have undergone major changes associated with rapid demographic displacements that altered the ecology and the distribution of vulnerable groups.

METHODS**:** Epidemiological studies conducted by the Venezuelan Ministry of Health, together with other researchers have provided prevalences of these diseases. Demographic data were provided by the National Statistics Institute.

RESULTS: Since the beginning of the control program of parasitic infections in 1936, these diseases were considered as the most important public health problems, affecting rural communities. Major investments in sanitary infrastructures decreased the prevalence of these endemic infections. However, internal migration from rural to urban areas (85% in 1936 vs 7% in 2011) associated with social and economic factors, led to significant ecological changes that allowed: the clearance of mollusks in large cities by increasing pollution of rivers previously infested with schistosomiasis snail vectors; the increase of anopheline breeding places in areas of gold mining; and the urban domiciliation of triatomines associated with ChD.

CONCLUSIONS**:** The increase of population density in cities with high poverty altered the ecology of the surrounding areas that favored triatomine domiciliation increasing the urban transmission of ChD and subsequent contamination of water bodies, eliminated planorbid snails, displacing the transmission to the periphery of those cities. The migration of people for economic reasons associated with increased anopheline breeding sites, has raised malaria transmission. These new epidemiological scenarios are some of the main challenges that the Venezuelan Health Ministry must face.

**An update of the oral transmission outbreaks of Chagas disease in Venezuela.**

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BACKGROUND: The first oral transmitted outbreak of Chagas disease (OTCHD) in the American Amazon region was published in 1967. From that time on, several micro-epidemics have been reported including the largest OTCHD affecting 103 persons in an urban school in Caracas in 2007. Here, we describe the general characteristics of OTCHD outbreaks in Venezuela.
METHODS: Epidemiological and entomological surveys, search of *Trypanosoma cruzi* in fresh and stained blood smears, culture and animal inoculation, as well as IgM and IgG determination by ELISA, Indirect Haemaglutination test, lytic antibodies and polymerase chain reaction (PCR) were used.
RESULTS: Since 2007, nine outbreaks have been detected from different parts of the country, being five from the north-central region and four of these in the capital area. Those OTCHD affected 245 persons (75% children) with 10 deaths (4% mortality). Homemade juices (guava, passion fruit, mango and pomarosa) have been incriminated as source of infection. *Panstrongylus geniculatus* is the vector found in the environment around houses. The two largest outbreaks were related with the school food, the others were familiar episodes. Follow-up after treatment of five outbreaks resulted in 70% persistence of litic antibodies and some positive PCR.
CONCLUSIONS: After the outbreak in Caracas where Chagas disease was not an endemic entity, at least one episode of OTCHD has been annually reported. Children are the most affected and acute illness finished in 4% mortality. Presence of infected *P. geniculatus* in urban areas facilitates contamination of beverages with nefarious consequences for the population.

**Dung removal dynamic of dung beetles (Coleoptera: Scarabaeinae) in response to macrocyclic lactone use on cattle ranches in the Mexican neotropics**

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BACKGROUND: The expanded use of macrocyclic lactones (ML) to treat endo- and ectoparasites in cattle in tropical regions can reduce dung beetle populations, thus interrupting the dung removal process in cattle pasture ecosystems.
METHODS: During the reproductive period (the rainy season) of two functional groups of dung beetles (paracoprid and telecoprid), we compared dung removal rates at ranches where ML are and are not used in the Yucatan, Mexico. Exclusion traps using 500 g of ML-free cattle dung were used. On each ranch two transects with six traps were set up (three to measure dung removal by dung beetles and the other three to measure dung weight loss from dehydration). The amount of dung removed by the two functional groups was measured after 24 h.
RESULTS: Dung removal rates were similar on ranches with and without ML use. Dung beetles removed 40.1 percent of all cow dung weighed. Paracoprids removed 87.46 percent and telecoprids 12.54 percent of all the dung that was removed.
CONCLUSIONS: Our results indicate that the ecological functions of dung beetles in the pastures studied do not appear to be affected by ML use, and that paracoprid species remove the most dung. Local migrations of dung beetles between the two types of ranches could be reducing the negative effect of ML on their populations. Ranches where cattle are raised without using ML, in a landscape where there are ranches that do use ML to control parasites in cattle, could serve as a source of healthy specimens that colonize areas where dung beetles are probably declining.
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**An immunomodulatory protein RH36 is relating to hard tick blood-feeding success and oviposition**

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BACKGROUND: An immunomodulatory protein RH36 was identified in the tick *Rhipicephalus haemaphysaloides*. The cDNA sequence of RH36 is 844 bp and it encodes a deduced 218 amino acid protein with a size of 24 kDa. Bioinformatic analysis shows that RH36 is high homology to the immunomodulatory protein p36 of tick *Dermacentor andersoni.*

RESULTS: The recombinant protein expressed in insect cell sf9 suppressed T-lymphocyte mitogen-driven in vitro proliferation of splenocytes as well as the expression levels of several cytokines such as IL-2, IL-12 and TNF-α. Furthermore, the proliferative response of splenocytes isolated from rRH36-inoculated mice was significantly lower than those from control mice, suggesting that rRH36 could also directly suppress immune responses in vivo. Interestingly, microarray analysis of the splenocytes showed that the expression of several immunomodulating genes was down regulated by rRH36 inoculation. Disruption of the RH36 gene with RNAi led to a 42.5% decrease in the tick attachment rate 24 hours after introduction in the rabbit ears and a 47.5% decrease in the engorgement rate of ticks. Unexpectedly, the RNAi also led to significant effects on tick oviposition. These effects of RH36 on blood feeding and ovipisition were further confirmed by vaccine tests using the recombinant protein.

CONCLUSIONS: These results indicate that RH36 is a novel member of immunosuppressant protein involved in tick blood feeding and ovipisition.

**Potential impact of climate variability on cutaneous leishmaniasis in endemic areas of Caldas, Colombia, 2006-2012**

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BACKGROUND: Leishmaniasis is still a public health problem in tropical countries, including Colombia. Among recently studied related factors on its epidemiology, climate change and variability are highly relevant.
METHODS: An ecological study assessing potential impacts of climate variability (ONI, rainfall, maximum, minimum and median temperatures and humidity, as well satellite images from imagery database NASA Earth Observations), on disease incidence rates was done at seven endemic municipalities of one department, Caldas, 2006-2012. Simple and multiple linear regression models were developed to establish potential associations between the climatic and the epidemiological variables analyzed at monthly level (significance $p<0.05$).
RESULTS: During this period, 1603 cases were reported (mean 4.1 cases/month, min 0-max 43), ranging from 0 to 362.32 cases/100,000pop. At regional simple linear regressions, there were associations between ONI ($r^2=0.026$; $p<0.001$) and rainfall ($r^2=0.049$; $p<0.001$) with incidence rates. At regional multiple linear regression maximum temperature was associated ($\beta=-9.485$; $p=0.040$). At municipal analyses, ONI was associated at 4 of 7 of them ($r^2\geq0.047$; $p<0.05$); rainfall at one ($r^2=0.047$; $p<0.05$); maximum and minimum temperature as well humidity was associated at one too ($r^2\geq0.362$; $p<0.001$).
CONCLUSIONS: Previous studies established influences of climate variability on cutaneous leishmaniasis in certain departments of Colombia, but there are not studies in western endemic areas such as Caldas. For this department, climate variability explained mildly at the model, its influence on the incidence rates across the period at department and municipal levels. With more available data from disease surveillance incorporating more microclimatic variables improved predictive models would be developed.

**Hookworm Infections in Cambodia and Lao PDR: Status and Issues**

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BACKGROUND: Geohelminths, in particular, hookworms are widely prevalent in developing countries, and status evaluation and control measures are needed. We performed a survey on geohelminths including hookworms among the people in Cambodia and Lao PDR from 2006 to 2011.
METHODS: The Kato-Katz fecal examination technique was applied to a total of 32201 (Cambodia) and 6178 (Lao PDR) schoolchildren and residents from 19 provinces in Cambodia and 8 provinces in Lao PDR. In some individuals adult worm collection was performed to identify the species of hookworms, by treatment with pyrantel pamoate and purging with magnesium salts.
RESULTS: In Cambodia, the overall prevalence of intestinal helminths was 26.2%, and hookworms were the most highly prevalent species with an average prevalence of 9.6% (ranged 1.0%-22.3% by province). Northwestern and southernmost areas appeared to be most highly prevalent with hookworm infections. In Lao PDR, the overall prevalence of helminths was 71.8%, and hookworms were the second most prevalent species with 27.8% prevalence (ranged 6.0%-47.7% by province). The most highly prevalent (55.6%) helminth in Lao PDR was *Opisthorchis viverrini*-minute intestinal flukes (*Haplorchis* spp. and lecithodendriid species). The majority of the hookworm specimens recovered from infected people was *Ancylostoma duodenale* and *Necator americanus*.
CONCLUSIONS: Hookworms were either the most or the second highly prevalent intestinal helminth species among people in Cambodia and Lao PDR. Because their reinfection velocity is very fast, only one-time mass deworming usually fails to control this infection. Repeated and sustained mass deworming programmes are needed to obtain a substantial reduction of the hookworm prevalence in these areas. Another issue is the difficulty of finding hookworm eggs in Kato-Katz smears which were kept longer than 6-12 hours after preparation.

**Molecular caracterization of *Trypanosoma cruzi* strains from sylvatic triatomines collected in domestic environment, in Espírito Santo State, Brazil**

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BACKGROUND: Chagas disease is a neglected illness caused by the *Trypanosoma cruzi* parasite, which widely affects American communities. This study attempted to identify *T. cruzi* genotypes circulating in Espírito Santo (ES) state, Brazil, which comprises a complex population.
METHODS: In ES, *T. cruzi* isolates samples were obtained from sylvatic triatomines, from June 2010 to May 2012 and DNA was extracted from 89 samples. The intergenic amplifications region of the calmodulin and TcSC5D gene were performed. The samples were sequenced and analyzed by Mega 5.05 software.
RESULTS: Seventy eight samples were amplified by the intergenic region of the calmodulin gene. However, 66 samples were sequenced, indentifying TcII (19.7%), TcII-like (63.6%), TcIII (9.1%) and TcIV (7.6%) lineages. Sixty two samples were amplified and sequenced by the TcSC5D gene, identifying TcI (1.6%), TcII (82.2%), TcIII (8.1%) and TcIV (8.1%) lineages.
CONCLUSIONS: Different *T. cruzi* populations were found in ES, the presence of TcII was predominate and some samples showed the TcI, TcIII and TcIV patterns, showing that the understanding of *T. cruzi* dispersion are still so far to be clear. Until now, we do not know which wild animals can act as reservoirs, performing a difficult correlation between the lineages and the sylvatic transmission cycle. Complementation of this study should be taken to a better understanding of the existence of these genotypes and the enzootic cycle of this parasite in this region.

**Current status of *Rhipicephalus microplus* resistant to ivermectin**

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BACKGROUND: Ticks and the diseases they transmit cause great economic losses to livestock in tropical countries. Non-chemical control alternatives include the use of resistant cattle breeds, biological control, farm management, and anti-tick vaccines. Chemical acaricides have played an essential role, and are used traditionally to control infestations in livestock, but intensive acaricide use has enabled the emergence of tick populations resistant to them. Populations of the cattle tick, *Rhipicephalus microplus*, single and multiresistant to organophosphates (OP), synthetic pyrethroids (SP), amitraz and fipronil have been reported worldwide including Mexico. Macrocyclic lactones (MLs) have been used to mitigate the negative effects of ticks, including tick populations resistant to other acaricides. In Mexico, the pharmaceutical industry reported from 2007 to 2009 that MLs are the most used antiparasitic drugs in ruminants (45-50% of the total market). In the MLs family, ivermectin is the most commonly used followed by moxidectin and doramectin. Ivermectin-resistant populations of *R. microplus* have been reported in Brazil, Uruguay and especially in Mexico (Veracruz and Yucatan). Although ivermectin resistance levels in *R. microplus* from Mexico were generally low in most cases, some field populations of *R. microplus* exhibited high levels of ivermectin resistance. Some tick populations exhibited a resistance ratio >10 at the lethal concentration of 50% and 99%.
CONCLUSIONS: Worldwide, many field populations of *R. microplus* are resistant to multiple classes of antiparasitic drugs, including OPs, SPs, fipronil, amitraz and ivermectin. Ivermectin-resistant populations of *R. microplus* have been reported in Latin America, especially in Mexico. Although ivermectin resistance levels in *R. microplus* from Mexico were generally low in most cases, some field populations of *R. microplus* exhibited high levels of ivermectin resistance. Strategies involving the early detection of resistance and the use of integrated tick control are recommended.

**Evaluation of molecular tests for the detection and monitoring of the protozoa of public health importance in water samples from Quindío Department (Colombia)**

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BACKGROUND: Infections by waterborne protozoan are one of the most common causes of disease in humans worldwide, either in developed and developing countries. In Colombia since 2007, a new regulation from the Health Minister makes obligatory the monitoring of protozoa in water for human consumption, but current methods are long to perform and expensive. Thus new affordable methods for this monitoring are urgently needed.
METHODS: We evaluate the limit of detection of PCR by testing serial dilutions of DNA from cysts of *Giardia*, oocysts of *Cryptosporidium*, cysts of *Blastocystis* or oocysts of *Toxoplasma.* Specificity was tested by using DNA of different protozoa or human, in each specific PCR assay. Many primers, physical and chemical protocols for DNA extraction and DNA amplification protocols were also evaluated. Finally, 46 samples taken before, during and after water plant treatment, were assayed with standardized conditions.
RESULTS: The best method of DNA extraction for water protozoa was mechanical lysis. Limit of detection was of 10 parasite forms for all PCR (*Giardia, Cryptosporidium* and *Toxoplasma*) excepting for *Blastocystis*, which had a limit of detection of 100 cysts. Water turbidity did not affect the PCR sensitivity. All PCR were specific when testing for other protozoa or human DNA. When field analysis of water samples were carried out with these protocols, the PCR assay was positive in: 12% for *Toxoplasma*, 4.2% for *Cryptosporidium*; 2% for *Giardia* and 0.5% for *Blastocystis*. The frequency decreases after plant treatment (excepting for *Toxoplasma*) but still detectable in some samples taken at home.
CONCLUSIONS: The PCR assay was sensitive, specific and of low cost. It detected parasite DNA in many points of the water collection, treatment and distribution system distribution. The PCR assays are useful for the monitoring of protozoa in drinkable water.

## *Toxoplasma gondii* infection in humans and animals in China

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Toxoplasmosis is a zoonotic infection of humans and animals, caused by the opportunistic protozoan *Toxoplasma gondii*, a parasite belonging to the phylum Apicomplexa. Infection in pregnant women may lead to abortion, stillbirth or other serious consequences in newborns. Infection in immunocompromised patients can be fatal if not treated. On average, one third of people are chronically infected worldwide. Although very limited information from China has been published in the English journals, *T. gondii* infection is actually a significant human health problem in China. In this presentation, we review the clinical features, transmission, prevalence of *T. gondii* infection in humans, as well as *T. gondii* prevalence in animals in China. Educating the public about the risks associated with unhealthy food and life style habits, tracking serological examinations to special populations, and measures to strengthen food and occupational safety are discussed.

# First report of *Cryptosporidium* spp. in white yaks in China

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BACKGROUND: *Cryptosporidium* is an enteric apicomplexan parasite, which can infect yaks, leading to reduce of milk production and poor weight gain. White yak (*Bos grunniens*) is a unique yak breed inhabiting only in Tianzhu Tibetan Autonomous County, Gansu province, northwestern China. The objective of the present study was to molecularly determine *Cryptosporidium* infection and species in white yaks.
METHODS: Seventy-six fecal samples from white yaks in Tianzhu Tibetan Autonomous County, Gansu province were collected. The small subunit ribosomal RNA (SSU rRNA) gene of each sample was amplified using nested PCR and sequenced. The *Cryptosporidium* species was determined by comparison of the obtained sequences with that of corresponding *Cryptosporidium* sequences available in GenBank by BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) and phylogenetic analysis with maximum likelihood (ML) using PAUP*.
RESULTS: The overall prevalence of *Cryptosporidium* infection in white yak was 5.26% (4/76). Species identification showed *C. andersoni* in one sample (collected in September), and *C. bovis* in three samples (one collected in November and two collected in September).
CONCLUSIONS: The present investigation revealed the existence of *Cryptosporidium* infection in white yaks in China, for the first time, and two *Cryptosporidium* species, namely *C. andesoni* and *C. bovis*, were identified. These findings extend the host range for *Cryptosporidium* spp., and also provide base-line information for further studies of molecular epidemiology and control of *Cryptosporidium* infection in these animals.

# The complete mitochondrial genome of *Gnathostoma spinigerum* bears a novel gene arrangement and supports the paraphyly of Spirurina

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BACKGROUND: Human gnathostomiasis is a neglected parasitic disease caused by *Gnathostoma* species (Nematoda: Gnathostomatidae). The *Gnathostoma spinigerum* infects a range of definitive hosts, including dogs, cats, tigers, leopards and humans. In spite of its significance as a pathogen, aspects of the molecular biology of this parasite remain poorly understood. Mitochondrial (mt) DNA is known to provide useful genetic markers for study in this area, but mt genome have been lacking for any members of the infraorder Gnathostomatomorpha.

METHODS: The complete mt genome of *G. spinigerum* was amplified and sequenced. The relationships of the specimen with selected members of the nematodes were assessed by phylogenetic analysis of concatenated amino acid sequence datasets by Bayesian inference (BI), Maximum likelihood (ML),Maximum parsimony (MP).

RESULTS: The complete mt genome of *G. spinigerum* is a circular double-stranded DNA molecule, with a size of 14,081 bp. This mt genome shows significant changes in mt gene order compared to all other nematodes studied to date. All phylogenetic analyses (BI, ML and (MP) based on the deduced amino acid sequences of all 12 protein-coding genes support the paraphyly of suborder Spirurina.

CONCLUSIONS: The novel mt genome should represent a new source of useful genetic markers for studying the population genetics and systematics of this parasite of humans and other animals.

# Characterization of the complete mitochondrial genome of *Setaria digitata* from water buffalo in China

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BACKGROUND: *Setaria digitata* is an important filarial nematode living in the peritoneal cavity of cattle and buffaloes. Despite its veterinary importance, the epidemiology, molecular ecology and population genetics of this parasite still remain unexplored.
METHODS: The complete mitochondrial (mt) genome of *S. digitata* was amplified in four overlapping long fragments using primers designed based on partial cox1, rrnS, cox2 and nad2 sequences. Phylogenetic re-construction of 13 spirurid species (including *S. digitata*) was carried out using Bayesian inference (BI) based on concatenated amino acid sequence datasets
RESULTS: The length of the complete mt genome of *S. digitata* is 13,814 bp, and this genome contains 36 genes (12 protein-coding genes, 22 transfer RNAs and 2 ribosomal RNAs) that are typically found in metazoans. The identity of the mt genomes was 99.7% between *S. digitata* from China and Sri Lanka, and the complete mtDNA sequence of *S. digitata* from China is slightly shorter (25 bp) than that from Sri Lanka. The phylogenetic analyses based on the deduced amino acid sequences of all 12 protein-coding genes support the Setariidae was a sister taxon to a clade containing the members of the Onchocercidae, including *B. malayi* and *D. immitis* (posterior probability = 1.00).
CONCLUSIONS: the present study determined the complete mtDNA sequences of *S. digitata* from China provides new molecular data for future studies of the comparative mitochondrial genomics and systematic of parasitic nematodes of socio-economic importance.

**Morphological and molecular characterization of trematode metacercariae found infecting the commonly edible fish and crab species in Manipur, Northeast India**

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BACKGROUND: Fish-borne trematode infections (FBTs) and Crustacea-borne trematodoses affect a large section of population, particularly in Asian countries. In India, centring in several mountainous regions of Northeast India including Manipur State, the natives customarily consume raw or improperly cooked freshwater fish and crabs that still sustain viable infective stage (metacercaria) of trematodes in their muscle tissue. Earlier studies have revealed suspected foci of paragonimiasis in the region. We aimed to investigate the spectrum of metacercarial diversity sustained by these edible intermediate hosts in Manipur, basing the study on morphological and DNA-based molecular characterization of the parasite taxa.
METHODS: Commonly edible fishes and crabs collected from various localities of the region were surveyed for recovery of metacercariae. Morphological identification was done using the standard protocols for light and scanning electron microscopy. For molecular characterization, genomic DNA was isolated from each metacercaria type; the nuclear ribosomal DNA larger subunit (LSR or 28S) and the inter-transcribed spacer 2 (ITS2) marker regions were PCR-amplified using appropriate primers. The sequences obtained were analysed for sequence similarity and phylogenetic study using bioinformatic tools viz. Bioedit software version 7.0.9.0 and MEGA5.
RESULTS: Fishes representing families Channidae and Heteropneustidae were found to harbour 5 types of metacercariae belonging to families Clinostomidae (2), Diplostomidae (2) and Allocreadiidae (1); among crabs, only *Barythelphusa lugubris masoniana* and *Potamiscus manipuriensis* were found infected with metacercariae representing *Paragonimus westermani, P. heterotremus* (Paragonimidae) and two *Microphallus* spp (Microphallidae). The sequence and phylogenetic analyses of rDNA28S and ITS2 supplemented and supported the morphology-based identification.
CONCLUSIONS: Among the metacercariae encountered in our study the two *Paragonimus* species have plausible zoonotic implications.

**Wildlife animals as a reservoir of nematodes from the genus *Trichinella* in the sylvatic cycle in Poland**

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BACKGROUND: Trichinellosis is a zoonosis caused by parasitic nematodes of the genus Trichinella. These parasites are maintained by a cycle involving wild omnivores and carnivores animals from reptiles to birds and mammals making them one of the world's most widely distributed group of nematodes. Human infections are documented yearly due to the consumption of raw pork from free-ranging and backyard pigs or wild boars.The aim of the study was to determine the complexity and current research of these parasites in wild carnivores and omnivores in Poland.
METHODS: The study was performed on 1820 wildlife collected in 2011-2014 in Poland. Muscle samples (diaphragm pillars, tongue and the lower part of the front legs) were examined individually by artificial pepsin-HCl digestion method. Genomic DNA was extracted from single larvae and identified at species level by multiplex polymerase chain reaction (multiplex PCR).
RESULTS: The overall prevalence in examined samples was found to be 7.6%. *T. britovi* was identified as a dominant species. The highest prevalence was observed in red foxes with 11.8% (94/798) and wild boars with 1.8% (14/793). The intensity of infection varied from 0.04 to 117 LPG.
CONCLUSION: Our results reveal that *Trichinella* spp. can invade the broad spectrum of sylvatic animals in Poland. The infection can be transmitted to wild boars and consequently can easily reach humans. This is especially essential in the term of very dynamic changes in populations of such animals as: foxes, raccoon dogs or the American mink. This research was partially supported by the NCRD Grant No. 12 0126 10 and project LIFE+ by Gleboki Brod Forest District (No. NEU-0744/LIFE-1/13/1).

**Is there any transmission of *Ashworthius sidemi* from wildlife animals to domestic cows?**

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BACKROUND: *Ashworthius sidemi*, a blood-sucking gastrointestinal nematode, is a primary parasite of Asiatic cervides, particularly sika deer (*Cervus nippon*). The introduction of this host species in European countries enabled the parasite to spread. The study done in Poland revealed that *A. sidemi* infections are common in bison, red, roe and fallow deer. Seeing the possibility that infections may be transmitted between wildlife and livestock, particularly sheep and cows, which may be grazed on the same pastures, the aim of the study was to confirm this hypothesis.Until now, the presence of *A. sidemi* in wildlife was confirmed during *post mortem* microscopic examination only. To facilitate an easier and more reliable diagnostic tool, a simple polymerase chain reaction (PCR) was developed to differentiate *A. sidemi*.
METHODS: The study was performed on fecal samples collected from European bison and cattle. After incubation, DNA of L3 larvae was identified to the *A. sidemi* level.
RESULTS: The amplified genomic DNA analyses revealed the presence of a segment of approximately 406 bp in both bison and cattle faecal samples.
Additionally these segments were sequenced and deposited in GenBank (KF414629.1, KF414630.1). The sequences showed a very high similarity to sequences already published in GenBank (EF467325).
CONCLUSIONS: This is the first evidence on *A. sidemi* in cattle. The results of the present study reveal that a simple PCR test for *A. sidemi* identification based on L3 DNA may be an effective way to diagnose invasion *in vivo*, without the need to perform post-mortem examinations. This research was supported by National Science Centre. Grant N N308 585740

**Canine granulocytic anaplasmosis – an update**

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BACKGROUND: *Anaplasma phagocytophilum*, the causative agent of canine granulocytic anaplasmosis (CGA), is implicated as emerging pathogen of dogs, cats, ruminants, horses and people worldwide.
METHODS: This review on CGA is based on literature and unpublished data.
RESULTS: Transmission in dogs occurs via *Ixodes* ticks and rarely via blood transfusions. Co-infections may occur with several tick-borne pathogens. Epidemiological studies evaluating the seroprevalence of *A. phagocytophilum* in dogs have been performed worldwide (e.g. in Germany 47%). Subclinical disease and possible silent elimination might be common. The most common clinical signs are acute onset of lethargy, inappetance, fever, and splenomegaly. Further signs are a tense abdomen, joint pain, bleeding, diarrhea, vomiting, polydipsia, lymphadenopathy, respiratory signs, limb edema, scleral injection/uveitis, and rarely CNS signs. The most consistent laboratory abnormality is thrombocytopenia, followed by anemia, lymphopenia, monocytosis, neutrophilia, and leukocytosis. Abnormal biochemistry findings include hypoalbuminemia, hyperglobulinemia, increased liver enzymes / bilirubin, and rarely azotemia. The diagnostic criteria for CGA are clinical signs / laboratory abnormalities together with 1) detection of morulae within neutrophils combined with a positive PCR test; 2) a four-fold increase or decrease in the antibody titer within 4 weeks**;** 3) a positive PCR test (in peripheral blood, buffy coat, bone marrow, CSF, synovial fluid or splenic tissue). Treatment of choice is doxycycline (5 mg/kg orally twice daily, for 2-3 weeks), most dogs show clinical improvement within 24 – 48 hours. (In-vivo effects have been described for rifampicin and chloramphenicol.) If immune-mediated disease (e.g. immune-mediated thrombocytopenia) is suspected, prednisolone can be carefully administered. The extent to which the pathogen induces chronic infection is unknown.
CONCLUSIONS: Prevention of tick-borne diseases such as CGA should be accomplished by maintaining strict tick control programs in endemic areas. A thorough daily inspection for and removal of ticks is recommended, in combination with regular application of residual acaricidal products.

**Canine granulocytic anaplasmosis – a clinical study (2006 – 2012)**

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BACKGROUND: Canine granulocytic anaplasmosis (CGA) is a tick-borne infectious disease of increasing importance in areas with high *Ixodes* populations. In North East Germany the seroprevalence for infection of dogs with *Anaplasma phagocytophilum* was 43%. Transmission in dogs occurs via *Ixodes* ticks and rarely via blood transfusions.
Aim of this study was the retrospective evaluation of medical records of dogs with CGA in regard to clinical and laboratory findings, therapy and course of disease.
METHODS: 974 dogs with clinical signs suspicious for anaplasmosis were tested with real-time PCR for *A. phagocytophilum* (2006 - 2012).
RESULTS: PCR testing was positive in 72 dogs; 9 dogs were excluded due to other diseases or incomplete medical records. The age of the 63 dogs ranged from 1 to 15 years; 33 dogs were male, 30 female; they belonged to 20 different breeds or were mixed-breed. 81% of the dogs were presented between May and August. Most common signs were acute onset of lethargy, fever, inappetence, a tense abdomen, and splenomegaly. Rarer findings were joint pain, surface bleeding, polydipsia, lymphadenopathy, polyuria, gastrointestinal, respiratory and/or neurological signs, and uveitis. Abnormal laboratory results included thrombocytopenia, anemia, lymphopenia, monocytosis, ALP elevation, hypoalbuminemia & hyperglobulinemia. In several dogs a platelet-bound antibody or Coombs` test was positive. Nearly all dogs received doxycycline over a time period of 2-3 weeks. In 3 dogs the treatment with doxycycline was discontinued due to side effects. 59 dogs recovered, 2 were euthanized; 2 were lost for follow-up.
CONCLUSIONS: Anaplasmosis was most often diagnosed in early summer and summer. The majority of dogs were presented with acute nonspecific clinical signs. However, in dogs with polyarthritis, hemorrhage, gastrointestinal, respiratory, neurological, ophthalmological signs and immune-mediated diseases, CGA should be on the list of differential diagnoses or potential triggering factors. The prognosis was good, most of the dogs recovered.

**Testing of dogs with meningitis and meningoencephalitis of unknown etiology for vector-transmitted microorganisms**

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BACKGROUND: In most cases of inflammatory central nervous system (CNS) disease in dogs, etiological infectious agents cannot be detected. Immunopathological studies suggest that an antigen may trigger an autoimmune response (“hit-and-run-hypothesis”) in some patients. In order to define the role of vector-borne pathogens in the etiology of meningoencephalitis of unknown etiology (MUE) and steroid-responsive meningitis-arteritis (SRMA), blood and cerebrospinal fluid (CSF) were analysed for such pathogens.
METHODS: 66 client-owned dogs were included in the prospective multicenter study over a two-year- period: 1) trauma group: dogs with non-inflammatory CNS disease (n=21), 2) dogs with MUE (n=22), 3) dogs with SRMA (n=23).
PCR was performed in blood and/or CSF for *A. phagocytophilum*, *E. canis*, *Bartonella* spp. and *Borrelia* spp. Serum antibodies against *E. canis*, *Bartonella* spp., Tick-borne encephalitis virus (TBEV) and *Borrelia burgdorferi* s. l. were determined. A qualitative eubacterial PCR was performed (CSF).
RESULTS: PCR testing in blood was positive for *A. phagocytophilum* in 4 and for *Bartonella* spp. in 1 dog (SRMA group). Serological / PCR analyses for *E. canis* were negative in blood of all dogs. There were no significant differences between the 3 groups regarding seroprevalence of *Bartonella* spp. and *B. burgdorferi* s. l.. Neither antibodies against TBEV in serum nor DNA of vector-transmitted pathogens in CSF were detected. *Pasteurellaceae* spp. DNA was detected in 3 dogs (trauma), suggesting contamination.
CONCLUSIONS: *E. canis* and TBEV seem to be rare causes of inflammatory CNS disease, in the current study neither DNA of *E. canis* nor antibodies against both pathogens were detected in dogs with MUE / SRMA. *Borrelia burgdorferi* s. l. is an unlikely cause of inflammatory CNS disease in dogs. No correlation was detected between infection with *Bartonella* spp. and MUE / SRMA. *A. phagocytophilum* and *Bartonella* spp. may play a role as trigger of a secondary immunopathy.

**Study of the intergenic region of the *Trichomonas vaginalis pfo a* gene in search of the iron responsive promoter elements.**

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BACKGROUND: The promoter region controls gene transcription. The *Trichomonas vaginalis pfo a* gene is positively regulated by iron at the transcriptional level and encodes for a hydrogenosomal pyruvate: ferredoxin oxidorreductase A (PFO A) enzyme. An *in silico* analysis of the *pfo a* 1132 bp Intergenic Region (IR) reported in the *T. vaginalis* genome sequence showed the presence of a sequence similar to the iron responsive core promoter described and characterized in the *ap65-1* gene. We hypothesized that these elements could work as an alternative iron responsive promoter that regulates the transcriptional activity of *pfo a* in *T. vaginalis*. To test this hypothesis, we cloned, sequenced, and analyzed the *pfo a* IR to experimentally identify the regulatory motifs present in this sequence.
METHODS: We designed specific primers to amplify different fragments of the *pfo a* IR by PCR. We cloned the ~750 and ~348 bp fragments into the pGEM-T Easy vector. Candidate clones were sequenced and an *in silico* analysis was used to identify regulatory elements or motifs.
RESULTS: In this study, we started to define the putative regulatory regions that could contain the elements necessary for iron response transcriptional activity. We found different regulatory elements known as Myb Recognition Elements (MREs). These elements have some differences to those previously described in *ap65-1* and were named MRE-like elements. We also found a sequence similar to the iron responsive core promoter and defined the positions of each motif based on the *pfo a* transcription start site previously defined by 5'-RACE experiments.
CONCLUSIONS: Our results suggest that the ~350 bp fragment of the *pfo a* IR could be the minimum promoter region necessary for the *pfo a* transcriptional regulation by iron. Work is in progress to demonstrate it.

**_Leishmania_ RNA Virus 1 modifies _Leishmania Viannia guyanensis_ biology**

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BACKGROUND: Some *Leishmania* species of the subgenus *Viannia,* found exclusively in the New World, are persistently infected with LRV1, a double-stranded RNA virus that belongs to the *Totiviridae* family. Studies in the 90's described LRV1 biology and prevalence, however Ives et. al (2011) have recently discovered a relationship between the presence of LRV1 and the development of mucocutaneous leishmaniasis. More specifically, they showed that virus genomic material is recognized by host TLR3 receptors during infection, triggering hyper-inflammatory reactions that can be responsible for severe disease outcomes. Thus far, the effect of LRV1 on *Leishmania* cells *per se* and how its genetic material is exposed by *Leishmania* to host cells is unknown.

METHODS AND RESULTS: By studying different clones of *L. guyanensis* M5313 strain containing variable amounts of LRV1, we established a direct relation between high viral loads and unhealthy parasite hallmarks, namely morphological alterations and low capacity to grow *in vitro*. Moreover, infected parasites showed substantial biological changes, such as compromised translation and altered total and exosomal protein profiles determined by mass spectrometry (MS). For instance, infected *Leishmania* showed a dramatic down regulation of GP63, but not GP63 mRNA levels. Unexpectedly, MS detected significant amounts of LRV1 major capside protein in exosome preparations derived from infected parasites, suggesting that viral particles were secreted along with these vesicles. When examining these preparations by TEM, we observed exosome-like vesicles frequently surrounding LRV1 particles, similarly to viral envelopes. This finding was further confirmed by different biochemical and molecular approaches.

CONCLUSIONS: Our results suggest that LRV1-infected *Leishmania* are not simply virus carriers, but parasites that are feasibly modified to be more pro-inflammatory to the mammalian host. In addition, we demonstrated for the first time that *Leishmania* actively secretes LRV1 along and within exosomes, opening discussions for a new mechanism used by the parasite to present viral material to the host.

**Comparative genetic diversity of *Trypanosoma cruzi* lineages in the Chaco region determined by multilocus microsatellite typing**

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BACKGROUND: The Gran Chaco region is considered hyperendemic for Chagas disease and a sustained reduction in prevalence has been difficult to achieve. The aetiological agent, *Trypanosoma cruzi* is characterised by high levels of genetic heterogeneity, currently being grouped into six phylogenetically lineages (TcI to TcVI). In this study we have used a multilocus microsatellite typing approach analysis to understand better the transmission dynamics of the parasite populations that circulate in this area.
METHODS: Eighty three cloned *T. cruzi* isolates were genotyped by 15 variable microsatellite loci, placed in six different chromosomes. Isolates were derived from different hosts and vectors across both domestic and sylvatic transmission cycles from localities within Argentina, Paraguay and Bolivia. Reference strains from Brazil, Colombia and Chile were included for comparison.
RESULTS: Sixty different multilocus genotypes were identified, TcIII being the most diverse. Within the domestic cycle two groups of TcII isolates were found circulating in the Paraguayan Chaco, both with multi-allelic profiles more related to Brazilian than Chilean strains. The locus (vicp2b) allowed differentiation of TcVI into two subpopulations, distributed among samples from Paraguay and Argentina. One predominant TcV multilocus genotype was found in isolates from Paraguay, Argentina and Bolivia. Sylvatic isolates were all TcIII but with diverse genotypic profiles, except for two indistinguishable samples from armadillos in Paraguay. A Neighbor-joining tree based on Distance Allele Shared ($D_{AS}$) values, revealed that TcIII from Paraguay grouped closely with Bolivian TcIII and separately from Colombian and Brazilian strains.
CONCLUSIONS: Microsatellite profiles from the domestic cycle indicate rapid clonal propagation of TcV and TcVI in the Chaco region, with the presence of two TcVI and two TcII subpopulations. The much greater genetic diversity among sylvatic TcIII suggests different mechanisms for maintaining such diversity compared to TcII/V/VI in the domestic cycle.
KEY WORDS: *Trypanosoma cruzi* - Chaco region - microsatellites
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**Impact of Chagas disease in Latin America***

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BACKGROUND: Chagas disease, caused by *Trypanosoma cruzi*, is an important public health problem in 21 Latin American countries and an emerging disease in non-endemic countries, due to increasing international migration. We present an overview of the burden, epidemiology and impact of Chagas disease on public health in Latin America.
METHODS: A literature review was conducted to obtain epidemiological studies on morbidity, mortality, and burden related to Chagas disease in Latin America. We searched in electronic databases (PubMed, SciELO, Scopus and LILACS) for reports published since 2000. Additional searches were performed in reference lists of selected reports, grey literature and technical reports of regional and national control programs, well as databases from the Pan American Health Organization (PAHO) and World Health Organization (WHO).
RESULTS: In Latin America, about 8-10 million people are chronically infected with *T. cruzi,* and 25-90 million people are at risk for infection. Recent studies estimated about 4.6 million people being infected in Brazil, and 5.5 million in Mexico. There are about 41,200 new cases by vector-borne transmission, and 14,400 newborns with *T. cruzi* infection annually. Congenital transmission ranges from 0% to 17% of children born to infected mothers in endemic countries. The number of Chagas disease-related deaths is estimated at 10.000-14,000. There are an estimated 546,000 to 806,000 disability-adjusted life years [DALYs]. The total economic toll attributed to the disease is estimated at over $7 billion USD/year.
CONCLUSIONS: Remarkable progress has been made in the prevention and control of Chagas disease in most Latin American countries, especially of the main routes of transmission (vector-borne and blood transfusion). However, the disease still represents a major public challenge in Latin America, with high socioeconomic and human health impact.
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**Limiting the use of anthelmintics through grazing management: a possible dream or a nightmare?**

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Gastrointestinal nematodes (GIN) parasitize grazing ruminants worldwide. Results in the last 30 years suggest that grazing management can reduce the need for anthelmintic treatment in small ruminants. Grazing management targets at least three objectives: a) dilute the risk of GIN infection, b) prevent auto-infection during a grazing event, and c) wait until a reasonable proportion of infective larvae naturally die. Under temperate conditions, dilution has been achieved through mixed grazing (two or more host-specific grazer species) and by having fewer animals per ha. Preventing auto-infection and allowing a decline in the infective larvae population have been achieved through controlling the length of the graze and rest periods. Most benefit in temperate climates has been obtained against *H. contortus*, with less benefit against *Trichostrongylus* spp and *Teladorsagia* spp. Although technically feasible, implementation of these strategies may be constrained by socio-economic limitations of farmers. Under tropical conditions, the use of mixed species grazing to dilute GIN infectivity has provided promising results and the rotational grazing systems seem technically feasible especially in the hot, humid tropical areas, where GIN infective larvae may die-off in weeks. However, insufficient studies support the viability of these practices under different humid and subhumid tropical conditions. There is a lack of information on the carrying capacity, nutritional value and infectivity of the vegetation in many tropical areas used for browsing. Grazing management should optimize nutrition of animals and growth and survival of the vegetation, rather than parasite control *per se*.

**Targeted selective treatment against gastrointestinal nematodes in sheep flocks from the humid tropics of Tabasco, Mexico**

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BACKGROUND: Gastrointestinal nematodes (GIN) cause economic losses amongst sheep flocks in hot, humid tropical areas of Tabasco, Mexico. A targeted selective treatment (TST) scheme was developed for the hot, sub-humid areas of Mexico, where it reduces the proportion of animals requiring AH treatment per year. It is unknown if such TST system is applicable for hot humid tropical zones. This study determined the treatment frequency resulting from a TST scheme that combines FAMACHA®, body condition score (BCS) and fecal excretion of GIN eggs in sheep from Tabasco, Mexico.
METHODS: Four sheep flocks with multidrug resistant GIN were selected by convenience. Drug resistance was evident for benzimidazoles (BZ), levamisole (LEV) and macrocyclic lactones (ML) amongst GIN populations of *Haemonchus, Trichostrongylus* and *Oesophagostomum*. Flocks were visited fortnightly during the rainy season (June to December 2013). The BCS and FAMACHA® was determined individually on approximately 100 ewes per visit in each farm. Ewes with FAMACHA® 4 - 5 (anemic) or BCS 1 - 2 (emaciated) were fecal sampled to determine their egg counts per gram of feces (EPG). Ewes with 750 EPG or more were treated with a combination of injectable drugs (BZ and ML).
RESULTS: From a total of 981 ewes, 65.5 % did not require deworming, 17.8% were dewormed once, 8% were dewormed twice, and 8.4% received three or more AH treatments.
CONCLUSIONS: The TST scheme reduced the number of ewes treated in the surveyed flocks. A large proportion of ewes remained without AH treatment. Most ewes received one treatment per semester and very few ewes received more than one AH treatment.

**Trichocystatin-3 (TC-3) is an endogenous cysteine proteinase inhibitor localized on the surface of *Trichomonas vaginalis***

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BACKGROUND: *Trichomonas vaginalis* has three genes that encode endogenous cysteine proteinase inhibitors. These inhibitors belong to the cystatin family, a large group of low molecular size proteins that inhibit papain-like CPs by obstructing the enzyme substrate access. This work is focused on the characterization of one of these inhibitors, Trichocystatin-3 (TC-3).
METHODS: We cloned and expressed the *tvicp-3* gene to obtain the recombinant protein and produce polyclonal antibodies in mice against TC-3r. These antibodies were used for western blot (WB) assays on total parasite extracts transferred onto nitrocellulose membranes and for indirect immunofluorescence assays with permeabilized and non permeabilized parasites for immunolocalization of TC-3 in *T. vaginalis.* Additionally, we used the recombinant TC-3 protein to test its inhibitory activity over protease-resistant extracts using a fluorogenic substrate for cathepsin-L and zymograms.
RESULTS: In WB assays the anti-TC-3r antibody recognized two protein bands of 22 and 55 kDa that do not correspond to the expected size (12 kDa). This protein is localized in the cytoplasm and on the surface of trichomonads. Moreover, TC-3 inhibited the proteolytic activity of *T. vaginalis* cathepsin-L-like CPs up to 40% and showed inhibitory activity mainly in the 65 kDa region on zymograms.
CONCLUSIONS: TC-3 is one of the endogenous cystatin-like CP inhibitors of *T. vaginalis* that could modulate the proteolytic activity of the cathepsin L-like CPs on the parasite surface.
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**Identification of epigenetic marks in trophozoites and cysts of *Entamoeba invadens***

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BACKGROUND: The intestinal parasite *Entamoeba histolytica*, the causative agent of amoebiasis, infects about 50 million people annually. Humans are infected by consumption of water or food containing cysts, the infective stage of the parasite. Upon infection, the cysts become trophozoites, the invasive form of the parasite. Therefore, encystation and excystation are essential mechanisms in the biological cycle of the parasite.
Encystation is a complex process involving intracellular rearrangements, changes in gene expression, protein synthesis and the formation of a chitin wall. The molecular mechanisms associated with encystation are not completely understood. The *E. invadens* experimental model has shown that acetylation and deacetylation of histones are involved in encystment. However, it is unknown which histones are acetylated and whether the nuclear architecture changes during encystation and excystation.
METHODS: Histones from trophozoites and cysts were obtained and analyzed by LC-MS/MS. WB assays were performed using commercial antibodies against epigenetic markers. Immunofluorescence and transmission electron microscopy in trophozoites and cysts of *E. invadens* were used to analyze the nuclear architecture.
RESULTS: In this study we found that histones H4 of *E. invadens* are acetylated in trophozoites and cysts and that those epigenetic markers associated with transcriptional activity are distributed in the nucleus. Furthermore, repressive markers are located at the perinuclear region in eukaryotic cells, while in E. invades are found mainly in the central region of the nucleus.
CONCLUSIONS: Our results suggest that the histone H4 is acetylated in several Lysine residues and that the nuclear architecture of E*. invadens* is different to eukaryotic cells.

# *In vitro* anthelmintic effect of the foliage from three plant species of the Annonaceae family against *Haemonchus contortus*

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BACKGROUND: Recent trials showed evidence of the anthelmintic (AH) effect of different Annonaceae plant extracts against *Haemonchus contortus.* However, there is no information about the lethal concentration (LC50%) of theses plants against *H. contortus* eggs and $L_3$. The objective was to determine the *in vitro* LC50% of methanolic and acetonic extracts from leafs of *Annona squamosa, A. muricata* and *A. reticulata* on the *H. contortus* egg hatching and $L_3$ exsheathment.
METHODS: Leafs of Annonaceae species were processed to elaborate the metanolic (ME) and acetonic extracts (AE). Egg hatching assays (EHA) and exsheathment inhibition assays (EIA) were performed for each of the 6 extracts. The concentrations tested were 150, 300, 600, 1200, 2400, 3600 µg/ml PBS with their respective positive and negative controls. Two isolates of *H. contortus* ($L_3$) were used: a benzimidazol (BZ) susceptible and a BZ resistant. The LC50% was determined using their corresponding probit tests.
RESULTS: Both extracts caused a significant dose-dependent AH effect on egg hatching or larvae exsheathment. The LC50% was dependent of the extract and the *H. contortus* isolate. The LC50% of the EHA ranged from 274.28 - 1284.09 µg/ml for the ME, and from 956.07 - 1610.93 with the AE. The LC50% of the EIA ranged from 373.07 - 1466.09 for the ME, and from 136.20 - 833.62 with the AE.
CONCLUSIONS: Both ME and AE showed clear AH effects; although, different LC50% against *H. contortus* eggs and $L_3$ larvae, were recorded.

# EFFECTS OF VASOACTIVE INTESTINAL PEPTIDE IN HUMAN AND EXPERIMENTAL INFECTION WITH TRYPANOSOMA CRUZI

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BACKGROUND: Vasoactive intestinal peptide, VIP, has gained great prominence due to its therapeutic potential ascribed to its ability of regulating the innate immunity, inhibiting antigen-specific Th1 cell responses and generating T regulatory cells. Additionally, VIP may act as a natural antimicrobial peptide, killing bacteria, fungi and infective forms of *Trypanosoma brucei*. Despite of the possible relevance that VIP may have during the course of Chagas disease, studies in this scenario remains poorly characterized in human and experimental infection by *Trypanosoma cruzi*. In this study we have evaluated the effects of VIP administration on the cardiac inflammatory infiltrate of mice experimentally infected with *T. cruzi*. Also, we determined the expression of VIP, in blood from patients with the indeterminate and cardiac forms of Chagas disease, and its association with the development of cardiomyopathy.
METHODS: C57BL/6 mice were infected with 5000 trypomastigotes of the VL-10 strain of *T. cruzi* and treated with intraperitoneal injection of VIP (13ug) for one month. Inflammatory and morphometric parameters were evaluated in serum and in the heart, respectively. In humans, plasma levels of VIP were evaluated by ELISA and correlation of VIP expression and clinical parameters were performed.
RESULTS: Our results indicate that although treatment with VIP was unable to prevent the cardiac inflammation triggered by *T. cruzi* infection, the observed inflammation in heart from treated mice was localized and lymphocytic while in untreated mice, the inflammation was more severe. In patients, our results demonstrated that low plasma levels of VIP were associated with cardiac morbidity in Chagas disease. Correlation analysis showed that low plasma levels of VIP were associated with worse cardiac function.
CONCLUSIONS: Our results corroborate the influence of VIP over the outcome of Chagas disease, inducing a more specific and controlled immune response in the inflammatory site in experimental model and in human patients.

**Detrimental effects of geldanamycin on adults and larvae of *Trichinella spiralis***

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BACKGROUND*:* Trichinellosis is a zoonotic disease affecting mainly the temperate regions. It can be associated with severe neurological, ocular, and cardiovascular complications and may end fatally. The treatment is a challenge for the physician, and the available therapy is far from ideal.
AIM: To evaluate the effect of heat shock protein (HSP) 90 inhibitor, geldanamycin, on the adult worms and larvae of *Trichinella spiralis*.
METHODS: This research comprised an in vivo study in which *T. spiralis*-infected mice were treated by two different doses of geldanamycin, thereafter larval count and pathological changes were determined in the muscles. Meanwhile, the in vitro study investigated the effect of two different concentrations of geldanamycin on adult worms and larvae of *T. spiralis* via transmission electron microscopy.
RESULTS: The in vivo study showed significant reduction of muscle larval counts under the effect of geldanamycin. Moreover, characteristic changes were noted as regards the parasite and the inflammatory response. The in vitro study revealed degenerative changes in the body wall of larvae and adults of *T. spiralis* under the influence of geldanamycin.
CONCLUSIONS: Heat shock protein (HSP) 90 inhibitor, geldamycin, seems to have detrimental effects on the adults and larvae of *T. spiralis*. It, or one of its derivatives, could be an adjuvant to anthelmintic therapy of trichinellosis, but more studies are warranted to establish its usefulness.

**_In vitro_ predatory activity of _Butlerius_ sp. (nematoda: Diplogasteridae) against _Ancylostoma caninum_ (nematoda: Ancylostomatidae) infective larvae**

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BACKGROUND: *Ancylostoma caninum* is a hematophagous nematode affecting canine and sporadically human beings causing anaemia and death. Recently, natural-nematode antagonists are gaining interest in workers mainly because of anthelmintic resistance is threatening animal health worldwide. *Butlerius* sp. genus is a predator nematode that has shown a high activity against *Haemonchus contortus* and other nematodes. The present study was aimed to evaluate the *in vitro* predatory activity of *Butlerius* sp. against *Ancylostoma canimum* infective stage.
METHODS: The confrontation predator/prey were assessed in faecal cultures elaborated into crystal containers (FCCC) with10 g of sterile sheep faeces mixed with polystyrene particles and water. Three series of FCCC each were distributed as follows: Serie 1, (predatory control with 200 *Butlerius* sp.). Serie 2, (prey control with 2000 *A. caninum*). Serie 3, (Predator/prey interaction, 200 *Butlerius* sp. and 2000 *A. caninum*) (n=7). FCCC were maintained at 28ºC and 45% relative humidity for 25 days. Nematodes were recovered from FCCC using the Baermann technique for 12 h. Means of recovered nematodes were compared among groups and a reduction percentage rate was estimated. Data were root square transformed $\sqrt{x+0.5}$ and a Student *t* test, was used.
RESULTS: *Butlerius* sp.shown an important predator behavior against *A. caninum*. Predation was visualized and photographied. The prey nematode population in FCCC was reduced in 98.7% ($p \leq 0.05$). An increasing in the *Butlerius* sp. population 275.5%, was recorded.
CONCLUSIONS: The high predatory activity shown by *Butlerius* sp. will be considered in further assays as a potential bio-control agent of ancilostomiasis.

# *In vitro* feeding habits of *Caloglyphus mycophagus* (Acarina: Acaridae) on the sheep parasitic nematode *Haemonchus contortus* ($L_3$) and the free living nematode *Panagrellus redivivus*

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BACKGROUND: *Haemonchus contortus* one of the most pathogenic parasite in sheep. Traditional control of this and other parasites is based in the administration of chemical drugs in sheep flocks, that cause anthelmintic resistance in the parasites and they can remain as environmental contaminants in soil, after been eliminated as active molecules through the animal feces. Recently, natural-nematode antagonists; including mites, are gaining interest as potential tools of nematode control. This research was aimed to evaluate the *in vitro* feeding behavior of the mite *Caloglyphus mycophagus* against *H. contortus* infective larvae ($L_3$) and a mixed stage population of *Panagrellus redivivus*.
METHODS: The interaction mite/nematodes was performed into Petri Dishes (2 cm diameter x 1 cm height) (n=10). One thousand nematodes of each specie and 2 mites were put into each plate, and incubated at room temperature (28 ºC) for 5 days. Proper controls with the assessed nematodes, without mites were used. Nematodes were recovered using the Baermann technique. Recovered nematodes were quantified and means were compared with their control groups. Results were expressed as a nematode reduction percentage.
RESULTS: Feeding behavior of *C. mycophagus* on either *H. contortus* ($L_3$) and *P. redivivus* was visualized and photographed. The *H. contortus* larvae population was reduced to 81%; meanwhile, 100% reduction was recorded with *P. redivivus*.
CONCLUSIONS: The results obtained suggest that the mite *C. mycophagus* can be considered in further assays as a potential candidate of biological control of sheep haemonchosis.

**Molecular detection of parasites affecting farmed fish**

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BACKGROUND: Many of fish species are farmed in grow-out cages, which can increase a risk of parasitic infections. Amoebic gill disease (AGD) is a condition caused by *Neoparamoeba perurans* affecting some species of cultured marine fish worldwide. Amoebic gill disease (AGD) is the most serious health problem of farmed Atlantic salmon in Tasmania. The only commercially available treatment is freshwater bathing. Epizootics of a range of different taxa of metazoan parasites, including blood flukes *Cardicola forsteri* and *Cardicola orientalis* have been documented in recent years among southern bluefin tuna ranched in southern Spencer Gulf, South Australia. Here we review development of the molecular detection methods for those parasites.
METHODS: All primes utilized in this study were designed with Primer Premier 5, BeaconDesignerTM7.8 (Premier Biosoft, CA, USA) and Geneious®6 software (http://www.geneious.com). Real-time PCR assays were performed using a CFX Connect Real-Time PCR Detection System (Bio-Rad, NSW, Australia)
RESULTS: Molecular detection methods were specific, which resulted in detection of new species of parasites. These methods were more sensitive than traditional detection techniques and allowed for analyses of biopsies, archival samples and environmental samples.
CONCLUSIONS: Molecular methods provide a specific and sensitive detection of parasites affecting farmed fish. The specificity allows for detection of parasites which cannot be easily identified using morphology. The result can be quantitative which allows estimation of infection load. Free living life stages can be detected in environmental samples. Molecular methods improve detection of parasites affecting farmed fish by providing rapid and differential quantitative results.

**Perturbing copper homeostasis is instrumental in early developmental arrest of intraerythrocytic *Plasmodium falciparum***

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BACKGROUND: Malaria continues to be a devastating disease. The elucidation of factors inducing the developmental succession/arrest of *Plasmodium falciparum* can provide crucial information about the developmental mechanisms of the parasite and may assist in the search for novel malaria medication. Based on information from genome-wide transcriptome profiling of various stages of *P. falciparum*, we investigated the importance of copper homeostasis for their developmental succession.
METHODS: The effects of perturbing copper homeostasis in *P. falciparum* were tested with relation to three aspects of the function of copper: 1) inhibition of copper-binding proteins, 2) copper-ion chelation, and 3) down-regulation of the expression of genes encoding copper-binding proteins with a specific growth-promoting factor.
RESULTS: Inhibition of copper-binding proteins with tetrathiomolybdate (TTM) caused irreversible cessation of growth of the parasite. The TTM arrested the parasite during trophozoite–schizont stage progression. The involvement of copper ions in developmental arrest was also detected using copper-ion chelation, implying a critical function of the reduced copper ion ($Cu^{1+}$) in the parasite during the early developmental stage. Chelation of $Cu^{1+}$ caused blockage of trophozoite progression from the ring stage. Profound growth arrest was detected in the parasite when it was cultured in a specific chemically defined medium. This developmental arrest was associated with down-regulated expression of genes encoding copper-binding proteins.
CONCLUSIONS: Profound early developmental arrest of *P. falciparum* was detected when copper homeostasis was perturbed. This may be applied as an effective antimalarial strategy.

**Imported cases of leishmaniasis in Australia between 2010 to 2014**

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BACKGROUND: Leishmaniasis is an imported disease in humans in Australia with an increase in cases occurring due to a rise in travel to endemic countries. Cutaneous leishmaniasis is the most common form of infection with an estimated 1.5 million infections annually world-wide. Although an animal host has been identified in Northern Australia, no known human cases have occurred. Due to leishmaniasis not being a common Australian disease, it is not generally thought of in an initial diagnosis for skin lesions. Therefore the aim of this study was to report the epidemiology of imported leishmaniasis into Australia, the countries infection was acquired from and the speciation of cases.
METHODS: One hundred and seventy-four suspected *Leishmania* infected tissue and bone marrow samples were submitted to the Department of Microbiology, St. Vincent's Hospital, Sydney, Australia between January 2010 and March 2014. DNA was extracted and submitted to conventional polymerase chain reaction (PCR) and speciation was confirmed by restriction fragment length polymorphism (RFLP).
RESULTS: There were 47 (27%) positive samples from 42 patients. Cutaneous leishmaniasis was the most common clinical presentation and two patients had visceral leishmaniasis. From the 42 positive patient's samples, 35 were able to be speciated and five different species were identified by RFLP. *L. tropica* was the most common species identified with 21 (50%) patients positive for this species. Infection with *L. tropica* was predominantly associated with travel from Afghanistan and Pakistan to Australia by defence force personnel, refugees and people who had visited family. There were four cases each of *L. major*, *L. donovain/infantum* and *L. braziliensis complex* and one case of *L. mexicana.*
CONCLUSIONS: These results show that even though human leishmaniasis is not present in Australia, there needs to be an increased awareness among clinicians about the possibility of *Leishmania* infection from people who have travelled abroad.

**Use of lactobacilli with probiotic potential as prophylactic treatment of murine brain toxoplasmosis.**

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BACKGROUND: The drugs of choice used in the treatment of toxoplasmosis are extremely aggressive and cause secundary effects in the patient. One of the major challenges today is to seek alternative treatments that are effective for this disease and also not generate sequels. This is where probiotics have emerged as a potential alternative. These are defined as "live microorganisms which when administered in adequate amounts confer the host health benefits and also have immunomodulatory properties." That is why the aim of this work was to demonstrate the protective potential of different species of lactobacilli against *Toxoplasma gondii*.
METHODS: 4 groups of NIH female mice were inoculated, each group received prophylactic treatment with PBS (n=11) *Lactobacillus casei* (n=11) *Lactobacillus pentosus* (n=11) and *Lactobacillus plantarum* (n=11) for 7 days prior to parasite challenge with *Toxoplasma gondii* strain Me49 (25 cysts), once the infection is followed by administering the lactobacilli daily until the first week post-infection, mice from each group were sacrificed (n=4) at the end of treatment (7 dpi) and at the end of post-infection 8 weeks (n=7). Parasite burden in the brain and serum cytokines (TNF-$\alpha$ and IL-10) in acute and chronic phase of infection were evaluated. The results were compared with the control group.
RESULTS: A significant reduction in parasite burden compared with the control group 58.4% and 52.2% in the groups treated with *L. casei* and *L. plantarum* was obtained respectively. During the acute phase of infection, the pro-inflammatory response (TNF-$\alpha$) was reduced with treatment of *L. plantarum*, while the anti-inflammatory response (IL-10) is reduced with the treatments of *L. pentosus* and *L. plantarum*. In the chronic phase of infection reduced the pro-inflammatory response (TNF-$\alpha$) in the groups treated with *L. casei* and *L. plantarum* was observed, these data correlate with the high levels of this cytokine and high parasite burden control group.
CONCLUSIONS: The results suggest that the strains of *Lactobacillus casei* and *Lactobacillus plantarum* are probiotics with non-specific anti-*Toxoplasma gondii* during the acute phase of the infection, allowing the possibility of being used as a complementary alternative treatment to the pharmacotherapy in this disease.

**Monogenean and aporocotylid digenean infections of cultured marine fishes**

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BACKGROUND: Mariculture is a rapidly developing industrial sector. Generally, fish are maintained in net cages with high density culture systems, which have advantages including relatively low construction and maintenance costs and easy management of fishes in cages. At the same time, this system has disadvantages to allow uncontrolled flow of sea water containing potentially infectious stages of fish parasites and provide suitable substrates for proliferation of some parasites. In such culture conditions, monogeneans and aporocotylid digeneans (blood flukes) are most important helminth parasites in terms of their pathogenicity and proliferation potential among caged fishes.
METHODS: The review is based on literary and unpublished data.
RESULTS: Monogenean infections induce respiratory and osmo-regulatory dysfunctions, and blood flukes cause asphyxiation leading to mass mortality due to accumulated eggs in the gills. Many monogeneans lay eggs with filamentous appendages, which entangle with culture nets. In infection of a blood fluke, *Cardicola opisthorchis*, in the heart of Pacific bluefin tuna, *Thunnus orientalis*, the intermediate host, *Terebella* sp. (Polychaetes, Terebellidae) propagates on ropes and floats attached to tuna cages. The infectious stages of monogeneans and blood flukes, oncomiracidium and cercaria, respectively, emerge in the vicinity of caged fishes. Prevention of infection with these parasites is difficult, as eradication of monogenean eggs and intermediate hosts of blood flukes from culture facilities is extremely difficult.
CONCLUSIONS: For monogenean infections, combinations of prophylaxis including modified culture techniques to avoid infection, selective breeding of resistant fish strains and general fish health management with freshwater bath and chemotherapy treatments should be developed. For control of *C. opisthorchis* infection, the only option is in-feed administration of praziquantel to treat infected fish. Biology of the intermediate host, infection cycle at tuna culture sites and treatment schedule need to be studied.

**Metronidazole treatment failure in clinical *Blastocystis* samples from Sydney, Australia**

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BACKGROUND: *Blastocystis* is the most common enteric protist found in humans. *Blastocystis* has a world-wide distribution with higher rates of infection seen in developing countries. Due to the self-limiting nature of *Blastocystis* infection, and the controversy over whether *Blastocystis* should be considered a pathogen or not, treatment is not always given. However, when antimicrobials are given, metronidazole is often the first-line treatment. There have been reports of metronidazole resistance and it has been suggested that some subtypes may be more resistant than others. Therefore the aim of this study was to look at treatment options for patients with chronic *Blastocystis* infection and comment on metronidazole resistance.
METHODS: Twelve clinical *Blastocystis* isolates from four common *Blastocystis* subtypes (ST1, ST3, ST4 and ST8) were tested *in vitro* against 12 commonly used antimicrobials (metronidazole, paromomycin, ornidazole, albendazole, ivermectin, trimethoprim- sulfamethoxazole, furazolidone, nitazoxonide, secnidazole, fluconazole, nyastatin and itraconazole). A further eighteen patients suffering from chronic *Blastocystis* infection with four different subtypes (ST1, ST3, ST4 and ST5) were followed as they underwent antimicrobial treatment.
RESULTS: All subtypes showed little sensitivity to the commonly used metronidazole, paromomycin and triple therapy (furazolidone, nitazoxanide and secnidazole) both *in vitro and in vivo*. Following treatment, resolution of clinical symptoms did not occur in the patients suffering from chronic *Blastocystis* infection and follow up testing revealed ongoing infection with the same subtype. Trimethoprim- sulfamethoxazole and ivermectin were the only antimicrobials that had a lethal dose for all of the subtypes.
CONCLUSIONS: This study highlights the lack of efficacy of several commonly used antimicrobial regimens for the treatment of *Blastocystis*, in particular metronidazole which is the first line treatment for *Blastocystis.* This study highlights the efficacy of other potential drug treatments including trimethoprim- sulfamethoxazole and ivermectin and suggests that current treatment regimens be revised.

# Genetic diversity of *Toxoplasma gondii* in China

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BACKGROUND*: Toxoplasma gondii* is a very successful parasite that can infect humans and virtually all warm-blooded animals with a worldwide distribution. China is a large country, the climate in China differs from regions to regions because of the country's highly complex topography, thus, the genetic diversity of *T. gondii* isolates could be different. In this presentation, we review the genetic diversity of *T. gondii* in China.
METHODS: Using multilocus polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), a number of *T. gondii* genotypes have been identified from animals (cat, pig, sheep, rabbits, wild birds, chicken, voles, plateau pika and Tibetan ground-tit) and humans in China, including ToxoDB#1, 3, 9, 10, 18, 20, 204, 205, 213, and 225.
RESULTS: ToxoDB#9 is the most prevalent in China. It was identified in cats in Beijing Municipality, Guangdong, Anhui, Guizhou, Shandong, Hubei, and Yunnan provinces, and it was also found in pigs in Guangdong, Henan, Yunnan and Anhui provinces. Therefore, ToxoDB#9 is a predominant lineage prevalent in Mainland China. ToxoDB#3 (the type II variant) was identified in cats in China. This type was found from sheep in Qinghai province, from birds in Xinjiang Uygur Autonomous Region, from sparrow in Lanzhou, Gansu province, and from pigs in Zhongshan, Guangdong province. ToxoDB#1 (the type II) was reported in humans, but it was also reported from cats in Yunnan province.
CONCLUSIONS: These findings indicate a high genetic diversity of *T. gondii* in China, with ToxoDB#9 (type China 1 lineage) being dominant in China.

**Development of an adjuvant therapy for severe malaria with the hemoglobin vesicle, an artificial oxygen carrier.**

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BACKGROUND: Severe anemia is one of the major causes of death in falciparum malaria. Blood transfusion treatment increases survival in patients of the disease. Because of logistic constraints and viral contamination of the blood supply, transfusions are frequently not practical in malaria endemic regions. A hemoglobin-vesicle (HbV) is an artificial oxygen carrier developed for use as a transfusion alternative, which is encapsulating a concentrated hemoglobin solution in a liposome. The HbV is free of infectious contamination, without blood type mismatching, may not require refrigeration for over 2 years. Herein we investigated the potential of HbV as an adjuvant therapy for severe malaria using rodent malaria models.
METHODS: The HbV suspension was prepared as reported previously (Sakai et al., 2004). Balb/c mice were inoculated with 1 x $10^6$ *Plasmodium berghei* (ANKA). Parasitemia and body weight were followed every day after inoculation. The HbV or saline infection was started in tail vein from 6-day after inoculation. To evaluate the effects of the HbV treatment, the rotarod performance test was carried out.
RESULTS: All the mice became quite anemic already at day 6 post inoculation when the HbV transfusion was started. No significant difference in parasitemia and body weight of the mice was observed between the HbV and saline treated groups after daily treatment until they died. However, we observed everyday that, immediately after its administration, the HbV treated mice became apparently more active comparing the saline treated mice. The riding time on the rotarod compared between the 2 groups revealed significant extension of the time in mice treated with the HbV.
CONCLUSIONS: The HbV transfusion may have improved the anemia and mitigated the complication of severe malaria in mice. The HbV has high potential as adjuvant therapy for human as well to save the lives of patients in the endemic areas.

**Molecular and genetic epidemiology of chloroquine resistant *Plasmodium falciparum* in Lao PDR under SATREPS project**

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BACKGROUND: SATREPS (Science and Technology Research Partnership for Sustainable Development) is a Japanese government program, a collaboration between the Japan Science and Technology Agency (JST) and the Japan International Cooperation Agency (JICA), that promotes international joint research targeting global issues, involving partnerships between researchers in Japan and researchers in developing countries. One of the most important fields/areas within the SATREPS is infectious disease control, and drug resistant malaria control in Laos is now included in the project as a primary issue. In this abstract, we report the distribution and frequency of a mutation in *Plasmodium falciparum* chloroquine resistance transporter (*pfcrt*) gene in codon 76 (K76T) which is associated with chloroquine (CQ) resistance.

METHODS: We analyzed 48 *P. falciparum* isolates in Xepon district, Savannakhet province, Lao PDR during Aug-Sept, 2013, and determined the frequency of the mutation in codon 72-76 in the gene by PCR and DNA sequencing methods.

RESULTS: 31 (64.6%) of the 48 isolates possessed CQ susceptible genotypes (CVMNK (4) and CVIEK (27): amino acid sequences of codon 72-76), while 17 (35.4%) showed CQ resistant genotypes (CVIET (7) and CVIDT (10). The CVIET is the major CQ resistant genotype reported in Thailand, and its wide distribution is confirmed in the Greater Mekong Sub-region (GMS). However, the CVIEK has thus far only been reported in Africa (Central Africa Republic and Sudan) hence, its specific distribution found in Savannakhet Province at high frequency is of keen interest and its epidemiological significance needs to be analyzed.

CONCLUSIONS: The clue to this mystery (the distribution of the CVIET genotype in wild type *P. falciparum* isolates) may be found by population genetic analysis using microsatellite markers flanking the *pfcrt* gene encoding the codon 72-76. Furthermore, analyzing population movements in the GMS could also lead to a better understand this epidemiological enigma.

**Genetic diversity of *Toxoplasma gondii* in China**

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BACKGROUND: *Toxoplasma gondii* can infect all warm-blooded animals including humans. Infection with *T. gondii* is probably the leading cause of posterior uveitis in humans and the most comment route of transmission is raw and undercooked meat from infected animals. *T. gondii* calcium-dependent protein kinase 1 (TgCDPK1) plays a critical role in direct parasite motility, host-cell invasion, and egress.
METHODS: We constructed a DNA vaccine expressing TgCDPK1 inserted into eukaryotic expression vector pVAX I and evaluated the immune protection induced by pVAX-CDPK1 in Kunming mice. Mice immunized with pVAX-CDPK1 intramuscularly and/or with a plasmid encoding IL-15 and IL-21 (pVAX-IL-21-IL-15). The immune responses were analyzed including lymphoproliferative assay, cytokine, antibody measurements, lymphocyte surface markers by flow cytometry and protective efficacy were measured as survival and cysts numbers after challenge 1 to 2 months post vaccination.
RESULTS: Immunization with pVAX-CDPK1 or pVAX-IL-21-IL-15 alone developed strong humoral responses and Th1 type cellular immune responses, and the significantly ($P < 0.05$) increase of both the percentages of CD4+ and CD8+ T cells compared with all the controls (blank control, PBS, and pVAX). Co-injection of pVAX-IL-21-IL-15 significantly increased humoral and cellular immune responses compared to the group of pVAX-CDPK1 or pVAX-IL-21-IL-15. Challenge experiments showed that co-administration of pVAX-IL-21-IL-15 and pVAX-CDPK1 significantly ($P < 0.05$) increased survival time (19.2 ±5.1 days) compared with pVAX-CDPK1 (17.3 ± 4.3 days) or pVAX-IL-21-IL-15 (12.0 ±2.0 days) alone, and pVAX-IL-21-IL-15 + pVAX-CDPK1 significantly reduced the number of brain cysts (72.7%) in contrast to pVAX-ROP13 (45.7%) or pVAX-IL-21-IL-15 alone (43.6%).
CONCLUSIONS: TgCDPK1 is identified to be a promising vaccine candidate for inducing a strong humoral and cellular response against *T. gondii* infection, and thus synergistic of mIL-21 and mIL-15 can induce non-specific immune responses, but also facilitate specific humoral as well as cellular immune responses elicited by DNA vaccine against acute and chronic *T. gondii* infection in mice.

# Application of Polymerase Chain Reaction for diagnosis of *Fasciola hepatica* in field-collected snails

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**Introduction:** *Fasciola hepatica* as a trematods that needs some fresh water snail intermediate hosts in which reproduce asexually and complete its life cycle.
In this study, Polymerase chain reaction (PCR) was used to detect *Fasciola hepatica* infection in the snail intermediate host.

**Methods:** In this study, 810 snails were collected from different parts of Dasht Room region in Kohgiluye & Boyerahmad province, south of Iran, new emerging focus for human fascioliosis. After snails identification, the snail shell was removed and DNA was extracted by phenyl chloroform isoamyl alcohol method. Yielded DNA amplified by PCR to identified existence of *Fasciola hepatica* cercaria in the snail body.

**Results:** Three genera including *Physa* (21.9%), *Planorbis* (11.8%) and *Lymnea* (66.3%) were detected among collected snails. Two species of *Lymnea*, *L.palustris* (15.4% out of 66.3%) and *L.truncatula* (50.9% out of 66.3%), were identified among this genus. No *Fasciola hepatica* DNA was identified within this snails.

**Conclusion:** According to the results of PCR method the collected snails, didn't infected with *Fasciola hepatica*. Although using complementary and also traditional methods for tracing *F.hepatica* cercaria in snail body is strongly recommended.

Key words: *Fasciola hepatica*, PCR, Snail

**Developing the therapeutic potential of the *Acanthocheilonema viteae* anti-inflammatory product, ES-62**

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BACKGROUND: ES-62 is the most abundantly secreted immunomodulator of the rodent filarial nematode *Acanthocheilonema viteae*. As a consequence of its anti-inflammatory properties, ES-62 can significantly suppress development of pathology in mouse models of asthma, cardiovascular disease, arthritis and lupus. This suggests that ES-62 has therapeutic potential, but as a large, potentially immunogenic glycoprotein, it is in reality unsuitable for use as a drug. Nevertheless, its anti-inflammatory activity relies on post-translational attachment of phosphorylcholine (PC), thus raising the possibility of designing small molecule analogues (SMAs) based around this active moiety.
METHODS: A library of over 100 novel SMAs was synthesized and screened for immunomodulatory activity *in vitro*, primarily by determining effects on cytokine production by macrophages exposed to PAMPs and mast cells activated via FcγRI. Active compounds were then tested in *in vivo* mouse models of autoimmunity and allergy.
RESULTS: Two sulphone-type SMAs, 11a and 12b, which were found to inhibit certain inflammatory responses of both macrophages and mast cells but to be non-cytotoxic, were selected for testing *in vivo* and, as with ES-62, suppressed development of pathology in collagen-induced arthritis (CIA) and ovalbumin-induced airway hypersensitivity and also proteinuria in the MRL/Lpr mouse, which spontaneously develops a lupus-like glomerulonephritis. Currently, we are comprehensively investigating whether the SMAs mimic ES-62 in molecular mechanism of action.
CONCLUSIONS: We provide proof-of-principle that safe, drug-like SMAs of the anti-inflammatory helminth product ES-62 can be produced for treating of autoimmune and allergic conditions.

# Cypermethrin resistance in Rhipicephalus (Boophilus) annulatus in Nour Township, North of Iran

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BACKGROUND: *Rhipicephalus (Boophilus) annulatus* is a major pest of domestic ruminants in North of Iran. The use of chemical acaricides is at the center of the national tick control strategy, however, pesticide resistance has led to operational failure. The aim of this study was to determine the susceptibility status of the tick to common acaricides.
METHODS: A multistage cluster random sampling method was used to examine 823 domestic animals (cow, sheep and goat) in three different geographical areas of plain/littoral, jungle and mountains. After species identification using appropriate keys, the fully engorged ticks were kept in an insectary under 28±2°C temperature, 78±5% relative humidity and 12:12 photoperiod until they laid eggs in about 4-10 days. Seventeen populations of R. (B.) annulatus ticks were tested with cypermethrin. The two-week old larvae were used for the Larval Packet Test (LPT) with Whatman#541 filter paper treated with the serial discriminating doses of cypermethrin. The mortality of the treatment and control replicates was scored after 24 hours. Probit analysis was employed to calculate the $LD_{50}$ and $LD_{99}$ of the populations and ANOVA-Tukey test was also used to compare the Probit analysis data between populations.
RESULTS: Population 75 showed a resistance ratio of 129 with cypermethrin when compared to the most susceptible population 23 at the $LD_{99}$ level, which is about 75-fold higher than the dose recommended by the formulating company. We documented for the first time a *R. (B.) annula*tus population in Iran with resistance to cypermethrin insecticide.
CONCLUSIONS: The resistance ratio of insecticide tested confirms operational failure with cypermethrin. Therefore monitoring the acaricide susceptibility status of the filed populations is the key to manage insecticide resistance by implementing resistance management strategies in the long run and also choosing alternative acaricides for the short term.

**Nutritional status and parasite infections: a study in schoolchildren and non-school children.**

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BACKGROUND: Malnutrition and parasite infections are common public health problem in developing countries. This interaction comprises cognitive processes and development.
METHODS: A cross sectional population-based study was undertaken to determine the relationship between nutritional status, intestinal parasites and demographic factors in a rural and urban area in Palmira, Colombia. A socio-demographic survey, anthropometric assessments and laboratory tests were performed in a population of school and non-school children. A 24-hour diet recall interview was also applied to their mothers.
RESULTS: A total of 667 children aged 5 to 14 years were assessed, 48% boys and 52% girls, 131 were non-school children. 19% of the children showed any level of malnutrition. The children Z scores were height for age -0.06 (±1.23) and weight for age – 0.26 (±1.17). Lipid profiles showed 11.5% children with high cholesterol levels and 1.7% with increased triglycerides levels. The mean hemoglobin concentration was 12.7 g% (±1.3) and was correlated with income and age. Eosinophilia was remarkably found in both school and non-school children (0-54% and 0-45% respectively). Parasite infections were found in 44.4% children, 54.6% in non-school children and 42.6% in schoolchildren. In non-school children parasites found were *E. histolytica* 43.5%, *G. lamblia* 26% and *Entamoeba coli* 13%. In school children infections were caused by *E. histolytica* 41.7%, *G. lamblia* 27% and *Entamoeba coli/E. histolytica* 8%. *A. lumbricoides* was < 1% and no hookworm infections were observed.
CONCLUSIONS: Lower mean Z score (height for age) was seen in non-school children when compared to school children; differences were also seen in hemoglobin, protein and HDL levels. Prevalence of parasite infections was high in both school and non-school children showing no significant difference, this may be explained by life style and habits of this population. Sanitation education and treatment of the parasitic infections must be reinforced in local health programs.

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# Trends in neurocysticercosis-related mortality in Brazil, 2000-2011

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BACKGROUND: Neurocysticercosis is the main cause of neurological disease by parasitic origin in humans, and an important public health problem in low and middle countries. We analyzed the epidemiological patterns of neurocysticercosis-related mortality in Brazil.
METHODS: We performed a nationwide study using official mortality data obtained from the Mortality Information System of the Brazilian Ministry of Health. We included all deaths in the country between 2000 and 2011, in which neurocysticercosis was mentioned on the death certificate as underlying or associated cause of death (multiple causes of death). We calculated crude mortality rates (per 1,000,000 inhabitants) by sex, age group, race/color, and region of residence. Trends over the 12-year period were assessed using Joinpoint regression models.
RESULTS: During the study period, 12,491,280 deaths were recorded. Neurocysticercosis was identified in 1,829 deaths, with 1,130 (61.8%) as the underlying cause and 699 (38.3%) as an associated cause. Average crude mortality rate was 0.82 deaths/1,000,000 inhabitants/year. Males (0.93 deaths/1,000,000 inhabitants/year), >70 years old (3.71 deaths/1,000,000 inhabitants/year), white race/color (1.01 deaths/1,000,000 inhabitants/year), and residents in the South region (1.17 deaths/1,000,000 inhabitants) had the highest rates. Mortality decreased significantly over the entire period (Annual Percent Change [APC]: -3.11%; 95% confidence intervals [CI]: -4.4 to -1.8), with different patterns between regions: an increase in the Northeast region, a decrease in the Southeast region, and stability in the North, South and Central-West regions.
CONCLUSIONS: This is the first nationwide population-based study on neurocysticercosis-related mortality in Brazil. Due to the absence of a national control program, and the fact that cysticercosis is not a reportable disease in Brazil, it remains a neglected health problem, with marked regional differences.

**Parasites as biological tags for fish stock assessment in the South West Atlantic**

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BACKGROUND: Marine fisheries in the South West Atlantic (SWA) are threatened by overfishing and under serious risk of collapsing. The SWA comprises a diversity of environments, possesses a complex oceanography and harbors a vast biodiversity that provide an enormous potential for using parasites as biological tags for fish stock delineation, a prerequisite for the implementation of control and management plans. Here, their use in the SWA is reviewed. Main evidences are derived from Argentine waters, where fish parasite assemblages are dominated by larval helminth species that share a low specificity, long persistence and trophic transmission, parasitizing almost indiscriminately all available fish species. Their ubiquity makes them promissory biological tags at higher scales, including fish assemblages and ecosystems.
METHODS: This review is based on literature and unpublished own data. The concept of biological tags is expanded from fish populations to communities and from local to regional scales, by mean of multivariate analyses across fish species and regions in the SWA.
RESULTS: Clear regional patterns of parasite distribution were observed at large scales, with recurrent clines in parasite assemblage structure across fish species, confirming their value as regional tags to identify fish assemblages and the masses of waters they inhabit and, consequently, as ecosystem indicators.
CONCLUSIONS: The advantages and constraints of the use of biological tags are analyzed and recommendations are given for future research. Essential information to delineate ecosystem boundaries for host communities can be obtained from parasite data, constituting a powerful tool to help the implementation of ecosystem-based approaches to fisheries management, the new paradigm for fisheries science. Holistic approaches, including parasites as biological tags for stock delineation will render valuable information to help insure fisheries and marine ecosystems against further depletion and collapse.

**Prevention and control of congenital Chagas disease: an update**

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BACKGROUND: Chagas disease is caused by the parasite *Trypanosoma cruzi*. It is a major cause of morbidity and mortality in Latin America, with 13,000 to 45,000 infected patients dying each year. New guidelines, approaches are in process to improve control, and prevention of congenital transmission.
METHODS: We reviewed bibliography related the theme of interest published or in process, mainly produced by teams of our Institute in collaboration with others, and experiences of other researchers.
RESULTS: There is a consensus that control of congenital transmission by diagnosis and treatment of infected newborns has shown to be highly effective when the infection is detected in a timely manner. Polymerase chain reaction (PCR) has shown to be useful to improve the opportunity of diagnosis in newborns after delivery with more sensitivity than micromethod or microhematocrit due to these have operator dependence, however, PCR needs an infrastructure which means a barrier to be solved in near future. Several projects are ongoing to obtain molecular commercial kits, feasible to be used in the Primary Health Care system, and assessed for the transfer to health systems. Other techniques, such as ELISA test using SAPA antigen proved to be useful to detect congenital infection within the first 3 months after birth using binomial samples (mother and child), or after 3 months of age, if child sample is only available. Regarding prevention, recent results are showing the benefit of trypanocidal treatment in girls or non pregnant women, to prevent congenital transmission to their newborns.
CONCLUSIONS:
Therefore, the availability of simple and accurate diagnostic methods that can be used at birth and can be easily implemented in PHC settings could provide tools for the timely treatment of infected newborns. Additionally, evidences are showing that congenital transmission could be prevented through treatment of infected women before they become pregnant.

**Response of a *Trypanosoma cruzi* isolate to clomipramine treatment in the acute and chronic phases and the effects upon mitochondrial activity.**

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BACKGROUND: Chagas disease (CD) treatment is controversial due to partial effectiveness, physicochemical problems and side effects of currently used drugs. For this, we evaluated the effect of Clomipramine (CLO), an inhibitor of *T. cruzi* trypanothione reductase, as an alternative therapy. *T. cruzi* invasion and replication produce reactive oxygen species that target mitochondria, modifying the energy supply and impacting in the genesis and progression of the cardiopathy. Additionally, clinical variability could also be explained by parasite genetic heterogeneity.
METHODS: Albino Swiss mice were infected with 50 parasites of an isolate from an endemic area in Argentina (SGO-Z12). It was characterized using two typing methods. Treatments were performed in the acute and chronic phases. For both moments, mice were divided into (n=20 in each group): not-infected (NI), infected non-treated and infected and treated with CLO (5mg/kg/day). Acute phase: CLO was administered for 30 days. Chronic phase, electrocardiographic abnormalities were criteria to begin treatment: CLO was administered for 60 days. Treatment effectiveness was measured by survival, parasitemia and qPCR. Mitochondrial function in myocardium and skeletal muscle was studied by determining the activity of complexes II and III (CII-CIII) of the respiratory chain.
RESULTS: SGO-Z12 isolate consisted of a mixture of lineages II and VI. Both treatments were effective in reducing parasitemia (qPCR), increasing survival and reestablishing skeletal muscle CII activity to NI values. CIII activity however, remained altered.
CONCLUSIONS: The diminished parasitemia in the treated groups probably improved their mitochondrial function, preserving the cardiac activity and therefore allowing a higher survival than non-treated groups.

# Screening for *Tripanosoma cruzi* antibodies and *Strongyloides stercoralis* antibodies in migrants to Italy coming from endemic areas

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BACKGROUND:Chagas Disease (CD) is an emerging infectious disease in Europe and outside endemic regions can be transmitted by blood transfusion, transplantation and by vertical route. The objective of this study was to evaluate the prevalence of *T .cruzi* and *S. stercoralis* antibodies among migrants coming from CD areas and living in Milan area, Italy. 270 serum samples were collected from South American people ( Bolivians, 47%, Peruans, 37%, Ecuadorians, 11%,El Salvador 4% and others 1%) from July to January 2014. All people were clinically asymptomatic and gave informed consent. Institutional Ethic Committee approved the study. Serum samples were assayed by three different methods: *DiaSorin LIAISON XL Murex Chagas* (CLIA), *Abbott Architect Chagas* (CMIA) and *BiosChile Chagas III* (Elisa). . All positives samples were assayed by another supplementary test ( Immunocromathographic test *Chagas (T.cruzi) ab rapid test* by Immunospark(USA) distributed in Italy by S.D.(Servizi Diagnostici). All 270 samples were assayed for *Strongyloides stercoralis* IgG antibodies by EIA *Strongyloides ratti* by Bordier Affinity Products distributed in Italy by Effegiemme.
RESULTS: 13% of the population sample resulted positive to *T. cruzi* antibodies. Of the all positives, 94% originated from Bolivia,3% from el Salvador and 3% from Argentina. The specific prevalence of the Bolivian group was 27% for El Salvador group was 11%and for Argentinian group was 25%. Of the positive results 9 were discordant: one positives only for the assay CMIA Architect, two were positives only for CLIA LIAISON XL, one was positive only for Elisa BiosChile and one was positive only for *Chagas (T.cruzi)ab rapid* test.Two results were negative only for BiosChile Elisa Test, two only for Liason LX test and tre rapid tests shown a faint positive band only after expired lecture time. Good correlation was shown between CLIA LIAISON XL and CMIA Architect results (R2= 0,965) (Show graphic). All the high-level positive results matched with the three tests (CMIA and CLIA S/CO > 5, Elisa >3). For borderline results were often discordance between the assayes results. 6 samples resulted positive for S.stercoralys antibodies and 3 were considered negative cut off results ≤ 20% S/C.O positive result value). 83% of the S. stercoralis positive samples were of Bolivian patients. Total prevalence for *S,stercoralis* ab positives was 2.5%.
CONCLUSIONS: Our preliminary results confirm a high rate of seroprevalence for *Trypanosoma cruzi* and *Strongyloides stercoralis* antibodies especially among people from Bolivia. Blood banks and Transplantations networks dealing with patients from Latin America should implement screening protocol for CD. Further studies would be useful for evaluate a possible link between specific antibody levels and clinical assessment

# A proteomic view at the endosymbiosis process in *Strigomonas culicis*

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BACKGROUND: *Strigomonas culicis*, a monoxenic trypanosomatid, shares a symbiontic relationship with a bacterium that lives in its cytoplasm. This bacterium can be removed by antibiotic treatment, originating an aposymbiontic strain. Comparisons between the strains are an interesting strategy to study the relationship of the symbiont and its host, and can also contribute to a better understanding of the evolutionary process involved in the origin of new organelles.
METHODS: A comparative analysis of the proteome of *S. culicis* wild and aposymbiontic strains was performed across different growth stages. The experimental setup consisted of two-dimensional gel electrophoresis (2DE) followed by MALDI-TOF/TOF and/or nano-LC-nano-ESI-LTQ/ Orbitrap protein identification. Quadruplicate 2DE were performed for each stage of cellular growth. Image analysis was performed for inter-strains comparisons providing a comparative differential analysis pairing each of the conditions, strain and stage of the cell growth, two by two. Statistical validation of the differentially abundant spots was conducted by LIMMA methodology accepting an FDR smaller than 5%.
RESULTS: Two-hundred and three spots displayed statistically significant shifts in abundance and were subjected to mass spectrometry identification. The modulation of differentially abundant proteins is higher in the aposymbiontic strain, being more prominent at the early log phase. Among the identified proteins are enzymes related to heme biosynthesis and structural proteins such as flagellar components, corroborating former biochemical and genomic data pointing that these proteins are more expressed in wild strains. Furthermore a total of 870 spots were unique to specific strain and growth stage conditions, and are currently being subjected to MS protein identification.
CONCLUSIONS: These results, associated to genomic data improve our understanding on the evolutionary process and the symbiotic relationship. On the other hand, the possibility of identifying a large number of metabolic pathways shared between organisms should provide further information about the biology of the parasite.

**Water buffaloes as sentinel animal population in the zoonotic schistosomiasis surveillance and control**

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BACKGROUND: The presence of animal reservoirs in *Schistosoma japonicum* infection has been a major obstacle in the control of schistosomiasis. Current control programs in endemic areas do not include animal surveillance. Guidelines for elimination including zoonotic surveillance coming from local ministries of health and the World Health Organization are needed to achieve possible elimination of schistosomiasis in many areas where the prevalence has been reduced to near elimination levels. Water buffaloes, considered one of the most susceptible animals and most exposed to *S. japonicum* infection, should therefore be assigned as the sentinel animal population in monitoring animal infections in near elimination areas.
METHODS: In this study, we investigated the importance of the water buffaloes in the elimination surveillance by testing samples from two areas in the Philippines, one with high endemicity (Calatrava, Negros Occidental) and the other with no cases of human schistosomiasis for almost 2 years (Talibon and Trinidad, Bohol). Serum and stool samples were collected in Calatrava (n=59) and Talibon-Trinidad (n=60); and tested using microscopy, stool PCR, SEA-ELISA, SjTPx-1 ELISA and Sj1TR ELISA.
RESULTS: Results showed that the prevalence of *S. japonicum* infection ranges from 1.69% (microscopy) to 49.15% (stool PCR) in the highly endemic Calatrava; and from 6.67% (microscopy) to 10% (stool PCR) in Talibon-Trinidad.
CONCLUSIONS: These results prove that in near elimination areas, human cases may be zero but animal infections persist, which continue to perpetuate transmission of schistosomiasis. Hence the importance of animal surveillance using water buffalos as sentinel group that would regularly monitored.

# *Haemonchus contortus* egg hatching inhibition by *Pleurotus ostretus* and *Pleurotus eringii* mycelia bio-molecules

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BACKGROUND: *Haemonchus contortus,* is a highly pathogenic nematodes affecting sheep. Edible fungi are considered as nutraceutics against many diseases including parasitosis. This research evaluated the *H. contortus* egg hatching inhibition by bio-molecules obtained from mycelia of *P. ostreatus* ECS-0152 and *P. eringii* ECS-01292 strains.

METHODS: Mycelia were incubated in whole wheat flour agar plates at 27ºC at the darkness for 7 days. Mycelia were crushed with 1%Tryton and PBS pH 7.4. Material was spinned down for 30 min at 4ºC. SDS-PAGE was performed to identify protein bands. Supernatant was used for assessing the egg hatching inhibition in 96 well plates (n=4). Ten microliters of water containing 100 *Haemonchus contortus* eggs and 90 µL of the corresponding strain were added to each well. *P. ostreatus* was used at 1.0 and 2.0 mg/mL; and *P. eringii* at 1.3 and 0.65 mg/mL concentration. A control series with water was used for comparison. Lectures were performed after 48 h.

RESULTS: Five proteins bands ranging between 13 to 150 kDa were identified in *P. eringii* and tree bands of 20 to 50 kDa in *P. ostreatus.* The egg hatching inhibition recorded was 97.18% and 97.04% for *P. eringii* at 0.65 and 1.3 mg/mL concentrations; respectively. *P. ostreatus* inhibited 62.34% the egg hatching at 2.0 mg/mL.

CONCLUSIONS: Crude extract from *P. eringii* showed the highest ovicidal effect. However, the effect showed by *P. ostreatus* is significant. Mycelia proteins from edible mushrooms could be good candidates as possible control agents of sheep haemonchosis.

**Curcumin induces apoptosis in *Schistosoma mansoni* adult worms *in vitro***

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BACKGROUND: Curcumin (Cur) isolated from the rhizome of the plant *Curcuma longa* has been shown activity *in vitro* and *in vivo* against *Schistosoma mansoni*. In addition, studies show that Cur induces apoptosis in several types of carcinogen cells and some parasites. However, the mechanism of action involving Cur in *S. mansoni* has not been characterized. In this study was evaluated some parameters involved with apoptosis in *S. mansoni* adult worms after incubation with curcumin *in vitro.*
METHODS: Adult worms were recovered by perfusion of mice, cultivated to different concentrations of Cur during 24 hours and the viability was analyzed using inverted microscopic. The apoptosis in adult worms was evaluated by transmission electronic, quantitative RT-PCR of specific transcripts related to apoptosis (Smcaspase3/7) and by caspase 3 activity. Also, the DNA fragmentation was evaluated by Tunel assay and agarose gel.
RESULTS: It was observed that Cur causes a significant reduction in viability of male and female parasites at 25 μM concentration. Parasites treated at 50μM of Curc showed typical apoptosis morphological such as vacuoles formation, swelling and disruption of mitochondrial membrane and chromatin condensation. In addition, the results showed an up regulation of mRNA expression of Sm caspase3/7 and an increase of caspase 3 activity in both male and female parasite. Also, it was observed several apoptotic nuclei and DNA fragmentation in male and female parasites.
CONCLUSIONS: Our results suggest that Curc induces apoptosis in *S. mansoni* adult worms and provide preliminary studies on its mechanism.
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**Study of the scientific production on leishmaniasis from Latin America**

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BACKGROUND: Leishmaniasis is a highly relevant neglected tropical disease. It has important consequences in affected populations, including a high fatality rate in its visceral form. It's present in Latin America, then is necessary to promote more research on it. A bibliometric assessment of the Latin American scientific production in leishmaniasis was done.
METHODS: Bibliometric study at SCI (1980-2013), MEDLINE/GOPUBMED (1802-2013), Scopus (1959-2013), SCIELO (2004-2013), LILACS (1980-2013). Different study types, characterized by years, city/country of origin, journals and more productive authors, by country, cites and H index.
RESULTS: At SCI, 2857 articles were found (17.7% of the total). Brazil was the highest producer (58.1%), followed by Colombia (9.9%) and Venezuela (5.6%); the region received 41186 citations, 54.2% of Brazil (H index=62), 12.1% Colombia (H index=30) and 4.5% of Venezuela (H index=25). At Scopus, there are 3681 (14.7% of the total), 53.2% Brazil, 6.8% Colombia and 6.0% Venezuela; 38.46% at Brazil were from Fundação Oswaldo Cruz; 30.6% of Colombia corresponded to Universidad de Antioquia; 31.34% at Venezuela were from Universidad Central de Venezuela. At Medline there are 4525 records (60.6% of Brazil). At SciELO there are 1068 records (67.5% Brazil). At LILACS there are 1740 records (56.0% Brazil).
CONCLUSIONS: Scientific production of Brazil predominates in the region, with one single institution generating more articles than Colombia and Venezuela together. Scientific production in bibliographical data bases, particularly regional, is still relatively low, and the disease neglected when compared to other tropical conditions such as dengue and malaria.

**Can protein supplementation reduce reliance on anthelmintics in small ruminant production systems?**

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BACKGROUND: A large body of evidence supports the view that protein supplementation can reduce worm burdens and faecal egg counts (FEC), and improve productivity of growing and periparturient sheep, infected with gastrointestinal nematode parasites. The magnitude of these effects may be dependent on protein level and composition. Recent studies indicate that FEC reduces to a greater extent upon supplementation with by-pass protein compared to rumen degradable protein. These outcomes would suggest that supplementation with by-pass protein could reduce reliance on anthelmintics (AH) for the control of parasitic gastroenteritis in ruminants.
METHODS: Grazing, parasitized lambs were either drenched and not supplemented, or left undrenched but were supplemented with a mixture of soybean meal and corn (16% protein, of which 40% by-pass) or fishmeal, soybean meal and corn supplement (19% protein, of which 80% by-pass), at a rate of 1% of body weight per day. Grazing, parasitized twin-rearing ewes were either not supplemented, or fed xylose-treated soybean meal (44% protein, of which 62% by-pass protein), at a rate of 400 g/head/day.
RESULTS: The undrenched lambs supplemented with the 16% protein supplement grew at the same rate as the non-supplemented, drenched lambs, despite having greater worm burdens. The 19% protein supplement reduced FEC by 50% and increased growth by 25%. Maternal supplementation reduced ewe FEC by 40%, increased lamb growth by 20% and reduced lamb AH usage by 33%, without impacting lamb FEC.
CONCLUSIONS: These studies support the view that by-pass protein supplementation can reduce reliance on AH and maintain or improve ovine resilience and resistance to gastrointestinal nematode parasites.

# Infection with carcinogenic liver fluke *Opisthorchis viverrini* modifies intestinal and biliary microbiome

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BACKGROUND: *Opisthorchis viverrini* is a fish-borne trematode endemic in East Asia. Following ingestion, the flukes locate to the biliary tract¸ where chronic infection frequently leads to cholangiocarcinoma (CCA). The precise mechanism(s) by which *O. viverrini* infection culminates in CCA is not known. One unexplored aspect is its influence on the host microbiome. In the Syrian hamster, infection with this pathogen reliably leads to CCA. Genomic

METHODS: DNAs of microbiota from colorectal contents and bile of hamsters and *O. viverrini* were examined in this model of fluke-induced CCA. Sequences of regions 7, 8 and 9 of prokaryotic 16S rRNA genes were amplified, pyrosequenced, operational taxonomy units classified, and analysis of community diversity undertaken.

RESULTS: Of ~1,000,000 sequences, 536,009 could be assigned to 20 phyla and 273 genera of bacteria or Archaea. Diversity analyses revealed that fluke infection perturbed the gastrointestinal tract microbiome, increasing Lachnospiraceae, Ruminococcaceae and Lactobacillaceae while decreasing Porphyromonadaceae, Erysipelotrichaceae and Eubacteriaceae ($p \leq 0.05$). In addition, >60 prokaryote species were detected in the biliary system, which confirmed bacteriobilia and a remarkable community associated with the parasites. These fluke-associated microorganisms included potential pathogens from the Enterobacteriaceae and Listeriaceae and others from external environments including cyanobacteria and Deinococci.

CONCLUSIONS: Given that opisthorchiasis is distinguished from other helminth infections by a robust inflammatory phenotype, with conspicuously elevated interleukin 6, and that inflammation of the biliary system leads to periductal fibrosis that is a precursor to CCA, the flukes as well as their microbiota might together drive this distinctive immune response.

**Transgenesis and functional genomics of schistosomes mediated by mammalian retroviruses**

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BACKGROUND: Draft genome sequences for the human parasites *Schistosoma japonicum*, *S. mansoni* and *S. haematobium* are now available. The schistosome genome encodes ~11,000 protein encoding genes for which the functions of few are well understood. Nonetheless, the new genes and novel non-coding RNAs represent potential intervention targets, and molecular tools are being developed to determine their importance.
METHODS: Over the past 15 years, noteworthy progress has been achieved towards development of tools for gene manipulation of schistosomes, including gene expression perturbation by RNA interference, and transient and stable transfection including transgenesis mediated by genome integration competent vectors. Retrovirus-mediated transgenesis is an established functional genomics approach for model species. It offers the means to establish gain- or loss-of-function phenotypes, can facilitate vector-based RNA interference, and represents a powerful forward genetics tool for insertional mutagenesis screens.
RESULTS: Murine leukemia virus (MLV) pseudotyped with vesicular stomatitis virus glycoprotein (VSVG) mediates somatic transgenesis in *S. mansoni*, and has been demonstrated to vector vertical transmission of integrated transgenes in *S. mansoni* leading the establishment of transgenic lines. In addition, MLV transgenes encoding antibiotic resistance allow the selection of MLV-transduced parasites cultured in the presence of the appropriate antibiotic.
Additional advances with site preferences and activities of transgenes, lines of transgenic schistosomes, antibiotic selection and enrichment of populations of transgenic worms, reporter gene activity will be presented.
CONCLUSIONS: These approaches provide a tractable means to manipulate the genome of the schistosome, and can be expected to also find utility in genetic investigations in other laboratories and for other helminth pathogens of important neglected tropical diseases.

**Barcode structure and principal component analysis of host and habitat specific camallanid populations in Indian carps and catfish off the Central West Coast and riverine ecosystems**

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BACKGROUND: The stochastic assemblages of population of camallanid nematodes were unique in marine fish of three different genera, two carps from marine environment off the Central west coast of Goa and one catfish from the Gangetic riverine ecosystem at Allahabad, U.P., India.
METHODS: Worms from 1448 marine carps (Lon73°50'09.87"E, Lat15°27'04.63"N; Alt 6m) (*Lutjanus malabaricus- 577* and *Johnius dussumieri-* 543) and catfish (*Mystus tengra*-328) during February, 2003-January, 2006 and May, 2012 - April, 2014 were included in this study. 18S rDNA gene analysis exhibited monophyletic assemblage of the two sequences of *Camallanus oxycephalus*, and the group of *P. cyathopharynx, Procamallanus laevionchus* and *Pro fulvidraconis* formed the outgroup.
RESULTS: A distant genetic variance (0.75-2.8) was concluded between the sequences of *Paracamallanus* sp. and the closer camallanids, recovered from catfish *M. tengra*. Restricted host specificity was usually seen but long term studies revealed susceptibility of camallanid worms to a wider host range after Tsunami (December, 2003). SEM revealed pattern of distribution of filitriches at the head differently from body surface. The appropriateness of Principal Component Analysis could be substantiated by the cumulative percentage of variances explained by carps and catfish – camallanid model that could be used as post-hoc measure. The stability of cumulative percentage, which progressed from 30.45 to 36.49%, suggested that the model became more relevant each year. The robustness of the applications within Principal Component Analysis is strengthened by the availability of five year's samples under natural conditions.
CONCLUSIONS: The worms of *Paracamallanus* sp. from *L. malabaricus* off Goa were unique to show monophyly on the basis of 18S rDNA, coi, ITS1 and ITS2 gene analyses. The synonymies under *Spirocamallanus istiblenni* (GU082495, GU170858, GU170863, GU170864), *C. oxycephalus* (GU170851, GU170853, GU082496), *Paracamallanus* sp. (GU082486, GU082489, GU082490, GU170855), and *C. cotti* (GU082508), whose accession numbers are available at GenBank, have been substantiated by molecular analyses.

**_In vitro_ assessing of _Ananas comosus_ liophylized juice and _Lespedeza cuneata_ aqueous and methanolic extracts against _Haemonchus contortus_ infective (L3) and histotrophic larvae (L4)**

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BACKGROUND: *Haemonchus contortus* seriously affect sheep. Some fruits and leguminous are considered as potential phyto-therapeutics. The *in vitro* effect of *Ananas comosus* liophylized juice (AnanLJ) and *Lespedeza cuneata* aqueous (LespAE) and methanolic extracts (LespME) against *Haemonchus contortus* infective (HcL3) and histotrophic larvae (HcL4), was evaluated.
METHODS: The larvae/extracts confrontation was carried out in 96 well plates. Two experimental stages were established: In stage 1, twelve treatments were settled: 1) Control (-) (water), 2) Control (+) (1% Ivermectin), 3) 1% DMSO, 4), 5) y 6) LespAE at 20, 60 and 100 mg/mL; respectivelly, 7), 8) and 9) LespMEat 20, 60 and 100 mg/mL respectivelly, 10), 11) and 12) AnanLJ at 80, 120 and 160 mg/mL, respectivelly. Eighty microliters of either AnanLJ or LespAE were deposited in each well and 20 µL of an suspension containing 200 HcL3 (with or without sheath) were deposited in each well (n=4). In second stage, a similar procedure was used; with the difference that 70 HcL4 were used instead HcL3. Plates were incubated at 25-29ºC and lectures were performed after 24, 72 and 120 h.
RESULTS: The AnanLJ caused 79-100% mortality against HcL3 and HcL4 at the highest concentration after 72 h. No important effect was found with LespME. LespAE showed a lethal effect close to 85% against sheathed L3 at 120 h ($p<0.05$).
CONCLUSIONS: AnanLJ and LespAE contain anthelmintic compounds and that could have an important implication as a possible control agent of the sheep haemonchosis.

**Phylogenetic analysis based on 18S rDNA, coi, ITS1 and ITS2 genes of *Scyphophyllidium* sp. and *Rhinebothrium maccallumi* and population distribution pattern in Indian sting rays and sharks with SEM infrastructure**

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BACKGROUND: The phylogenetic analysis of worms of *Scyphophyllidium* sp. collected from *Rhincodon typus* during May, 2001 to July, 2003 (N=592), during July, 2008 to June, 2012 (N=395) and February, 2012 to April, 2014 (N=346), and of *Rhinebothrium maccallumi* (AM124476) from *Neotrygon kuhlii* from February, 2012 to April, 2014 (N=240) off the coast of Goa in India represented monophyletic interrelationship among the former to include two sequences (GQ265700 and GQ265702) in a single clade on the basis of 18S rDNA, coi, ITS1 and ITS2 genes, while the third (GQ265701) was associated with it.
METHODS: The host-specific variations in the infectivity by different tetraphyllid species of elasmobranchs in Arabian Sea were recorded. The worms yielded evidence of distributional variety that could be correlated with the size and weight of fish.
RESULTS: The genetic distance depicted by the individual sequences of *Scyphophyllidium* sp. and *R. maccallumi* from other tetraphyllids in the neighbour joining tree were appreciable (0.04-1.5). The negative binomial distribution could explain ($P < 0.001$) the dynamics of cestode populations in Indian sharks. The consistent pattern of positive association of size of tapeworms with the size of marine fish indicated variations in fecundity that also showed association with seasonal periodicity. The increasing size of worms provided greater opportunity of frequent access to available resources that eventually determined growth of parasitic organisms inside the body of elasmobranch fishes. The ultratopography revealed typical differentiable pattern of distribution of filitriches on the scolex and the surface of proglottides. The pace of occupation of available space was also determined by the size ultimately because this was instrumental to restrict the maximum size attained by the tapeworms.
CONCLUSIONS: Therefore, one of the important factors, to determine the carrying capacity within the environment of a particular host which the latter could sustain, turned out to be the size of worms.

# Asymptomatic infection and Malaria epidemiology in Brazil: a systematic review on the possible role of specific antibodies

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BACKGROUND: Asymptomatic malaria is prevalent in malaria endemic regions and has become a serious cause for concern as efforts are increasing towards eliminating the parasite. Interestingly, asymptomatic malaria is not only limited to regions of high transmission where exposure-related immunity is expected to develop; it has also been reported in the low transmission including the Brazilian Amazon Region. The study of naturally acquired immune response in populations exposed to low levels of transmission could provide important information for learning how to improve the management of the disease including the optimal design of a vaccine.
METHODS: A systematic review of the published literature was conducted. Only original research papers were included. Previous studies on humoral immune responses, mainly IgG to *Plasmodium falciparum* and/or *Plasmodium vivax* blood stage antigens and its relationship with degree of exposure, infection status and disease expression in subjects living in the endemic Brazilian Amazon region were eligible.
RESULTS: Eighteen studies met the inclusion criteria. Major differences were observed in IgG subclass distribution of specific antibodies from symptomatic infected patients, asymptomatic parasite carriers, and non-infected subjects living in epidemiologically diverse areas across the Amazon Basin.
CONCLUSIONS: Given the importance of this topic to malaria control in Brazil there are few published studies. The protective role of specific IgGs in naturally acquired immunity could be considered in spite of the unstable transmission levels in Brazil. However, published evidence of the protective role of antibodies to *P. falciparum* or *P. vivax* blood stages is still conflicting.

**Reduction of the *Haemonchus contortus* infective larvae (L3) population on Italian Ray Grass pots by spraying *Butlerius* sp. (nematoda: Diplogasteridae) as a possible model of bio-control of hemonchosis**

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BACKGROUND: Haemonchosis affects sheep flocks worldwide. Anthelmintics are dramatically loosing their efficacy. *Butlerius* sp. is a nematode that captures and feeds on prey nematodes. The reduction of the *Haemonchus contortus* (L3) population on Italian Ray Grass pots by spreading *Butlerius* sp. was assessed as a model of study.
METHODS: Two experiments were simultaneously performed; one was carried out under lab conditions and the second under outdoor conditions. In both experiments four treatment/series of 10 Ray grass pots were established as follows: Series 1 (Control *Butlerius*) received 600 adult *Butlerius* sp; Serie 2 (Control HcL3), received 1500 HcL3; Series 3 (Control water); Series 4, (Interaction predator/prey), received 5 mL of an aqueous suspension containing 600 adult *Butlerius* sp. plus 1500 HcL3 (n=10). Pots in experiment 1 were maintained into the lab and pots in experiment 2, were put outside the lab on a green area close the lab. Metereological parameters were recorded. Pots in both experiments were watered everyday to avoid dryness. Pots remained in incubation for 15 days. Larvae were recovered using the Baermann technique for 12 h. ANOVA and Tukey test were used to analyze data.
RESULTS: In the experiment 1 (under lab conditions) 97% reduction of the HcL3 was recorded and 60% under field conditions.
CONCLUSIONS: Spraying the predatory nematode *Butlerius* sp to Ray grass pots reduces the HcL3 population either at the lab conditions and even under field conditions. These results could have a future implication in research focused to find an alternative method of control of the sheep haemonchosis.

**Study of the proteins of the telosomic complex in *Plasmodium falciparum.***

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BACKGOUND: Telomeres are DNA-Protein complexes that stabilize chromosome ends. From yeast to higher eukaryotes it has been observed that telomeres bind a large number of proteins that constitute the telosomic complex. The telosomic complex is responsible for carrying out many functions essential for cell viability. However, despite functional conservation between organisms, there are some differences between the proteins that are part of this telosome complex between species. Given that some proteins of this complex are species-specific, these proteins could represent potential targets for drug/vaccine design.
This work is focused on the identification and characterization of proteins of the telosome complex in *Plasmodium falciparum*.
METHODS: We searched for the number of proteins that could interact directly with telomeres through southwestern and DNA-UV crosslinking assays. Additionally, we obtained nuclear proteins associated to a probe that contains telomere repeat sequences of P. falciparum through oligonucleotide pull-down assays. These samples were processed by LC-MS/MS to identify the proteins that bound to the telomere. Of 63 Proteins we choose two candidates, based in the domains annotated to these proteins and its possible function predicted by the apicoalign server, to investigate their localization through immunofluoresence assays and their role in the telomere biology of P. falciparum through knock-down assays.
RESULTS: To date we identified at least 6 proteins that interact directly with the telomeres in *P.falciparum*. However, only two of them, named PfAP2t and PfKu70, seem to co-localize with telomeres *in vivo*. PfAP2t is a specific protein of this parasite, whilst PfKu70 is present in other organisms.
CONCLUSIONS: Our results suggest that PfAP2t and PfKu70are part of the telosomic complex in *P. falciparum*. We propose that since Pf2AP2t is a specific telosomic protein of *P. falciparum* it could be considered a potential target for drug design.

**_In vitro_ anthelmintic effect of _Carica papaya_ Latex and _Cocos nucifera_ water against _Haemonchus contortus_ infective (L3) and histotrophic larvae (L4)**

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BACKGROUND: Chemical drugs are loosing their anti-parasitic effect. The *in vitro* effect of *Carica papaya* Latex and *Cocos nucifera* water against *Haemonchus contortus* infective (L3) (HcL3) and histotrophic larvae (L4), (HcL4), was evaluated.
METHODS: *Carica papaya* latex and *Cocos nucifera* water were concentrated and lyophilized. Confrontation was performed into 96 well plates. Two experiments were performed. 1) Two hundred HcL3 into 20 µL of water were deposited into each well (n=4) and 80 µL of the following products were added: 1) Control water; 2) Control Ivermectin; 3) *C. nucifera* at 120 mg/mL; 4) *C. papaya* at 80 mg/mL. Lectures were performed at 24, 48 and 72 h. Sheated (HcSL) and exsheated larvae (HcEL), were evaluated. 2), Fifty microliters of PBS containing 50 HcL4 were deposited per well and 100 µL of the following products were added: 1) Control: PBS (pH=4); 2) control: Ivermectin; 3) *C. nucifera* at 120 mg/mL; 4) *C. papaya* at 120 mg/mL.
RESULTS: *C. papaya* latex did not show an important activity against HcL3. *C. nucifera* water showed 39 and 76% mortality against HcSL and HcEL; respectively at 48h. The highest activity of *C. nucifera* was 97.9 and 98.6% at 72 h with HcShL and HcEL; respectively. A moderate activity (34.3% and 42.9%) was recorded with *C. papaya* and *C. nucifera*; against HcL4, respectively. After 72 h, *C. papaya* latex and *C. nucifera* water showed 54.2% and 100% mortality; respectively ($p<0.05$).
CONCLUSIONS: Coco water contains bio-compounds with an important lethal activity against *H. contortus* both HcL3 and HcL4.

**Migration inhibition and activity of encysted larvae of *Toxocara canis* from a product formulated ivermectin controlled released in in mice with CD-1 strain induced infection.**

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BACKGROUND: *Toxocara canis* is an intestinal nematode common in dogs with zoonotic potential requires continuous control strategies.
METHODS: A controlled released formulation with 3.5% ivermectin against larvae of *Toxocara canis* in mice CD-1 was evaluated. ( groups of 10 mice CD-1 were used inoculating 7 of of them with 500 larvae eggs, leaving 30 days before applying the first treatment with 200µg/Kg subcutaneously; after this time the control group non inoculated nor treated, as well as the positive control and the group 1 (inoculated and treated once). Were killed; later groups 2 and 3 were sacrificed at 60 and 90 days. Brain, heart, lungs, liver, kidneys and striated muscle were obtained from every single animal. Every organ was subjected to artificial digestion in order to measure the larvae condition in the sediment under microscopic conditions. 90 days later, a second treatment was performed and groups 4 and 5 were slaughtered 30 and 60 days after and subjected to the same sequence. To define the migratory the migratory inhibition potential, a group of animals was treated and 30 days later was inoculated with 500 larvae eggs, this group was killed 30 days after and studied under the same sequence. Obtained data were processed using analysis of variance and the Tukey test.
RESULTS: Efficacy percentage was obtained following Wescot equation. An inhibition of 94.25% and a significant decrease of larvae was obtained since the first treatment with 64.52% up to a maximum of 93.13 in the second. For brain and muscle samples there was an inhibition of 83.5% and 95.6% respectively with an initial removal of 43.7% for brain to 65.3% for muscle and final elimination of 92.03% and 90.05 % respectively.
CONCLUSIONS: The obtained results show advantage over other principles and strategies reducing the frequency of preventive treatments with 94.25% of inhibition.

**Ivermectin antiparasitic activity in repeated dose monthly interval against larvae in mice CD1 *Toxocara canis*.**

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BACKGROUND: *Toxocara canis* is an parasite of canine high incidence, pathogenicity and zoonotic potential that requires continuous monitoring.
METHODS: In this study the antiparasitic activity of ivermectin was evaluated against larvae of *Toxocara canis* using three repetitive doses at monthly intervals. 60 mice CD1 strain divided into 6 groups were used, one uninoculated and untreated five groups were inoculated with 600 viable larvae eggs *Toxocara canis* in the five inoculated groups was allowed to evolve infection 30 days, after that period an inoculated group was left untreated and the remaining four were treated with three repeated doses of 200 µg/Kg of subcutaneously ivermectin at monthly intervals. 30 days after the first treatment the negative control group the positive control and one of three treatment groups were sacrificed; the remaining lots were sacrificed 90, 120 and 150 days post treatment. Brain, heart, lungs, liver, kidney and skeletal muscle were removed from each animal. Each organ was subjected to artificial digestion to quantify larvae by microscopic observation of the sediment. The results of the count were organized and analyzed statistically with the method of analysis of variance and Tukey test, the percentage of efficiency was obtained by the equation Wescot generally and specifically for brain and skeletal muscle.
RESULTS: The treated groups showed significant decrease of larvae from the first treatment with 30.5 % up to 83.4 % at last, to the brain was found from the first treatment by 28 % to 77.3 % reduction and skeletal muscle 30.6 % to 87.8 % in the fourth treatment.
CONCLUSIONS: When comparing the results with studies of recent years, the total reduction of 83.4 % of the larvae is within the range of effectiveness observed so far.

**Clinical and epidemiologic characteristics of adult patient´s cohort with chronic Chagas' disease in Colombia, 2000-2012.**

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OBJECTIVES: To determine clinical, electrocardiographic, echocardiographic and radiological abnormalities, in Colombian infected with *T. cruzi* at diagnosis.
METHODS: We performed univariate, bivariate and multivariate statistical analysis. The associations were expressed in odds ratios (OR)
RESULTS: In total, 431 patients were included, 56.6% women, ages ranged from 18-72 years (mean 46.4 ± 11.8 years). Clinically, 28% had chest pain and dyspnea 24%. The most common electrocardiographic findings were: sinus bradycardia 22.3% and right bundle branch block 13.5%. Changes in 24-hour Holter were observed in 113 patients, the premature ventricular complexes, was the most frequent abnormality in 84.0%. Cardiomegaly was identified in chest radiography in 43 patients. Regarding the severity of the disease, 237 patients were classified in Kuschnir 1 and 42 in Kuschnir 2. The factors associated with cardiac symptoms were: age ≥ 65 years (OR 4.36; 95% CI 1.04-18.03), previous medical history (OR 2.58; 95% CI 1.24-5.35) and cardiomegaly (OR 3.82; 95% CI 1.48-9.85). Potentially protective factors were: male gender (OR 0.33; 95% CI 0.14-0.74) and to have a family history of *T. cruzi* infection (OR 0.40; 95% CI 0.19-0.83). Electrocardiographic abnormalities with: FE ≤55% (OR 3.04; 95% CI 1.19-7.72); cardiomegaly (OR 3.01; 95% CI 1.18-7.62). Cardiomegaly with: age ≥54 years (OR 2.91; 95% CI 1.29-6.59), presence of abnormal ECG (OR 3.52; 95% CI 1.44-8.58) and male gender (OR 0.38; 95% CI 0.16-0.90).
CONCLUSIONS: Our results show a Chagas cardiomyopathy established in half the patients in the cohort. The abnormalities found are consistent with this disease. The women´s prevalence has an important role in the risk of vertical transmission. The male gender was a protective factor for heart symptoms and cardiomegaly. This association has not been reported so far and further research is required to support a possible association.

**Safety profile of nifurtimox in the treatment of adult with chronic Chagas' disease**

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BACKGROUND: Nifurtimox is one of the approved drugs to treat Chagas disease, but safety profile data in adults are scarce. We aimed to assess nifurtimox tolerance and safety in a cohort of adult patients with chronic Chagas' disease.
METHODS: Observational, retrospective follow-up study conducted by review of medical records of adults with chronic Chagas disease who were treated with nifurtimox at National Institute of Health from June 2000 through December 2012. Eligible patients received nifurtimox at 7-9 mg/kg/day for 60 days, with regular medical and biological follow-up. Survival curves were constructed for time to treatment interruption (Kaplan–Meier method). The occurrence of Adverse Reactions Drug (ADRs) as a risk factor for treatment interruption was analyzed using a parametric time-to-event model. The associations were expressed in relative times (TR)
RESULTS: In total, 85 patients were treated with nifurtimox. Ages ranged from 18 to 67 years (mean 42.0+11.1 years); The dose ranged from 360 to 780 mg/day (mean 539.5 + 88.4 mg/day) and the duration of treatment ranged from 9 to 60 days (mean 53.6 + 13.2 days; median 60.0 days). Among the patients who received nifurtimox, 67 had ADRs and 59 of these interrupted treatment. Regarding the severity of ADRs, 39 had mild, 19 had moderate and 1 had a severe ADRs. In terms of causality of ADRs, 24 (35.8%) as probable, 27 (40.3%) as possible, and 16 (23.9%) as definite. Gastrointestinal symptoms predominated followed by neurological disorders. The Kaplan–Meier curves stratified by the severity of ADRs showed differences in mean survival times between the intensity of the adverse reaction and the interruption of nifurtimox. According to the conventional model Generalized Gamma, male patients have longer survival times rather than female (TR 4.52; 95% CI 3.29-6.20; P <0.001); likewise, patients older than 65 years have lower survival times than those who younger (TR 0.25; 95% CI 0.15-0.41 P <0.001); similarly, patients with subsidized regime have lower survival times than those with contributive regime (TR 0.40; 95% CI 0.28-0.58 P <0.001); in addition, the dose of nifurtimox higher than 8 mg / kg / day reduces survival times (TR 0.35; 95% CI 0.24-0.50 P <0.001); and similarly, the presence of more than three ADR decreases survival times (TR 0.26; 95% CI 0.18-0.36 P <0.001).
CONCLUSIONS: Our results suggest that nifurtimox treatment in adults infected with *T. cruzi* is safe. Despite the high rate of ADRs, they were associated with low morbidity and in few cases the interruption of treatment was required. It is recommended to consider the risk factors found in this research to reduce interruption of treatment due to ADRs.

**Association between serum IgE and IgG levels in malaria-helminth co-infection in Colombia.**

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INTRODUCTION**:** In tropical regions, overlapping distribution of various parasites, including *Plasmodium spp.* and helminths results in a high rate of co-infection. Interactions between these parasites can have an influence on the human immune system.
OBJECTIVE**:** to assess the associations between serum IgE and IgG levels in patients with uncomplicated *falciparum* malaria (case) and controls without malaria and compared in the absence and presence of co-infection with helminths.
METHODS**:** a case-control study in Colombia. Serum IgE and IgG levels and the prevalence of helminths infection in both groups were determined. Conditional logistic regression was performed for high/normal IgG (normal IgG 700-1600 mg/dL; high >1600 mg/dl), and for high/normal IgE (IgE normal for age 3 to 16 years <280 IU/ml and high IgE >280 IU/ml; for age >16 years, normal <200 IU / ml and high> 200 IU/ml), which considered the matching of cases and controls, adjusting for the presence of helminths, and a multiplicative interaction between helminths and malaria.
RESULTS**:** In total 224 patients were included, 63 cases and 161 controls. As for the cases were found; infections with *Ascaris lumbricoides* 25.37%, *Trichuris trichiura* 26.87% and *Hookworm sp.* 35.82%. With controls were observed, a higher percentage of *A. lumbricoides* infection in 31.64% and lower infection rates for *T. trichiura* 20.90% and *Hookworm sp* 17.42%. The associations between high/normal IgG were: with to be case (OR 2.19, 95% CI 0.81-5.90); to have helminths (OR 1.51, 95% CI 0.65-3.51) and malaria-helminth interaction (OR 0.53, 95% CI 0.11-2.58 ). For high/normal IgE were: to be case (OR 0.71, 95% CI 0.15 to 3.29), to have helminths (OR 1.66, 95% CI 0.39-6.97) and malaria-helminth interaction (OR 1.70, 95% CI 0.10-27.1).
CONCLUSIONS**:** The results showed no significant associations between serum IgE and IgG levels with to have uncomplicated malaria by *P. falciparum*, to have helminths or to have malaria-helminth co-infection. However, it is important to note that for IgG an apparent antagonistic effect of malaria-helminth co-infection was observed. by contrast to IgE, a synergistic interaction was evident. Thus, the total effect of to be a case in malaria-helminth co-infection was: OR: 1.21 (0.71 * 170 = 1.21) and the total effect of to have helminths in malaria-helminth co-infection was: OR : 2.8 (1.70 * 1.66 = 2.8). We suggest future research to assess specific immune response in order to elucidate the possible implications of co-infections on the humoral immune response.

# Molecular detection of *Entamoeba moshkovskii* for the first time beside *Entamoeba histolytica*, and *Entamoeba dispar*, in cohort of diarrheic Egyptians

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BACKGROUND: Amebiasis is infection by *Entamoeba* (*E.*) *histolytica*, regardless of associated symptomatology. The presence of separate but morphologically indistinguishable nonpathogenic species from *E. histolytica* lead us to use multiplex PCR assay to detect and genetic differentiate *Entamoeba* species; *E. histolytica, E. dispar* and *E. moshkovskii*, in diarrheic stool of cohort of Egyptians.

METHODS: A total of 504 human fecal samples were collected from diarrheic/dysenteric patients attending Beni-Suef University Hospital Outpatient's Clinics. Fecal samples were examined for intestinal parasites including *Entamoeba* species microscopically using direct wet smear and concentration methods and were subject to multiplex PCR for detection and differentiation of *E. histolytica*, *E. dispar*, and *E. moshkovskii*.

RESULTS: The overall coproscopic prevalence of *Entamoeba* species was (20.4%) that surpassed molecular prevalence (10%). Using multiplex PCR *E. histolytica* was less prevalent (1.4%) followed by and *E. moshkovski* (3.3%), *E. dispar* (4.6%) was the most prevalent species, with mixed *Entamoeba* infection was detected in 0.8% of cases.

CONCLUSIONS: *E. moshkovskii* was reported for the first time among Egyptians populations with coexistence with *E. histolytica*. The *E.histolytica* was much less prevalent and 6 times lower than non-pathogenic amoebae. Coproscopy for detection of *E. histolytica* showed limited diagnostic performance with false-positive and false negative results. There is a need to implement PCR assays as a practical non-microscopic method that can differentiate between amoeba infections to determine the true prevalence of *E. histolytica* for its better management and control.

**Inflammasome activation by DNA carrying immunecomplexes from malaria patients.**

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BACKGROUND: Each year, at least 200 million people become infected with *Plasmodium vivax* or *P. falciparum,* which cause a severe febrile illness that contributes to social and economic instability in developing countries. High levels of pyrogenic cytokines such as IL-1β, TNF-α and IFN-γ produced during infection by *Plasmodium* exacerbate host immune responses causing various debilitating symptoms. The studies performed *in vivo* and *in vitro* suggest that Toll-like Receptor (TLR) 9 is critical for the onset of this early inflammatory response in malaria. This project focuses on how parasite DNA is carried to the inner cellular compartment and activates TLR9. We observed the presence of high circulating levels of immune complexes (ICs) (by C1q ELISAS) and parasite DNA (by qPCR) during acute episodes of malaria. We also detected high levels of both IgM and IgG anti-dsDNA in individuals undergoing acute malaria episodes. Furthermore, ICs from malaria patients were found to contain primarily parasite derived DNA. Evaluating the immunostimulatory activity, we observed that ICs induced high levels of TNF-$\alpha$ and IL-10 (measured by CBA) as well as caspase-1 activation (by immunoblot) and IL-1$\beta$ (by ELISA) release by peripheral blood mononuclear cells (PBMCs) and purified monocytes from healthy donors. When monocytes were primed with IFN$\gamma$, stimulation with ICs resulted in a switch of cytokine profile, similar to monocytes from malaria patients that produce high levels of IL-1β and TNF-α, but low IL-10 levels. Importantly, cytokine production by PBMCs stimulated with ICs was inhibited with the TLR7/TLR9 antagonist (ER-6446, EISAI Pharmaceuticals), but not the TLR4 antagonist.
CONCLUSIONS: Hence, ICs are important shuttles for carrying parasite DNA to inner compartment of innate immune cells, resulting in activation of nucleic acid sensing TLR9 and signaling for inflammasome assembly. Altogether, our results support the hypothesis that ICs are important components of the systemic pro-inflammatory response and pathogenesis of malaria.

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**Biological characterization and genotyping of *Trypanosoma cruzi* isolates from Oaxaca, México.**

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BACKGROUND: Chagas' disease is caused by the parasite *Trypanosoma cruzi*. The parasite strains are very heterogeneous and have been classified into six discrete typing units (DTU from TcI to TcVI) that present differences in their morphology of blood forms, virulence, and ability to induce injury, susceptibility to chemotherapeutic agents, antigenic constitution and infectivity.
METHODS: The present study was conducted to obtain and characterize seven *T. cruzi* isolates obtained from triatomine infected feces collected in Oaxaca, Mexico. The growth rates, metacyclogenesis, morphometric characteristics and infectivity in culture cells and mice were evaluated in all the isolates. The genotype of the seven isolates and three reference strains was determined by the amplification of three molecular markers: non-transcribed spacer of mini-exon genes, divergent domain of 24Sα rRNA and size-variable domain of the 18S rRNA sequence.
RESULTS: Similar levels of growth rates and morphometric characteristics were observed in the isolates. However, differences in parasite transformation and *in vitro* and *in vivo* infection efficiency were identified. The genotyping analysis indicated that all the isolated parasites correspond to TcI, but one of them was mixed with a TcV population.
CONCLUSIONS: *T. cruzi* isolates obtained from triatomine collected in Oaxaca show distinct biological features. However, they all are classified as TcI. Interestingly one of the isolates was mixed with TcV, a DTU not reported before en Oaxaca.

**Feeding behavior of triatomines captured in Oaxaca, Mexico.**

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BACKGROUND: Chagas disease is caused by *Trypanosoma cruzi*, which is transmitted by reduviid hematophagous insects from the Triatominae subfamily. These insects feed on blood from a wide range of hosts, and the study of their feeding behaviors has been used to understand the ecology and epidemiology of *T. cruzi*. Since many potential vectors exist, the importance of their presence within a particular region depends greatly on its feeding habits and host preference, beside the ecology of the area. Therefore, determining the source of the blood meals help to understand, the natural feeding patterns of the vector species, identify potential reservoirs, improve the knowledge of Chagas disease epidemiology and for the design of rational vector control strategies in a specific area. Despite this, very little is known about the feeding behavior of triatomines in Mexico, especially in highly endemic states such as Oaxaca.
METHODS: In this study, feeding sources and *T. cruzi* infection indices were determined in 139 triatomines captured in domestic and peridomestic areas of Oaxaca, using heteroduplex assays of cytochrome b and PCR, respectively.
RESULTS: Heteroduplex findings revealed that the main source of meal is human's blood, followed by blood from poultry and domestic animals. The *T. cruzi* infection indices were 27 %, being *Triatoma barberi* the most infected vector specie.
CONCLUSIONS: The finding of infected triatomines whose main source of blood meal is humans, indicates a high transmission risk of Chagas disease in Oaxaca.

# Molecular and Functional Characterization of the RNA Binding Capacity of the EhCFIm25 Protein from *Entamoeba histolytica*

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BACKGROUND: The poly(A) tail formation at mRNA 3´UTR is a fundamental process for gene expression regulation in eukaryotes. In human, the CFIm25 protein is an essential factor of the pre-mRNA 3′UTR processing machinery. Notably, its binding to the UGUAN sequence in 3´UTR regulates the recruitment of other polyadenylation factors, the selection of the cleavage site and the size of the poly(A) tail, thus contributing to mRNA integrity, transport, stability, and translation. *Entamoeba histolytica,* the protozoan responsible for human amebiasis, has a conserved polyadenylation machinery that includes the EhCFIm25 protein which is a RNA binding protein that interacts with the poly(A) polymerase in this pathogen. Here, we aimed to identify the amino acids that are important for the RNA binding capacity of EhCFIm25
METHODS: Amino acids were chosen from multiple sequence alignment of homologous CFIm25 proteins by ClustalW software and literature review. Selected residues were replaced by alanine by site-directed mutagenesis assays and their relevance for RNA binding was assessed through REMSA using the recombinant wild type and mutant EhCFIm25 proteins and the 3´UTR of the *EhPgp5* gene as a probe. The folding of wild type and mutant proteins was also evaluated by Molecular Dynamics (MD) and Circular Dichroism (CD).
RESULTS: *In silico* analyses showed the conservation of Leu135 and Tyr236 amino acids (in EhCFIm25) among homologous proteins from diverse organisms. Their independent change to alanine totally abolished the formation of the RNA/protein complex in REMSA, indicating that both Leu135 and Tyr236 amino acids are essentials for the RNA binding capacity of EhCFIm25. MD and CD experiments revealed that the secondary/tertiary structure is not affected in mutant proteins.
CONCLUSIONS: Our *in vitro* and *in silico* strategy evidenced the relevance of conserved Leu135 and Tyr236 amino acids in EhCFIm25 protein for RNA binding activity. Further experiments currently in progress will help to elucidate the functional role of this protein in the formation of poly(A) tail at the 3' end of mRNA in *E. histolytica.*

# Winter and summer larval cestodiasis on krill *Nyctiphanes simplex* (Hansen) in the Gulf of California, Mexico

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BACKGROUND: Krill cestodiasis has been historically studied worldwide through incidental findings (often just one infected host) and/or from non-quantitative sampling efforts. Thus, the ecology and effect of parasite infection is poorly understood. This work shows cestode infection on krill collected during winter (January) and summer (July) of 2007 in the Gulf of California, Mexico.

METHODS: From systematic samples collected with bongo nets we characterize parasitic infection estimating, prevalence, intensity, diversity, distribution, internal (histology) and external (SEM) morphology of four types of cestodes.

RESULTS: *Nyctiphanes simplex* accounted for 64 % (winter) and 84 % (summer) of all species of krill collected in both cruises being infected by four types of cestodes (sensu lato) Trypanorhyncha: *Hemionchos*, Tetrarhynchobothriidae, Tentaculariidae. and Tetraphyllidea incertae sedis. Cestod intensity was typically 1 except for Tetraphyllidea (intensity > hundredths); all them hosted in the hemocoel. Cestodes had higher prevalence during summer (0.23 to 14.2%) than during winter (0.03 to 8.8%). *Hemionchos sp*. had the highest prevalence and abundance infecting hosts from the larval furcilia phase to adulthood. Females were most frequently parasitized including some ovigerous females suggesting that, cestode infection does not necessarily end in castration. Tentaculariidae and Tetraphyllidea (discovered in August 2012) likely caused the most virulent infection with typical pathology. The spatial distribution of hosts with cestodiasis (prevalence) indicates that locations with large prevalence were highly heterogeneous and it does not necessarily coincide with centers of high host abundance.

CONCLUSIONS: Contrary to our expectations *N. simplex* was rarely infected by acanthocephalans, trematodes and nematodes and highly parasitized by cestodes. Cestodes of krill probably infect and complete their life cycle infecting elasmobranches and baleen whales (this latter consume high amounts of krill) and this will be investigated in the predators that inhabit the Gulf of California in the near future.

# Gliding motility of *Babesia bovis* merozoites

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BACKGROUND: *Babesia bovis*, a causative agent of a zoonotic babesiosis transmitted by tick, is an intraerythrocytic protozoa of the phylum Apicomplexa. Parasites belong to this phylum invade their target host cells with a unique active process known as a gliding motility, which has been known for tachyzoite stage of *Toxoplasma gondii* and sporozoite stage, but not merozoite stage, of malaria parasites. However, it was not visualized how *Babesia* merozoites target and invade the host red blood cells (RBCs) under microscope, and it was also not known if this parasite exhibits gliding motility or not.
METHODS: In this study, we employed time-lapse video microscopy to trace the parasite expressing fluorescent protein from the merozoite egress from the infected RBC until completion of the RBC invasion. In addition formation of the parasitophorous vacuole during the invasion and its breakdown were also observed using membrane-stained bovine RBCs.
RESULTS: We found the gliding motility of *B. bovis* merozoites was similar to the helical gliding motility of *Toxoplasma* tachyzoites. The trails left by the merozoites were visualized by indirect immunofluorescence assay using antiserum against parasite surface protein. Inhibition of gliding motility by actin filament polymerizer or depolymerizer indicated that this movement was driven by actomyosin-dependent motor system. Also, we revealed that the parasitophorous vacuole formed during invasion was broken-down within ten minutes after invasion.
CONCLUSIONS: We revealed that *B. bovis* exhibits gliding motility. Merozoites of both *Babesia* parasites and malaria parasites invade into and grow in the host RBCs, but the behavior of released merozoites in the blood are unexpectedly different, former motile and the latter not. Gliding motility of *Babesia* parasites may serve as a previously unrecognized pathogenic factor.

**Efficacy and target of endoperoxide N-89 and N-251 against larval stage *Schistosoma mansoni***

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BACKGROUND: Schistosomiasis is an important parasitic disease in South-America, Asia and Africa. Praziquantel is used as a main drug for schistosomiasis, but in consideration of its drug-resistant strains, development of new drug is needed. Recently, some papers reported several antimalarial drugs, such as artemisinin derivatives, also have killing effects against schistosomes. Moreover, N-89, synthesized as antimalarial drug based on endoperoxide structure of artemisinin, had antischistosomal effect in vivo. In this study, we evaluated the effect of N-89 and N-251 with hydroxyl groups on its side chains against larval stage *Schistosoma mansoni* (schistosomulum) and investigated the targets of these drugs inside the schistosomula.
METHODS: In order to check the efficacy of N-89 and N-251, we treated the mechanically transformed schistosomula with each drug. The survival rate was calculated by larval count under the microscopy at 7 days of the treatment. To confirm drug uptake, we observed co-localization of rhodamine-conjugated N-251 and organelle markers. To examine what the effect was induced by N-251 against schistosomula, we observed ultrastructure of the treated schistosomula at 2h and 16h after treatment under the transmitted electron microscopy (TEM).
RESULTS: Both N-89 and N-251 had killing effect against schistosomula rather than artemether, artemisinin derivative (EC50=N-89 : 19.9μM, N-251 : 12.9μM, artemether : 174.9μM). The accumulation of rhodamine-conjugated N-251 was observed in organelles stained by LysoTracker (acid organelle marker). Treated schistosomula with N-251 for 2hrs, the number of acid organelles increased compared to control group, while they almost diminished at 16h after treatment. Furthermore, the damages of lysosome-like organelle/some vesicles inside the glands were observed in the treated schistosomula, but less around the other areas.
CONCLUSIONS: N-89/N-251 had poweful antischistosomal effect in vitro. They targeted to the acid organelles and damaged them, and finally may kill schistosomes.

**Metronidazole induces DNA damage in *Giardia duodenalis***

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BACKGROUND: Metronidazole (Mtz) has been historically used for the treatment of Giardiasis. Up to date, the mechanisms involved in Mtz resistance in *G. duodenalis* have involved reduction of the PFOR enzyme activity necessary for generation of Mtz radicals which forms adducts with proteins like thioredoxin reductase, α-β giardins and possibly DNA damage, resulting in the trophozoite death. In this study we aimed at determining whether Mtz generates DNA double strain break (DSB) in *G. duodenalis* trophozoites. By other hand H2AX phosphorylation is a well-known marker for DSB DNA damage.
METHODS: The DL50 of Mtz in the trophozoites of *G. duodenalis* WB strain clone C5 was determined by sub-culture in liquid media method. A TUNEL assay was carried out to detect general DNA damage and then specific DSB damage was assessed by determining phosphorylated H2AX through western blot analysis in G. duodenalis.
RESULTS: The Mtz produces a 5 change-fold of the H2AX phosphorylation and DNA damage as observed by TUNEL assay.
CONCLUSIONS: Our results demonstrated that Mtz treatment produces DSB damage in *G. duodenalis* WB strain clone C5. These results open possibilities to explain Mtz resistance in regard to DNA repair machinery.

**Freshwater anisakids of zoonotic significance with epigenetic variations**

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It has not been common till recent years to find infestations by anisakid larvae in freshwater fish of Northern India. But appearance of these causative agents of anisakiasis commenced in 1998, and soon adult worms of *Rostellascaris spinicaudatum* (Malhotra and Anas, 2001) were recovered in plenty in catfishes of the Gangetic riverine ecosystems (Lon82$^0$06'04.80"E, Lat25$^0$16'06.92"N, Alt64m). The genetic distances of the latter from the co-occurring larvae of *Contracaecum* sp. and that of *Anisakis* Type III larvae were 1.76 and 1.637, respectively. The analysis of 1$^{st}$ Internal Transcribed Spacers of rDNA gene revealed that the larger clade of morphologically closer *Iheringascaris* adult & its larvae exhibited a sparse 84% affinity for alignment with *Rostellascaris* from fish of River Ganges. The economic and health significance of the worms of Anisakidae are known worldwide. The larvae of *Anisakis* Type III were encountered during 2013, in catfish, *Rita rita* that inhabited upstream freshwater river Ganges near Fatehpur, U.P. The occurrence of such larvae has frequently been reported from the coastal dolphins worldwide, and the riverine stretch between Allahabad to Fatehpur is commonly inhabited by riverine dolphins since past century. Therefore, further investigations would be required to establish mechanism of their transport from coastal to freshwater zones in Northern India. Though the occurrence of clusters of sunflower pre-cloacal papillae brought these closer to *Goezia bangladeshi* (Akther et al., 2004) of Goeziinae, their alignment with sequences of *Anisakis* Type III larvae, reported from marine fishery, established predominance of epigenetic variations in larval characteristics of these exotic variety in freshwaters. Since these Anisakis typical Type III larvae were never reported from freshwater gangetic fish during the past century, the characteristics of bifurcated, bulbous intestinal ceca and sunflower pre-cloacal papillae on 'cactus' tail would require further analysis to work out concrete evidence of their phylogenetic interrelationships.

**Genome-wide investigation of target genes of a *Plasmodium* AP2 family transcription factor AP2-O**

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BACKGROUND: Malaria parasites have 27 genes encoding the AP2 (Apetala2) family of DNA-binding proteins, the only known transcription factor for this parasite to date. We previously reported that a member of the AP2 family of proteins, designated as AP2-O (Apetala2 in ookinete), is responsible for stage-specific transcriptional regulation in the *Plasmodium berghei* ookinete, which is a motile stage that invades the mosquito midgut. AP2-O has a single AP2 DNA-binding domain and binds to the promoter region of target genes, thereby inducing genes involved in mosquito midgut-invasion. In this study, to identify the role of AP2-O in ookinete development and, more generally, to investigate the basic features of transcriptional regulation in malaria parasites, we attempted genome-wide identification of AP2-O target genes using ChIP-Seq (chromatin immunoprecipitation followed by high-throughput sequencing).

METHODS: ChIP-seq was performed using transgenic *P. berghei* expressing GFP-tagged AP2-O. AP2-O targets were predicted on the basis of the distance from each gene to AP2-O binding sites identified by ChIP-seq.

RESULTS: ChIP-seq data revealed that AP2-O specifically bound to upstream genomic regions of more than 500 genes including all ookinete genes previously reported, suggesting that approximately 10% of the total genes are directly regulated by AP2-O. They were involved in distinct biological processes such as morphogenesis, locomotion, midgut-penetration, protection against mosquito immunity, and preparation for subsequent oocyst development.

CONCLUSIONS: AP2-O organizes distinct processes of ookinete biology as a master transcription factor. This direct and global regulation by AP2-O provides a model of gene regulation and may explain the paucity of sequence-specific transcription factors in apicomplexan parasites.

**Signaling the genome of ancestral *Leishmania*: A look into Ras family GTPases.**

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BACKGROUND: The digenetic genera *Leishmania* are thought to have arisen from a monogenetic ancestor, *Leptomonas,* with estimated divergence of one million years. *Leishmania* is a eukaryotic pathogen of socio-economic importance. Our recent report of co-existence of *Leptomonas* in clinical isolates of *Leishmania donovani* from India calls for development of entirely new strategies for the treatment of this neglected parasite disease.
METHODS: Utilizing next generation sequencing technology we have explored the genomes of the two clinical isolates from India to answer a wide range of evolutionary and pathological questions. Whole genome sequencing confirmed one isolate to be *L. donovani* while the other was confirmed to be *Leptomonas* like. With the availability of complete genome sequences with us of these Kinetoplastid species *Leishmania* and *Leptomonas* representing important evolutionary branch points, we have analyzed their complete genomes for Ras protein superfamily namely Rab GTPases.
RESULTS: Cellular organization and signaling in both unicellular and multicellular organisms is heavily influenced by the Ras superfamily of small GTP-binding proteins, which maintain a structurally and mechanistically preserved GTP-binding core despite considerable divergence in sequence and function. Our lab is involved in determining therapeutic strategies to inhibit the function of Rab GTPases. Rab protein families also constitute important evolutionary events. We observed a noticeable decrease in the number of Rab superfamily orthologues in *L. donovani* is which is probably due to the loss of ancestral genes. *Also g*ene duplication events seem to have produced an accumulation of paralogous sequences, which probably results in variation between these species analysed in terms of the numbers of each Rab proteins
CONCLUSIONS: Ancestral diversification in terms of Rab protein superfamily could be discerned between ancestral *Leishmania.*

**Ceragenin CSA-13 on infection with *Leishmania* (*L.*) *mexicana* in the search for new therapeutic agents against cutaneous Leishmaniasis**

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BAGROUND: The toxicity of drugs currently used against leishmaniasis, and the development of resistant parasites demonstrate the importance of evaluating new therapeutic agents with leishmanicidal activity safe and effective. Previous studies demonstrated in vitro antileishmanicidal activity of the Ceragenin 13 (CSA-13) a Cationic Steroid Antibiotic against *Leishmania (L.) mexicana.* Therefore, we evaluate the effect of CSA-13 on *L. (L.) mexicana* infection in a murine model.
METHODS: $10^3$ amastigotes of L. (L.) mexicana (MHOM/BZ/BEL21) were inoculated in the footpad of female BALB/c mice, 3 weeks post-infection CSA-13 was applied to groups of mice at various doses (4, 8, 16, 32, 54, 72, and $10x10^3$ $5x10^3$ ng/g body weight/day) subcutaneously, intralesional, for 3 consecutive days. We evaluated the progress of the disease for 10 weeks by measuring the parasite load in the cutaneous lesions using the limiting dilution technique and the footpad swelling (lesion size) was determined weekly by digital morphometry using tpsDig2 program. Additionally we assessed the influence of CSA-13 on the weight of experimental animals and blood chemistry parameters. RESULTS: A reduction of the parasitic load in the groups of mice treated with 4, 8, 16, 32, 54 and 72 ng/g weight/day CSA-13 was evidenced, after 3 weeks post-treatment, with respect the control group (PBS), we also determined that the dose 4 and 8 ng/g weight/day of CSA-13 were more effective in terms of decreasing the size of the cutaneous lesions in BALB/c mice infected with *L. (L.) mexicana*. Moreover, the CSA-13 did not affect the weight of BALB/c mice but causes a slight alteration of blood levels of transaminases, and blood glucose amylase.
CONCLUSIONS: Ceragenin 13 proven to decrease lesion size and parasite load. This antibiotic might represent a useful tool in combination with new drugs against cutaneous leishmaniasis. Funded by: CDCH UCV PSU-09-7878-2009/1; FONACIT G-2005000375 MS

**The role of nucleoside transporters during asexual phase of *Plasmodium berghei* parasites**

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BACKGROUND: Protozoan parasites rely on the host for the supply of purine nucleosides because they are unable to synthesize purine rings *de novo*. Nucleosides and nucleobases are transported across the parasite plasma membrane by nucleoside transporters (NTs) of *Plasmodium* spp. The *P. falciparum* (*Pf*) genome sequencing project revealed four nucleoside transporters, PfNT1, PfNT2, PfNT3 and PfNT4. In the genome of *P. berghei* (*Pb*) ANKA which is a rodent malaria parasite, the orthologues of PfNT1, PfNT2 and PfNT4 were identified. However, the roles of PbNT1, PbNT2 and PbNT4 in *Pb* ANKA remain unclear.
METHODS: To investigate the roles of PbNT1, PbNT2 and PbNT4, we generated *pbnt1*–disrupted *Pb* ANKA (Δ*pbnt1* parasites), *pbnt2*–disrupted *Pb* ANKA (Δ*pbnt2* parasites) and *pbnt4*–disrupted *Pb* ANKA (Δ*pbnt4* parasites), and evaluated the effect of *pbnt1*, *pbnt2* or *pbnt4* disruption on the outcome of infection with *Pb* ANKA.
RESULTS: We showed that the rapid increase of wild-type *Pb* ANKA (WT parasites) in mice early in infection was significantly inhibited by disruption of *pbnt1*. Moreover, Δ*pbnt1* parasite-infected mice showed neither cerebral paralysis nor cerebral haemorrhage, and all mice spontaneously recovered from infection. By contrast, mice infected with *pbnt2* or *pbnt4* parasites showed features similar to those of mice infected with WT parasites.
CONCLUSIONS: We demonstrated that the high virulence of *Pb* ANKA in the asexual phase is suppressed by disruption of *pbnt1* but not *pbnt2* or *pbnt4.*

**Helminths suppress streptozotocin-induced diabetes via STAT6- and IL-10- independent mechanisms**

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BACKGROUND: There is experimental and epidemiological evidence supporting microbial or parasitic infections protect hosts from type-1 diabetes (T1D).
METHODS: Here we investigated anti-diabetic effects of *Schistosoma mansoni* (Sm) and *Heligmosomoides polygyrus* (Hp) in an inducible T1D model: multiple low-dose streptozotocin (STZ)-induced T1D in mice. Male C57BL/6 mice were infected with Sm cercariae (6weeks prior) or Hp infective larvae (1week prior) to the STZ injection. To induce T1D, 50mg/kg/day of STZ were injected to the infected mice during 5 sequential days. Blood glucose levels were weekly monitored. The pancreatic islet size was analyzed by HE staining. Gene expressions in the pancreas or in the pancreatic lymph nodes (LN) were measured by real-time PCR.
RESULTS: Both Sm and Hp partially but significantly suppressed hyperglycemia induced by STZ injection. Degradation of pancreatic islets in the STZ-injected mice was partially prevented by helminth infections. Expressions of pro-diabetic mediators (IL-1beta and TNF-alpha) were lowered by the infection. Regarding Th2 cytokines, IL-4 and IL-13 expressions were increased whereas IL-10 expression did not change in the infected mice. Moreover, M2 macrophage marker genes were up-regulated in the infected mouse PLN. As in wild-type (WT) mice, Sm and Hp reduced hyperglycemia in STAT6 KO mice and in IL-10 KO mice.
CONCLUSIONS: The results obtained suggest that IL-4/IL-13 and IL-10 signaling are not essential for the anti-diabetic effects of the helminths.

# *Plasmodium knowlesi* thioredoxin peroxidase 1 binds to nucleic acids and has RNA chaperone activity

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BACKGROUND: Malaria parasites are highly sensitive to oxidative stress but live in a pro-oxidant rich environment containing oxygen and iron. Therefore, their antioxidant defenses are crucial for their survival and being considered as an interesting target for antimalarial drug development. Peroxiredoxins (Prxs) is a ubiquitous family of antioxidant enzymes and play a vital role in the reduction of reactive oxygen species in malaria parasites. *Plasmodium knowlesi* like *P. falciparum* can cause severe and fatal disease and it is being considered as emerging human malaria parasite in Southeast Asia. Better understanding of basic biology of *P. knowlesi* is crucial for control of the disease. In this study, we cloned and characterized thioredoxin peroxidase 1 (PkTPx-1) which is a member of Prx family from *P. knowlesi*.
METHODS: PkTPx-1 gene was cloned and recombinant protein (rPkTPx-1) was produced by heterologous over-expression in *Escherichia coli*. rPkTPx-1 was used for evaluation of enzymatic activity and polyclonal antibody production.
RESULTS: Using rPkTPx-1, its antioxidant activity was confirmed in a mixed function oxidation assay where PkTPx-1 prevented nicking of plasmid DNA by hydroxyl radicals. In addition, it was found that rPkTPx-1 was able to bind to nucleic acid. Moreover, rPkTPx-1 had RNA chaperone activity in a nucleic acid melting assay indicating new function of PkTPx-1 other than antioxidant activity. Using specific polyclonal antibodies against rPkTPx-1, it was indicated that PkTPx-1 is expressed in the cytoplasm of the parasite.
CONCLUSIONS: Altogether, these results suggest that PkTPx-1 not only protect the parasite from adverse effects of reactive oxygen species but also has RNA chaperone activity.

**Wheat germ cell-free protein synthesis system (WGCFS): a breakthrough for the post-genome vivax malaria research**

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While malaria mortality is mainly due to the infection of *Plasmodium falciparum*, *P. vivax* remains to be a huge burden to the communities in Asia, Pacific, and Central/South America. The development of vaccines against *P. vivax* is an essential component toward malaria elimination, however, there are only a few vivax malaria vaccine candidates being tested in clinical trials. The reason for the limitation on *P. vivax* research is mainly due to the difficulties in both *P. vivax* parasite culture in vitro and the lack of efficient recombinant protein expression platform. In order to facilitate the post-genome vivax research, we applied the wheat germ cell-free protein synthesis system (WGCFS), which is greatly advantageous over *E. coli* based cell-free system in obtaining high yield of quality recombinant proteins. One of the applications of the WGCFS on vivax research is an antibody profiling against arrays of vivax parasite recombinant proteins. We cloned a few hundreds of *P. vivax* blood-stage specific genes, successfully expressed > 90% of the proteins as soluble recombinant proteins using WGCFS, and probed the protein array with human sera from vivax infected individuals. Profiling antibodies in sera that differ in immune status against the proteins helped being able to discriminate antibody patterns between malaria-naïve and infected sera and hence identification of antigens. These data that recombinant *P. vivax* proteins were immunoreactive to human antibodies suggest that the folding of the WGCFS expressed proteins were similar to that of the native *P. vivax* antigens and hence these proteins are amenable for future vaccine and serological marker studies. We also present a characterization study of a novel erythrocyte-binding protein of *P. vivax*, PvMSP1P, identified using WGCFS. PvMSP1P is a novel surface antigen of *P. vivax* merozoite that binds to erythrocytes and it might play an important role in the merozoite

invasion process.

**Treatment failure, drug resistance and VL control in the Indian subcontinent: lessons learnt from a multidisciplinary research project (Kaladrug-R).**

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BACKGROUND and METHODS**:** We conducted a multidisciplinary study (Kaladrug-R) to monitor drug resistance and treatment response in visceral leishmaniasis (VL) in the Indian subcontinent (ISC), focusing on Miltefosine (MIL), the drug currently used in the VL elimination programme, and antimonials (SSG) the former drug, now abandoned in the region.
RESULTS**:** We found a substantial decline in the effectiveness of MIL (up to 20 % relapse within 12 months), and children were more at risk for MIL relapse due to underexposure. The observed decline of MIL effectiveness could not be related to *in vitro* defined MIL resistance, but did correlate with increased parasite infectivity.
Mathematical modelling and *in vitro*/*in vivo* studies indicated that commonly present SSG-R parasites in the ISC were more fit (increased infectivity and better host manipulation skills) than their sensitive counterparts, highlighting the importance of assessing the legacy of decades of SSG-use. The increased infectivity observed in both SSG-R parasites and parasites from MIL-relapsed patients indicates that this may be a common mechanism to survive treatment of the host.
The whole genome of 203 *L. donovani* clinical isolates was sequenced, providing unexpected information about (i) the parasite's resistome and (ii) an unprecedented view on the evolutionary history of that species over the past epidemics in the ISC.
In addition, *in vitro* induction studies showed that resistance to paromomycin was easily induced at the *in vitro* amastigote level.
A mathematical modelling approach was applied to better understand the dynamics of VL and highlighted the impact of asymptomatically infected individuals on *L. donovani* transmission. Besides chemotherapy, vector control thus appears to play a major role to achieve VL elimination.
CONCLUSION**:** Our research demonstrates the complexity of the problem of treatment failure and drug resistance in VL and highlights the need of multidisciplinary approaches to tackle it.

**Towards unbiased molecular biology of Protozoa: an integrative approach to investigate the survival and adaptation skills of *Leishmania donovani***

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BACKGROUND: Although *L. donovani* in the Indian subcontinent (ISC) is a genetically homogeneous parasite population, phenotypic diversity at the level of drug susceptibility exists. We aimed to assess the genetic diversity of *L. donovani* in ISC and how it may have impacted parasite survival skills.
METHODS: The genomes of 203 *L. donovani* clinical isolates from the Indian subcontinent were analyzed and LC-MS metabolomics was performed on a small subset of strains.
RESULTS: The main population in our sample set (Core-191) was genetically homogeneous, but contained 6 genetically distinct sub-populations and a variety of admixed strains. Sub-population ISC005 deserves particular attention as it contains parasites that are *in vivo* resistant to SSG. ISC005 is estimated to have radiated around 50 years ago, after a major population bottleneck (DDT campaign) and at times when the SSG efficacy decline was first reported. We find a series of genomic adaptations in ISC005, such as characteristic SNPs, indels in the aquaporin 1 gene, copy number of episomes or entire chromosomes. Among the genes concerned by SNPs or CNVs, many are hypothetical proteins. To generate more hypotheses, we complemented this genomic analysis with a metabolomics comparison between parasites of ISC005 and other groups. This revealed changes at the level of the membrane and oxidative stress defense in ISC005 parasites. Although these could not be directly related to specific genetic changes yet, they show which pathways are prone to serve major roles in ensuring parasite adaption.
CONCLUSION: We found compelling insights into the presence and retrospective emergence of different genetic lineages of *L. donovani* in the ISC and how it may have impacted their survival up to now. Single cell genomics and annotation improvement efforts will be crucial to have a more unbiased view into the exact (regulatory) mechanisms by which *Leishmania* ensures its survival.

**Aneuploidy in natural *Leishmania* populations: chromosome chaos or adaptive strategy?**

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BACKGROUND: Aneuploidy has been extensively demonstrated in *Leishmania* under experimental conditions (stress, drug pressure…), but the frequency and the significance of this phenomenon in natural populations of trypanosomatids is yet to be defined.
METHODS: In the frame of three running projects on whole genome sequence diversity, we explored the occurrence of aneuploidy among clinical isolates of *L. donovani* (203 lines sequenced, Indian sub-continent), *L. braziliensis* (53 lines, Peru and Bolivia) and *Trypanosoma congolense* (45 lines, sub-Saharan Africa). Ploidy was estimated at cell-population level, by analyzing sequencing read-depth and allele frequency in heterozygote SNPs.
RESULTS: In *T. congolense*, deviations from disomy were not encountered in any of the 11 chromosomes. In sharp contrast, aneuploidy is frequent in the 2 *Leishmania* species: (i) chromosome 31 was tetrasomic in all strains and (ii) 33 and 26 chromosomes showed a variable ploidy in *L. donovani* and *L. braziliensis* respectively. However, some near-diploid isolates (all disomic chromosomes, except for number 31) are observed: 20 and 30% in both species respectively. In *Leishmania*, aneuploidy is species- and chromosome-specific. In *L. donovani*, the same chromosomes show a higher frequency of aneuploidy in natural and experimental conditions. Gene ontology enrichment shows major differences between aneuploidy and stable chromosomes. Besides aneuploidy, *Leishmania* has other strategies for gene dosage, like long and linear subtelomeric episomes and short circular episomes.
CONCLUSIONS: Aneuploidy is likely an adaptive strategy, driven by selective pressure on the chromosomal gene content, as shown in experimentally induced drug resistance.

**Molecular epidemiology of *Leishmania donovani* in the Indian subcontinent: agreement between data from whole genome sequencing and historical reports**

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BACKGROUND: Molecular epidemiology of visceral leishmaniasis (VL) in the Indian sub-continent (ISC) was long hindered by the lack of resolution of molecular markers. Next generation sequencing is now providing the ultimate solution to this problem.
METHODS: We sequenced the whole genome of 203 *L. donovani* clinical isolates collected in the last decade in India, Nepal and Bangladesh. Population and demographic analyses were made with STRUCTURE and BEAST respectively.
RESULTS: We identified two completely distinct (45,743 SNPs) lineages: (i) the 'Yeti-12' is homogeneous (5628 SNPs), tend to originate from an atypical, highland VL focus in Nepal; (ii) The 'Core-191' is endemic in the lowlands and likely emerged around 1850, corresponding to the first reported VL outbreak (Garo Hills); this lineage is relatively homogeneous (2,418 SNPs), but was split in 6 defined and 2 mixed groups. Our Indo-Nepalese sample likely emerged around 1900 in India, West of Koshi river, close to the site of the reported outbreak of Purnea in 1899. Within several of the defined groups, a recent radiation occurred about 50 years ago, probably during the first VL epidemics after the DDT campaign. This radiation was concomitant with the reported decrease in efficacy of antimonials; one genetic group (ISC005) emerged in India and shows several dramatic molecular adaptations relevant for antimony resistance. Population structure is essentially clonal, but patterns of hybridization have also been detected. Simple PCR assays are now available to track the main genotypes of the ISC.
CONCLUSIONS: Approaching the deadline of the VL elimination programme, it is critical to follow up the spatial and temporal distribution of *L. donovani* populations to better understand their impact on VL epidemiology.

**Molecular phylogeny of Phyllobothriidae (Cestoda: Tetraphyllidea)**

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BACKGROUND: Tapeworms of the family Phyllobothriidae are parasitic in the spiral intestine of elasmobranchs (sharks, rays and skates) as their final hosts. Systematics of this family is confusing due to the lack of morphological characteristics and poor condition of the specimens including types. There are many problematic genera and species. We performed molecular analysis to clarify the taxonomy and phylogeny of Phyllobothriidae.
METHODS: A partial sequence (1911 bp) of 18S rDNA was obtained for 39 species collected from 26 species of elasmobranchs in the coastal waters of Japan and 3 species from GenBank. A total of 42 species analyzed covers 13 genera. Phylogenic trees were constructed with the maximum likelihood method (ML) and maximum parsimony method (MP).
RESULTS: The ML and MP trees showed that four genera including doubtful species, *Phyllobothrium*, *Crossobothrium*, *Monorygma*, and *Marspiobothrium*, were polyphyletic. Especially, *Phyllobothrium* was separated into 4 species groups. *Phyllobothrium biacetabulatum* was located in a clade of the order Rhinebothriidea. *Phyllobothrium* sp. 1 was separately located at basal Phyllobothriidae. *Phyllobothrium squali* and *Phyllobothrium* sp. 2 formed a clade together with *C. longicolle* and *M. megacotyla*. The type species *P. lactuca*, and *P. serratum* and *Phyllobothrium* sp. 3 were in another clade, and the host sharks of these 3 species belonged to Triakidae.
CONCLUSIONS: Molecular analysis showed many polyphyletic genera suggesting the family Phyllobothriidae needs taxonomical revision.

**Administration of soybean 15-lipoxigenase crude extract decreases amoebic liver abscess formation in hamsters.**

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BACKGROUND: Amoebic liver abscess (ALA) formation is characterized by an increased production of leukotrienes (LTs), which are 5-lipoxygenase products (5-LOX). Subsequent modification of LTs by 15-LOX generates lipoxins (LXs), mainly $LXA_4$, which has important anti-inflammatory properties. It is known that administration of soybean (*Glycine max*) 15-LOX crude extract (*Gm*-LOXs) generates endogenous $LXA_4$. The aim of this study was to analyze the effect of the administration of *Gm*-LOXs on both 5-LOX and 15-LOX protein expression, and on $LXA_4$ plasmatic levels during ALA formation.
METHODS: Groups of hamsters were infected with *E. histolytica* (HM1:IMSS strain) and treated with *Gm*-LOXs (100,000 U/day). Animals were sacrificed at 2, 4 and 7 days post-infection (p.i.), livers and abscesses were dissected to calculate damage percentage. Plasmatic levels of $LXA_4$ were determined by ELISA assay; 5-LOX and 15-LOX expression was analyzed by immunohistochemistry and image analysis.
RESULTS: At 2 and 4 days p.i. 15-LOX expression was higher than that observed for5-LOX; at 7 days p.i., 5-LOX expression was greater than 15-LOX, suggesting a higher production of LTs. Plasmatic levels of $LXA_4$ were substantially higher in animals treated with *Gm*-LOXs in comparison with levels from non-treated animals. *Gm*-LOXs administration was able to reverse the increase in 5-LOX expression at 2 and 4 days p.i., decreasing the ALA size by 17 and 9% respectively compared to ALA observed in non-treated animals; higher concentrations of *Gm*-LOXsare needed to decrease liver damage at 7 day p.i.
CONCLUSIONS: Results suggest that endogenous $LXA_4$ biosynthesis constitutes a novel means to modulate inflammation in this infection. (*FOMIX-2008-C01-92074*).

**Genotyping of rare clinical forms isolates of *Leishmania tropica* from Bam county as the main endemic region of anthroponotic cutaneous leishmaniasis (ACL) in Southeast Iran**

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BACKGROUND: *Leishmania tropica* is the main causative agent of ACL in the many Old World endemic regions including Iran. Some unusual clinical forms have been reported among ACL patients. This study investigated the *L. tropica* ITS sequences polymorphism in these forms.
METHODS: Based on microscopic examination, 26 smear preparations from different rare clinical forms including; relapse (n=3), sporotrichoid (n=5), lupoid (n=5), resistant to treatment (n=4) and treatment failure (n=9) cases were selected from smears collection which had been provided in the 2007-2012 in Bam Leishmaniasis Control Center. Species identification was performed by kDNA-PCR using LiR and 13Z primers. ITS MG sequences of *L. tropica* isolates were amplified by LITSR (El tai *et al.* 2000) and LITS-MG primers (Ghatee et al. 2013) and then analysed by sequencing.
RESULTS: All 26 samples were identified as *L. tropica*. Almost all 26 rare clinical isolates were from haplotype A which is the dominant haplotype in southeast Iran. Only one lupoid and one resistant isolate showed different single nucleotide polymorphism (SNP) in a microsattelite repeat and identified as haplotype L and haplotype R, respectively. Both variations occurred in ITS1 region while no variation was observed in 5.8s or ITS2 regions. Survey of patients' history with resistant lesions confirms the non-competent immune system backgrounds for ¾ of patients.
CONCLUSIONS: Homogeneity was observed among rare clinical forms isolates of *L. tropica* in this region. Of course low polymorphism may be due to resolution power of ITS marker. Microsatellite multilocus typing (MLMT) or kDNA-RFLP approaches are offered to investigate probable correlation between each clinical form occurrence and background genetic variations, although resistance to treatment may be due to host immunity condition.