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Cytokine and innate factors expression analysis in Pelibuey lambs after challenge with *Haemonchus contortus*

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BACKGROUND: Immune response caused by the different antigenic challenges of the gastrointestinal nematode (GIN) life stages, may elicit changes in the gene expression pattern of lymphocytes. In order to describe the immune mechanisms involved in the control of nematode populations within the host, the gene expression profile of lymphocytes from peripheral blood was assessed in Pelibuey lambs experimentally infected with *Haemonchus contortus*.
METHODOLOGY: Blood samples were obtained to extract the leucocytes on the day of infection (at 0 h and 4 h), at 48 h and 14 days post infection (PI) with *H. contortus*. The expression profiles of IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, FCERL1A, GPX and SOD1 were described using Real Time RT-PCR. Statistical analysis was carried out by using the REST® program.
RESULTS. Among this gene panel, classical T_H2 cytokines (IL-4, IL-5), and some innate factors (SOD1 and GPX) were significantly up-regulated early in our study (at 4 hours PI). Also, on day 2 PI (48 h) Pelibuey lambs exhibited a significant up-regulation of T_H1 (IL-2 and IL-8) and a Th2 (IL-4) cytokines, reaching their maximum significant expression ratio compared with day 0. Also, the eosinophil count at 48 h PI was positively correlated with the EPG count (P=0.05), which suggests high eosinophils activity in those lambs with more larvae established. In contrast, on day 14 PI, the gene expression pattern was decreased with a significant down-regulation of inflammatory cytokines (IL-6 and IL-8), while no other differences were observed in the other genes.
CONCLUSIONS: The immune response against *H. contortus* primary infection in Pelibuey could be driven by both T_H1 and T_H2 responses in conjunction with innate factors comprising an early activation (4 h and 48 h PI). Meanwhile, Pelibuey lambs seemed to reduce their inflammatory response against *H. contortus* on day 14.

Lethal activity of *Artemisia cina* and NOSODE (homeopathic products) against *Haemonchus contortus* eggs

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BACKGROUND: The nematode *Haemonchus contortus* causes severe damage in the abomasal mucosa of ruminants. Anthelmintic drugs are the main control against *H. contortus.* However, resistance problems have reduced the efficacy of anthelmintic drugs. Recently, homeopathic products have shown nematicide damage. The main goal of this study was evaluated the lethal activity of *Artemisia cina* (an ethanolic extract from plant) and a NOSODE of *H. contortus* (crude extract from adult stages) as homeopathic products against *H. contortus* eggs.
METHODS: The egg hatch test (EHT) was performed using *H. contortus* isolate from FES-Cuautitlán, UNAM, (Coles et al., 1992). Homeopathic products *Artemisia cina* 30CH and *H. contortus* NOSODE 30 CH were obtained from a commercial lab (Millenium Lab, México). The EHT was performed in 96-well plates and incubated at room temperature for 48 h. Five treatments were performed, 1) *Artemisia cina* 30CH, 2) *H. contortus* NOSODE 30 CH, 3) water (negative control), 4) 70% alcohol (homeopathic control) and 5) albendazole (0.03ugmL^{-}1, positive control). The efficacy of the homeopathic products was analized using the Statgraphics program.
RESULTS: The EHT showed inhibition of parasitic eggs as follows: 91,57% albendazole, 72,95% *Artemisia cina* 30CH, 16,15% *H. contortus* NOSODE 30CH, 20,15% alcohol and water at 2,47%. Significant statistical difference (*p>0.05*) was observed between the means of the five treatments with at 95.0%. I.C. Also, parasitic eggs of *H. contortus* treated with *A. cina* showed degeneration of parasitic egg contents with apparent membranous integrity.
CONCLUSIONS: *H. contortus* lethal damage of *A. cina* 30CH showed reduction of the egg hatching in more than 70%. Contrary to these results, the nosode of *H. contortus* showed low parasitic damage on parasitic eggs, suggesting the inefficacy of this homeopathic product. Because of technological advances and the importance that examining anti-parasitic agents based on phytochemical as homeopathic medicine has gained, it is required more studies of the available plant material in future studies.

Ion and Nutrient Transport in Bloodstream Malaria Parasites

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BACKGROUND: Erythrocytes infected with malaria parasites have increased permeability to many solutes, but the precise mechanism was unclear. Patch-clamp studies suggested one or more ion channels, but the number of channels and their molecular origins have been debated. The physiological roles served by the permeability and whether it can be targeted for development of therapies were also unknown.
METHODS: We introduced the use of electrophysiological methods for the study of membrane transport in malaria parasites. A transmittance-based assay has been developed and used to execute several high-throughput screens for transport inhibitors. We have also used *in vitro* selection to generate transport mutants. DNA transfections, biochemical studies, and parasite growth inhibition using physiological and non-physiological conditions have also been developed and used.
RESULTS: These studies implicate an unusual ion channel known as the plasmodial surface anion channel (PSAC) in the increased transport of both inorganic and organic solutes. Inhibitor screens have identified specific and potent compounds that block PSAC. Inhibitors that act against specific genotypes have enabled linkage analysis in two genetic crosses, leading to the identification of a single locus on parasite chromosome 3. DNA transfections, molecular studies of PSAC mutants, and biochemical studies have implicated the *clag* multigene family in this transport activity. Growth inhibition studies implicate an essential role in parasite nutrient acquisition.
CONCLUSIONS: PSAC activity is determined by parasite proteins inserted into the host erythrocyte membrane. The unusual properties of the channel, conservation of the *clag* genes in malaria parasites, and absence of mammalian orthologs suggest that this channel may be an excellent target for development of antimalarials.

Effects of climate change on avian malaria in Alaska

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BACKGROUND: With Arctic sea ice coverage at a record low, and considerable melting of permafrost, host-pathogen relationships in the far north are rapidly changing. Studies suggest that with warmer habitat, vector distribution and parasite prevalence may increase due to favorable thermal conditions. Additional studies provide evidence suggesting that avian malaria and other protozoan parasites may increase in prevalence and northerly distributions with global warming. Here we report the first evidence of *Plasmodium* transmission in Alaska bird populations. In addition, we determine the prevalence of co-infections of haematozoan parasites in Alaskan birds. We develop predictive models for how global climate change will affect the distribution of avian malaria and provide data on bioclimatic associations with prevalence of co-infection and distribution.
METHODS: We collected blood samples from 47 bird species, both resident and migratory, over a latitudinal gradient in Alaska. Through random forest models, we analyzed the prevalence of co-infection from each parasite group based on patterns observed at the collection sites.
RESULTS: Molecular screening revealed higher prevalence of haematozoa (53%) in Alaska than previously reported. *Leucocytozoon* had the highest diversity, prevalence, and prevalence of co-infection. We found temperature, precipitation, and tree cover to be the primary environmental drivers that show a relationship with the prevalence of co-infection. We found both residents and hatch year birds infected with *Plasmodium* as far north as 64°N, providing clear evidence that malaria transmission occurs in these climates. Based on our empirical data, we make the first projections of the habitat suitability for Plasmodium under a future-warming scenario in Alaska.
CONCLUSIONS: The results provide insight on the impacts of bioclimatic drivers on parasite ecology and intra-host interactions. This has implications for understanding infectious diseases in an ever-changing environment.

Shading of culture cage reduces the infection of skin fluke *Neobenedenia girellae*

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BACKGROUND: *Neobenedenia girellae* has a broad host range and cause problems in various finfish aquaculture. Today's control measures focus on removal of attached worms by bathing fish in freshwater (FW) or H_2O_2 and the oral administration of praziquantel (PZQ). However, these treatments require labor, time and costs. Moreover, bathing treatment can be a great stress to fish. We aim to develop a new method to reduce infection using larval positive phototac behavior.
METHODS:.We conducted a 3 month experiment to compare *N. girellae* infection between juvenile chub mackerel *Scomber japonicus* kept in open cage and those kept in a shaded cage (98% lower light intensity). We sampled fish from each group every 5 days and counted the worms on individual fish. Fish had to be treated either by FW bath or PZQ when the mean worm intensity exceeded 20 or 10 worms/fish, respectively. Change in the infection rate and frequencies of treatments were compared between the groups.
RESULTS: For the open unshaded cages, a total of 3 times FW bath or 6 times PZQ treatment was conducted. On the other hand, no treatment was required for the shaded cages. The overall mean *N. girellae* intensity in the shaded group was less than half of that of unshaded cage despite the absence of post-infection treatment.
CONCLUSIONS: Our results indicate that shading effectively prevents *N. girellae* infection and help to reduce the frequency of post-infection treatments. Shading can be a simple, easy and effective method to lower the infection of *N. girellae* and may also be useful for other parasites which possess positive phototaxis.

Seizure outcome in patients with neurocysticercosis

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BACKGROUND: Up to 80% of patients with neurocysticercosis (NCC) present with seizures or epilepsy. Seizure outcome is therefore an important component of outcomes in patients with NCC.
METHODS: The literature was searched for articles that reported seizure outcome, at a minimum mean follow up of 2 years or more, in patients with NCC. The results are presented as a discussion of the important papers that have reported seizure outcome in patients with NCC.
RESULTS: There are only few studies that have reported long-term seizure outcome in patients with NCC. Seizure outcome in patients with NCC is variable. Recurrence of seizures is common following a first seizure and in those with a persistent lesion in the brain, although, seizures are usually easily controlled with one or two anti-epileptic drugs (AEDs). Following resolution of the lesion in a patient with a solitary cysticercus granuloma (SCG), the risk of seizure recurrence after withdrawal of AEDs is low (around 15%). However, those with multiple parenchymal lesions, calcific lesions, multiple seizures or breakthrough seizures have a higher rate of recurrence and may need administration of AEDs for longer periods. Intractable epilepsy is rare in patients with NCC and surgery for intractable epilepsy is infrequently required in these patients.
CONCLUSIONS: Prognosis for seizures in patients with NCC is variable. Patients with NCC require AEDs for varying periods of time following the first seizure. AEDs should at least be administered till the resolution of the brain lesion on imaging studies. In some patients AEDs will be required for several years.

Epidemiology of cysticercosis in India

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BACKGROUND: Cysticercosis is endemic in India as seen from several reports of the disease from hospitals all over the country. However, there are only few population-based studies of cysticercosis from India.
METHODS: The literature was searched for recent (after the year 2000) community based epidemiological studies of cysticercosis from India. The results are reported as i) prevalence of neurocysticercosis (NCC) as a cause of epilepsy; ii) prevalence of asymptomatic NCC; iii) seroprevalence of cysticercosis in the community; and iv) prevalence of porcine cysticercosis
RESULTS: There are only about 10 recent community based studies of cysticercosis from India. i) NCC has been reported to the cause of around 30% of all cases of active epilepsy; ii) asymptomatic NCC was only evaluated in a pig-rearing community in north India and was seen in around 15% of individuals; iii) seroprevalence of cysticercosis ranged from around 6% to 22% varying with the region of the country and the methodology used; and iv) porcine cysticercosis was reported in around 2% to 26% of living pigs or carcasses that were tested.
CONCLUSIONS: Epidemiological studies confirm the high prevalence of cysticercosis both among humans and pigs in India. NCC is the cause for nearly one third all cases of active epilepsy in India.

Cloning and characterization of phosphoenolpyruvate carboxykinase in *Raillietina echinobothrida* (Cestoda: Cyclophyllidea)

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BACKGROUND: Phosphoenolpyruvate carboxykinase (PEPCK; EC 4.1.1.32) is a rate-limiting glycolytic enzyme in *Raillietina echinobothrida* (Cestoda: Cyclophyllidea), whereas it has a gluconeogenic role in its vertebrate host (*Gallus domesticus*). The enzyme plays a weighty role at the PEPCK/PK branch point in oxidation of glucose in the cestode parasite and is considered to be an anthelmintic target. In order to find out the functional differences, PEPCK from the cestode parasite (rePEPCK) was cloned and characterized and compared with its host counterpart (hPEPCK).
METHODS: CDS of the rePEPCK was obtained using rapid amplification of cDNA ends (RACE) and the rePEPCK ORF was cloned and over-expressed in *E. coli*. The over-expressed enzyme was purified and characterised.
RESULTS: Tris-HCl buffer (50 mM, pH 7.4) was found to be suitable buffer for PEPCK in the parasite and its host rather than acetate buffer. The rePEPCK also showed a standard Michaelis-Menten kinetics with low *K*m (46 µM) for PEP in compasion to its host. Mn^{2+}, not Mg^{2+}, was found to be the appropriate divalent cation for its optimal activity, whereas other metal ions (Ni^{2+}, Hg^{2+}, Ca^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+} etc) showed less activity. HCO_3^- at concentrations of 20 mM and 8 mM were required for optimal activities of rePEPCK and hPEPCK respectively. GDP was also found to be effective nucleotide compared to other nucleotides for rePEPCK activity. In order to find out some effective modulators, isoflavones from *Flemingia vestita* were also tested on the rePEPCK activity and their *Ki* were determined.
CONCLUSIONS: From the present study, it has been found out that there is a significant functional difference between the activities of rePEPCK and hPEPCK. Hence, rePEPCK could further be exploited for its pharmaceutical studies for anthelmintic action.

Effect of bacteriocinogenic and probiotic bacteria on cellular immunity and parasite *Trichinella spiralis* infection in mice

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BACKGROUND: Probiotic bacteria in the host's gut confer a health benefit to the host and play an important role in development and maintenance of the immune homeostasis among mucosal intestinal and systemic immune responses. Probiotics can kill or inhibit pathogens by strain-specific mechanisms relying on competition, molecule secretion and immune induction, and have also a potential to control parasite infections.
METHODS: Bacteriocinogenic and probiotic strains of different origin (*Enterococcus faecium* EF55, *E. faecium* AL41, *E. faecium* 2019-CCM7420, *Lactobacillus fermentum* AD1-CCM7421, *Lactobacillus plantarum* 17L/1) were administered daily in dose of 10^9cfu/ml in 100 µl and mice were infected with 400 larvae of *T. spiralis* on 7th day of treatment.
RESULTS: A protective effect against parasite worm burden in the intestine was observed only in mice treated with bacteriocinogenic strains of *E. faecium* (reduction cca 40 %). Stimulation of the host immune response (proliferative activity of T cells, CD4 subpopulation, metabolic activity of macrophages) resulted in reduction of muscle larvae with the highest efficacy (reduction cca 70 %) in mice treated with *E. faecium* AL41, followed by *L. fermentum* AD1-CCM7421 and *L. plantarum* 17L/1 treatment.
CONCLUSIONS: The results indicate that examined probiotic strains might provide a strain-specific protection against parasites not only in the gut, but also in host tissues. Immunological interactions between probiotic strains, parasite and host cells need to be investigated more in details.
The work was supported by the Project "Competence centre for biomodulators and nutritional supplements - Probiotech (ITMS-26220220152)".

Immunomodulatory effects of probiotic bacteria on phagocytosis and respiratory burst activity of blood polymorphonuclear leukocytes in mice infected with *Trichinella spiralis*

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BACKGROUND: Probiotic strains confer a beneficial property to the host as immune stimulation, protection against pathogens; have the capacity to control intestinal parasite infection but also some nongut infection. However, molecular mechanisms mediating the beneficial effects are as yet poorly understood. Phagocytosis and respiratory burst are two of the most important functions of leukocytes and essential for the elimination of invading pathogens. Phagocytosis is a complex process mediated through various cell surface receptors and enzymes activated by reactive oxygen species produced in the process of respiratory burst.
METHODS: Bacteriocinogenic and probiotic strains of different origin (*Enterococcus faecium* AL41, *Eneterococcus durans* ED26E/7, *Lactobacillus fermentum* AD1-CCM7421, *Lactobacillus plantarum* 17L/1) were administered daily in dose of 10^9cfu/ml in 100µl and mice were infected with 400 larvae of *T. spiralis* on 7th day of treatment.
RESULTS: The results indicate phagocytic and metabolic activity of blood leukocytes is inhibited at weeks 3 and 4 post *T. spiralis* infection (pi), i.e. in the time of massive blood migration of newborn larvae into the host muscles. The administration of bacterial strains with a probiotic effect prevented the inhibition at week 3 pi and stimulated phagocytosis and respiratory burst of blood leukocytes that could contribute to a decreased larval migration and a destruction of newborn larvae and then reduced parasite burden in the host.
CONCLUSIONS: The highest protective effect against *T. spiralis* infection was induced by strains *E.durans* ED26E/7 and *L.plantarum* 17L/1.
The work was supported by the Project "Probiotech (ITMS-26220220152)".

Multiple routes of phosphatidylethanolamine biogenesis ensure the membrane integrity in *Toxoplasma gondii*

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BACKGROUND: *Toxoplasma gondii* requires significant quantities of phospholipids to ensure membrane biogenesis during intracellular tachyzoite proliferation. Phosphatidylethanolamine (PtdEtn) is one of the most ubiquitous phospholipids in pro-end eukaryotes, and the second most abundant in *T. gondii*.
METHODS: We identified and characterized the pathways for PtdEtn synthesis in *T. gondii* using a variety of biochemical and genetic methods. Enzymatic activity was verified by heterologous expression in yeast and metabolic labeling of *T. gondii* tachyzoites. Genetic knockouts were generated to investigate the relative importance of *de novo* PtdEtn synthesis. Furthermore, we studied the import of phospholipids across the parasite membrane using fluorescent tracer lipids.
RESULTS: We show that *T. gondii* expresses two distinct phosphatidylserine decarboxylases localized in the parasite mitochondrion (*Tg*PSD1mt) and the parasitophorous vacuole (*Tg*PSD1pv), respectively. In addition the parasite encodes an active CDP-ethanolamine pathway in the endoplasmatic reticulum. The latter can partially compensate for the loss of *Tg*PSD1mt. The secreted *Tg*PSD1pv enzyme appears to be dispensable for the parasite growth. In addition, axenic *T. gondii* tachyzoites are capable of incorporating tracer PtdEtn and phosphatidylserine in an energy- and protein-dependent manner, suggesting an active phospholipid transport across the plasma membrane.
CONCLUSIONS: These results demonstrate an exceptional compartmentalization and plasticity of PtdEtn synthesis and phospholipid transport in *T. gondii*, which likely ensures a flexible membrane biogenesis in dissimilar nutritional milieus.

Travel and Tourism are drivers for trichinellosis

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BACKGROUND: Acquiring trichinellosis while travelling abroad is not a new phenomenon (see Mc Auley et al, 1991) and imported cases are regularly reported worldwide. Cases contracted abroad and reported by the French National Reference Centre for *Trichinella* (NRCT) are analyzed here.
RESULTS: Since 1998, 28 imported cases, representing 37% of all cases, were reported to the NRCT, with a mean annual incidence of 2 cases. Between 1975 and 1998, 40 imported cases represented only 1.5% of all identified cases but with a comparable mean annual incidence of 1.6 cases. Incidence of imported cases could even have decreased since 1998 as the number of international travelers increased during that period. Since 1998, most cases were acquired in Canada from bear meat (hunters). Some cases were acquired in West Africa from warthog meat, in Laos from pork and one case, in Algeria, was due to jackal meat consumption.
DISCUSSION: These imported cases are most likely to occur in countries where the habit of eating raw meat is common and may reveal a high transmission in some regions where the disease is or had become unknown (e.g. Senegal, Laos…). Backpackers, adventure travelers or hunters will certainly be at higher risk and should be informed about the risks of eating raw meat (pork, game or reptile meat) and should be discouraged from illegally importing potentially infected meat which could introduce the parasite in *Trichinella*-free areas.
CONCLUSIONS: Travelers can be could indicators of the emergence of the parasitosis in a given country. Imported cases are good indicators of the epidemiology of the disease in countries where the original infection occurred.

A survey of urinary and intestinal schistosomiasis infection among primary school pupils of selected schools in Agwu L.G.A., Enugu state, Nigeria

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BACKGROUND: The purpose of this study was to know the prevalence of both urinary and intestinal schistosomiasis among selected primary schools which include: Central Primary School Agbaogugu, Akegbi Primary School, Ogbaku Primary School, Ihe Primary School and Owelli-Court Primary School in Agwu Local Government Area, Enugu State Nigeria between November 2012 to October 2013.

METHODS: Sedimentation method was used in analyzing the urine samples, and combia-9 test strips were used in testing for haematurea, the stool samples were parasitologically analyzed using the formal ether technique. A total of six hundred and twenty samples were collected from the pupils, which include 310 urine samples and 310 stool samples.

RESULTS: Out of the 310 urine samples examined, 139(44.84%) were infected with urinary schistosomiasis. While out of 310 stool samples examined, 119(38.39% were infected with intestinal Schistosomiasis. Children between 12-14years were the most infected with both urinary and intestinal schistosomiasis with prevalence of 45(14.84%) and 48(15.48%) respectively. Children between 3-5years were the least infected with both urinary and intestinal schistosomiasis 30(9.68%) and 25(8.06%) respectively. Infections among schools show that pupils from Akegbi Primary School had the highest prevalence of infection for both urinary and intestinal schistosomiasis 37(11.94%) and 32(10.32%) respectively. The total prevalence of urinary schistosomiasis infection using combi-9 test strips was 181(58.39%). The egg intensity for both urinary and intestinal schistosomiasis were 154 eggs and 96 eggs respectively. The study also revealed that 55(27.5%) of the total number of pupils sampled had mixed infections of both urinary and intestinal schistosomiasis.

CONCLUSIONS: The results of the study revealed high prevalence of both urinary and intestinal schistosomiasis infections in Agwu L.G.A Enugu State among the pupils. We therefore, recommend that the infected pupils should be treated. Proper health education on the mode of transmission and the dangers of the infections should be carried out among the population.

In vivo assessment of Closantel ovicidal activity in *Fasciola hepatica* eggs

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BACKGROUND: Fasciolasis is a zoonotic parasitic disease caused by *Fasciola hepatica*. Closantel is a flukicide and has been shown to have an efficacy *in vivo* e *in vitro* against mature and immature stages, does not exhibit ovicidal activity *in vitro* and the ovicidal effects *in vivo* at present are undetermined. Egg hatch assay (EHA) is a laboratory tool used to determine a given parasite's resistance to extant drug therapy remains a safe and reliable technique. In the present work the EHA was used to evaluate *in vivo* the ovicidal activity of Closantel on fluke eggs.
METHODS: Sheep parasitized with liver flukes (*Cullompton* strain) were treated with Closantel (10 mg/kg bw) (oral and subcutaneous) stunned and exsanguinated at 12, 24, y 36 hs. Post treatment (pt). *F. hepatica* eggs were collected from bile. Eggs obtained of sheep without treatment were used as control. After a period of incubation (25 °C, 15 days in dark environment), hatching was induced for two hours of exposure to light, quenched with the addition of formalin. Hatching was assessed sorting the eggs into two groups: embryonated (E) and unembryonated (U). The test (n= 3) was expressed as percentage of total eggs analyzed.
RESULTS: The result obtained for control was 89,5% ± 2,12 for (E) and 10,5% ± 2,12 (U). The average of both ways of administration (without significant differences among them) at 12 h pt was 84,5% ± 0,71 (E) and 15,5% ± 0,71 (U); 24 h pt was 86,75 ± 3,18 (E) and 12,75% ± 3,18 (U) and 36 h pt was 32,5% ± 3,54 (E) and 67,5%± 3,54 (U).
CONCLUSIONS: Our results suggest that Closantel affect *in vivo* the normal development of the eggs. As one of the first effects, this drug affects the performance of the trematode's reproductive physiology.

Genetic analysis comparison of Glutathione S-Transferase in *Fasciola hepatica* of different hosts. Its implications on the phenomenon of anthelmintic resistance

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BACKGROUND: Fasciolasis is a zoonotic parasitic disease caused by *Fasciola hepatica* and its control is mainly based on the use of triclabendazole (TCBZ). Parasite resistance to different anthelmintics is growing worldwide, including the resistance of *F. hepatica* to TCBZ. In fasciolosis exist the phenomenon of cross-infection in which the coexistence of cattle, sheep and pigs allows the parasitic passage between species. These passages determine changes in adaptation to the new environment in which could be encouraged the resistance phenomenon. Glutathion S-Transferases (GSTs) in *F. hepatica* are major detoxification enzymes in adult helminthes. Recently we have demonstrated that the fluke TCBZ resistant (*Sligo* strain) expressed significatively major GST metabolic activity compared to that from susceptible flukes (*Cullompton* Strain). That enhanced metabolic obtained activity correlates with overexpression of mRNA for GST and the presence of two transversionals mutations in the *gen*GST in *Sligo* strain (TCBZ-R). In this work we comparatively characterized the gene for *F. hepatica* GST (*gen*GST) and the expression of the corresponding mRNA from three different hosts (sheep, cattle and pig) compared against *Sligo* (TCBZ-R) strain and *Cullompton* (TCBZ-S) strain.
METHODS: Purification of total RNA was using Trizol ®. The RT-PCR was performed with SIGMA-TRI REAGENT ® (T 9414) kit. The comparison analysis of *gen*GST sequences of cattle, sheep and pigs were performed using CLUSTAL2.
RESULTS: The flukes from the three host species expressed identical to the corresponding *gen*GST TCBZ-S (*Cullomptom* strain). The expressed *F. hepatica* GST RNA transcripts from all three host species showed significant differences (pig 2>bovine1.7>sheep1).
CONCLUSIONS: These differences in expression are directly related to the degree of resistance of different hosts against fasciolosis contributing to the understanding of the mechanisms that generate resistance in different definitive hosts of this disease.

Heterogeneity of *Anopheles* species composition, population densities and malaria transmission patterns in two Yanomami areas, Brazil.

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BACKGROUND: Studies on the determinants of malaria in indigenous communities are scarce. In this study we evaluated entomological aspects to provide important information on how local malaria transmission is sustained in remote indigenous areas.
METHODS: We conducted a longitudinal entomological survey over 19 months in two distinct Yanomami areas, collecting in 4 communities of each region during three consecutive days. Mosquito collections were done throughout dry, wet and transitional seasons, using CDC light traps and a protected-human-baited trap in the intra-, peri- and extradomiciliary environments.
RESULTS: A total of 3,097 anophelines were collected, belonging to 9 species. *An.oswaldoi s.l* was the predominant species (43%), followed by *An.darlingi* (23%) and *An.intermedius* (14%). *An.darlingi* was collected in 4 out of 8 communities, however only in one locality biting in high numbers (2.64 bites/ hour). Considering all anopheline species, the mean Human Biting Rate (bites/ person/ hour) was much higher in the peridomiciliary (1.99) and extradomicilary (1.78) environments than inside the indigenous dwellings (0.04).
CONCLUSIONS: A marked heterogeneity of entomological parameters was observed in these 2 indigenous areas. Only in one community indoor malaria transmission by *An.darlingi* may be important, pointing out the importance of peri- and extradomiciliary transmission in these Yanomami communities. In hotspots of malaria incidence of indigenous areas, it would be advised to provide entomological information at a micro-scale level, with the main goal of implementing an effective vector control strategy per locality.

Bioequivalence study comparing the first recombinant vaccine against Hydatidosis produced in Argentina and the Australian formulation.

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BACKGROUND: The EG95 recombinant vaccine has been developed for prevention of hydatidosis in intermediate host (Heath-Ligthtowlers, 1995).
In Argentina, procedures have been incorporated to adjust the recombinant EG95 protein expression and the selection of adjuvant formulations. Subsequently, Providean Hidatil EG95 was approved as the first recombinant vaccine for veterinary use in 2011.
The efficacy of Providean Hidatil EG95 was compared with the australian formulation and the bioequivalence was demonstrated in a field trial carried out in Chubut since 2009.

METHODS: Three groups of sheep (n=10) were immunized subcutaneously on day 0-30 and boostered 465-1400 dpv. Group 1 was immunized with Providean Hidatil EG95 (50µg-EG95 plus Montanide ISA 70) and Group 2 received the australian vaccine (50µg-Eg95 plus 1 mg QuilA). Control sheep received no antigen. Total IgG titers against EG95 were tested individually on day 0, 30, 45, 465, 730, 1000, 1300, 1400 and 1430 using a validated ELISA.
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RESULTS: Providean Hidatil EG95 induced high titers of specific antibodies in sera from immunized sheep that persisted for more than one year after 2 dosis and two years after booster on day 465. Furthermore, titers did not show significant differences with those elicited by the australian vaccine.

CONCLUSIONS:
The results demonstrate that Providean Hidatil EG95 is bioequivalent to the australian vaccine. The correlation between EG95 titers and protection will be discussed.
The production of the EG95 vaccine is a fact in our country. The vaccine was included in the National Hydatid Control Programme as a new tool to protect intermediary hosts.

Game and human trichinellosis: experience from a recent outbreak

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BACKGROUND: Various wild omnivores (e.g. wild boar) and carnivores (e.g. bear) intended for human consumption are susceptible for infection with zoonotic *Trichinella* spp. The clinical outcome of human infection after exposure usually depends on various determinants such as the *Trichinella* species found in the sylvatic cycle, larval burden in the meat, processing parameters, consumption behavior as well as time point of diagnosis and anthelmintic treatment.
METHODS: Data from trichinellosis cases related to game meat consumption were analyzed with special regard to an autochthonous outbreak in Germany which occurred in 2013 after consumption of sausages produced from wild boar meat.
RESULTS: Game associated trichinellosis cases are often reported from consumption of raw or undercooked wild boar meat containing *T. spiralis* or other relevant species. During the outbreak in Saxony (Germany) in 2013, more than 100 persons were exposed after eating raw wild boar sausages. Parts of wild boar meat with approximately 10 *T. spiralis* larvae per g were processed to 1050 sausages containing 0-125 larvae. 95% of the exposed persons consumed sausages within 4 days after retail and 26% ate two sausages or more. Of 82 exposed persons who could be followed up, 14 (17%) developed *Trichinella* specific antibodies and myalgia and/or periorbital swelling.
CONCLUSIONS: To reduce the risk of wild boar meat for public health, *Trichinella* meat inspection must comply with given standards. Consumers should be informed about the risk of eating raw or undercooked game meat/products and physicians should be aware of diagnostic and therapeutic features especially in regions where trichinellosis is a rare disease.

Humoral responses in *Rhodnius prolixus*: bacterial feeding induces differential patterns of antibacterial activity and antimicrobial peptides genes expression in the midgut.

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BACKGROUND: The triatomine, *Rhodnius prolixus,* is a major vector of *Trypanosoma cruzi*, the causative agent of Chagas disease in Latin America. It has a strictly blood-sucking habit in all life stages, ingesting large amounts of blood from vertebrate hosts from which it can acquire pathogenic microorganisms. In this context, the production of antimicrobial peptides (AMPs) in the midgut of the insect is vital to control possible infection, and to maintain the microbiota already present in the digestive tract.
METHODS: In the present work, we studied the antimicrobial activity of the *Rhodnius prolixus* midgut *in vitro* against the *Gram-positive* and *Gram-negative* bacteria *Escherichia coli* and *Staphylococcus aureus*, respectively. We also analysed the abundance of mRNAs encoding for defensins, prolixicin and lysozymes in the midgut of insects orally infected by these bacteria at 1 and 7 days after feeding.
RESULTS: Our results showed that the anterior midgut contents contain a higher inducible antibacterial activity than those of the posterior midgut. We observed that the main AMP encoding mRNAs in the anterior midgut were for lysozyme A, B, defensin C and prolixicin while in the posterior midgut lysozyme B and prolixicin predominated. *S. aureus* and *E. coli* ingestion induced the enhanced expression of different *R. prolixus* AMP in comparison to naïve insects.
CONCLUSION: Our findings suggest that *R. prolixus* modulates AMP expression genes upon ingestion of bacteria with patterns of expression that are distinct and dependent upon the species of bacteria infecting. Thus, AMPs induced by *S. aureus* infection could be regulated by Toll pathway in *R. prolixus*, while AMPs induced by *E. coli* infection could be under IMD pathway control.

Keywords**:** *Rhodnius prolixus*, Antimicrobial peptides, Bacteria, mRNA modulation

A granulin growth factor secreted by the carcinogenic liver fluke, *Opisthorchis viverrini*, and its role in wound healing and carcinogenesis

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BACKGROUND: The human liver fluke, *Opisthorchis viverrini*, infects 9 million people throughout South-East-Asia and is a major cause of cholangiocarcinoma (bile duct liver cancer). In fact, as many as one-sixth of infected patients will develop liver cancer. The mechanisms by which the parasite causes cancer are multi-factorial, but one process is the secretion of mitogenic parasite proteins into the bile ducts, driving cell proliferation and creating a tumourigenic environment.

METHODS: Using proteomics and transcriptomics to characterize the *O. viverrini* secretome, we identified *Ov*-GRN-1, a homologue of the human growth factor, granulin. Previously we demonstrated potent growth factor activity (nanomolar) of recombinant *Ov*-GRN-1 on human biliary cells using the xCELLigence system.

REDULTS: Here we show that *Ov*-GRN-1 induces wound closure *in vitro* and results from *in vivo* wound healing experiments are ongoing and will be presented. *Ov*-GRN-1 stimulated angiogenesis (blood vessel formation) *in vivo*, a process critical for cancer development. Internalization of recombinant *Ov*-GRN-1 to biliary epithelial cells induced dramatic changes in protein expression. Indeed, and in support of our hypothesis, many of the cellular proteins that were upregulated in response to *Ov*-GRN-1 were associated with cell growth and cancer, whereas the downregulated proteins were associated with tumour/proliferation suppression. Finally, silencing of *Ov-grn-1* gene expression in adult worms using RNA interference resulted in reduced mitogenicity of biliary cells by *O. viverrini* excretory/secretory products.

CONCLUSION: Our novel findings contribute to the understanding of host-parasite interactions, and begin to address the mechanisms by which this parasite causes such a devastating form of cancer.

Non-encapsulated *Trichinella* species and their enigmatic epidemiology

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BACKGROUND: *Trichinella* species whose larvae do not encapsulate after muscle cell differentiation have been described as *T. pseudospiralis*, infecting mammals and birds, and *T. papuae* and *T. zimbabwensis*, infecting mammals and reptiles. In the last 20 years, worldwide reports on non-encapsulated species in animals have increased. The aim of this work was to explore the epidemiological patterns of these elusive zoonotic pathogens.
METHODS: Meta-analysis of data on *Trichinella* species published in the scientific literature and from the website of the International Trichinella Reference Center (www.iss.it/site/Trichinella/index.asp.).
RESULTS: *Trichinella pseudospiralis* has been isolated from 14 mammalian species (92 isolates) and from 7 avian species (7 isolates) of Asia, America, Australia, and Europe. Over the last 10 years, *T. pseudospiralis* accounted for 1.8%, *T. spiralis* 51.3% and *T. britovi* 46.8%, of 1677 isolates from wild boar of Europe. *Trichinella papuae* has been isolated from 2 mammalian species (10 isolates) and from 1 reptilian species (11 isolates) of Asia and Australasia; and *T. zimbabwensis* from 1 mammalian species (1 isolate) and 2 reptilian species (17 isolates) of Africa.
CONCLUSIONS: The increasing number of reports of these *Trichinella* species is related to the widespread use of the more sensitive digestion tests. The number of mammals tested for these parasites has been much higher than that of birds and reptiles. It follows that the role played by birds and reptiles in the epidemiology of non-encapsulated species, cannot be established yet. The cosmopolitan distribution of *T. pseudospiralis* suggests a role of birds in the spread of this parasite, but the identification of genetically different populations in different continents suggests also genetic isolation. The restriction of *T. papuae* and *T. zimbabwensis* to tropical regions indicates reptiles (crocodiles) as major reservoir.

Neglected Tropical Diseases: Emerging Trematodiases in India: the present scenario

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BACKGROUND: Of worm infections affecting mainly the tropical world, trematodiases are major zoonoses, endemic in Central and Southeast Asia. In India the major trematodiasis- causing flukes parasitise the liver (*Fasciola gigantica*, *Opisthorchis noverca*), pancreas (*Eurytrema pancreaticum*), intestine (*Fasciolopsis buski*, *Artyfechinostomum sufrartyfex*), and lung (*Paragonimus* spp), the intestinal and lung flukes being especially significant as potential zoonoses in the Northeast. Under the Government-coordinated initiatives, genomic studies of zoonotic helminths are envisaged with a view to focus on control strategies. In the first phase we applied Next Generation Sequencing (NGS) and bioinformatics technologies to explore the genes transcribed in *F. buski*, *A. surfratyfex* and *P. westermani*.
METHODS: We amplified and sequenced the nuclear ribosomal and mitochondrial DNA regions, designed species-specific primers and carried out molecular characterization using Bayesian and phylogenetic analysis tools. Herein, we describe high-throughput sequencing and bioinformatics pipeline for whole genome, mt genomics and transcriptome analysis utilizing short-read NGS platforms viz. Ion Torrent and Illumina. The transcripts were blasted against all platyhelminth UniProtKB and annotated using gene ontology, molecular function and biological processes.
RESULTS: All chosen marker regions allowed an accurate in-silico distinction of the parasite taxa. The mt genome of *F. buski* (14118 bp) is the shortest trematode mitochondrial genome sequenced till date, closely resembles that of *Fasciola hepatica* and its gene order tallies with that in other trematodes. For transcriptome, transcripts were assembled and genes annotated, which allowed an analysis of RNAi pathway and energy metabolism.
CONCLUSIONS: The mtDNA NGS and transcriptome data generated through our study would help in deciphering biological characteristics of metazoan parasites and provide an enormous resource for development of suitable diagnostics and therapeutic molecules.

Temporal fluctuations in the prevalence of *Taenia solium* cysticercosis in pigs in Mbeya Region, Tanzania

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BACKGROUND: Porcine cysticercosis is a serious agricultural problem in sub-Saharan Africa where pigs are raised, but the temporal distribution has received almost no attention. This study aimed to describe the fluctuations in prevalence of porcine cysticercosis in an endemic area over time.

METHODS: Three cross-sectional surveys were carried out in Mbeya Region, Tanzania; the first two approximately six months apart (March/April 2012 and October/November 2012) and the last approximately eight months later (July/August 2013). Based on known presence of porcine cysticercosis a census was conducted in 22 villages attempting to include all non-pregnant more than two-month old pigs. Jugular vein blood was collected and analysed using Ag-ELISA. In each survey between 800-1000 serum samples were collected.

RESULTS: The first survey revealed cysticercosis prevalence of 15% (n=822, 95%CI: 13-18%), and in the 6-month follow-up, the prevalence had significantly increased to 24% ($p<0.001$, χ^2-test, n=812, 95%CI: 21-27%). In the 14-month follow-up the prevalence had dropped to 20% ($p=0.053$, χ^2-test, n=998, 95%CI: 18-23%).

CONCLUSIONS: Compared with a previous study conducted in the same area in 2007, this was a reduction in prevalence of approximately 35%. Several factors may have contributed to the observed fluctuations. In December 2010 the study area suffered a decimating outbreak of African swine fever, which might have changed the composition of the production systems, affecting cysticercosis prevalence. Fluctuations in porcine cysticercosis prevalence could also be a result of seasonal variation in local crop production practices. Also, as the Ag-ELISA assay used is not species specific, variation in transmission of *T. hydatigena* could potentially influence the results. The observed fluctuations contradict a theoretical model which predicts a stable equilibrium. Further studies are needed to determine whether the prevalence of porcine cysticercosis has an endemic equilibrium, or in fact go through fluctuations with or without the presence of the factors described in this study.

Development of a new antileishmanial phytomedicine.

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BACKGROUND: Antimonial drugs were initially developed a hundred years ago to treat cutaneous leishmaniasis (LC). However, not many alternative drugs are available in endemic areas. We develop a new antileishmanial product with immunoregulatory activity.

METHODS: Chemical analysis of solvent extract was performed by mass spectrometry. Parasite killing was evaluated by flow cytometry and electron microscopy in *L. donovani* promastigotes and by Giemsa staining in infected macrophages previously treated with plant product. IL-12 levels were measured by ELISA assay in supernatants of macrophages treated with plant product. Infected Balb/c mice were treated with low doses of plant extract.

RESULTS: Chemical analysis identified phytochemical steroids. The extract is effective to destroy promastigotes and amastigotes *in vitro* and levels of IL-12 were increased in supernatants of macrophages treated *in vitro* with low doses of plant product. This product is effective to significantly reduce the load of parasites in spleen and liver of infected mice as compared to untreated controls.

CONCLUSIONS: Our group developed a new product, which presents immunoregulatory activity, effective to treat mice infected with *L. donovani*.

Reduced responsiveness of *Onchocerca volvulus* to ivermectin: Prospects for monitoring ivermectin resistance using genetic markers

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BACKGROUND: Ivermectin (IVM) has been used annually or biannually for up to 25 years to control *Onchocerca volvulus*, the causative agent of River Blindness. In recent years, there has been evidence of sub-optimal responses of this parasite to IVM, in some human populations in Ghana and Cameroon that have been under IVM mass drug administration (MDA) for many years, manifested as a more rapid repopulation of skin with microfilariae, after IVM treatment and as female worms containing live stretched microfilariae, *in utero*, despite recent (80 – 90 days) IVM treatment, compared to historical responses to IVM treatment and to populations that respond well to the MDA. This change in response to IVM may affect transmission levels and possible morbidity, and appears to be an early indication of IVM resistance development. Drug resistance has a genetic basis and is a result of more individuals, in the parasite population, being better able to produce and transmit progeny (having a drug resistant genotype), despite the drug treatment, than is found in the unselected population. It is important to be able to monitor for such genetic differences in order to maintain the progress towards elimination of onchocerciasis.
METHODS: We have compared genetic polymorphism using Next Generation sequencing, across the whole genome, between populations that showed sub-optimal responses to IVM, and good responding populations and relatively IVM-naïve populations, of *O. volvulus* from Cameroon and Ghana.
RESULTS: Following the initial detection of differences in the extent of polymorphism in pools of worms, from these different IVM response categories, we have conducted analysis, by Sequenom, on individual adult parasites to assess genotype frequencies.
CONCLUSIONS: Statistical analyses were performed to identify loci that could be used as markers for monitoring for suspected IVM resistance in *O. volvulus* in the control programs.

New Understanding and Molecular Diagnosis of Anthelmintic Resistance in *Haemonchus contortus*

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BACKGROUND: Anthelmintic resistance has become a serious problem for the control of *Haemonchus contortus* and other parasitic nematodes in livestock. Conventional methods for monitoring for anthelmintic resistance, such as fecal egg count reduction tests are insensitive, expensive and slow and are often only used in research settings. It has been known for some time that mutations in the *β-tubulin* gene can confer benzimidazole resistance.
METHODS: Genetic analyses have been applied to sheep farms to detect benzimidazole resistance in *H. contortus*.
RESULTS: Recently, we have discovered mutations in the *dyf7* gene confer macrocyclic lactone resistance in *Caenorhabditis elegans* and *H. contortus*. For levamisole resistance in *H. contortus*, the deletion of a 63 bp indel in the *acr8* gene causes truncation of the Acr8 subunit of the levamisole receptor and levamisole resistance.
CONCLUSIONS: This new information allows us to apply genetic tests to detect resistance to all of the common broad spectrum anthelmintics used to control *H. contortus* in livestock. The availability of new genetic methods for monitoring anthelmintic resistance offers considerable advantages over conventional in vivo and in vitro methods for assessing anthelmintic resistance.

Molecular characterization of BRF1, a subunit of Pol III transcription factor TFIIIB, in *Trypanosoma brucei*

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BACKGROUND: BRF1 is a subunit of the RNA polymerase III (Pol III) transcription factor TFIIIB, together with TBP and BDP1. BRF1 is essential for Pol III activity and, hence, for cell survival. A putative orthologue of BRF1 in *Trypanosoma brucei* (TbBRF1) has been found, but definitive identification and characterization are still lacking.
METHODS: Sequences of several BRF1 orthologues were aligned with ClustalΩ to identify specific BRF1 domains. TbBRF1 conditional knock-down cell lines were generated using a tet-inducible RNA interference (RNAi) system. Growth curves were performed to analyze cell viability, and mRNA and protein decrease was confirmed by Northern-blot and Western-blot, respectively. The effect of TbBRF1 depletion on Pol III transcription was analyzed by nuclear run-on assays.
RESULTS: We found that TbBRF1 contains the typical conserved domains: a zinc finger motif and two TFIIB-related regions. In the knock-down cell line, RNAi induction effectively reduced the TbBRF1 mRNA and protein levels. As expected, ablation of TbBRF1 led to a growth arrest. Run-on assays showed that TbBRF1 is necessary for Pol III-mediated transcription.
CONCLUSIONS: TbBRF1 contains the conserved domains found in other BRF1 orthologues. TbBRF1 participates in Pol III transcription in *T. brucei* procyclic forms and is needed for cell viability.

Current diagnosis of *Opisthorchis viverrini* and biomarkers of hepatobiliary disease and cholangiocarcinoma in an endemic community in Thailand

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BACKGROUND: The liver fluke, *Opisthorchis viverrini*, a zoonotic fishborne trematode (FZT), is a primary cause of hepatobiliary diseases (HBD) and cholangiocarcinoma (CCA) in southeast Asia. Globally, CCA is known to have high mortality and no early diagnosis is available. In order to achieve a goal in reduction of CCA by eliminating the liver fluke, improved and better methods for diagnosis of liver fluke infection as well as biomarkers for screening CCA are essential for the success of the campaign.
METHODS: Sampled subjects were recruited from an endemic community of opisthorchiasis in Khon Kaen, northeast Thailand. For diagnosis of the liver fluke infection, we developed monoclonal antibody-based antigen enzyme-linked iimunosorbent assay (Mab-ELISA) to detect *O.viverrini* antigen in feces and urine specimens from field collected samples. The standard parasitological method i.e. formalin-ethyl acetate concentration method (FECT) was used as a reference. Assessments of urinary antibody and DNA adduct (8-hydroxy-2-deoxyguanosine or 8-oxo-dG) as biomarkers of HBA and CCA were performed using abdominal ultrasonography as a reference method.
RESULTS: The new Mab-ELISA antigen detection method for urine and feces yielded similar positive rates of *O.viverrini* comparable to that by FECT. The antigen levels in both urine and feces correlated with intensity of the liver fluke infection. Between 41-47% of egg negatives were antigen positive. Analyses of urinary IgG specific to *O.viverrini* showed that the level was associated with the risk of HBD and CCA. DNA adduct in urine was also associated with the risk of HBD and CCA.
CONCLUSIONS: Our results suggest that detection of specific antigens are promising methods for diagnosis of the liver fluke infection, particularly urinary antigen detection which is more practical for field condition. Parasite-specific-antibody as well as DNA adduct in urine samples are potential morbidity markers for HBD and CCA. With further improvement of these screening methods into, for instance, a strip-test kit should enhance the elimination of parasite and CCA control program.

Bioinformatics analysis of Phosphoenolpyruvate carboxykinase (PEPCK) from *Raillietina echinobothrida, a cestode parasite of domestic fowl*

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BACKGROUND: Phosphoenolpyruvate carboxykinase (PEPCK) is an important glycolytic enzyme in helminth parasites including cestodes; in contrast the enzyme has a gluconeogenic role in the host animal. Earlier studies have revealed that the activity of this enzyme is altered by genistein, a natural phytoestrogen in the cestode, *Raillietina echinobothrida*; a common intestinal parasite of domestic fowl, *Gallus gallus.* Herein, we compared the properties of the parasite PEPCK with those of other parasites and higher vertebrates including human.

METHODS: The *R. echinobothrida* enzyme (rePEPCK) sequence was obtained by using rapid amplification of cDNA ends (RACE); the enzyme properties were studied using ProtParam, NCBI BLAST, RCSB Protein Data Bank etc. Genistein and its analogues were docked in the active site of rePEPCK using Glide extra-precision (XP), version 5 (Schrodinger).

RESULTS: The rePEPCK sequence (2166 bp) was obtained by joining the 3'end, middle sequence and 5' end and submitted to NCBI GenBank (KC252609). Properties (molecular weight, total number of amino acids, isoelectric point etc.) of rePEPCK closely resemble the characteristics of the PEPCK enzyme from the related species. The BLAST report shows 85% similarity with the partial CDS of *Taenia asiatica* PEPCK and 90% similarity with the partial protein sequence of *Taenia twitchelli* PEPCK. The rePEPCK protein sequence shows 56% similarity with the complete sequence of *Homo sapiens* PEPCK (cytosolic). Docking studies identified a few amino acid sites involved in genistein binding.

CONCLUSIONS: Our study results suggest that PEPCK from the parasite may be a potential anthelmintic drug target and thus be used in further pharmacological studies.

Praziquantel induced ROS generation leads to apoptosis in *Raillietina echinobothrida*

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BACKGROUND: Praziquantel used as a broad spectrum anthelmintic for veterinary animals as well as for humans since 1970. Keeping in mind that the precise mechanism of action has not been understood fully, the present study was conducted to find out the probable mechanism of action in the poultry tape worm *Raillietina echinobothrida*.
METHODS: Cestodes were exposed to 1 µg of the drug praziquantel per ml of PBS *in vitro* for different time intervals (3h, 6h and 9h) along with control and observed using TEM, TUNEL staining, chromosomal condensation and caspase 3/7 fluorescent detection. In addition, alterations in the major antioxidant enzymes like GSH, GST and SOD associated with the ROS generation were also analysed.
RESULTS: Ultrastructural changes in cell organelles like nucleus, nuclear envelope, mitochondria cristae and formation of apoptotic bodies throughout the cytoplasm in praziquantel treated worm, indicate characteristic features of typical apoptosis. DAPI and TUNEL stained sections showed severe chromosomal condensation and apoptotic nuclei in the treated worms. We observed a decrease in the mitochondrial membrane potential and increase in active caspase-3/7expression in the treated worm. In addition, it is also observed that praziquantel causes alteration in the major antioxidant enzymes (GSH, GST and SOD) associated with the ROS generation.
CONCLUSIONS: The present study revealed that the drug praziquantel generates ROS, leading to apoptotic cell death through biochemical and structural alterations in the cestode *R. echinobothrida.*

Establishment of urinary parasite-specific antibody detection for diagnosis of human strongyloidiasis

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BACKGROUND: Serodiagnosis of strongyloidiasis has higher sensitivity than the conventional parasitological method and requires blood sample for analyses. Here we establish a protocol for IgG detection in urine specimens by enzyme-linked immunosorbent assays (ELISA) and assesses its value in the diagnosis of strongyloidiasis in comparison with the conventional serum-based ELISA.
METHODS: Indirect enzyme-linked immunosorbent assay protocol was developed to detect *Strongyloides*- specific IgG in urine specimens. Receiver operating characteristic analysis was used to obtain a cutoff point for serodiagnosis. The conventional serum-based ELISA system was simultaneously performed to analyse the matched specimens originated from the same subjects. Serum, faeces and urine were collected from each individual subject from endemic areas of strongyloidiasis in northeast Thailand and processed for diagnosis. Within the sample of 149 subjects, 41 (27.5%) were positive for strongyloidiasis by agar plate culture (APCT) and formalin-ethyl acetate concentration techniques (FECT). Performances of urine and serum-based ELISA were evaluated using the parasitological methods as a reference.
RESULTS: The urine-based ELISA for diagnosis of strongyloidiasis had sensitivity and specificity of 82.9% and 100%, respectively. The sensitivity and specificity for serum were 92.7% and 100%, respectively. The seroprevalence by urine ELISA was 62.4% and that by serum ELISA was 65.8%. The urine ELISA showed no cross reaction with *Opisthorchis viverrini*, *Taenia*, *Trichuris* and hookworms. In the serum ELISA, no cross reaction was found in *Angiostrongylus cantonensis*, *Taenia* and hookworms.
CONCLUSIONS: Urinary ELISA for serodiagnosis of strongyloidiasis showed similar efficacy to serum ELISA and both methods are more sensitive than standard parasitological diagnoses. Since collection of urine is non-invasive and easy to do, the urine ELISA is suitable for diagnosis as well as mass screening of strongyloidiasis prior to a standard confirmatory test

Perturbed IL7 receptor-signaling on T cells in chronic Chagas disease

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BACKGROUND: We have previously demonstrated that the immune system in subjects with chronic *T. cruzi* infection display features common to other persistent infections with signs of immune exhaustion. Alterations in cytokine receptor signal transduction have emerged as one of the cell-intrinsic mechanisms of T cell exhaustion.
METHODS: Herein, we analyzed the expression of IL-7R components (CD127 and CD132) on $CD4^+$ and $CD8^+$ T cells. IL-7-dependent signaling events in PBMCs from patients at different clinical stages of chronic Chagas heart disease were also evaluated by flow cytometry upon stimulation with recombinant human IL-7.
RESULTS: Subjects with no signs of cardiac disease showed a relative decrease in $CD127^+CD132^+$ and a reciprocal gain of $CD127^-CD132^+$ $CD8^+$ T cells compared to uninfected controls whereas the expression of IL-7 receptor components in patients with cardiac disease did not differ from uninfected controls. IL-7-induced phosphorylation of STAT5, as well as Bcl-2 and CD25 expression were lower in *T. cruzi* infected subjects compared with uninfected controls. Increased basal levels of STAT5 phosphorylation were associated with poor responses to IL-7 in patients with severe cardiomyopathy. The majority of IFN-γ-producing $CD4^+$ T cells in response to *Trypanosoma cruzi* antigens comprise effector memory T cells that express CD127.
CONCLUSIONS: The present study highlights perturbed IL-7/IL-7R T cell signaling through STAT5 as a potential mechanism for T cell exhaustion in chronic *T. cruzi* infection.

Mediators of microneme secretion in Apicomplexa

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BACKGROUND: Host cell invasion is an essential step in the propagation of obligate intracellular parasitism by the Apicomplexa. Underpinning this process is the sequential release of apical secretory organelles termed the micronemes and rhoptries. Microneme secretion precedes rhoptry secretion and is an essential step in egress from infected cells and subsequent gliding and invasion. Despite its importance, the precise signalling pathways sustaining this process are not currently fully defined. An increase in intracellular calcium levels, likely in response to parasite PI-PLC activation at the plasma membrane, activates calcium-dependant protein kinases (CDPKs) that subsequently phosphorylate specific substrates, ultimately leading to microneme secretion.
METHODS & RESULTS: To better understand this essential process, we have investigated the events downstream of PI-PLC activation at the plasma membrane of *T. gondii*. Specifically we have focussed on the generation of phosphatidic acid (PA) from PI-PLC-derived diacylglycerol (DAG). In eukaryotic cells PA plays a variety of roles in signalling, membrane curvature and protein recruitment triggering exocytosis. Given its concomitant up-regulation during invasion-associated PI-PLC signalling, we hypothesised a role in microneme discharge. Importantly, specific inhibitors that block production of PA, or prevent its dephosphorylation to DAG critically impact on microneme secretion. The enzymes involved in this process have been identified and rudimentarily characterised. In connection with this signalling pathway, we have identified and characterised an essential apical PH domain containing protein binding to PA (APH). This protein is acylated at the surface of the micronemes and plays a crucial role in attachment/invasion and egress from infected cells.
CONCLUSIONS: PA is a signaling molecule that participates in microneme secretion and APH appears to act as mediator of PA sensing possibly taking part in organelle fusion at the plasma membrane. Overall this work contributes substantially to the knowledge base for regulated organelle secretion in Apicomplexa.

Studies on different species of plant parasitic nematodes that attack vegetable crops grown in Afikpo north L.G.A Ebonyi state, Nigeria

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The study on the prevalence of different species of plant parasitic nematodes that attack vegetable crops grown in Afikpo North L.G.A., Ebonyi State, Nigeria was carried out between October 2011 to September, 2012. A total of 200 soil, roots, stems and leaf samples were collected from different farm sites in Afikpo North L.G.A. which include; Amasiri, Akpoha, Enohia, Afikpo town, Unwana, Ndibe, Ibii Ozizza and Kpogirikpo. Nematodes were extracted and isolated from 100g soil sample using a simple bucket sieving method with the aid of a strainer. This was done in order to recover actively migrating nematodes. A total of seven (7) different species of nematodes were recovered, which include; endoparasitic and ectoparasitic nematodes. They were four endoparasitic nematodes, recovered, they are; *Meloidogyne incognita* with the highest prevalence of 45 (37.50%), *M. hapla* with a prevalence of 32 (26.67%), *Pratylenchus spp*. (migratory) with a prevalence of 25 (20.83%) and *Heterodera* spp, with a prevalence of 18(15.00%). The other three (3) species recovered were ectoparasitic nematodes, they include, *Xiphinema* spp. with the highest prevalence of 20(41.67%) which is a migratory nematode; *Dolichorus* spp. (also migratory) with a prevalence of 16(33.3%) and *Trichodrous* spp. with a prevalence of 12(25.00%). However, the following vegetable crops such as okro, cucumber, garden eggs, alfalfa, tomatoes, pepper, fluted pumpkin, etc. where found to be attacked by nematode species from the study area. There is need for further researches in order to come up with cheap, but effective nematode management technique especially for the benefit of the rural farmers. Keywords: *Nematodes, vegetables, Meloidogyne incognita, Trichodorous species, Heterodera species*

Challenges for the control of congenital toxoplasmosis in Southamerica

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BACKGROUND: Congenital toxoplasmosis is not only more prevalent but it seems to be more severe in South America compared to other parts of the world.
METHODS: Bibliographic search with terms: toxoplasmosis and South America in PubMed and Scielo. Analysis of our own unpublished data.
RESULTS: A meta-analysis published in 2013 in the Bulletin of World Health Organization estimated the global annual incidence of congenital toxoplasmosis to be 190,100 cases (95% credible interval, CI: 179,300-206,300). High burdens were seen in South America and in some Middle Eastern and low-income countries. The frequency of congenital toxoplasmosis as determined by newborn screening programs in South America varies in rates of 0.6% reported in Colombia to 0.01% in some regions of Brazil. This frequency is at least 10 times higher for most of the studies performed in South America than those reported in Europe and United States, but in addition cases in South America are more symptomatic. Although the clear justification to implement control programs, there exist reluctance to perform systematic prenatal diagnosis by private and public health assurances companies, due to the high costs of serological screening and the debate about the efficacy of treatment during pregnancy. To face the controversy in diagnosis and treatment options during pregnancy, the Colombia´s Ministery of Health along the Colombian Association of Infectious Diseases and the Colombian Association of Gynecologists developed the world's first evidence based recommendations for diagnosis and treatment of toxoplasmosis during pregnancy. The methodology was based on GRADE evaluation. The official guidelines were launched in 2013 and included the economical evaluation of one, three or monthly testing during pregnancy. The results of this analysis indicated that depending of health resources availabilit, a monthly testing can be economically cost-effective. Available at: http://gpc.minsalud.gov.co/Documents/Guias-PDF-Recursos/Embarazo/GPC_Prof_Sal_Embarazo.pdf
CONCLUSIONS: Implementation of evidence-based guidelines could result in reduction of the huge socio-economical impact of congenital toxoplasmosis in South America.

Virulence, immune response and ocular toxoplasmosis

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BACKGROUND: Ocular toxoplasmosis is the most important cause of posterior uveitis in the world. There exists an important impact on quality of life of patients suffering this disease.
METHODS: Intraocular measurement of cytokines, lymphoproliferative assays and determination of the cytokine profile and molecular and serotyping studies of infecting strains in patients with ocular toxoplasmosis and controls.
RESULTS: We recently provide evidence that ocular toxoplasmosis is more severe in South American patients compared to French patients and we demonstrated that this was linked to the type of strain. There were also a significant correlation between the profile of cytokines in aqueous humor and severity of the disease (greater size of scars, higher inflammatory response). Intraocular IFN-γ and IL-17 expression was lower, while higher levels of IL-13 and IL-6 were detected in aqueous humor of Colombian patients. Additionally, immune response in human toxoplasmosis after *ex vivo* antigenic stimulation was Th1- or Th2-skewed, depending on a patient's clinical condition. Colombian ocular toxoplasmosis patients' immune response was Th2-skewed, regardless of the nature of antigen stimulus. We also found in other study of 20 Colombian patients, that the virulent allele of *Toxoplasma* ROP18 in ocular toxoplasmosis was correlated with severe ocular inflammatory response.
CONCLUSIONS: Altogether, these results indicate that some South American strains cause more severe OT due to an inhibition of the protective effect of IFN-γ.These findings afford new research avenues to look how to revert the Th2 deviated immune response in patients with severe forms of ocular toxoplasmosis.

Innate Resistance against *Toxoplasma gondii*: An Evolutionary Tale of Mice, Cats, and Men

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BACKGROUND: The evolutionary importance of any intermediate host for *Toxoplasma* is a function of the frequency with which it contributes to the transmission of the parasite or, in other words, is prey for felines. As a result, rodents are significant intermediate hosts, and their immune system adapted to better cope with *T. gondii* infection. By contrast, many vertebrates, as humans, which are not regularly part of the felines' food chain are considered accidental intermediate hosts and play little or no part in parasite evolution. Recent studies have revealed remarkable species specificity of the Toll-like receptors (TLRs) TLR11 and TLR12 and the IFN-inducible GTPases (iGTP) that are essential elements in the immune control of *Toxoplasma* in mice, but not in humans.
RESULTS: Our studies suggest that TLR11 and TLR12 emerged in rodents as *T. gondii* sensors that more efficiently initiate IL-12-dependent IFN-γ production, leading to a rapid activation of iGTPs and parasite elimination. Intriguingly, despite the wide parasite spread in nature, only a limited number of mammal species encode functional TLR11 and TLR12 genes, which include rodents and other orders of small mammals that are potential prey for cats and other felines. Although one can imagine the evolutionary pressures that gave rise to TLR11 and TLR12, why these genes are absent or downgraded to noncoding status in human genome is still unresolved? Biological and evolutionary aspects of these findings for the *T. gondii* host-pathogen relationship and for human disease will be discussed.

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Single nucleotide polymorphisms in innate immune genes associate with clinical outcome of acquired and congenital toxoplasmosis.

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BACKGROUND: The Toll-Like Receptor (TLR) pathway is of critical importance in mouse resistance to experimental toxoplasmosis. Our main hypothesis is that polymorphisms in genes encoding TLRs and related signaling molecules associate with human susceptibility to either congenital or acquired ocular toxoplasmosis.
METHODS: Haplotypes-tagging single-nucleotide polymorphisms within various innate immune genes were genotyped.
RESULTS: We observed an association of different single nucleotide polymorphisms (SNPs) in *TLR9* (rs187084) and *UNC93B1* (rs308328) and the clinical outcome of acquired ocular disease, whereas two SNPs (rs1461567 and rs4251513) in *IRAK4* gene were associated with congenital disease. Importantly, UNC93B1 mutant as well as IRAK4 KO mice had an impaired immune response and were highly susceptible to experimental infection with *T. gondii.* In contrast, we found no association of SNPs in *TLR2, TLR4* and *Mal/TIRAP* (an adaptor required for TLR2 and TLR4 function) and the outcome of toxoplasmosis. We also found an association of a SNP in *NOD2* (SNP rs3135499) with acquired ocular disease. Unexpectedly, we found an increased production of interleukin 17A (IL-17A) by CD4+CD45RO+T-bet−IFN-γ−T-helper 17 cells from patients with ocular toxoplasmosis.
CONCLUSIONS: Altogether, our results suggest that NOD2 influences the production of IL-17A by CD4+ T lymphocytes and also contributes to the development of ocular toxoplasmosis.

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Presence of anti-*Toxocara canis* antibodies in equine.

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BACKGROUND: *Toxocara canis* is a cosmopolitan parasite lodged at the small intestine of canine, for what the toxocariosis is an infection caused by larvae of the kind *Toxocara* spp, being *T. canis* or *T. cati*, the principal causal agents of the disease, constituting this way a sanitary problem widely spread in the whole world, for the multisystemic affectation that they produce. The infection is acquired by the accidental ingestion of embryonated eggs that are in soils, fomites or contaminated food, enclosed in the hair of puppies, etc. In this study the presence of anti-*Toxocara canis* antibodies was evaluated in equine as paratenic hosts.
METHODS: 96 blood samples of horses were used chosen at random. 5 ml took of blood of the jugular vein of every individual, the samples were deposited in pipes Vacutainer® by separating gel and were transported to a temperature of 4-6 °C to Parasitología's Laboratory of the National Institute of Pediatrics. The level evaluation of antibodies anti-*Toxocara canis* was realized using antigens of excretion / secretion of *Toxocara canis,* with the commercial kit SCIMEDX *Toxocara* Microwell Serum ELISA, the reading of the optical densities Victor Wallac K120 carried out in a spectrophotometer, using a point of court of 0.3 diopters for the positives. To compare the seroprevalence between kinds there was in use Fisher's exact test, the association between the seropositivity to *Toxocara canis*, was evaluated by means of the Ji-square test.
RESULTS: A seroprevalence was situated to *T. canis* of 13.82 % in the analysed horses. One did not present statistically significant difference (*P*=0.40) between females 14.81% (4/27) and males 13.43% (9/67) positives.
CONCLUSIONS: The equine ones in his paper like guests paratenic present antibodies anti *Toxocara canis*, which generates a source of infection for human beings and other animals.

Sylvatic triatomines in the Paraguayan Chaco. Epidemiological Challenges.

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BACKGROUND: Since 2011 when *Triatoma infestans* was found in sylvatic areas of the Chaco central several evidences have shown that sylvatic triatomines invade indigenous dwellings. However after post spraying *T. infestans* population detected is more related to residual insects that survived inside walls and ensures of sprayed dwellings. Other sylvatic species frequently detected invading peridomicile areas is *Triatoma sordida*. In this study we discuss the epidemiological importance of both species in the Chaco region.
METHODS: The study was carried out at 10 Leguas indigenous community. Triatomines were manually captured during daylight hours with the help of NERO, a 9 month-old gray German shepherd male dog. The specimens were placed individually in plastic containers and transported live to the laboratory and were then preserved in 70% ethanol for subsequent DNA extraction from legs. Feces were checked microscopically for possible trypanosome infection.
RESULTS: A total of 70 triatomines was located by NERO (22 *T. infestans*, 30 *T. sordida* and 18 *T. guasayana*). All specimens were captured alive from vegetation such as dry branches, hollow or standing trees as quebracho blanco (Aspidosperma), palo santo (*Bulnesia sarmientoi*) and dried cactus (*Stetsonia coryne*). *T. sordida* was captured most often in peridomestic environments and in sylvatic areas, and never was found invading or colonizing houses. *T. infestans* was captured in domestic, peridomestic and in sylvatic areas and confirmed by the mitochondrial cytochrome B gene analysis.
CONCLUSIONS: In peridomiciles and domiciles only *T. infestans* was found infected. In sylvatic areas there was a predominance of *T. sordida* in relation to *T. infestans*, and although both species were found in the same period of time they were never found sharing the same habitat. None of the specimens of any species examined were infected with trypanosomes. These features decrease *T. sordida* potential involvement in the *Trypanosoma cruzi* transmission.

Living Labs, spaces for open innovation and technology transfer. An alternative for social and economic problems to control Chagas disease.

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BACKGROUND: Living Labs model concentrates its effort to support actors, providing a neutral space in which stakeholders could know and co-develop innovations in real-world contexts and proposes five basic principles for the operations of the aforementioned living labs: courage, openness, realism, influence and sustainability. This study shows a living lab and project management model through a participatory approach for the development of a indigenous community in the Paraguayan Chaco.
METHODS: The study was carried out through 4 steps: 1) Description of the stakeholders and the perception of the problem, 2) Relationship with stakeholders and participatory approach to deepen the problem. 3) Preparation of the project and involvement of the research community. 4) Research-action.
RESULTS: To make possible the collection of rainwater, zinc roofs with gutters that collect water in tanks were implemented. This was the option chosen by the community, but they also experienced other modalities such as collection tents and access to safe water for human consumption through locally made ceramic filters. In a village, a model house was built with walls and roofs that help to eliminate access points for the transmitting vector of Chagas disease, in addition to using the roofs as water collectors for the drought season. The blocks were built with a manual machine for cement-soil. The building was finally used as a health post in the village, on the initiative and request of the indigenous community itself.
CONCLUSIONS: With the application of new knowledge and obtainment of products, the community has achieved the building of a house model with soil-cement blocks and training to replicate it; training in building for installation of tanks and gutters, and testing of new ideas for water collection; building of organic vegetable gardens for areas of high water stress, and use and training of ceramic filters for safe water consumption.

Patients infected with *Leishmania mexicana* have altered NK-cell response

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BACKGROUND: *Leishmania mexicana* causes localized (LCL) or diffuse cutaneous leishmaniasis (DCL). The cause of dissemination in DCL remains unknown, yet NK-cells possibly play a role in activating leishmanicidal mechanisms. We had previously shown that *Leishmania* lipophosphoglycan (LPG) is a ligand for TLR2, activating human NK cells.

METHODS: NK cells of LCL and DCL patients were stimulated with *Leishmania* LPG. TLR2, TLR1, TLR6 expression was analyzed by FACS; IFN-γ and TNF-α production by ELISA and immunecomplexes between TLR1 and TLR2 or TLR6 and TLR2 were analyzed by immunoprecepitations. Gene expression was analyzed by real-time PCR.

RESULTS: NK numbers and effector mechanisms differed between both groups of patients: DCL patients showed reduced NK-cell numbers, diminished IFN-γ and TNF-α production and lower TLR2, TLR1 and TLR6 expression, as compared to LCL patients. The altered protein expressions found in NK cells of DCL patients correlated with their down-regulation of IFN-γ gene expression, and to a lesser extent, those of TNF-α and TLR2, in LPG-stimulated and non-stimulated cells, as compared to LCL patients. NK cells in female patients produced higher IFN-γ levels throughout the disease progression. TLR2 expression diminished in both genders with prolonged disease evolution and age. We show the activation pathway of LPG binding to TLR2 and demonstrated that TLR2 forms immunocomplexes with TLR1 and TLR6, when binding LPG. The analysis of NK cells in DCL lesions corroborated the results obtained in blood NK cells, showing reduced NK-cell numbers, randomly scattered within the lesions and diminished cytokine production, which contrasts with those of LCL lesions, where NK cells produced high levels of IFN-γ and TNF-α and were found within organized granulomas.

CONCLUSIONS: Disease severity in DCL patients is related to a reduction of NK-cells numbers and their effector mechanisms, evidenced by low TLR expression and cytokine production. (CONACyT-102155; PAPIIT IN215212)

Novel aspect of immune suppression in leishmaniasis

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BACKGROUND: *Leishmania* lipophosphoglycan (LPG) is a molecule that has been used as a vaccine candidate, albeit with contradictory the results. Unsuccessful protection could be related to suppressed T cell responses.
METHODS: We analyzed the expression of inhibitory receptor PD-1 in $CD8^+$ and $CD4^+$ lymphocytes and it's ligand PD-L2 in macrophages of BALB/c mice immunized with various doses of *Leishmania mexicana* LPG and stimulated *in vitro* with different concentrations of LPG. We also analyzed these molecules in *Leishmania mexicana* infected mice.
RESULTS: *In vitro* stimulation with 100 µg LPG enhanced the expressions of both the inhibitory receptor PD-1 in $CD8^+$ cells and its ligand PD-L2 in macrophages. Additionally, activation molecules CD137 were reduced in $CD8^+$ cells. The modulation of PD-1, PD-L2 and CD137 correlated with the amount of LPG used. We further analyzed the expression of these molecules in mice infected with $1x10^4$ or $1x10^5$ *L. mexicana* promastigotes and stimulated *in vitro* with LPG. Infection with $1x10^5$ parasites significantly increased PD-1 expression in $CD8^+$ and PD-L2 in macrophages. When these $CD8^+$ cells were further stimulated *in vitro* with LPG, simulating a second exposure to parasite antigens, the PD-1 expression increased significantly more, in a dose dependent fashion.
CONCLUSIONS: *Leishmania* LPG inhibits $CD8^+$ T lymphocytes and macrophages in a dose-dependent fashion and according to the parasite load, leading to enhanced expression of PD-1. Functional inactivation of $CD8^+$ cells can have critical consequences in leishmaniasis, since these cells are crucial for disease control. These results call for evaluations of potential immunogens, specifically where CD8 cells are required, since inhibiting molecules can be induced after certain thresholds of antigen concentrations. We propose that the analysis of PD-1, PD-L1 and PD-L2 are a useful tool to monitor the optimal dose for vaccination candidates. (CONACyT-102155; PAPIIT IN215212)

The analysis of exosome-like vesicles from *Schistosoma japonicum* treated with erythrocytes

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BACKGROUND: Recently, the secreted vesicles from cells are known to have roles of cell-cell communications and immune evasions against host cells in protozoan parasites. Especially, exosome-like vesicles are mostly reported as an important mobile carrier to other cells, because they have a large amount of miRNA, lipid, and protein. In the present study, to observe the mechanisms of communications and immune evasions of *Schistosoma japonicum*, belong to platyhelminth parasite, we analyzed the exosome-like vesicles derived from schistosomes. However, we did not almost collect the secretory vesicles from cultured adult worms of *S. japonicum*. Now, we evaluate the exosome-like vesicles from the cultured worms treated with erythrocytes, because it is reported that the productions of exosomes required lipid metabolisms, especially, sphingomyelin to ceramide.
METHODS: We collected paired adult worms of *S. japonicum* form the infected mice. The worms were treated with mouse erythrocytes for 1 days, 3 days, or 7 days. After culture, the supernatants were collected and ultracentrifuge was performed. The vesicle fraction was observed under an electron microscopy, and total RNA was extracted from this fraction to analyze by Bioanalyzer, and to detect specific miRNA of *S. japonicum*. Furthermore, we evaluated the inhibitory effects of exosome-like vesicles productions using GW4869 (n-SMase inhibitor), and BAPTA-AM (chelators of intracellular Ca^{2+})
RESULTS: A large amount of exosome-like vesicles was observed in the supernatant from the erythrocyte-treated worms, and small RNA fraction and schistosome-specific miRNA was also found in exosome-like vesicle fractions. However, the productions of exosome-like vesicles were not inhibited by GW4869 and BAPTA-AM.
CONCLUSIONS: The adult worms of *S. japonicum* secrete the exosome-like vesicles after the stimulations of the erythrocyte uptake. So, they may use the secreted vesicles for the male-female communications at adult stage, because they begin to take the erythrocyte in the portal vein of the liver before the sex maturation.

Isolation, identification and biological characterization of nematophagous fungi from water buffalo (*Bubalus bubalis*) and soil from the Mexican southeast

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BACKGROUND: Gastrointestinal nematodes (GIN) affect the sheep industry. Nematophagous fungi (NF) trap and destroy nematodes and they are considered as a tool against GIN. This study was aimed to isolate, identify and characterize NF from feces of water buffalo (*Bubalus bubalis*) (WB) and from soil from the Mexican southeast.
METHODS: WB feces from 25 animals from Veracruz and soil from 12 Municipalities from Tabasco, Mexico, were collected. NF were isolated using the “sprincle” technique. identification was based on identification keys. NF were evaluated in water agar plates containing the fungal cultures of 10 days of age (n=6). Fifty microliters of water containing 500 *Haemonchus contortus* (L3) (*Hc*L3) were added to each plate and incubated at room temperature for 7 days. *Hc*L3 were recovered using the Baermann technique. Mean of recovered *Hc*L3 in fungal plates was compared with a proper control to estimate predation. Data were analized using ANOVA and Tukey test.
RESULTS: Four isolates were obtained from feces: *Arthrobotrys oligospora, var microspora* (4-276; 269 and 50-80 strains) and *A. oligospora, var. oligospora* (48-80 strain) and 5 NF were obtained from soil: *A. musiformis* (Bajío 3, Yumca and Macuspana strains) and two NF identified as *A. oligospora* (Comalcalco and Jalapa de Méndez, strains). Predation ranged from 85.9 and 100% and 55.5 and 100% ($p \leq 0.05$) for isolates from feces and soil, respectivelly.
CONCLUSIONS: The NF isolated showed good predatory activity and they will be evaluated as potential biological control agents of GIN.

Basophils protect against gastrointestinal helminths by Fc-receptor mediated release of IL-4/IL-13

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BACKGROUND: Basophils are functionally related to mast cells, can be activated by the high-affinity receptor for IgE (FcεRI) and release several effector molecules including the cytokines IL-4/IL-13 which play an important role for protective immunity against gastrointestinal helminths. Basophils contribute to protective immunity against secondary infections with gastrointestinal helminths and ticks. However, the molecular mechanisms how basophils confer this function have not been resolved.
METHODS: We generated mixed bone marrow chimeras with donor cell from basophil-deficient Mcpt8Cre mice and IL-4/IL-13-deficient or Fc-receptor deficient mice so that all basophils in these chimeras lacked expression of IL-4/IL-13 or Fc receptors while all other cell types were not affected. These chimeras were subjected to sequential infections with *N. brasiliensis* and *Heligmosomoides polygyrus*, two murine models for human hookworm infection.
RESULTS: We observed that protective immunity against secondary infection with *N. brasiliensis* or *H. polygyrus* was largely dependent on basophil-derived IL-4/IL-13. Activation of basophils via the FcRg chain, which is the main signaling component of FcεRI and FcγRIII, was required for this effect. By using mice that lack IgE or IgG1 we could further show that IgE but not IgG1 played the major role for basophil activation
CONCLUSIONS: By using two different gastrointestinal helminth infection models, we could demonstrate that IgE-mediated activation and release of IL-4/IL-13 from basophils accounts for faster worm expulsion and reduced fecundity during secondary infection. This finding could help to design vaccines against hookworms that promote differentiation of long-lived IgE-producing plasma cells which secrete IgE antibodies for prolonged sensitization of basophil against worm antigens.

Functional analysis of Apicomplexa-producing plant hormone

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We previously reported that *Toxoplasma gondii* produces the plant hormone, abscisic acid, and used it as a key molecule for the cell differentiations. Furthermore, many Apicomplexan parasites possess the plastid, apicoplast. Since the apicoplast is evolutionary thought to originate from the 2nd endosymbiosis of ancestral red algae, it suggests the existence of conserved plant hormone set in the parasites, but it has not been examined.

Here we show that a rodent malaria parasite, *Plasmodium berghei*, produces a plant hormone, salicylic acid (SA). SA is immune-relating hormone of land plant, and it existed in the parasites lysate at extremely high concentrations. To unveil the function of this molecule, we transfected a human malaria parasite, *P. falciparum*, with *nahG* gene, a SA degrading enzyme from *Pseudomonas* sp., and successfully established the SA deficient mutant. Although we did not find any changes in growth, the mutant significantly decreased the concentration of prostaglandin E_2 (PGE_2). *P. falciparum* is known to actively synthesize PGE_2, and that is believed to modulate the host immunity as an adaptive evolution of *Plasmodium* spp. to the infecting milieu. The data of parasite SA and PGE_2 suggested the influence of SA not to the *in vitro* parasite growth but to the immune-alternation of the host.

Next we established the *in vivo* experimental system by *P. berghei* transfectant with *nahG*. The mice infected with the *nahG*-transfectant were changed the mortality significantly. Both of Evans-blue leakage assay and brain histology showed the enhanced cerebral malaria phenomenon in mice infected with the transfectant. We also found that both plasma PGE_2 and Th1 type cytokines were changed in infections of the transfectant comparing to the control. These data suggested that SA of *Plasmodium* spp. modulates the immune function of the host as a new pathogenic factor of this threatening parasite.

Alternative control for helminth infections in ruminants: a reality?

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BACKGROUND - Anthelmintic resistance in ruminants threatens the sustainability of the sheep and goat industries throughout the world, and for the cattle industry the problem is increasing. The exacerbation of this problem over the last decade has provided the impetus for research into non-chemotherapeutic parasite control alternatives.
METHODS - The use of targeted treatments (TT), targeted selective treatments (TST), grazing management, host genetic resistance, helminth vaccines and biological control can all be helpful for preserving anthelmintic effectiveness, by reducing the reliance on anthelmintics.
RESULTS - Both TT and TST have been shown to be able to reduce anthelmintic use with negligible, if any, effects on animal performance. Methods for grazing management are well known, while selection for resistance in the host and vaccines towards helminths appear promising. However, there are few studies showing the effects of using these approaches in combination. Although some of these options provide practical benefits if adopted today, or exciting prospects for the future, a major mentality shift by the farming industry will be required to put these principles into practice. In this presentation, two questions will be discussed (1) What are the prospects for TT, TST and vaccine control? and (2) What is needed to implement these control systems?
CONCLUSIONS – We have now tools to reduce the reliance on anthelmintics, however, effective dissemination of this information is required to ensure that farmers are aware of these new tools, and how to apply them, so that they can be implemented into existing herd/flock management strategies.

Kissing-bugs, and the risk of trypanosomiasis in SE Asia

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Most species of kissing-bug (Hemiptera, Reduviidae, Triatominae) occur in the Americas, where they are well-known as vectors of *Trypanosoma cruzi* – causative agent of Chagas disease (American trypanosomiasis). This infection is not yet recorded from SE Asia – except in previously-infected american immigrants – but the kissing-bugs themselves are of increasing nuisance in many SE Asian cities. At present, the main nuisance is due to bite reactions – which are sometimes serious enough to give fever, and in at least one case resulted in death attributed to anaphylactic shock. The most widespread of these kissing-bugs, *Triatoma rubrofasciata*, is a competent vector of *T.cruzi* in Latin America, and is also frequently infected with a rat trypanosome – *T.conorhini* – which is not yet known to infect other domestic animals or humans. The sudden increase in reported bite reactions to *T.rubrofasciata* may reflect host switching after the drastic reductions in urban chicken populations in response to fear of avian influenza over the last decade.

Host cell invasion by *Trypanosoma cruzi* extracellular amastigotes: recent findings

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BACKGROUND: Differently from the metacyclic and bloodstream trypomastigotes, cellular invasion by *T. cruzi* extracellular amastigotes (EAs) is mainly dependent on host cell actin cytoskeleton. Few studies have shown the role of either host or parasite components in this process.

METHODS: HeLa cells, LIMP-2 KO fibroblasts, and EAs from the G strain were used and interactions analyzed by confocal and electron microscopy and immunochemical approaches.

RESULTS: Regarding host factors, HeLa cells cortactin depleted, a key regulator of actin dynamics, presented reduced invasion by EAs. Cortactin is recruited to and colocalizes with actin filaments and protein kinase D1 (PKD1), a cortactin upstream kinase, at EA invasion sites. EAs induced phosphorylation/activation of PKD1 and extracellular signal-regulated kinases (ERK), but not Src family protein tyrosine kinases. β-glucocerebrosidase, a lysosomal protein associated to LIMP-2, also influenced EA invasion, possibly by modifying membrane fluidity through ceramide generation. Mevalonate kinase (TcMvk) is secreted by the parasite and induces host signaling. Recombinant TcMvk positively modulated internalization of *T. cruzi* EAs whereas inhibited invasion by metacyclics in pre-treated HeLa cells. EAs also secrete Ssp-4-rich vesicles that may associate forming trails, also visible on the parasite surface. Fractionation of EAs supernantants indicated that TcMvk and Ssp-4 are secreted with vesicles or in vesicle-free forms. Finally, initial results indicate that these secreted fractions can modulate host cell invasion by EAs.

CONCLUSIONS: All together these results corroborate that EA-host cell interaction is a multifactorial process regulated by both cellular and parasite factors.

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Seroepidemiology of *Toxoplasma gondii* in dogs and cats in the regions of Grande Vitória, Espírito Santo, Brazil

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BACKGROUND: Toxoplasmosis, infection by the protozoan *Toxoplasma gondii,* is widely prevalent in humans and animals throughout the world. The seroprevalence in companion animals, such as dogs and cats, can be used to assess environmental contamination by the parasite, since they are exposed to similar risks to which man is subjected.
METHODS: The prevalence of infection caused by the *parasite* was evaluated in the regions of Grande Vitória, state of Espírito Santo. We evaluated serum samples from 378 dogs and 79 cats from Centers for Zoonosis Control (CCZs) and temporary shelters, as well as epidemiological data on origin, sex, breed and age of each animal. IgG anti-*T. gondii* antibodies were analyzed by Enzyme-Linked Immunosorbent Assay (ELISA) and Indirect Immunofluorescent Antibody Test (IFAT).
RESULTS: The seroprevalence in dogs was 39.4% (149/378) by ELISA and 38.1% (142/373) by IFAT, while the frequency of antibodies in cats was 15.2% (12/79) by ELISA and 7.6% (6/79) by IFAT. Risk factors associated with canine infection were stray origin and equal to or higher than a year old. Risk factors associated with feline infection was related to the sex of cats, with females showing higher chance of infection. The evaluation of the results of the dogs revealed excellent agreement between techniques ($\kappa = 0.82$), with no statistically significant differences ($p = 0.377$). Among the cats, despite agreement between both tests ($\kappa = 0.63$), there was differences according to statistical analysis ($p = 0.041$).
CONCLUSIONS: The results demonstrate a high contamination of the environment by the parasite, suggesting high level risk of human infection and other animals. This is the first study to determine frequency of antibodies anti-*T. gondii* in dogs and cats in Espírito Santo.

Impact of *Plasmodium vivax* uncomplicated and complicated malaria in Latin America

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Morbidity and mortality burden of malaria, particularly in children, represents a public health threat also in those countries from regions such as South East Asia and Latin America with moderate to low levels of transmission. Malaria mortality in these areas has been mainly attributed to *P. falciparum*, but its direct and indirect burden has not well defined. These patterns are increasingly causing concern in some countries. Although *P. falciparum* is justifiably regarded as the greater menace because of its high mortality, widespread antimalarial drugs resistance and its dominance on Africa, malaria due to *P. vivax* has also placed significant burdens on health, longevity and general prosperity of large sections of the human population. The debilitating impact of *P. vivax* malaria remains high, unacceptable and preventable for well over one billion inhabitants of the planet. Complicated and even fatal cases of malaria due to *P. vivax* have been increasingly reported in the medical literature. In Latin America the burden of mortality due to malaria, although decreasing, is still significant. Powerful antimalarial campaigns in the region directed mainly to *P. falciparum* achieved a significant reduction of mortality in the last century. Evidence suggests that *P. vivax* can imposes a significant burden of mortality that may have resulted from its interaction with other diseases and conditions, although this largely neglected, compared to *P. falciparum*. These and other epidemiological issues will be discussed during this conference, focused in Latin America.

Globalization of a Latin American parasitic zoonosis: Chagas Disease

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Chagas disease is a parasitic zoonosis endemic in Latin America, caused by a protozoan organism, *Trypanosoma cruzi*, and transmitted by triatomines, particularly from genus *Triatoma*. Discovered over 100 years ago by Carlos Chagas this disease had an intense vectorial intervention, particularly in South America, where control programs reduced significantly its transmission and seroprevalence. However, in the context of globalization and migration, this condition emerges as global public health problem now seen in regions such as Europe, North America, Asia, Australia, where people, even asymptomatic, from different countries of Latin America are carriers of the infection. For these reasons, in these recipient regions of the World, where significant number of cases are seen, is important to keep in mind and to know about the clinical and epidemiological aspects, as well diagnostic and therapeutic implications of this parasitic disease. Even more, new modes of transmission (oral borne) may also be implying that human beings are not the only potential risk in non-endemic regions, but also contaminated food can be a source of the parasite. Currently some Andean countries locations represented the places where highest seroprevalences are reported (e.g. Bolivia), however countries such as Venezuela and Brazil have also reported recently many outbreaks of oral acute disease. Given the migration patterns, countries with more reported cases of Chagas disease imported are Spain and United States of America, but followed by Canada, Portugal, France, Italy, Switzerland and England among others. In this context clinical and epidemiological suspicion, blood bank surveillance and testing looking for antibodies against *T. cruzi* are of utmost importance in cities where significant number of Latin American migrants would be seen. Although *T. cruzi* infection diagnosis is not easy, even commercially-developed test are available for rapid patient assessment, however, PCR-based techniques are preferred for confirmation. Therapy in Chagas disease is only considered efficacious in acute forms, although treatment during chronic forms is controversial. Drugs approved for its treatment still remains in the couple benznidazole and nifurtimox, although a long line of drugs (many of them currently used for other indications) are experimentally active against the parasite. Implications of disease and migration into public health and infectious disease practice outside Latin America will be discussed.

Mapping malaria in municipalities of the Coffee-Triangle Region of Colombia using Geographic information system (GIS)

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BACKGROUND: In other geographical settings, use of GIS for development of epidemiological maps in malaria has been extensively used, however not in the Coffee-Triangle region of Colombia, an area of three departments and 53 municipalities with endemic areas of disease for *P. vivax*, *P. falciparum* and *P. malariae*. Then, we developed such maps.
METHODS: Surveillance cases data (2007-2011) were used to estimate annual incidence rates using reference population data, on above described etiological agents of malaria (cases/100,000 pop) to develop the first maps of malaria in the 53 municipalities of the coffee-triangle region of Colombia (departments Caldas, Quindio, Risaralda). GIS used was Kosmo® 3.1. Thirty thematic maps were developed according municipalities, years and parasite etiology, as well on uncomplicated and complicated cases.
RESULTS: Between 2007-2011, 6582 cases were reported (6478 uncomplicated and 104 complicated) (77.8% from one department, Risaralda), for a cumulated rate of 269.46 cases/100,000pop. Among uncomplicated cases, 5722 corresponded to *P. vivax* (234.25 cases/100,000pop), 475 *P. falciparum* (19.45 cases/100,000pop), 8 *P. malariae* (0.33 cases/100,000pop) and 273 mixed (*P. falciparum/P. vivax*) (11.18 cases/100,000pop). Highest rate was reported in the less developed and more rural municipality of one department (Pueblo Rico, Risaralda) with 5.77 cases/1,000pop (717 cases in 2009).
CONCLUSIONS: Burden of disease is concentrated in one department (over 75% of the cases of the whole region). Use of GIS-based epidemiological maps allow to guide decisions-taking for prevention and control of a public health problem that still represents a significant issue in the region and the country, particularly in children.

Metabolomic analysis of *Giardia intestinalis*

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BACKGROUND: Metabolomics can generate a detailed profile of low-molecular-weight metabolites in biological systems, giving insights into the parasite-host metabolic interaction. Separation and identification techniques used include GC-MS, LC-MS, and NMR spectroscopy. Metabolomic studies hold great promise for the improved understanding and control of *Giardia*. Previous work on *Giardia*'s has focused on specific pathways and although NMR-based analyses of metabolic end products have been performed, no comprehensive profile of intra- or extracellular metabolic products or intermediates has been performed. The data generated by this approach not only give compound identity but also concentration. This approach can be used to compare different isolates of the same organism or used to evaluate the effects of change on these systems. Limitations to this approach include significant variation within samples that can be overcome with internal standards as well as variation among samples that requires large numbers of replicates to be analysed. In addition, the approach is only as good as the library of compounds available.
METHODS: We have analysed the variation in metabolites utilised and produced by 5 genotyped isolates of *Giardia intestinalis*.
RESULTS: Data from this study show that although there is significant variation between isolates it is clear that the overall profiles of utilisation and production are similar. However, the metronidazole resistant isolate JKH-1 exhibits an altered pattern indicating a greater flux through the carbamoyl phosphate pathway and a concomitant reduction in glycolysis. CONCLUSIONS: A more detail analysis of other resistant isolates is required however this may suggest reduced flux through pyruvate: ferredoxin oxidoreductase and thus reduced drug activation is important in resistance.

Phenotypic and genotypic characterization of *Haemonchus* spp. and other gastrointestinal nematodes resistant to benzimidazole in infected calves from tropical regions of Campeche State, México

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The aim of this study was to identify the presence of anthelmintic resistance to benzimidazole (BZ) in gastrointestinal nematodes (GIN) from naturally infected calves in tropical regions of Campeche State of Mexico. The faecal egg count reduction test (FECRT) was conducted on 10 livestock localized in the Carmen, Candelaria, Champotón, Escárcega and Palizada municipalities of Campeche. The assessed anthelmintic was albendazole (Valbazen, Pfizer). Infected calves were allocated into two groups: control and treated on each livestock. The number of eggs excreted per g of faeces was estimated by McMaster technique at 0 and 14 days pre- and post-treatment, respectively. Morphometric larvae (L_3) identification was conducted through faecal culture and taxonomic keys per group. The presence of BZ resistance polymorphisms on *Haemonchus* were determined by Allele Specific (AS-PCR) at codon 200 and by end-point PCR technique at codons 200, 198 and 167 from the isotype 1 of the β-tubulin gene. Genomic DNA template was performance from a pool of L_3 species, previous BZ treatment. Morphometric identification results showed five L_3 species before treatment, *Haemonchus* spp, *Cooperia* spp., *Trichostrongylus* spp., *Ostertagia* spp. and *Oesophagostumum* sp., and two L_3 species were identified after BZ-treatment (*Haemonchus* spp and *Cooperia* spp.). GIN resistance to BZ was observed on three farms out of 10, and seven GIN populations were susceptible by FECRT. Molecular analysis results showed BZ resistance problem on *Haemonchus* spp. population (*rr or Sr*) associated with nucleotide changes at codon 200 (T*T*C to T*A*C) by AS-PCR. Mutations at 167 (T*T*C to T*A*C) and 198 (G*A*A to G*C*A) codons were not identified in any *Haemonchus* population. Resistance to BZ was found in *Haemonchus* spp. and other GIN from grazing cattle in 10 livestock from Campeche State.

Transcriptional analysis of 5S ribosomal RNA genes in *Leishmania major*

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BACKGROUND: 5S ribosomal RNA (rRNA) genes are transcribed by RNA polymerase III (Pol III). Transcription factors TFIIIA, TFIIIB and TFIIIC are also required for synthesis of 5S rRNA. Little is known about Pol III transcription in *Leishmania major*. Neither TFIIIA nor TFIIIC have been identified in this parasite, which contains only 11 copies of the 5S rRNA genes.
METHODS: Bioinformatic analyses were done to identify internal promoter sequences and to determine putative secondary and tridimensional structures of the rRNA. RACE experiments were performed to locate transcription start and termination sites. EMSA, Southwestern and Northwestern assays were done to identify proteins that bind to the 5S rRNA gene and to the transcript.
RESULTS: Internal promoter elements are conserved in *L. major* 5S rRNA genes. Also, the secondary and tridimensional structures of 5S rRNA are highly conserved in relation with other eukaryotes. As an example, helix III 3D contains two unpaired adenines that in other organisms are needed to transport the rRNA to the nucleolus. We found that the transcription initiation site corresponds to the first nucleotide of the gene, and that transcription ends in a tract of Ts located downstream of all the 5S rRNA genes, showing that processing is needed in the 3´ region. EMSA assay showed the specific union of a low-weight protein (or proteins) to the 5S rRNA gene. Southwestern and Northwestern assays revealed that nuclear proteins bind specifically to 5S rRNA gene and its transcript.
CONCLUSIONS: Transcription initiation and termination sites are conserved in 5S rRNA genes in *L. major*. Proteins that specifically bind to these genes have been identified.

Epidemiological survey on malaria infections in western lowland gorillas inhabiting Dzanga-Sangha Protected Areas, Central African Republic

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BACKGROUND: African great apes are infected with several *Plasmodium* species, including the precursor of human parasite *Plasmodium falciparum*. However, the ecology of these pathogens in gorillas is not studied. This study designed to determine the prevalence, diversity and individual traits underlying the incidence of malaria infection in gorillas.
METHODS: We analysed 131 gorilla faecal samples using nested PCR to amplify approximately 930bp fragment of Cytochrome b (*Ctyb*). All positive amplicons were sequenced bidirectional to identify *Plasmodium* species. A total of 95 human blood samples were included to examine the possibility of cross transmission between gorillas and human.
RESULTS: Prevalence of *Plasmodium* spp. was high in both gorillas and humans (32% and 44.2% respectively). *P. praefalciparum*, *P. alderi* and *P. blacklocki*, as well as *P. vivax* and *P. ovale* were present in gorilla samples. Only *P. falciparum* was detected in human samples. No evidence of cross transmission between gorillas and human was observed. We found no association between sex and *Plasmodium* infections, however younger gorillas individuals ≤ 6 years were more susceptible to infections.
CONCLUSIONS: longitudinal monitoring of *Plasmodium* infections in gorillas is required in order to draw precise ecological aspects of the pathogens.

Sceneries of Chagas disease transmission in Brazil Amazon

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BACKGROUND: The Brazilian Amazon Region covers 4,871,500 Km^2, accounting for 58% of the Brazilian territory, and 67% of the Pan-Amazon. Chagas disease in the Brazilian Amazon Region had always been considered to be an enzootic disease of wild animals until Shaw et al (1969) described the first four cases of humans infected with *T.cruzi* in Belém of Pará, from a small outbreak most liked caused by oral transmission.
METHODS: Revising the risks of endemic Chagas disease in Brazilian Amazon we found (Coura & Junqueira 2012): (i) there is an extensive reservoir of wild mammals infected with *T.cruzi* represented by 38 species of six order: Marsupialia, Chiroptera, Edentada (Xenarthra), Carnivora and Primata; (ii) 16 species of wild triatomines, 10 of them infected with *T.cruzi*, have been found in Brazilian Amazon; (iii) several outbreaks of acute and numerous chronic and fatal cases of Chagas disease have been described in that region; (iv) the uncontrolled deforestation and risks of adaptation of wild triatomines to human dwellings and (v) the increasing migration of people with domestic animals infected with *T.cruzi* from endemic areas to the Amazon Region.
RESULTS AND CONCLUSIONS: Four sceneries of Chagas disease transmission can be observed in the Amazon Region (Coura et al 2013): (i) **enzootic infection** of wild mammals transmitted by sylvatic triatomines and/or by cannibalism; (ii) **anthropozoonosis**, an accidental human infection by *T.cruzi* when man invades the wild ecotope or when triatomines and wild reservoirs of *T.cruzi* (marsupials) invade human dwellings; (iii) acute disease caused by **oral transmission** of *T.cruzi* through infection of food contaminated with faeces and/or urine of wild triatomines or with the odoriferous secretions of marsupials, thus causing epidemic outbreaks of acute Chagas disease and (iv) **professional disease** from plant extraction workers, especially those who works with extraction of piassava fibers, attacked by wild triatomines.

Wild, peridomestic and domestic cycles of Chagas disease

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BACKROGUND. The wild cycle of Chagas infection has existed in nature for millions of years. Some accidental human cases might have occurred at the time when mankind lived in caves, but evidence of human infection has so far only been found in mummies from 4,000 to 9,000 years ago. Although triatomines have been known since the XVI century, their adaptation to human dwellings began with the agricultural cycle and was intensified during the livestock cycle, through increasing deforestation and removal of the wild animals that were the food source for triatomines. To survive, triatomines gradually adapted to areas surrounding human dwellings and the interiors of these dwellings, and as a result underwent genetic simplification. METHODS. The transmission of Chagas disease involve four situations: **enzootic disease** of wild animals to an **anthropozoonosis** when mankind invaded wild ecotopes and became infected. In addition, when wild animals and vectors invade human domiciles, man became infected by means of vector transmission or through food contamination due to the excreta of vectors or marsupials. When wild triatomines adapt to areas in and around human dwellings and Chagas infection starts to be exchanged between domestic animals and humans, as is the case of endemic areas for Chagas disease, the situation is classified as a **zoonosis**. Finally, the infection can be characterised as an **zooanthroponosis**, meaning an infection that is transmitted from man to domestic animals and from these to wild animals. RESULTS AND CONCLUSIONS. The ecoepidemiology of Chagas disease involving more than 100 species of mammals and 150 species of triatomines, potential reservoirs and vectors of *T.cruzi*, and more of 90 millions of persons exposed to the infection from the Southern of United States to Southern of Argentina and Chile, including wild, peridomestic and domestic cycles, which interchanges in different environments.

Cytokine gene polymorphisms in *Blastocystis* carriers

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BACKGROUND: *Blastocystis,* a human parasite, has been associated to the development of irritable bowel syndrome (IBS). Since some gene polymorphisms might have a role in the pathophysiology of this syndrome, in which the expression of some cytokines is unregulated. The objective of this study was to assess the role of single nucleotide polymorphisms (SNPs) for interleukin (IL) -6, 8, 10 and tumour necrosis factor-alpha (TNF-α) in IBS patients and controls, with or without *Blastocystis* infection.
METHODS: After giving written consent, 45 patients with symptoms of IBS according to the Rome III criteria and 45 controls were enrolled. DNA was extracted from peripheral blood white cells for SNP analysis at positions -174 for IL-6; -251, +396, +781, +1633 for IL-8; -1082, -819, -592 for IL-10, as well as -238 and -308 for TNF-α
RESULTS: IL-8+396(G) and IL-10-1082 (A) alleles (p=0.0437 and p=0.0267, respectively), as well as their homozygous genotypes (p<0.0001 and p=0.0039, respectively) and IL-8+781(CT) (p=0.0248) were significantly overrepresented in patients with IBS in comparison with controls. IL-8+396(GG) genotype was relevant because it was associated to IBS (p<0.0001), to *Blastocystis* (p=0.0025), and to IBS–*Blastocystis* (p=0.0272). In the latter binomial association, this genotype presented a high contribution (etiological fraction=0.452) and a risk >fourfold to develop IBS. IL-8+781 (T) and IL-10-592 (C) alleles were also associated to *Blastocystis* and to IBS–*Blastocystis*, respectively (p=0.0448 and p=0.0166). Haploview analysis revealed linkage disequilibrium in TNF-α (p<0.0001); however, none of the SNPs for IL-6 and TNF-α were found to be significantly related with IBS or with *Blastocystis*.
CONCLUSIONS: Our results suggest that some IL-8 and IL-10 SNPs could change individual susceptibility increasing the relative risk in the development of IBS in *Blastocystis* carriers.

Can operational research help guide the control and elimination of schistosomiasis?

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BACKGROUND: In 2001 World Health Assembly (WHA) Resolution 54.19 encouraged countries to control morbidity due to schistosomiasis through mass drug administration (MDA) of praziquantel (PZQ). In 2012 with WHA Resolution 65.21, elimination of schistosomiasis was called for where feasible. These WHA Resolutions, plus the WHO Roadmap 2020 for NTDs and the London Declaration of January 2012, coupled with Merck-Serono's pledge to donate 250 million PZQ tablets/year and delivery funds from USAID, DFID and others, stimulated essentially all endemic countries in sub-Saharan Africa to develop national MDA plans for schistosomiasis. The fight against schistosomiasis is in a critical period with tremendous opportunities and significant challenges. How do we achieve the most effective results with MDA? What tools do we need to do this better? How will we know when to move from morbidity control to elimination? What combinations of interventions, beyond MDA, are needed for elimination?
METHODS: The Bill & Melinda Gates Foundation-funded Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) has programs in more than 24 institutions in 19 countries to try to answer these very practical questions through multiple large field-based operational research studies, parallel intervention evaluations, and development of better diagnostics.
RESULTS: There is progress on evaluation of a point-of-contact, urine-based schistosome antigen (CCA) detection assay as a mapping tool for the prevalence of *Schistosoma mansoni*, and the multiple large field-based intervention studies are now yielding preliminary findings on comparative MDA strategies.
CONCLUSIONS: This presentation will summarize the current status of the operational research by SCORE that is designed to provide much-needed answers for national program managers so they can most effectively plan and implement these critical public health programs.

RNA Polymerase III transcription in *Trypanosoma brucei*: characterization of the negative regulator Maf1

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BACKGROUND: RNA polymerase III (Pol III) synthesizes essential RNA molecules, such as tRNAs and 5S rRNA. Maf1 is a negative regulator of Pol III transcription conserved from yeast to human. It physically interacts with Pol III and with transcription factor TFIIIB. The function of Maf1 is regulated by phosphorylation of specific amino acids. By *in silico* analysis we identified two Maf1 genes in the *T. brucei* genome.
METHODS: To analyze the function of Maf1, RNA interference (RNAi) knock-down cell lines were generated. The growth of the cells was examined after tet induction. Northern and Western blots were carried out to evaluate the levels of Maf1 mRNA and protein, respectively. Nuclear run-on experiments were performed to study the effect of Maf1 depletion on transcription in *T. brucei* procyclic forms.
RESULTS: Bioinformatic analysis confirmed the presence of the three previously-reported conserved motifs on Maf1 and allowed the identification of 14 putative phosphorylation sites. A clear reduction in the amount of Maf1 mRNA and protein was observed in the knock-down cell line. Interestingly, the induced cell line showed a ~30% increase in cell growth rate compared to the uninduced line. A substantial increase in Pol III transcription was found in the induced culture.
CONCLUSIONS: Our data show that Maf1 is a repressor of Pol III transcription in *T. brucei.*

In silico and _in vitro_ interactions between myosin B and MTIP of _Plasmodium falciparum_.

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BACKGROUND: Gliding motility in Apicomplexan parasites is driven by a myosin-based machine named glideosome. In *Plasmodium falciparum*, myosin A (PfMyoA) is associated with the myosin tail domain interacting protein (MTIP) and both have been involved in gliding motion. This complex PfMyoA-MTIP is present in both motile and invasive parasite stages and is widely conserved across *Plasmodium spp.* Recent evidence from our laboratory suggests that *P. falciparum* myosin B (PfMyoB) would have redundant functionality with PfMyoA. The aim of this study was to build a docking model of MTIP and the PfMyoB tail domain using computational tools, and to analyze *in vitro,* protein-protein interactions between a recombinant protein with the PfMyoB tail domain and a whole recombinant MTIP.
METHODS: For bioinformatics, a 3D model of PfmyoB was made by method of *threading* and a pdb archive was taken from pdb.org for MTIP. Docking was made and adjusted by PatchDock server. For *in vitro* interactions, a soluble PfMyoB tail domain recombinant protein was produced as a fusion protein with maltose binding protein (MBP). A whole recombinant MTIP was also produced as a soluble fusion protein with gluthation-S-transferase (GST). As controls, we generated soluble recombinant proteins for MBP and GST. All these proteins were purified by affinity chromatography and a far western blot assay was made with PfmyoB as bait. Thus, PfmyoB was immobilized in PVDF membranes and incubated with MTIP as prey. Controls included MBP, BSA and TBS buffer on the bait membrane and incubations with GST as prey also.
RESULTS: An *in silico* interaction model was generated for PfmyoB and MTIP. Far western blot experiment show that these proteins can interact *in vitro*.
CONCLUSIONS: Our results support the hypothesis of functional redundance of PfmyoA and PfmyoB. This research was supported by Colciencias, project 1101-521-28729.

Identification of determinants of visceral leishmaniasis by proteomic comparison of atypical *Leishmania donovani* strains

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BACKGROUND: Leishmaniasis is a neglected tropical disease caused by *Leishmania* protozoa. Two main forms are found in the Old World, self-healing and self-limited cutaneous leishmaniasis, and the potentially fatal visceral leishmaniasis, with parasite dissemination to the liver, bone marrow and spleen. The *Leishmania donovani* species complex is the causative agent of visceral leishmaniasis worldwide. However, several thousand cases of cutaneous leishmaniasis caused by *L. donovani* have been reported in Sri Lanka, while visceral leishmaniasis is rare there.
METHODS: Two clinical isolates were obtained from Sri Lanka, one from a cutaneous leishmaniasis patient (CL) and one from a visceral leishmaniasis patient (VL). In addition, we performed *in vivo* selection experiments in BALB/c mice with the CL isolate to select for parasites with restored capacity to infect visceral organs. The *in vivo* developed strain (IV) had a 25-fold increase in liver parasite burden and a 45-fold increase in the spleen, compared to the initial infection. Label-free proteomic comparison of whole cell proteomic lysate and exosome composition was performed for these three strains.
RESULTS: Certain biological processes such as translation, biosynthetic processes, antioxidant protection and signalling are elevated in visceral strains. Conversely, biological processes associated with transport and trafficking are elevated in the cutaneous strain.
CONCLUSIONS: These processes may be especially important to determine leishmaniasis disease phenotype. In addition, comparison between the human VL strain and the mouse-selected IV strain indicates that the processes that govern visceral leishmaniasis may differ between humans and mice.

The first prospective registry of Cystic Echinococcosis: the Italian RIEC with an European future

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BACKGROUND. Cystic Echinococcosis (CE) is endemic in Eastern and Southern Europe, Italy included. However, its real burden is largely unknown due to the lack of efficient reporting systems designed to take into account the peculiar features of this disease. In Italy, only a yearly summary of regional data is required by the health authorities and the prevalence and incidence of human CE are hugely underestimated because only hospitalized cases are registered, while the majority of CE cases are diagnosed and managed on an outpatient basis. Furthermore, no official data are transmitted to European authorities. The neglect of CE also results in general lack of knowledge on its diagnosis and clinical management outside referral centres, with consequent heterogeneity in clinical practices and often unnecessary procedures with associated risks and costs.
METHODS. In 2012 the Istituto Superiore di Sanità (ISS - Italian National Health Institute – Rome) in collaboration with the University of Pavia, WHO Collaborative Centre for the Clinical management of Cystic Echinococcosis, implemented the Italian Registry of Cystic Echinococcosis (RIEC). This is a prospective multicenter registry of CE patients visited from January 2012 in Italian health centres that adhered voluntarily. RIEC is accessible on the website of ISS since October 2012 with the aims of: indicating the burden of CE in Italy; bringing the importance of this infection to the attention of health authorities; encouraging public health policies geared toward its control; stimulating research on CE. Moreover, it provides an useful tool for patients follow-up and evaluation of therapeutic interventions.
RESULTS AND DISCUSSION. As of February 2014, 346 patients were enrolled in 11 centres, figures largely outnumbering the national reports of many endemic European countries. We will discuss updated results and challenges of RIEC, that is the template for the European Registry of CE, to be implemented within the FP7-HERACLES project.

In vitro larvicidal effect and _in vivo_ antihelmintic effect of _Oxalis tetraphylla_ (Oxalidaceae) hydroalcoholic extract against _Haemonchus contortus_ in lambs

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BACKGROUND: Haemonchosis seriously affects the sheep industry. The _in vitro_ larvicidal effect and the _in vivo_ antihelmintic effect of _Oxalis tetraphylla_ hydroalcoholic extract against _Haemonchus contortus_ in lambs were evaluated.
METHODS: Two experiments were performed to investigate the _in vitro_ larvicidal activity of the extract against sheated (ShHcL3) and exsheated (ExShHcL3) larvae, and the nematode egg reduction population in faeces of lambs orally treated with _O. tetraphylla_ hydroalcoholic extract (HE). The _in vitro_ assay was carried out into 96 well plates (n=3), at 24 h and 48 h. In the second experiment, three groups of 9 sheep were established as follows: Group 1) _O. tetraphylla_ HE (20 mg/kG BW); group 2) Water (+ control); group 3) Levamisole IM (7.5 mg/kg BW). Treatments were applied everyday for 8 days. The number of eggs per g of faeces (epg) and the PCV values were recorded.
RESULTS: The _in vitro_ assay showed 80.9 and 86.5% mortality in ShHcL3 after 24 and 48 h, and 97 and 99% mortality in ExShHcL3. The _in vivo_ assay showed variable both epg and PCV values; however, the average reduction of the epg attributed to _O. tetraphylla_ HE was 45.6% ($p<0.05$). No statistical difference in the PCV values was observed.
CONCLUSION: The _O. tetraphylla_ HE contains bio-active compounds with anthelmintic activity and they will be considered in future assays as possible control agents of the sheep haemonchosis.

Field evaluation of methods of *Coccidia* infection control in some poultry farms in Egypt

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BACKGROUND: Commercial broiler chickens farms have shown a great growth in the world in the last few years. Nowadays the problem of infectious diseases represents an important incident of losses among all species of birds including chickens. Among of those diseases are the parasitic ones specially *coccidia* infection.
METHODS: A total of 576 broilers from 9 broilers chicken farms in Dakahlia Governorate,Egypt were investigated, each farm was visited three times per cycle 1st at 12 day of age; 2nd at 22 days of age and 3rd at 35 day of age. The fecal samples were collected, prepared and examined microscopically for *Eimeria* spp. The oocysts were counted and identified. Nine groups of chickens were treated with Dirlazuril, Narrasin/micrabazin, Diclazuril, Amprolium, Toltrazuril (Bayox) and Paracax.
RESULTS: The infection rate with Eimeria spp. in poultry farms in Dakahlia Governorate, Egypt was 30% and the *Eimeria* species were *E. necatrix; E. tenella; E. acervulina* and *E. mitis* regarding to the seasonal dynamics the results showed that the highest incidence was in winter season (55.55%), while the lowest incidence was in summer season as (7%). The results cleared that the tissue specificity and gross lesions were very helpful in preliminary diagnosis especially of *E. acervulina* and *E.tenella.*The study recorded the results of application of different anti-coccidial drugs and vaccine and compared between them.
CONCLUSIONS**:** It was concluded that the detected Eimeria spp. were 4 species; the highest incidence was in winter and the lowest one was in summer season, also stated that the best control program that improve the live body weights, weight gain, feed consumption, feed conversion ratio and reduce the mortality rate and economic losses.

Usage and storage of fish parasite data sets for stock delineation

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BACKGROUND: When preparing parasite data for analysis, developing a summary table listing the parasite categories and, for each area, the number of fish examined and the mean parasite abundance is valuable. The parasite categories should differentiate taxa as finely as possible, preferably to species level. If space permits, standard deviations or standard errors of abundances can be included, though these may be only a guide, as parasite data rarely approximate a normal distribution. Prevalence data is informative in some cases while intensities appear to be of little value in stock delineation. An additional column giving the correlation coefficient for abundance versus fish age can reveal clues to the likely residence time of a parasite in the fish and whether an adjustment needs to be made for fish age prior to analysis. Fish age is best estimated from annual bands in calcified structures (e.g. annuli in otoliths) or from a suitable proxy such as otolith weight rather than fish length because of the asymptotic nature of growth frequently observed in fishes. After selecting appropriate parasite taxa, analyses of both individual taxa and multi-parasite datasets using a combination of analytical methods are recommended. Nonparametric methods such as analysis of similarities (ANOSIM), permutational multivariate analysis of variance (PERMANOVA) and cluster analysis are valuable because they do not require the normality and homogeneity of variances that constrain parametric approaches. Finally, when researchers have finished with their data it would be desirable to make the raw data available to others, e.g. through a cloud website. This not only pre-empts statistical queries from reviewers and readers but comparisons with future data sets may provide valuable long term insights into fish and parasite populations.

Standardization of an experimental model of neurocysticercosis by surgical implantation of oncosphere (*Taenia solium*) in the central nervous system of pigs.

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BACKGROUND: Neurocysticercosis is a disease that still affects humans, principally in countries of Latin America, Asia and Africa. Although great advances in diagnosis, treatment and prevention have been made, some problems persist particularly regarding the treatment of patients affected by parasites located extraparenchymal. Experimental models are relevant to better understand the disease and to evaluate new treatments. Unfortunately, for the moment, experimental models of neurocysticercosis using *Taenia solium* do not exist.

METHODS: Activated *Taenia solium* oncospheres were inoculated by surgery in the central nervous system of pigs at the level of the subarachnoideal space of the convexity. Two doses of oncospheres were inoculated: 500 and 1000. Detection of specific antigens and antibodies (ELISA) was made before and every 21 days after infection. Animals were sacrificed four months after infection and their brains were macro- and microscopically examined.

RESULTS: All the pigs included exhibited cysticerci at necropsy. Efficiency of infections was between 2.5% and 4.5%, depending of the dose of oncosphere inoculated. The pigs remained neurologically healthy during all the experiment and developed an antibody response. Most of the parasites were in caseous/calcified stages, surrounded by an important inflammatory reaction.

CONCLUSIONS: The results presented here will permit to design protocols to evaluate cysticidal and antiinflammatory treatments. This could be of great relevance to improve treatment of human neurocysticercosis.

Partial purification and characterization of pyruvate kinase in a cestode parasite, *Raillietina echinobothrida*

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BACKGROUND: Pyruvate kinase (PK; EC 2.7.1.40) is one of the regulatory enzymes of glucose metabolism in helminth parasites. Though, PK and phosphoenolpyruvate carboxykinase (PEPCK) share the same substrate (PEP), PK is reciprocally regulated at PK/PEPCK branch point of the cestode parasite and therefore considered to be an anthelmintic target. In this study, we characterized the enzyme from a cyclophyllidean cestode *Raillietina echinobothrida*, an intestinal parasite of domestic fowl, *Gallus domesticus*.

METHODS: PK was partially purified from the live parasite using ammonium sulfate {$(NH_4)_2SO_4$} precipitation and DEAE-sephacel column chromatography methods. The purified enzyme was characterized spectrophotometrically at 340 nm.

RESULTS: HEPES buffer (50 mM, pH 7.4) was found to be the better suited buffer for PK as compared to Tris-HCl, Imidazole and acetate buffers. The enzyme also showed a standard Michaelis-Menten kinetics with low *K*m for its substrate. KCl (150 mM) was found to be the appropriate monovalent cation for its optimal activity, whereas other metal ions (CsCl, NaCl, LiCl etc) showed less activity. $MgSO_4$ (30 mM) and ADP (1.5 mM) are required for optimal activity of PK in the parasite. In order to find out various modulators for the enzyme, K_i for genistein and daidzein were determined.

CONCLUSIONS: Results of our study suggest that PK has a significant functional difference from PEPCK in the parasite. Its differing regulation could be further exploited for studying its role in anthelmintic action.

Monitoring the control of human soil-transmitted helminthiasis: are we ready?

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Given the worldwide upscale of mass drug administration programmes, there is need for a monitoring system that allows programme managers, policy-makers and donors of the drugs to assess whether the objectives are being met and, if necessary, to correct the implementation strategy. However, mass drug administration programmes implemented to control soil-transmitted helminths (STH), are currently poorly monitored, and one of the main reasons for this lack of monitoring systems is the absence of a framework that guides health-care decision makers in designing surveys. First there are a lot of aspects to be considered, including choice of metrics (e.g. prevalence *vs.* infection intensity), sampling strategy, sample size (number of schools, and number of subjects), sampling effort (number of stool samples per subject, number of examinations on the same stool sample), examination strategy (individual examination *vs.* examination of pooled stool samples), and diagnostic methods (e.g., qPCR, Mini-(FLOTAC), Kato-Katz thick smear, and McMaster egg counting method). Second, given the large variation in epidemiology of STH both between and within countries, health care decision makers will need recommendations that are specific for their field conditions. Moreover, they will probably need to adapt the study design in function of time, as the epidemiology of infections will change over consecutive rounds of drug administration. Third, due to restriction in both financial and technical resources, health care decision makers will aim for the most cost-effective study design.
Faced with similar challenges, veterinary parasitologists have developed novel tools to determine impact of infections and to monitor the short and long term efficacy of anthelminthic drugs. Given the apparent similarities, these tools will also find applications in the control of human STH. We will illustrate how these tools can contribut to the field of STH in terms of diagnosis, monitoring of efficacy, STH-host reactions and identification of sources of contamination.

Tannin-containing nutraceuticals: an alternative mode of control of gastro-intestinal nematodes in ruminants

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Parasitic infections with gastrointestinal nematodes (GINs) remain a major pathological threat in outdoor production systems of livestock. Up to now, the control of these parasitic diseases essentially relied on the use of commercial, anthelmintic (AH) drugs. However, resistance to these AHs is nowadays widespread amongst worm populations in small ruminant farms across the world. Results obtained over the last 20 years, relying on both *in vitro* and *in vivo* studies, indicate that bioactive, tanniniferous fodders, when used as nutraceuticals, represent a valuable option to complement and/or to replace commercial AHs for the control of GINs in domestic ruminants both in cold temperate, Mediterranean or tropical areas. A well-targeted use of such tannin containing fodders and resources requires a better knowledge on the modes of action against the worms. This means to better understand (1) the changes caused at the various parasitic stages and (2) the nature and concentration of the active tannin molecules that are most effective in terms of AH activity. Such basic information is important for any development and industrial implementation.

Delivery of host-derived haem is essential for tick reproduction (membrane feeding in action)

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BACKGROUND: Ticks are blood-feeding arthropods transmitting a wide array of pathogens. Being obligatory haematofagous organisms, ticks may utilize haemoglobin hydrolysis for triple-nutrient acquisition, namely: amino acids, haem, and iron. Haem acquisition and transportation was hypothesized to be important for ovaries maturation and embryo development as ticks were shown to be unable of synthetizing haem molecules *de novo*.
METHODS: Using an *in vitro* feeding system, we managed to establish differentially fed groups of ticks (haemoglobin-containing and haemoglobin-depleted diets) to dissect particular metabolic fates of each of the nutrient. We confirmed/complemented the new findings by RNAi analyses.
RESULTS: For the first time, to our knowledge, we managed to establish differentially fed sets of adult ticks. We found out that, surprisingly, ticks do not need hemoglobin for successful feeding. Then, deprivation in haem availability leads to profound impairment in larvae hatching. Also, we identified individual genes that code for haem transporter through haemolymph and storage protein in ovaries. Finally, we noted that haem is likely not the source of iron ions for tick metabolism.
CONCLUSIONS: We managed to carry out comparative analyses based on differentially fed ticks. Our results describe the necessity of haem transportation into ovaries upon tick feeding to facilitate ticks´ reproduction.

Adaptation, proteome evolution and parasitism in nematodes

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BACKGROUND: Parasitism among nematodes has occurred in multiple, independent events. Deciphering processes that drive species diversity and adaptation are keys to understanding parasitism and advancing control strategies. Studies have been put forth on morphological and physiological aspects of parasitism and adaptation in nematodes; however data is now coming available to investigate adaptation and therefore parasitism at the genomic level.
METHODS: Herein we explore the relationship between changes in protein families and protein domains over the course of metazoan evolution and the relationship between these changes and the ability and/or result of nematodes adapting to their environments. Change, as defined by birth/death and duplication/deletion events within protein families and domains was analyzed using proteomes of 9 metazoans including data from *Trichinella spiralis* and two outgroup species.
RESULTS: Among the three major metazoan groups i.e. vertebrates, arthropods, and nematodes, both birth and death events coincided with species adaptation and diversity; however, the rates of birth/death events in protein families and domains varied largely among the different lineages. Our results show that parasitism and adaptation was accompanied by changes in proteins involved in sensory perception, metabolism, and transcription/translation, and by protein family expansions in functions related to morphology and body development.
CONCLUSIONS: Our data suggest that gene loss occurred in conjunction with nematode specialization resulting from worms adapting to well-defined, environmental niches. Further, we observed evidence of independent lateral gene transfer events involving conserved genes that may have played a role in the evolution of nematode parasitism.

Histochemical and biochemical alteration of tegument and major neuroregulatory enzymes of *Hymenolepis diminuta* upon treatment with *Cassia* plants

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Abstract

BACKGROUND: Resistance towards many anthelmintic drugs and severe side effects of them opened the gateway towards alternative anthelmintic research. Exploration of traditional medicine system became very popular recently. Botanicals are being used to fulfill the lacuna of effective anthelmintics. In our study, alcoholic extract of leaves of *Cassia angustifolia* Vahl., *C. alata* Linn., and *C. occidentalis* Linn. (Caesalpinaceae) were adjudged on rat tapeworm *Hymenolepis diminuta* for anthelmintic efficacy *in vitro*. Morphological alterations on the tegument of treated parasites were pronounced through SEM and TEM.

METHODS: Both biochemical and histochemical studies of the major tegumental enzymes like acid phosphatase (Acpase), alkaline phosphatase (Alkpase), adenosine triphosphatase (ATPase) and 5'nucleotidase (5'-Nu) and neuroregulatory enzymes like Acetylcholinesterase (AChE) of the parasite after treatment were observed and compared with drug praziquantel.

RESULTS: Biochemical studies indicated a decline in the tegumental enzymes activities after treatment. Interestingly *C. angustifolia* and *C. occidentalis* showed greater decline in enzyme level than that of praziquantel. Histochemical localization of above enzymes confirmed the reduction of their activity upon exposure with different *Cassia* extracts. Depletion of staining intensity of AChE confirms the immobilizing effect on parasites upon treatment with crude extract and the biochemical alteration further confirmed the reduction in the AChE activity.

CONCLUSIONS: Thus our results suggest that all three tested plants have anthelmintic efficacy on the parasites. Activity of these enzymes on the physiology of the parasites is our future target.

Phytochemical constituents and bioactivities of the extracts of *Cassia alata* Linn.: A review

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BACKGROUND: Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. *Cassia* species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values. *C. alata* is of high therapeutic value for anti-microbial, anti-fungal activities. It also possesses antiulcer, analgesic, anti-inflammatory, contraceptive, antiplasmodial, insecticidal and larvicidal activities. They are rich sources of polyphenols, anthraquinone derivatives, flavonoids and polysaccharides. *C. alata* is an important source of secondary metabolites, notably anthraquinone derivatives. Its leaves have been reported to contain pharmacologically active substances, including 1, 6, 8- trihydroxy- 3-methyl anthraquinone (emodin), emodic acid, flavonoid- luteolin, hydroxyanthracene and ethyl ester.
METHODS: Literature survey
OBSERVATIONS: The hexane, chloroform and ethyl acetate extracts of the leaves of *C. alata* have been tested for their antimutagenic, antifungal, analgesic, anti-inflammatory and hypoglycaemic activities. Phytochemical screening of the leaves and roots of *C. alata* revealed the presence of some bioactive components, which have been linked to antimicrobial properties. The effects of water, methanol and chloroform extracts on some pathogenic bacteria showed that the plant parts can be used to treat infections caused by them. Anti-inflammatory activities of heat-treated *C. alata* leaf extract and kaempferol 3-O-gentiobioside (K3G) isolated from *C. alata* as an abundant flavonoid glycoside were studied by comparing their activities with the activities of sun-dried *C. alata* leaf extract.
CONCLUSIONS: The available information in the literature on the bioactivities of the active constituents of the extracts of *C. alata* shows that the plant contains compounds with strong pharmacological activities of potential clinical relevance. There is an increasing body of evidence that *C. alata* is an important potential source of new pharmacological agents that may be beneficial in the prophylaxis and therapy of human and livestock diseases.
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Challenges for malaria elimination in the Brazilian Amazon

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Brazil responds for almost half of the cases of malaria in the Americas. *Plasmodium vivax* has become the major parasite and recently the goal of *Plasmodium falciparum* elimination has been discussed at the Brazilian Ministry of Health. Despite the reduction in the number of cases, historically this decrease does not seem to be sustainable and not many areas are able to achieve elimination. The major challenges for the sustained control are malaria in indigenous populations, malaria in borders areas and the lack of new technologies, which impact the vivax malaria burden. Primaquine is used in the seven-day regimen (30mg/day) systematically in all microscopy-confirmed vivax cases, however compliance may not be optimal. Tafenoquine is a new 8-aminoquinoline under phase III clinical trial and the possibility of a single dose (300mg) for the radical treatment opens new venues for the discussion of elimination, altogether with other major challenges such as the emerging *P. vivax* chloroquine resistance, the recognition of asymptomatic cases and the vector control with the use of impregnated bednets.

Influence of toxoplasmosis on human behavior, personality and health

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BACKGROUND: *Toxoplasma* infects about one third of the world population. The life-long presence of dormant stages of *Toxoplasma* in the brain and muscular tissues of humans is usually considered asymptomatic from the clinical point of view. In the past 20 years we have learned that so called “latent toxoplasmosis” has large impact on human hosts, influencing behavior, personality, physiology, morphology and health. Infected subjects have an increased dopamine, testosterone (men) and decreased tryptophan levels. It has been suggested that many symptoms of latent toxoplasmosis are mediated by changes in concentration of these molecules. The most important effect of the increased level of dopamine is an increased proneness of infected subjects for schizophrenia. Increased frequency of infected subjects was observed in schizophrenia patients. Moreover, *Toxoplasma*-infected subjects express more severe symptoms of schizophrenia and decrease of gray matter density in brain. In a recent ecological correlation study, we searched for associations between prevalence of toxoplasmosis and specific disease burdens.
RESULTS: The study performed on 88 countries showed that among neuropsychiatric disorders epilepsy and obsessive-compulsive disorder expressed the strongest association with prevalence of toxoplasmosis. However, positive association also existed with bipolar disorder, alcohol use disorder and schizophrenia. Among other disorders, very strong associations were observed with ischemic heart disease and some forms of cancer, e.g. prostate cancer, breast cancer and leukemia.
CONCLUSIONS: Our results suggest that manipulative activity of *Toxoplasma* or various side effects of latent toxoplasmosis could have a serious impact upon the mortality and morbidity of many common disorders.

Fasciola hepatica fatty acid binding protein inhibits TLR4 activation by targeting CD14-coreceptor and suppresses the inflammatory cytokines induced by LPS *in vitro* and *in vivo*

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BACKGROUND. The parasitic fluke *Fasciola hepatica* induces strong Th2 and T-regulatory immune responses while simultaneously suppress Th1-driven immune responses to bystander microbial infections. The parasite achieves this by secreting a large number of molecules from which cathepsin proteases; theoredoxin and glutathione *S*-transferase have been the most studied. Various proteomic studies have identified fatty acid binding protein (FABP) on the tegumental surface of parasite or in the excretory-secretory products. FABP play important roles in the parasite's nutrition and appear to be also important molecule for inducing cross-immunity against *F. hepatica* and *Schistosoma* species. Although there are numerous studies exploring the vaccine potential of *F. hepatica* FABP, there are no studies directed to investigate whether FABP could interact with the toll-like receptors of antigen presenting cells and consequently play any role in the innate immune response.
METHODS. We purified native FABP and evaluated its effect in a mouse model of septic shock. We also studied its mechanism of action in vivo and in vitro.
RESULTS. A single Intraperitoneal injection of FABP completely protected mice against septic shock. FABP was found to reverse the NF-κB activation induced by LPS 4h after its onset in vitro. FABP also suppressed the production of inflammatory molecules TNFα, IL1β, IL12p40 produced by macrophages in mice exposed to LPS, thus acting as antagonist of TLR4. FABP achieve its anti-inflammatory effect by targeting the CD14-coreceptor, and suppressing the ERK-phosphorylation, which is a common molecule for various TLR pathways. Since CD14 is also required for the activation of TLR2, TLR4 and TLR7/8 it can't be rule out that FABP might suppress the activation of these receptors by a similar mechanism.
CONCLUSSIONS. The observation that FABP exert its anti-inflammatory effect via targeting CD14, which is a co-receptor for various TLRs open doors for using this molecule as a new class of drug against endotoxemia.

Chronic and Innate Immunity Stimulation with T. cruzi vesicles from different strains

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BACKGROUND: *Trypanosoma cruzi*, the etiological agent of Chagas Disease, presents a broad range of surface antigens able to elicit distinct patterns of macrophage activation. It has been demonstrated that GPI-mucins, the most abundant surface antigen in trypomastigotes, can be also found on parasite-derived microvesicles (Ves). T. cruzi-derived surface glicoconjugates (GPI-mucins) display a "strain-specific" ability to induce NO and cytokine production by macrophages (Soares *et al*., 2012). However, it is still unknown if "strain-specific" Ves also trigger similar immunological responses. In this study, we evaluated the *in vitro* pro-inflammatory properties of Ves from Y, Yu-Yu, CL-14 and Colombiana strains on macrophage and spleen cells.
METHODS: Thioglycollate-elicited peritoneal macrophages from WT, TLR2-/- and TLR4 -/- mice and spleen cells from chronic *T.cruzi* infected Swiss mice were evaluated. Cells were primed with INF-γ (25U/ml) and exposed to strain-specific Vesicles (1, 5 and 50 ug/mL) and controls (LPS, LPG and *T. cruzi* 5:1 and Total Antigen). Following incubation, NO and cytokines were measured in the supernatants by Griess reaction and CBA-multiplex flow cytometry, respectively. Additionally, MAPKs (ERK, p38 and JNK) were evaluated after incubation with microvesicles from all strains using J774.1 macrophages.
RESULTS: In WT mice, a strain-specific stimulation pattern, with YuYu and CL-14 strains displaying a more potent pro-inflammatory profile (NO, IL-6 and TNF-alpha production) via TLR2 was observed. In mice spleen cells, a potent pro- and anti-inflammatory response, induced a production of NO, IL-6, IL-2, INF-γ and TNF-alpha. The vesicles from Y and CL-14 strains induced anti-inflammatory response, IL-10, IL-4 and IL-5. Although all Ves were able to differentially stimulate macrophages and spleen cells, they were able to activate ERK, p38 and JNK, where Colombiana strain exhibited a gradual activation profile.
CONCLUSIONS: These data reinforce the relevant role of *T. cruzi* surface molecules in the pro-inflammatory events underlying the immunopathogenesis of Chagas disease.

Prevalent HLA class II alleles in Mexico City confer resistance to the development of amebic liver abscess.

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BACKGROUND. In Mexico the incidence rates of amebic liver abscess (ALA) are particularly high in northwestern states, compared to the central region. These could be related to host genetic factors, partially responsible for resistance or susceptibility to the development of ALA. Due to its biological function in the immune response, polymorphisms in genes of the HLA class II molecules are relevant candidates to study.
METHODS. We studied 77 patients with diagnosis of ALA from the state of Sonora and Mexico City. Genomic DNA was extracted from peripheral blood. To establish the genetic identity of both populations, 15 STRs, were analyzed by PCR-multiplex, and the allelic frequencies of HLA studied by PCR-SSO. Frequencies obtained were compared statistically with their control groups (126 individuals).
RESULTS. We found only in the population of Mexico City, the *HLA-DQB1*04* allele is associated with protection against ALA development; it was less frequent in patients (0.083 vs 0.234, respectively; p=0.004, pc=0.02, OR=0.30, 95 % CI=0.10-0.75). While *HLA-DRB1*08* allele and the *HLA-DRB1*08/-DQB1*04* haplotype showed a tendency of protection.
CONCLUSIONS. This suggests that prevalent alleles in the population of Mexico City may be associated with protection against development of ALA.

Neurocysticercosis: The effectiveness of cysticidal treatment could be influenced by the host immunity

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BACKGROUND: Neurocysticercosis is still endemic in most non-developed countries of Latin America, Africa, and Asia. Anti-helminthic drugs (AHD) are generally effective and rapidly destroy parenchymal cysticerci. In contrast, an important heterogeneity in the response to AHD exists when parasites are extra-parenchymally located. The present study was designed to evaluate whether differences in the immunological profile of the patients are involved in the diversity of the response to AHD.
METHODS: Responder (reduction greater than 50% in the parasite burden following AHD treatment) and non-responder (NR) patients to AHD treatment were included. A global gene expression microarray and a cytokine analysis were made and their results were compared between Responder and NR patients.
RESULTS: Microarray pre- and post-treatment comparisons showed that a total of eighteen immune-related genes were up-regulated in the five responder patients with respect the expression profile seen in the four non-responder subjects. The function of up-regulated genes exerted pro-inflammatory (RORγC, Sema4A, SLAMF3, SLAMF6), anti-inflammatory (TGFβ, TNFRSF25, TNFRS18, SLAMF1, ILF2), or immunomodulatory effects (CXCL2, RUNX3, SLAMF9, TGFBR3). Cytokine analysis (ELISA) performed in sera, CSF and PBMC supernatants showed that responder patients (N=39) present higher CSF IL-17A levels (*P*=0.04) and higher supernatant IL-6 levels (*P*=0.03) than NR patients (N=26) 60 days after treatment.
CONCLUSIONS: These results suggest a possible association between a pro-inflammatory profile and the response to treatment, and thus the possible participation of the host immunity in the effectiveness of AHD treatment.

Biochemistry and proteomic study of *Angiostrongylus costaricensis* adult worms

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BACKGROUND: *Angiostrongylus costaricensis* is a nematode that causes abdominal angiostrongyliasis, a widespread human parasitism in Latin America. Experimental information on *A. costaricensis* proteins is limited, and only mitochondrial genome has been published. This study aimed to characterize the proteolytic activity of and shotgun proteomic analysis of *A. costaricensis* adult worms.
METHODS: Crude extracts and excretory/secretory proteins of nematode were assayed using fluorogenic substrates and zymography. Male and female extracts were also processed by the LC-MS/MS technique to identify the corresponding peptides.
RESULTS: Gelatinolytic activity was detected at a neutral pH, with optimal activity observed at alkaline pH. Proteolytic activities were observed in female secretome, male and female worms lysate in solution with DTT. Secretome from female worms activity was effectively inhibited by PMSF and ortophenantroline, indicating the involvement of serine proteases and metalloproteases. The mechanistic class of the gelatinases from male and female extracts could not be precisely determined using traditional class-specific inhibitors Using shotgun proteomic approach more than 400 proteins in both gender were identified using PEAKS 6 software. Several of the most abundant proteins identified were cytoskeleton-associated proteins, proteins involved in energy metabolism and anti-oxidant proteins. Proteases were also identified, such as cathepsin B-like cysteine proteinase 1 and aspartic protease.
CONCLUSIONS: A systematic analysis of the proteome of this nematode is important not only for understanding this parasite's invasion mechanism(s), but also for the discovery of potential drug targets and new preventative and therapeutic strategies.

Blastocystıs spp. ınfectıon ın a famılıal Medıterranean fever patıent

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BACKGROUND: In many epidemiologic surveys Blastocystis is the most frequently isolated parasite with a higher prevalance in underdeveloped countries. A variety signs and symptoms ranging from intestinal to cutaneous disorders are observed. Familial Mediterranean Fever (FMF) is a hereditary autoinflammatory disease characterized by epizodic fever and inflammatory polyserositis which could lead to a variety of gastrointestinal manifestations such as recurrent abdominal pain, diarrhea, ascites and bleeding.
METHODS: A patient had suffered from FMF since 1994 who had abdominal pain, diarrhea attack and dermatologic signs like maculopapular rush. She admitted to the our hospital with these symptoms. Her stool examination was performed three times in our laboratory with wet saline mounts and in iodine preparations.
RESULTS: Blastocystis spp is detected using standart clinical parasitologic techniques including wet saline mounts and in iodine preparation. We saw significant number of cysts in her stool (ie >5 cysts per high powered field).
CONCLUSIONS: Diarrhea and abdominal pain are the most common blastocystis infection's symptoms. Also these symptoms are observed in FMF patients. Even bloody diarrhea could be considered an initial symptom of FMF. Both FMF and Blastocystis infection may be simultaneously. As in our patient, resolution of dermotological signs and recovery of gastrointestinal symptoms were monitored after metranidazol treatment and eradication of parasite from stool. FMF and whether a relationship between Blastocystis infection hasn't been studied previously. A large series of scientific studies on this subject is recommended.

Prevalence of Water and Food Born İntestinal Parasitic Diseases in Children in Eskisehir and Surroundings Areas

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BACKGROUND: Intestinal parasites are one of the leading causes of waterborne outbreaks but usually ignored. They are affecting millions of people in developing and developed countries. Microscopic diagnosis is standard methods for human intestinal parasites. This study, investigate that presence of water and food-borne intestinal parasites in children to Eskisehir and surroundings area.
METHODS: Stool specimens were obtained from 0-12 age old 1049 children. All samples after the macroscopic examination, inoculation culture media, prepared by formalin ether sedimentation methods and included in microscopically examination. Furthermore, specimens were stained with Trichrom and the modified Ehrlich Ziehl Neelsen method and evaluated for *Cryptosporidium spp.*, *Cyclospora spp*. and *Microsporidia spp*.
RESULTS: The distribution of parasites which were detected with at least one of the methods in stool samples as follow; *Blastocystis hominis* 11%, suspected amoeba cyst and trophozoits 6.7%, *Microsporidia spp* 7.1%, *Cryptosporidium spp* 3.7%, *Giardia intestinalis* 1.5%, *Ascaris lumbricoides* 0.1%.
CONCLUSIONS: Our study emphasizes that water and food born intestinal parasitic infection are still an important public health problem. Interventions including health education on personal hygiene to the student sand to the parents, especially to mothers are required.

Prevalence of intestinal Parasites in Primary School Students in Rural Areas and the effect of Health Education in interference of parasites level in Eskisehir

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BACKGROUND: The aim of this study was to determine the prevalence of intestinal parasites among elementary school students and the effect of health education on this prevalence. We detected presence of intestinal parasites in primary school students and investigate to the effect of parasitic diseases and hygiene education level of parasitism in children rural areas of Eskisehir.
METHODS: A cross-sectional research which is conducted two training sessions in mobile education primary school. At the first stage, students were trained in parasitic disease and hygiene, conducted a questionnaire and investigated. The presence of intestinal parasites investigated; native iodine, formalin-ethyl acetate, cellophane tape methods. Following year, re-investigate the presence of intestinal parasites. The results were evaluated statistical methods.
RESULTS: The prevalence of intestinal parasites, 1 and 2 period respectively 32.3%, 23.3%. We found 18.18% *Blastocystis hominis*, 6.1% *Giardia lamblia* 6.4% of 109 samples *Enterobius vermicularis*, (0.9%) *Pentatrichomonas hominis* by cellophane tape. There was no statically difference of presence of parasites ($p>0.05$) in both studies. The parasitic characteristics of the persons examined was higher in the presence of parasitic who have animals, do not kindergarten, under 10 age and doesnt regularly wash their hands after using the toilet ($p<0.05$).
CONCLUSIONS: We considered to be more effective of education, diversification of educational materials according to socio-demographic and cultural characteristics of children and continuity of activities and the provision of practical training would be useful.

In vitro antiplasmodial activity of biosynthesized silver nanoparticles using *Morinda citrifolia* (Noni) against *Plasmodium falciparum*

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BACKGROUND: The utilization of various plant resources for the biosynthesis of metallic nanoparticles is called green nanotechnology, and it does not utilize any harmful chemical protocols. The present study reports the plant mediated synthesis of silver nanoparticles using the plant leaf extract of *Morinda citrifolia* (Noni), which acts as a reducing and capping agent. The aim of the present study was to assess the anti-plasmodial activity of synthesized AgNPs against the malarial parasite, *Plasmodium flaciparam*.

METHODS: The obtained nanoparticles were characterized using UV-visible spectroscopy; EDX (energy-dispersive X-ray), SEM (Scanning electron microscope), XRD (X-ray diffraction) and Fourier transform infrared (FTIR) analysis. The efficacy of green synthesized AgNPs at different concentrations (25, 50, 75 and 100µg/ml) were tested on *P. flaciparam*.

RESULTS: Synthesized AgNPs particles were confirmed by analysing the excitation of surface plasmon resonance (SPR) using UV–vis spectrophotometer at 422 nm.The scanning electron micrograph showed structures of spherical, cubic shape, and the size range was found to be 40–60 nm. The EDX spectra showed the purity of the material and the complete chemical composition of the synthesized AgNPs. XRD study shows that the particles are crystalline in nature with face centered cubic geometry. The FTIR analysis of the nanoparticles indicated the presence of proteins, which may be acting as capping agents around the nanoparticles. Biosynthesis of nanoparticles may be triggered by several compounds such as carbonyl groups, terpenoids, phenolics, flavonones, amides, proteins, alkaloids and other reducing agents present in the biological extract. The parasitic inhibition was dose-dependent. The synthesized AgNPs showed significant anti-plasmodial activity when compared to aqueous leaf extract of *M. citrifolia*. The maximum efficacy was observed in synthesized AgNPs against *P. falciparum* (IC_{50}=100 µg/ml; 100%) respectively.

CONCLUSIONS: This method is considered as a new approach to control the malarial parasite, *P. flaciparam*. Therefore, this study provides first report on the anti-plasmodial activity of synthesized AgNPs using *M. citrifolia* against *P. flaciparam*.

Effect of 17-β estradiol and progesterone on *Toxoplasma gondii* infection in astrocytes *in vitro*.

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INTRODUCTION: Toxoplasmosis is a disease caused by an obligate intracellular parasite called *Toxoplasma gondii*. In humans, proliferating tachyzoites have been detected in glial cells in patients developing toxoplasmic encephalitis. The progesterone and 17-β estradiol have different effects on infection, may exacerbate or reduce parasite replication, however the participation of these hormones in astrocytes infected is no known. The aim of the present study is to investigate the effect of 17-β estradiol and progesterone on *Toxoplasma gondii* infection in astrocytes *in vitro*.
METHODS: Astrocytes were obtained from rat cortex and were pre-treated with 17-β estadiol and progesterone at concentrations (10, 20, 40 and 80 nM/mL) for 48 hours, then were infected 24 hours with 14,375 *Toxoplasma gondii* tachyzoites. The effect of hormones on *Toxoplasma gondii* infection in astrocytes was evaluated by immunocytochemistry using anti-*Toxoplasma* antibody to identify the parasite and anti-GFAP to identify astrocytes. Cellular viability was measured with MTT.
RESULTS: The 17-β estradiol increased the number of intra and extra cellular parasites at concentrations of 20 and 80 nM/mL versus control. Progesterone decreased the number of intra and extra cellular parasites at all concentrations compared to the control. Finally, the parasite viability was reduced to concentrations of 20, 40 and 80 nM/mL of 17-ß estradiol by MTT.
CONCLUSIONS: 17-β estradiol exacerbates *Toxoplasma gondii* infection at 20 and 80 nM/mL, while that progesterone at 10, 20,40 and 80 nM/mL had a reduced effect on *T. gondii* proliferation in astrocytes infected.

Genetic structure and molecular phylogeography of *Aedes aegypti* (Diptera: Culicidae) in Colombia

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BACKGROUND**:** *Aedes aegypti* is the primary vector of the dengue virus (DENV1-4) in the world. In Colombia, despite its epidemiological importance, little is known about its population structure and phylogenetic relationship with evolutionary lineages reported in the Americas and Africa. Therefore, the present work is a spatiotemporal analysis of the genetic structure and phylogeography of this vector in Colombia.
METHODS**:** Between 2012 and 2013, a total of 298 specimens collected in different seasons in three cities of different biogeographic regions of Colombia were analyzed by fragments of 830 bp and 400 bp of mitochondrial COI and ND4 genes respectively. Diversity parameters and genetic structure were estimated among differentness isolates of time and space. The phylogeographic analyses include sequences of individuals from North, Central and South America, as well of West and East Africa.
RESULTS**:** A total 160 haplotypes were observed in the data concatenated COI-ND4. Although low genetic differentiation was observed over time, a high genetic structure was observed between regions due to changes in frequency of two highly supported genetic lineages. Lineage 1 associated with West African populations was found in all regions throughout the year, while the Lineage 2 associated with East African populations was found only in the Andean and Caribbean region in specific times. Environmental factors such as temperature and use of chemical insecticides showed to decrease the genetic diversity of the populations analyzed.
CONCLUSIONS**:** This work demonstrates a spatial genetic structure in populations of *Ae. aegypti* of Colombia. It suggests the West African lineage to be the ancestral in the country, and associated with cities with high incidence levels of dengue cases and the use of chemical insecticides. The East African lineage is considered to be recently introduced in the Andean and Caribbean region, and is associated with cities having low dengue incidence and little use of chemical insecticides.

Seroepidemiological survey of Amoebiasis in elementary school children in Eskisehir/Turkey

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BACKGROUND: *Amebiasis*, is a common parasitic infection in all over the world, which is caused by *Entamoeba histolytica*. In developing countries it is still an important public health problem, and the differential diagnosis of infected persons should be done as soon as possible. In our study was determining the seroprevalence of intestinal amoebiasis in elementary school children.

METHODS: We collected 977 stool samples in 5-14 age group in elementary school children. All stool samples were examined macroscopic and wet mount microscopic examination and culture for *Entamoeba histolytica* cyst or trophozoites. We were performed additional tests (ELISA and PCR methods) on samples with diarrheal and suspected microscopy.

RESULTS: We worked 977 fecal samples, by the method of direct microscopic examination of *E. histolytica / dispar* prevalence of 83 (23.6%), and 45 of them (12.8%) of them with trichrome stain, 32 (9.1%) of them with the culture method, 13 (3.7%) were determined by ELISA, 4 (1.1%) were molecular method and found to be positive. by molecular method 7 (2.0%) fecal sample is found to be positive of *E. histolytica*, of which 4 (1.1%) of them with direct microscopy, 2 (0.6%) of them with trichrome stain, 2 (0.6%) of them with the culture method, and 1 (0.3%) were found to be positive by ELISA. In only one sample is positive with all diagnostic methods which were we used.

CONCLUSIONS: Various prevalence rates of *E. histolytica* infection had been published by different researchers. The rates vary for the different locations, states, and the types of patients surveyed and the source of the survey materials. Improved sanitation, personal hygiene and deliberate policy by government for rural community health concern will indeed prevent fecal contamination of food and water.

Genotype diversity of the circumsporozoite protein of *Plasmodium vivax* in different malaria foci of Mexico

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BACKGROUND**:** Information of *Plasmodium vivax* genotype variability at different malaria foci will contribute to understand transmission dynamics and useful for epidemiological surveillance. The circumsporozoite gene (*csp*) presents two main central repeat units (CRU) vk210 and vk247, and further genotypes are resolved mainly by CR and carboxyl end variability.

METHODOLOGY**.** Methanol fixed and stained thick blood smears with *P. vivax* from different malaria foci (F1, Chihuahua-Sinaloa; F2, Durango-Nayarit-Jalisco; F3, Pochutla, Oaxaca; F4, Palenque-Ocosingo, Chiapas) were obtained, 2010-2012. Total DNA was used to analyze the *csp* gene by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) using specific primers and digestion by AluI and BstI enzymes. PCR fragment size and RFLP patterns were visualized on agarose gels.

RESULTS**:** Csp displaying CRU-vk247 was predominant in all malaria foci (83% and three genotypes were resolved vk247-I, -II and –III. Other 31 samples (17%) had CRU-vk210, and four further genotypes were resolved: A, B, D, and G. In F1 focus, one major (vk247-I; 92.8%) and two less frequent genotypes were detected (vk210B and vk210A). The F2 focus had vk247-I (96.8%), and genotype vk247-III present in one isolate, and one mixed: vk247/vk210. In F3, all parasites had vk274-I, whereas in F4, various genotypes were detected; vk247-I (44.4%), vk210A (33.3%) and vk247-II, vk210B, vk210D, vk210G and one mixed genotype infection (Vk210A/B) were less frequent. All these genotypes, except Vk247-II and -III, and vk210G were previously reported in the Soconusco area of Chiapas, Mexico.

CONCLUSION**:** Variation of the *csp* genotype and its frequency at different malaria foci reflect residual genotypes likely transmitted in Mexico for decades prior to *P. vivax* focalization. The epidemiological and biological implications of these results will be discussed.

The cysteine proteinase TvCP4 from *Trichomonas vaginalis* has two regions with different function

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BACKGROUND: *Trichomonas vaginalis* has multiple proteinases mainly of the cysteine type (CPs). TvCP4 is a CP with homology to cathepsin L, positively regulated by iron, and immunogenic in patients with trichomonosis. This CP is synthesized as a precursor of 305 amino acids, containing the prepro fragment and the mature enzyme domain with the typical catalytic triad (C25, H159, N175) of papain-like family of clan CA. Our goal in this work was to analyze the function of these two regions of TvCP4 from *T. vaginalis*.
METHODS: We cloned, expressed, and purified the recombinant TvCP4 prepro region (ppTvCP4r) and performed enzyme inhibitions assays using trichomonad protease resistant extracts and fluorogenic substrates for cathepsin L and legumain CPs and compared the 2-DE zymograms of ppTvCP4r-treated with control untreated parasite extracts to identify the target CPs. For the mature TvCP4 protein, we produced a polyclonal antibody (anti-TvCP4r) and used it for immunolocalization of TvCP4, haemolysis, and *in vitro* secretion assays to determine its function in *T. vaginalis* virulence.
RESULTS: Our results showed that ppTvCP4r is an inhibitor of the proteolytic activity of cathepsin L-like CPs. Additionally, TvCP4 is localized in the cytoplasm and at the parasite surface mainly in iron-rich conditions, involved in haemolysis of human erythrocytes and detected in vaginal secretions of patients with trichomonosis.
CONCLUSIONS: The ppTvCP4r is a specific inhibitor of the proteolytic activity of some *T. vaginalis* CPs from Clan CA that could be also useful to block papain-like CPs from other organisms. TvCP4 is the first surface CP positively regulated by iron described as a *T. vaginalis* virulence factor involved in hemolysis.

GLOWORM: An international consortium providing new solutions to mitigate global change associated consequences of worm infections in European livestock farming

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GLOWORM is an EU funded consortium of 10 academic institutions and 4 SME companies from across Europe, comprising a multitude of disciplines such as parasitology, epidemiology, molecular biology, farming systems and GIS. A major constraint on the effective control and management of helminth parasites in livestock is the lack of rapid, high-throughput, routine diagnostic tests to assess the health status of individual animals and herds and to identify the parasite species responsible for disease. A number of diagnostic assays are being developed and evaluated and/or validated within the GLOWORM consortium. For example a luminex®-based serological multiplex assay for the simultaneous detection of antibodies directed against *Fasciola*, *Dictyocaulus* and *Cooperia* in milk samples has been developed and will be applied within the project for epidemiological surveys in key European dairy regions to provide spatial distribution and risk maps. Another aim of the GLOWORM project is the development of transmission models to better understand how climate and management influence parasite epidemiology that could be used for more targeted control. Models have been developed for *Fasciola hepatica*, *Ostertagia ostertagi*, *Cooperia oncophora*, *Teladorsagia circumcincta* and *Haemonchus contortus*, integrating the nematode life cycle with host immunity, farm management, and grass growth patterns. Early point validation of the model components is promising and once validated, further enhancements will involve the incorporation of farm management data and climate projections to explore the impact of climate change on the seasonal transmission of gastrointestinal nematodes infecting livestock in Europe. Modelling will also permit evaluation of the efficacy of strategic worm control and grazing management practices against a backdrop of changing climate and farm management practices. To provide a database for spatial analysis and modelling of gastrointestinal parasite distribution, surveys on the prevalence and abundance of parasites based on faecal sampling of sheep are being carried out in Switzerland, Ireland and Italy.

Prolongation of cardiac allograft survival by recombinant SAG1 of *Toxoplasma gondii* is associated with up regulation of CD4+CD25+Foxp3+ regulatory T cells*

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BACKGROUND: Organ transplantation has been widely accepted as the treatment of choice for end-stage organ failure. Although the application of immunosuppressive drugs has contributed significantly to the success of allograft survival, side effects resulted from immunosuppression or drug toxicity also markedly impact the quality of life of recipients. Therefore, there is an unmet clinical need for novel immunmodulatory drugs in transplantation to mitigate the development of graft injury, chronic allograft damage and premature graft loss.
METHODS: Our current study demonstrated injection of rSAG1 at 4 d before transplatation could prolong graft survival in a murine model of cardiac transplantation. Flow cytometry studies showed an increased CD4+CD25+Foxp3+ regulatory T cell population in splenocytes from rSAG1-exposed receipts mice.
RESULTS: This effect coincided with low expression of interferon-gamma (IFN-γ), interleukin(IL)-4 and IL-17 while increased production of IL-10, transforming growth factor-beta (TGF-β) and IL-12 by splenocytes in the rSAG1-exposed receipts. Depletion of regulatory T cells abrogated the prolonged allograft survival induced by rSAG1 and this effect was associated with decreased expansion of regulatory T cells, along with higher levels of IFN-γ, IL-12 and IL-17 and lower levels of IL-4, IL-10 and TGF-β produced by spenocytes.
CONCLUSIONS: Exposure to rSAG1 before cardiac transplantion may induce prolonged survival of allogeneic cardiac allografts and this protective effect is related to upregulation of CD4+CD25+Foxp3+ regulatory cells. Further study shoud be developed to clarify the detailed mechanism of the rescue therapy for acute rejection in mouse cardiac transplantation and may be effective in human organ transplantation in the future.
Financial support was obtained from the National Natural Foundation of China (project grant number 81000739)

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Financially supported by the National Natural Foundation of China (project grant number 81000739).

A combined proteomic and immunological analysis of excretory-secretory products of *Schistosoma japonicum* adult worms and eggs

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BACKGROUND: Excretory-secretory products (ESPs) are the majority of worm components, which can be directly recognized by host immune system and sequentially, induce the development of specific antibodies or other immunoresponse.
METHODS: In our current study, a high throughput proteomic approach combined by MS with Two-dimensional gel electrophoresis (2-DE) was used to identify living-adult worms and living-eggs ESPs. The immunoreactions of ESPs with rabbit sera collected at two or six weeks after *S.japonicum* infection have been also studied.
RESULTS: By 2-DE, we identified 166 protein spots from adult ESPs and 138 protein spots from egg ESPs. Of these spots, 48 and 34 spots were larger and clear, which may indicate the abundant proteins of *S.japonicum*. Twelve protein spots were identical in both eggs and adult worms, 24 spots were stage-specifically expressed in adult worms and 16 spots were egg-specifical. After the further MALDI- TOF/TOF analysis, CBL, homeobox, CBN-MOP protein and putative myosin of *Brugia malayi*-origin could be recognized by infective sera of two weeks. Dicer-2 protein and glycolated oxidase were reactive with sera of six weeks post infection. An enolase sorted from egg antigens was found to strongly react with early-infected sera. In addition, adult and egg ESPs were immunoprecipitated with purified IgG of *S. japonicum* infected rabbit, which was covalently immobilized on a CNBr-activated sepharose beads. The bound peptides were then identified by MALDI-TOF-TOF MS. The results were retrieved in MASCOT database.
CONCLUSIONS: Seven peptide fragments were identified in adult and egg ESPs, respectively. Among the seven fragments, three peptide fragments were identical in adult and egg ESPs.

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Parasite specificity of *Haemonchus contortus* and *Haemonchus placei*

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BACKGROUND: *Haemonchus contortus* in sheep produces a long chronic infection and immunity is not easily elicited other than after prolonged repeated infection. In contrast, bovine strains of *H. placei* are apparently unable to persist in environments grazed only by small ruminants.
METHODS: The patent periods of infection of both species was evaluated in lambs after a single infection with 4,000 infective larvae (L3) or in groups of lambs serially infected with either *H. placei* or *H. contortus*.
RESULTS: After a single infection, animals shed a large number of eggs in the faeces for several months with the highest counts, with means higher than 3,000 eggs per gram of faeces between 24 and 106 days and between 38 and 73 days post infection with *H. contortus* and *H. placei*, respectively. Lambs serially infected with *H. placei* and then challenged with the same species presented the most intense immune response with the highest levels of anti-parasitic immunoglobulin and number of inflammatory cells in the abomasal mucosa. As a result, this group had the lowest rate of parasite establishment (2.68% of the 4000 L3 given), but this phenomenon did not occur in animals single challenged with *H. placei*, in which the rate of establishment was relatively high (25.3%). However, when the animals were previously serially infected with *H. placei* and then challenged with *H. contortus*, no evidence of significant protection was observed (establishment of 19.18%).
CONCLUSIONS: After a single infection, both *H. contortus* and *H. placei* may survive over one year inside of a host sheep. In contrast, a strong immune response to *H. placei* occurs in lambs after serial infections.