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Phylogenetic analysis of *Trypanosoma brucei* and *Trypanosoma evansi* in naturally infected cattle in Nigerian

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BACKGROUND: In continuing efforts to better understand the genetics of bovine trypanosomosis resistance, we assessed genetic diversity of *T. brucei* and *T. evansi* in naturally infected Nigerian cattle using repetitive DNA and internal transcribed spacer 1 of rDNA sequences and compared these sequences to species in other countries.
METHODS: Species specific primer set (TBR 1& 2) and Universal primer set (ITS1 CF &BR) targeting the internal transcribed spacer 1 region of rDNA and repetitive sequences, respectively were used to amplify the DNA of trypanosomes in naturally infected cattle. The PCR products with expected band sizes were purified from the gel and sequenced uni-directionally. The obtained sequences were analyzed and used to construct phylogenetic trees using neighbor-joining algorithm of the phylogeny program of MEGA 5.05.
RESULTS: Repetitive DNA sequences in both species ranged from 161bp to 244bp and 239bp to 240bp for *T. brucei* and *T. evansi*, respectively, while the ITS1 rDNA sequences length range from 299bp – 364bp.The mean GC content of ITS1 rDNA sequences was 33.57% and that of repetitive sequences were 39.9% and 31.1% for *T. brucei and T. evansi*, respectively. Sequence alignment revealed both *T. brucei* and *T. evansi* repetitive DNA sequences to be more polymorphic than ITS-1 rDNA sequences, with moderate points of deletion and insertions. Phylogenetic analysis of *T. brucei* separated it into two clades. *T. evansi* repetitive DNA sequences clustered tightly within the *T. brucei* clade while the ITS-1 rDNA sequences of *T. brucei* were clearly separated from *T. theileri* and *T. vivax* individually used as outgroups.
CONCLUSIONS: This study suggest that ITS-1 rDNA sequences may not be suitable for phylogenetic differentiation of the Trypanozoon group and strongly supports that *T. evansi* may be a phenotypic variant of *T. brucei* with potential implications for designing prevention and therapeutic strategies.

Differential serum IFN-gamma (IFN-γ), interleukin 10 (IL-10) and cardiac troponin I (cTnI) expression in natural bovine trypanosomosis in Nigerian

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BACKGROUD: Response to African animal trypanosomoses involves Type-I and/or Type-II immune response or a combination of both. Interferon gamma (IFN-γ) and interleukin-10 (IL-10) are the main cytokines produced in Type-I and Type-II immune response, respectively. Most of the reports on the immune responses of animals to trypanosomosis are experimental murine models and no report is available on immune responses of cattle naturally infected by trypanosomes as well as data on serum cardiac troponin I(cTnI) level in infected cattle. In this study, we report for the first time the serum level of IFN-γ, IL-10 and cTnI in naturally infected cattle using enzyme linked immunosorbent assay method (ELISA).
METHODS: The sera collected from 58 cattle naturally infected by trypanosomes (*T. brucei brucei, T. congolense and T. vivax*) were used to assess the level of cytokines and cTnI using ELISA technique. The obtained values were correlated with the levels of parasitaemia and the determined packed cell volume (PCV) using Pearson correlation method.
RESULTS: Of the 58 samples that tested positive for trypanosomes (*T. brucei*, 24.1%; *T. congolense*, 32.8% and *T. vivax*, 43.1%) by microscopy, 50 samples consisting of 12.1%, 37.9% and 36.2% were confirmed as *T. brucei, T. congolense-savanah* strain and *T. vivax,* respectively by PCR. There was increase serum levels of the IFN-γ and the cTnI of infected than non-infected cattle. The increases were not significantly different ($p < 0.05$) from the serum levels of non-infected cattle except the IL-10 level that was significantly ($p < 0.05$) lower than those of non-infected cattle. There was positive correlation between the PCV and IFN-γ in *T. brucei* infection while in T. vivax infection, there was negative and positive correlation between PCV and parasitaemia and, parasitaemia and IFN-γ.
CONCLUSIONS: This study suggests that the three species of trypanosomes detected elicits secretion of proinflamatory cytokine, IFN-γ and cTnI. The significantly lower level of IL-10 than the IFN-γ in this study suggests that IFN-γ may be involved in early response to trypanosomosis than the IL-10 which might be playing suppressor/regulatory role on the IFN-γ.

Prevalence and socio-economic factor of Intestinal protozoa in primary schools in Zawia city, Libya

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BACKGROUND: Intestinal parasites affect an estimated 3.5 billion persons and cause clinical morbidity in approximately 450 million (WHO, 2000). Some intestinal parasites have their major impact on children, while others infect all age groups but have their most profound effect on adults (Zakai, 2004).

METHODS:This study was conducted in Eight primary school were selected by systematic random sampling using the master list of the schools In Zawia city- Libya. Questionnaires were also distributed to check for relationship between infection, type of sewage system and sources of drinking water. A total of 605 random fresh stool samples were collected from children aged between 6 and 14 years. Stool examination was done by direct and formalin ether technique.

RESULTS: In total 64 (10.6%) cases were found with intestinal protozoa in their stools. The most common protozoa was *Entamoeba coli* 22 (3.6%), *Entamoeba histolytica* found in 19 (3.1%) cases and *Giardia lamblia* in 11 (1.8%) cases. Double infection was seen in only three samples. The results showed that there was a significant relation between the prevalence of intestinal protozoa and the sources of drinking water ($p < 0.05$). Also there was statistical significance between intestinal protozoa infection and location of school [Urban /Rural] ($p < 0.05$). The low prevalence of intestinal protozoa among the study groups reflects the outstanding health and hygienic care in primary schools visited.

CONCLUSIONS: This study showed that the prevalence of intestinal parasites among primary school children in Zawia city- Libya is lower than others cities. In the present study, the children in rural schools had a significantly higher prevalence of intestinal parasites than urban schools.

Loiasis : Knowledge,Attitude and Perception amongst Inhabitants of Okigwe Zone, Imo-State Nigeria.

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METHODS: A cross- sectional survey amongst 38 communities in Okigwe zone of Imo-State,Nigeria were sampled on local terminology (knowledge),attitude and perceptions of loiasis infection.Questionnaires, key informants and interviews were employed to collect data. A total of 1,325 questionnaires were administered to the respondents who volunteered in the study Area.

RESULTS: Igbo language is the common language within the zone. Eyeworm is commonly known as “Ari anya” which means “Ari”(worm) and “Anya”(Eye).Other names include “Nwangwu”,”Nwa-ari,”Nwatoke”, ”Nsi-anya” while Calabar swelling is commonly known as “Afufu”. The perceived causes/transmission of clinical Manifestation of loiasis in Okigwe zone showed that highest number attributed the cause of eyeworm with poor personal hygiene 79(12.1%), for Calabar swelling,they attributed it to old age 202(30%).On whether the disease can be prevented or not, majority of the respondents didn’t make any comment,meaning they were not aware of the infection. For Eyeworm 192(29.4%)believed it can be prevented while for Calabar swelling 206(31%) respondents believed it cannot be prevented.The respondents were also asked whether relationship exists between Eyeworm and Calabar swelling,highest number 1,244(94%)of respondents believed that no relationship exists between the two. Their response towards clinical manifestation of loiasis were appauling. Majority of the respondents were not bothered for both eyeworm 416(83.5%) and Calabar Swelling 236(64.3%)respectively.Their attitude towards clinical manifestation of loiasis showed they were influenced by prevailing traditional superstitious and socio-cultural norms in the communities. They also have low level knowledge of causative agent of loiasis. These depicted a gross unawareness to the causes of loiasis infection.

CONCLUSIONS: There is urgent need, therefore, for a health education campaign with emphasis on participatory learning and action methodologies into the intended drug administration (MDA) being considered for Onchocerciasis elimination. Futher to enlighten them on the severe infection caused as a result of co-infection of *Onchocerca volvolus* and *Loa loa* infections.

The Biting Rate of *Chrysops spp* and Transmission Potential of Loiasis in Okigwe Zone of Imo-State Nigeria.

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METHODS: An entomological survey of *Chrysops spp* were conducted in 38 communities in Okigwe zone Imo-State, Nigeria to determine the biting rates and Annual transmission Potential (ATP) and transmission dynamics of loiasis by *Chrysops* vector. Fly catches were made using human bait method.

RESULTS: A total of 972 flies were caught with 837(86.1%) alive and dissected.The results obtained showed that *Chrysops* vector were more abundant during rainy season than dry season. The daily and seasonal biting cycle of *Chrysops spp* showed two peaks of activities,8-11am and 2-5pm.Of these,215(25.7%) were parous while 622(74.3%) were nulliparous.An infection rate of (9.7%) was recorded in the study Area.

CONCLUSIONS: The findings suggests a strong existence of Loiasis in Okigwe zone. It further suggests that Control strategy adopted by African Programme for Onchocerciasis Control (APOC) will be hindered. Close monitoring of Loiasis and Onchocerciasis are advocated. This could also have significant implications for the concept of severe Adverse Reactions (SARs) due to co-infection of *Loa loa* and *Onchocerca volvolus*.

Bayesian geostatistical model-based estimates of geospatial distribution of soil-transmitted helminthiasis and albendazole treatment requirements in Nigeria

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BACKGROUND: The control of soil-transmitted helminthiasis (STH) in Nigeria, using preventive chemotherapy, has become imperative in the light of global fight against Neglected Tropical Diseases (NTD). We provide for the first time Bayesian model-based risk maps to facilitate planning, targeting of control activities and surveillance.
METHODS: Disease data were derived from STH surveys carried out in 2011. The data were geo-referenced and collated in a geographical information system (GIS) database for the generation of STH point prevalence maps. Bayesian geostatistics methods using advanced variable selection with remotely sensed environmental covariates, was used to model the spatial risk of STH for Nigeria.
RESULTS: STH is currently endemic in 20 of 36 states of Nigeria. Hookworm infection was found in 482 (86.8%) locations covering 20 states, ascariasis is endemic in 305 (55%) locations in 16 states and trichiuriasis is endemic in 55 (9.9%) locations in 12 states. ascariasis and hookworm infection are co-endemic in 16 states, while the three species are co-endemic in 12 states. The prevalence range for ascariasis was 1.6% to 77.8%, 1.7% to 51.7% for hookworm and 1.0% to 25.5% for trichiursis. Model-based predicted prevalence of ascariasis, ranged from 0.1% to 82.6% with a mean prevalence of 2.9% (95% confidence interval (CI): 2.90-2.93%), while hookworm infection ranges from 0.7 to 51.0% with a mean prevalence of 7.9% (95% confidence interval (CI): 7.86-7.91). Land surface temperature and dense vegetation are the significant covariate influencing the spatial distribution of STH in Nigeria. Prevalence estimates adjusted for school-aged children in 2011, showed that ascariasis is <10% in all the 36 states including Abuja, while hookworm infection is >10% in 8 states and <10% in 29 states.
CONCLUSIONS: It was estimated that 40.1 million school-aged children are at risk of STH in Nigeria, requiring 80.2 million albendazole tablets annually for treatment.

First report of a SNP in the codon 200 of the β-tubulin gene of *Ancylostoma caninum*

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BACKGROUND: The mass drug administration, especially with benzimidazoles, corresponds to the main control method to the disease caused by members of the hookworm's family. However, this strategy can lead to selection of drug resistant strains. Mutations in codons 167, 198 and 200 of isotype 1 β-tubulin gene have been associated with benzimidazoles resistance in some nematodes. The mutation at codon 200 has been detected in human's hookworm, but so far has not being reported in *A. caninum*. The aim of this study was to perform a scan of polymorphisms at codons 198 and 200 of isotype 1 β-tubulin gene in *A. caninum* collected from two different regions in Brazil.
METHODS: Three hundred eleven worms were collected from dogs originated from Centers for Zoonosis Control from two different States. To analyze the codon 200, a molecular tool based on ARMS-PCR was developed. Plasmids, to be used as controls for the absence and presence of mutation, were constructed.
RESULTS: The mutation at codon 200 was observed only in one of the localities analyzed and in a low frequency (0,8%). Samples were subjected to sequencing to validate the technique developed and also to scan the region comprising the codon 198. The sequencing validated the technique developed and showed no correlation between the mutations.
CONCLUSION: This is the first report that determines the presence of the mutation in the codon 200 of the β-tubulin gene in *A. caninum*. Although the mutation was found in a low frequency, these data reinforce the need for monitoring for benzimidazole resistant in hookworms population, given the emergence of the problem in other species.

Molecular detection of *Capillaria philippiensis*, An emerging zoonosis in Egypt.

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BACKGROUND: Human infection with *Capillaria philippinensis* is accidental, however it may end fatally if not diagnosed and treated in the proper time. The first case was detected in the Philippines in 1963 but later reported in other countries around the world, including Egypt.

METHODS: In this report, molecular diagnosis using a specific Nested PCR for detection of *C. philippinensis* in faeces is described based on the amplification of small ribosomal subunit. *C. philippinensis*-specific PCR method have been successfully developed in this study.

RESULTS: Copro-DNA of the *C. philippinensis* positive samples gave no cross-reaction with human DNA or the DNA of any other parasites tested using our efficient and specific PCR test. The *Capillaria* DNA sequence has been submitted to genbank with the access number KF604920.

CONCLUSIONS: This method can be very useful for improvement of early diagnosis of cases, and also to understand the different environmental routes of transmission by detection of *C. philippinensis* DNA-stages in the possible fish intermediate hosts and reservoir animal host, helping to improve strategies for surveillance and prevention of human disease.

A case report of canine leishmaniasis, Korea

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BACKGROUND: There has been no report of canine leishmaniasis in Republic of Korea. Therefore we report *L. infantum* infection in a dog which was originated from Korea.
METHODS: A dead 26 months old male mixed dog was submitted. He was born and raised in Korea and has showed decreased appetite and weight loss before death. Necropsy, histopathological examination, electron microscopy, and *in situ* hybridization and PCR to detect *L. infantum* were performed.
RESULTS: At necropsy, dog showed the generalized lymphadenomegaly, and spleen was enlarged two times than normal size. Histopathologically, granuloma was observed in spleen, lymph nodes, liver, kidney and large intestine.The granuloma was consisted of many macrophages with intracytoplasmic *Leishmania* amastigote, and some plasma cells and neutrophils. Also in lymph nodes and spleen, severe follicular atrophy was seen. In kidney, severe diffuse membranous glomerulonephritis and diffuse interstitial fibrosis were detected. And *in situ* Hybridization revealed many amastigotes of *L. infantum* in liver, kidney and spleen. Transmissible electron microscopy detected many amastigotes of *L. infantum* with flagellum in the cytoplasm of macrophages in liver and spleen. Tested all tissues including heart, lung, liver, spleen, stomach and kidney were positive for conventional *Leishmania* spp. PCR and *L. infantum* was confirmed by real-time PCR.
CONCLUSIONS: Based on gross lesion, histopathological lesions, *in situ* hybridization, electron microscopy and PCR, our case was diagnosed as canine leishmaniasis. This is first case in Korea.

The challenge of human and black fly migration in onchocerciasis elimination in Africa

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BACKGROUND: Human onchocerciasis, transmitted by black fly vectors is a severely disabling filarial disease that is endemic in Africa south of Sahara, where it impedes national and individual development, makes fertile land inhospitable, impairs intellectual and physical growth and exacts a huge cost in treatment and control. As result of these, onchocerciasis has been subject to control efforts for more than five decades. These control efforts starting with vector control using rapidly biodegradable insecticides was followed with the 1987 discovery of ivermectin (Mectizan®) and the unprecedented decision of Merck & Co. Inc to donate the drug free, resulting in the strategy of annual ivermectin distribution programme (IDP). The launching of African Programme for Onchocerciasis Control (APOC) in 1995 opened a new chapter in the control with the sustainable community directed treatment with ivermectin (CDTI). This is to the extent that till date, over 60 million eligible people have been treated in 26 African countries. This long-term mass ivermectin distribution in endemic African countries, together with sustained political commitment of government, APOC, Non-Governmental Development Organizations (NGDOs) and endemic communities have shown promise in elimination of onchocerciasis. Results of epidemiological studies from endemic areas in African countries have shown that large-scale treatment with ivermectin has stopped further infections, especially in areas where there were high percentage of treatment coverage. This development has given hope and shown that onchocerciasis elimination is feasible in Africa. The great mobility of human population in and out of endemic areas under CDTI due to civil wars, voluntary and forced migration, nomadic herdsmen seasonal movement as well as seasonal migration of black fly vectors have posed critical challenge in the elimination process. CONCLUSIONS: This work brings to focus the implications of these; and at the same time attempts to create understanding of this challenge, with the aim to improve and sustain the onchocerciasis elimination process in endemic Africa countries.

The zoonotic risk of *Ancylostoma ceylanicum* isolated from stray dogs and cats in Guangzhou, south China

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BACKGROUND: Canine and feline hookworm infection is endemic in many countries with zoonotic transmission representing a potentially significant public health concern. However, there are limited data available on the zoonotic transmission of canine and feline hookworms in China. This study was conducted to evaluate the zoonotic risk of *Ancylostoma ceylanicum* isolated from stray dogs and cats in Guangzhou, south China.

METHODS: A primer pairs CAF/CAR was designed to amplify complete ITS sequences of obtained *A. ceylanicum*. The results were compared with fourteen ITS reference sequences of human-derived *A. ceylanicum* registered in GenBank, and phylogenetic trees were established by using NJ and ML methods..

RESULTS: The sequence similarity of three dog-derived and five cat-derived *A. ceylanicum* with fourteen human-derived *A. ceylanicum* were 96.8%~100% and 97.8%~100%, respectively. Phylogenetic analysis placed *A. ceylanicum* isolated from dogs and cats in same group with *A. ceylanicum* human isolates.

CONCLUSIONS: Due to the ability of *A. ceylanicum* to cause a patent infection in humans, the zoonotic risk arising from dog and cat reservoirs to communities in this region should be determined.

ACKNOWLEMENTS: This work was supported by grant from National Natural Science Foundation of China (grant no. 30972179 and 31272551). We thank the humane shelter' personnel for helping us in collecting all of the samples.

Genotyping *Giardia duodenalis* isolates from dogs in Guangdong, China based on multi-locus sequence

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BACKGROUND: *Giardia duodenalis* is one of the most common intestinal protozoan parasites, which is ubiquitous in mammals. To date, glutamate dehydrogenase (gdh), triosephosphate isomerase (tpi), β-giardin (bg) and small subunit ribosomal DNA (16S rRNA) genes have been widely used to identify *G. duodenalis* assemblages. However, the currently available primers may preferentially amplify some assemblages, multi-locus sequence typing is recommended.
METHODS: A total of 216 dogs' fecal samples were collected in Guangdong, China. The positive samples examined by microscopy were identified as the assemblages (or sub-assemblages) of *G. duodenalis* by using normal or nested PCR based on four genetic loci (tpi, gdh, bg and 16S rRNA). The phylogenetic trees were constructed with MEGA5.2 by using neighbor-joining method.
RESULTS: 9.7% (21/216) samples were found to be positive; moreover, ten samples were single infection (seven isolates assemblage A, two isolates assemblage C, and one isolate assemblage D) and eleven samples were mixed infection where assemblage A was the predominant, which was potentially zoonotic.
CONCLUSIONS: The choice of gene loci can influence the amplification result. Multi-locus sequence typing is more exact than single or double locus (loci) in genotyping, which provided a foundation on molecular identification and genetics of the *G. duodenalis* in dogs.This work was supported by grant from National Natural Science Foundation of China (grant no. 30972179 and 31272551). We thank the humane shelter' personnel for helping us collect all of the samples.

Multilocus Genotyping of *Giardia duodenalis* isolates from cats in Guangzhou, China

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BACKGROUND: *Giardia duodenalis* commonly causes enteric infections in humans as well as mammals worldwide. However, there have been no reports on the zoonotic potential of *G. duodenalis* from cats in China. The objective of this study was to genetically characterize isolates of *G. duodenalis* and determine if zoonotic potential of *G. duodenalis* could be found in cats from urban and suburban environments in Guangzhou, China.
METHODS: Among 102 stray cat's fresh fecal samples from Guangzhou, China, 30 fecal samples were collected in Baiyun district(urban), 72 in Conghua district (suburban). *G. duodenalis* specimens were examined using light microscopy, then the positive specimens were subjected to PCR analysis using glutamate dehydrogenase (gdh), triosephosphate isomerase (tpi), β-giardin (bg) and small subunit ribosomal DNA (16S rRNA) genes, subsequent sequencing. Sequences were analyzed by the phylogenetic trees constructed using MEGA5.2.
RESULTS: 9.8% (10/102) cats' fecal samples were found to be positive, 10% (3/30) in Baiyun district, 9.7% (7/72) in Conghua district. Among the ten positive samples, nine samples were single infection (eight isolates assemblage A and one assemblage F) and one sample was mixed infection with assemblage AI and assemblage C. Isolates of assemblage A were the predominant in our study, which was potentially zoonotic.
CONCLUSIONS: These findings has not only confirmed the high prevalence of *G. duodenalis* in stray cats, but has also shown, for the first time, that zoonotic assemblages/sub-assemblages occurred frequently in cats living in urban and suburban environments in China.This work was supported by grant from National Natural Science Foundation of China (grant no. 30972179 and 31272551). We thank the humane shelter' personnel for helping us in collecting all of the samples.

SCHISTOSOMIASIS AND NON HODGKIN LYMPHOMA: A CASE REPORT FROM OWERRI NIGERIA

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BACKGROUND: We report a very rare case of an association of urinary schistosomasis and non-Hodgkin lymphoma in a 30 years old male patient diagnosed and managed for non-Hodgkin lymphoma.

METHODS: The patient initially presented with weight loss, weakness, night sweat and generalized lymphadenopathy. Lymph node biopsy revealed high grade Non Hodgkin lymphoma. Chemotherapy was commenced immediately and after three cycles, the lymph nodes were noted to have regressed progressively but relapsed due to the patient's inability to get additional chemotherapy. The patient refused referral to a center with supportive management for high dose chemotherapy for rescue regimen.

RESULTS: The patient's condition 15 weeks post treatment was observed to have deteriorated with bilateral pedal edema possibly due to lymphatic obstruction and infiltration, abdominal swelling, massive scrotal and penile edema. The patient looked chronically very ill and pale. There was bleeding from the scrotal sac which appeared to be perforated. Further laboratory tests were recommended, scrotal pack was put in place while other management strategies were continued. Schistosomiasis was not suspected. The patient reported back 1 week later with severe abdominal pains, severe pains while passing urine as well as clotted blood in the urine, bloody watery stool and general weakness. The 30 years old young man looked like a 60yrs old man with swollen legs, scrotum and abdomen. When sent to the laboratory for urinalysis and other laboratory tests, the patient passed out adult worms confirmed to be *Schistosoma heamatobium* .

CONCLUSIONS: He confirmed he had passed out the worms earlier before coming to the hospital. Two days later the patient died.

STATUS OF ONCHOCERCIASIS I N ENDEMIC COMMUNITIES ALONG THE IMO RIVER BASIN SOUTH EAST NIGERIA

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BACKGROUND: Community directed treatment with Ivermectin (CDTI) has been ongoing for almost two decades in onchocerciasis hyper-endemic communities of Imo and Abia States Nigeria. This study looks at the current status of onchocerciasis in some hyper-endemic communities located along the Imo River Basin, a known breeding site for the Simulium vectors. The focus of this study include skin microfilarial load after several years of treatment, adult worms status, physical and ocular conditions of the subjects.

METHODOLOGY: Ethical considerations were prioritized with written consents obtained from the State Ministry of Health, traditional rulers, as well as the individuals. Two Local Government Areas (LGA) of Abia State; Isiukwuato and Umunneochi LGAs were selected and within each, four communities. In Imo State Okigwe LGA was selected and nine communities. Skin snipping under maximum safety conditions using corneo-sclera punches were taken from the consenting individual. Qualified medical personnel in the research team did physical, ocular examinations and nodulectomy. The excised nodules were subjected to histological examination.

RESULTS: The overall microfilariae prevalence in the communities of Abia State was found to be 26.0% with a community microfilariea load (Cmfl) 0.28mf/mg while that of the Imo State communities was found to be 18% with a Cmfl of 0.22mf/mg. Severe dermatological conditions of onchocerciasis were not observed in the selected communities. Only dermal conditions such as Leopard skin or depigmentation (5.6% -Abia State; 20% - Imo State), Nodules (4.2% - Abia State; 7% - Imo State), Lizard skin or Atrophy (0.2% - Abia State, 15% - Imo State) were observed. Impaired vision was noted in 4.2% subjects in Abia State and 17% in Imo State. Hanging groin was not seen in the subjects from Abia State while 3% was recorded for subjects from Imo State. Sections of adult worms on histological slides showed degenerated worms throughout while 63.4 % of subjects claimed they had nodules but their nodules had disappeared.

CONCLUSIONS: The persisting microfilaria load indicates continual transmission. The dermal conditions have however improved considerably. State of the adult worms in the excised nodules as well as verbal reports on dissolution of nodules confirm the efficacy of Ivermectin and further indicate that the observed microfilaridermia must be from new infections. Continual usage of Ivermectin will play a major role in the elimination of the disease. Pregnant women and children under 5 (exclusion criteria for Ivermectin treatment) remain reservoirs for transmission of onchocerciaisis, especially in endemic communities such as the communities selected for this study with subjects in very close proximity with the breeding sites of the black fly vectors. Despite the limitations of the community directed treatment with Ivermectin, elimination of onchocerciasis is still achievable.

CD8$^+$ T cells are preferentially activated during primary low dose *Leishmania major* infection but are completely dispensable during secondary anti-*Leishmania* immunity.

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BACKGROUND: Leishmanization, which is the deliberate injection of live virulent parasites, is the only known procedure that protects humans against the disease. However, this practice has been abandoned due to concerns for safety. However, with recent renewed interest in this old practice, it is important to determine what dose of live parasites is sufficient to induce protection with minimal side effects. Secondly, we previously showed that CD8$^+$ T cells are required for optimal immunity to low dose infection with *L. major*. However, whether parasite dose also affects the initial expansion of T cell and secondary immune responses remains unclear.

METHODS: Using in vivo *Leishmania major* infection model we assessed whether the initial infection dose affects the quality and magnitude of secondary anti-*Leishmania* immunity. We also used *in vitro* proliferation and flow cytometry to investigate the contribution of CD8$^+$ T cells in secondary anti-*Leishmania* immunity.

RESULTS: We found that while LD infection resulted in enhanced proliferation and IFN-g production by CD8$^+$ T cells, HD infection predominantly induced proliferation and IFN-g production by CD4$^+$ T cells. Despite this differential induction of T cell responses, both LD and HD infected mice were protected against secondary virulent *L. major* challenge. Interestingly, while depletion of CD4$^+$ cells in mice that healed LD and HD infections led to loss of resistance following secondary challenge, depletion of CD8$^+$ cells had no effect.

CONCLUSIONS: Collectively our results show that LD and HD infection induced comparable protection and although CD8$^+$ T cells are preferentially activated and contribute to primary anti-*Leishmania* immunity following low dose infection, they are dispensable during secondary (challenge) immunity to this parasite.

Ultrastructural analysis of posterior polar endocytic phagocytosis by *Leishmania* amastigotes

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BACKGROUND: Mechanisms for procurement of nutrients by *Leishmania* differ according to its stage-specific requirements. Mechanistic insight into phagocytosis by protists and developmental regulation of endocytosis was achieved by investigation of ultrastructural interaction of cutaneous and visceral *Leishmania* isolates from Egypt with their host cell cytoplasm.
METHODS: Two leishmanial isolates were used. One was from a patient's cutaneous lesion acquired in northern Sinai (SH), and the other was obtained from pools of livers and spleens of stray dogs from Agamy resort in Alexandria (D3). Isolation and maintenance of promastigote and amastigote stages was extracellularly in Offut's medium and intracellularly in laboratory out bred male Syrian hamsters. Culture pellets and biopsies 1 mm thick from various hamster tissues were fixed in cold 2.4% gluteraldehyde in 2% Ca cacodylate buffer (pH 7.2). The specimens were processed for electron microscopy and ultrathin sections were cut on a LBK Reichard ultra microtome and post stained with uranyl acetate in lead citrate-solution. Sections were examined in a Zeiss EM 952 electron microscope.
RESULTS: In amastigotes the subpellicular microtubules, which form the cytoskeleton support for the pellicular membrane, showed pronounced free endings at the posterior pole of the parasites, still covered by the plasma membrane. The posterior area deficient of microtubules formed a cup-like invagination of variable depths according to the parasite's endocytic activity. Evidence of phagocytosis by tissue forms of both isolates is exhibited by the similarity of homogenous bodies engulfed in the posterior invaginations and the host cell cytoplasm.
CONCLUSION: The exhibition of endocytic phagocytosis by intracellular *Leishmania* supports the concept of a common origin of protists from a phagocytic cell ancestor, and is evidence that phagocytosis, as a criterion, is not secondarily lost in the genus.

Pathophysiology of *Acanthamoeba* keratitis

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BACKGROUND: *Acanthamoeba* keratitis (AK) is a sight threatening infection of the corneal surface and is caused by a free living protozoan parasite related to the genus *Acanthamoeba*. It is the parasite that eats eye.
METHODS: The pathology of AK infection involves the pathogenic cascade: a series of events that includes the production of several pathogenic proteases that degrades basement membrane and includes cytolysis and apoptosis. This leads to the dissolution of the collagenous corneal stroma. The mechanism of *Acanthamoeba* adhesion to corneal surface may involve interaction of mannose binding glycoprotein on surface of corneal epithelium. There is evidence that a collagenolytic enzyme could have a role in the generation of the ring like stromal infiltration.
CONCLUSION: a review on AK is presented in the poster, it includes ocular manifestations with photographs related to ocular lesions and pathogenic cascade are presented in the poster as well.

Malaria and haematologic parameters of pupils at different altitudes along the slope of Mount Cameroon: a cross-sectional study

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BACKGROUND: Haematologic abnormalities are features in *Plasmodium falciparum* infection, and anaemia is an inevitable outcome. This study examines the influence of malaria status and altitude on haematologic parameters in school-aged pupils.

METHODS: A cross-sectional study was conducted among 728 school pupils aged between four and 15 years at three different altitudinal ranges along the slope of the Mount Cameroon region. The investigative methods included the use of questionnaire, clinical evaluation and laboratory investigations. Blood sample collected from each child was used for the preparation of blood films for detection of malaria parasites and assessment of malaria parasite density as well as full blood count determination using an automated haematology analyzer.

RESULTS: The prevalence of malaria in the study population was 33.8% and 64.2% (158/246) of these were asymptomatic (AM). Pupils in lowlands had a significantly higher ($P < 0.05$) prevalence (95% confidence interval, CI) of malaria (60.6%, CI = 54.6–65.9%) than those in middle belt (29.1%, CI = 23.9–34.8%) and highlands (7.7%, CI = 6.1–9.8%), while those in middle belt had significantly higher geometric mean parasite density (475) than those in lowlands (233) and highlands (388). The prevalence of malaria was significantly higher in children that presented with fever (40.4%, CI = 33.8–47.2%) when compared with afebrile subjects (31%, CI = 27–35.2%). Pupils with AM had a higher prevalence of leucopaenia (43.7%, CI = 35.8–51.8%), microcytosis (27.2%, CI = 20.5–34.9%), hypochromasia (27.8%, CI = 21–35.5%) and thrombocytopaenia (14.9%, CI = 8.9–22.8%) when compared with those with clinical malaria (CM). All mean haematological parameters were comparable in pupils with CM and AM, except for the mean white blood cell (WBC) counts. Pupils with AM had significantly lower ($P = 0.02$) mean WBC counts ($5.1 \pm 2.5 \times 10^9$/L) than those with CM ($5.9 \pm 2.3 \times 10^9$/L). Age, altitude and malaria parasitaemia was of significant influence on several haematological parameters.

CONCLUSIONS: Altitude influenced the distribution and density of malaria parasites and was of confounding influence on the haematologic profiles. These results highlight the insidious effects of AM on the haematologic components.

Genetic approaches for controlling transmission of mosquito-borne diseases: focus on malaria

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Genetics-based approaches have been proposed to control transmission of vector-borne pathogens. Strategies for both population suppression and population replacement of mosquitoes have benefitted from the development of transgenesis, site-specific recombination and targeted effector molecules. Advances in the development of vector-based genetic control strategies for preventing malaria parasite and dengue virus transmission have resulted in tools that in laboratory trials either suppress completely mosquito populations or render them incapable of transmitting the pathogens. The latest research will be presented on the development of a flightless-female strain for population suppression, a parasite resistance gene and DNA insulator activity for preventing the transmission of the human malaria parasite, *Plasmodium falciparum* by the Asian vector mosquito, *Anopheles stephensi*.

Parasites of the cockroach, *Periplaneta americana* (Insecta: Blattidae) in Lagos, Nigeria with special reference to *Raillietiella gehyrae* and the experimental infection of albino rats with cystacanths of *Moniliformis moniliformis*

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BACKGROUND: A previous study on the parasites of cockroaches was conducted with one of the objectives to know if the cockroach serves as an intermediate host for the pentastomids (tongue worms) in Lagos. One hundred cockroaches examined failed to incriminate them as the intermediate hosts. This study is a further attempt to see if the cockroach serves as the intermediate host of the pentastomids. Another was to determine the species of Acanthocephala infecting the cockroaches as a follow-up to the previous study.
METHODS: Two hundred (200) cockroaches were collected from various locations in Lagos metropolis between May 1983 and April 1985. They were dissected, examined for parasites, and processed using standard methods.
RESULTS: Twelve (5.0%) of the cockroaches were infected with the larvae of *R. gehyrae.* They were recovered from more male (8 of 12) than female (4 of 12) cockroaches. This is a geographic record and accomplished an objective which was not achieved in the previous study. Twenty-one (10.5%) cockroaches were infected with cystacanths of Acanthocephala. They were recovered from more females (15 of 21) than males (6 of 21). Of 9 laboratory bred albino rats force-fed with 15 cystacanths respectively, one female harbored 2 male and 2 female adult acanthocephalans identified as *Moniliformis moniliformis* recovered 8 weeks post infeceiion. Also, the following parasites were recovered: *Nyctotherus ovalis* (*Plagiotomidae: Ciliophora) (1.0%), Gregarina blattarum* (Eugregarinaria: Telosporea) (6.5%), the nematodes *Leidynema appendiculata* (3.0%), *Hammerschmidtiella diesingi* (17.5%), and Thelastoma sp. (2.5%) (Thelastomatidae: Nematoda).
CONCLUSION: It is now evident that cockroaches serve as intermediate hosts of the pentastomid *R. gehyrae* in Lagos, Nigeria and that the cystachants recovered from the cockroaches in a previous study and the present one are those of *M. Moniliformis*. It is recommended that more studies of this nature be conducted in other cities and/or states of Nigeria.

In vitro stressing factors alters de TCA cycle and the morphology of _Taenia crassiceps_ cysticerci

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BACKGROUND: *Taenia crassiceps* cysticerci are a well known experimental model to *T. solium* cysticercosis. These cysticerci have been explored in several different aspects, for example, morphology, morphological response to anthelminthic drugs, biochemical aspects and responses to drugs. There are factors found within the host such as glucose alterations and insulin intake that may interfere in the parasite's growth and metabolism. Therefore the aim of this study was to determine the morphological and biochemical alterations *in vitro* induced in *T. crassiceps* cysticerci by the presence of glucose, insulin, albendazole and praziquantel isolated and in association.

METHODS: The cysticerci were culture for 24 hours in supplemented RPMI culture medium added to two different concentrations of glucose, insulin, albendazole and praziquantel. The morphometrical analysis was performed through the Image J programme, and the biochemical one through HPLC.

RESULTS: The exposure to the stressing factors lead to: decrease in growth (insulin and glucose), blockage of growth (albendazole, insulin and glucose) and shrinking (praziquantel, insulin and glucose) of the cysticerci. The metabolic effects are related to a decrease in the TCA cycle metabolites due to the drug's mode of action, interestingly the praziquantel, insulin and glucose association enhanced the drug's mode of action.

CONCLUSIONS: This data may lead to cautious handling of cysticercotic patients who may present diabetes and use of insulin as the treatment may lead to increased alteration of the parasite's metabolism and greater side effects on cases of neurocysticercosis, as the parasite will die more quickly and secrete even more metabolites into the extracellular medium.

Identification of immunodominant epitopes of juvenile stages of *Fasciola hepatica* as candidate immunogens.

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BACKGROUND: Fasciolosis is a parasitic disease prevalent in temperate regions of the world and affects a large number of species, including humans. A control measure is the use of triclabendazole, however, resistance has already been reported. That is why new alternative for control are required. An alternative is the identification of molecular targets in the parasite as candidates for vaccination with the use of phage display technology.

METHODS: Both libraries with filamentous phages exhibiting random peptides 12 and 7 amino acids fused to the minor coat protein (pIII) of bacteriophage M13 were used. *L. humilis* snails infected with miracidia of *F. hepatica* to obtain metacercariae which were then used to infect rabbits and obtain anti-adolescarias antibodies. The IgG were purified, quantified and evaluated its reactivity. Nejs antigen was obtained by excystation of metacercariae. The peptides were selected with purified IgG through biopanning. Eluted phage from the last round of selection were amplified, qualified and individual clones were selected and were characterized by indirect sandwich ELISA.

RESULTS: Thirty-four individual clones were selected; eighteen clones were selected using the combinatorial library of 12 amino acids (12-mer) and sixteen clones through the combinatorial library of 7 amino acids (7-mer). Through affinity anti-adolescaria IgG of *F. hepatica* eight clones showed higher reactivity.

CONCLUSIONS: The eight identified as more reactive can be used as candidates for vaccination in definitive hosts, as well as for diagnosis.

Networks of post-transcriptional control in trypanosomes

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BACKGROUND: In African trypanosomes, transcription is polycistronic, and mRNAs are excised by *trans* splicing and polyadenylation.
METHODS: We measured the rates of *trans* splicing and mRNA decay in two life cycle stages of trypanosomes, the bloodstream and the procyclic form, by transcription inhibition and RNASeq. Many trypanosome mRNAs are spliced within a few minutes of synthesis. Some show simple exponential decay, but mRNAs with short half-lives tend to show initial fast degradation, followed by a slower phase. These are often degraded by a deadenylation-independent pathway that depends on the 5'-3' exoribonuclease XRNA. Many longer-lived mRNAs shows initial slow degradation followed by rapid destruction: the slow phase probably reflects gradual deadenylation. Developmentally regulated mRNAs usually show regulated decay rates. Rates of mRNA decay are quite good predictors of steady state levels for short trypanosome mRNAs. In contrast, longer mRNAs are less abundant than expected from their decay rates.
RESULTS: Mathematical modeling results indicate that co-transcriptional degradation is important in determining mRNA levels, and can explain the loss of long or slowly-processed mRNAs. To find proteins that can regulate mRNA translation and decay, we conducted a high-throughput screen, and identified over 200 potential regulators.
CONCLUSIONS: In addition to known regulators and components of the degradation machinery, we found translation factors, proteins with RNA-binding domains, and numerous proteins that had not previously been linked to mRNA metabolism, including proteins of unknown function and metabolic enzymes. A quarter of these novel regulators were found to have RNA-binding ability using a targeted proteome array.

Mucosal innate immune response toward *Entamoeba histolytica*

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BACKGROUND: *Entamoeba histolytica* (*Eh*) is an extracellular protozoan parasite of the human colon, which occasionally breaches the intestinal barrier. Eradicating ameba that invades is essential for host survival. A defining but uncharacterized feature of amebic invasion is direct contact between ameba and host cells. This event corresponds with a massive pro-inflammatory response. To date, pathogen recognition receptors (PRR) that are activated by contact with viable *Eh* are unknown.
METHODS: We quantified inflammasome activation in macrophages using well-defined pharmacological inhibitors of the nlrp3 pathway and in macrophages that were genetically deficient for inflammasome components. Mechanistic studies investigated if the danger signal ATP could trigger the inflammasome by blocking the P_2X_7 receptor and by enzymatic depletion of extracellular ATP. Inflammasome activation was visualized in real-time using confocal microcopy with cell-permeable caspase-1 probes.
RESULTS: Here we show that the innate immune system responds in a qualitatively different way to contact with viable *Eh* versus soluble ligands produced by viable or dead ameba. This unique *Eh* Gal-lectin contact-dependent response in macrophages was mediated by activation of the inflammasome. Soluble native Gal-lectin did not induce inflammasome activation, but was sufficient for transcriptional priming of the inflammasome and non-inflammasome-dependent pro-inflammatory cytokine release.
CONCLUSIONS: Our results suggest that the inflammasome is a pathogenicity sensor for invasive *Eh* and identify for the first time a PRR that specifically responds to contact with intact parasites in a manner that accords with scale immune response to parasite invasion.

Lactobacilli administered concurrently with an attenuated vaccine *vs.* babesiosis in cows stimulates significantly IgG1 antibodies anti-*Babesia bovis* and anti-*B. bigemina*.

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BACKGROUND: The effect of *Lactobacillus casei* administered along with a live attenuated vaccine vs. bovine babesiosis (VAC) on induction of IgG1 and IgG2 antibodies to *Babesia bovis* and *B. bigemina* was evaluated. We have previously reported that *L. casei* increases the efficiency of VAC, under controlled conditions and under extreme conditions in the field and that *L. casei* triggers the rapid differentiation of monocytes into macrophages *in vitro*; however, the levels of IgG1 and IgG2 antibodies to *Babesia bovis* and *B. bigemina* are not known in vaccinated animals.
METHODS: Twenty-one dairy cows were allocated into three groups (seven animals per group): unvaccinated, vaccinated with VAC, and vaccinated simultaneously with VAC and *L. casei* (VAC-LC). All animals were kept in a babesiosis endemic area at Tlalixcoyan, Veracruz. Anti-*Babesia* antibodies were assessed by the indirect fluorescent antibody test (IFAT) in sera from bovines of an endemic babesiosis area before (day 0) and after vaccination (days 15 and 30).
RESULTS: At days 15 and 30 after vaccination the average levels of IgG1 to *B. bovis* and to *B. bigemina* were significantly higher in VAC-LC group than those levels observed in VAC and control groups ($P < 0.01$); while levels of IgG2 were similar in VAC and VAC-LC groups but higher than in the control group ($P < 0.01$). These results are consistent with the finding that bovine dendritic cells stimulated *in vitro* promote IgG1 production.
CONCLUSIONS: It was concluded that the efficiency improvement of VAC, in part is due to the *L. casei* boost of IgG1 over IgG2 antibodies to *Babesia bovis* and *B. bigemina* when lactobacilli are inoculated simultaneously with this vaccine.

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Proteolytic activity of *Arthrobotrys musiformis* and its nematicide effect *in vitro* against *Haemonchus contortus* larvae 3.

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BACKGROUND: *Arthrobotrys musiformis* is a fungus nematophagous used for biological control against *Haemonchus contortus* larvae. The aim was to identify, purify and characterize at least one *Arthrobotrys musiformis* extracellular protease activity in vitro against larvae 3 (L_3) of *Haemonchus contortus*.
METHODS: *A. musiformis* was isolated and cultured from different substrates collected in Mexico, was grown on solid medium and liquid. For extracellular proteases, the fungus liquid was cultured for 2 weeks at room temperature under constant agitation. Thereafter, this medium was filtered and the mycelium was discarded, bioassays were conducted to determine nematicide activity against larvae 3. The liquid medium is passed through an ion exchange chromatography and after a hydrophobic interaction chromatography. Fractions were collected with proteolytic activity were concentrated and determined the optimum conditions thereof (temperature, pH, inhibitors).
RESULTS: *A. musiformis* was collected in Puebla and Veracruz, had a percentage of 44% of entrapment against *H. contortus* L_3. *A. musiformis* produced intracellular and extracellular proteases in the solid medium and in liquid medium and even in the absence of nematodes. In the filtrate medium proteases were identified and after passage of the columns were collected fractions nematicidal 85 and 77% of L_3 of *H. contortus*, at 48 hours, had difference with the control (medium without fungus). There proteolytic activity with albumin and gelatin. The proteolytic activity was highest at 37°C at pH 8. The PMSF inhibited the activity so that it could be serine proteases. There was more activity with gelatin as substrate.
CONCLUSIONS: This study showed that there nematicide effect of *A. musiformis* against *H. contortus* and at least there is an extracellular protease. The better you know the nature of *A. musiformis*, will have more accurate information on their possible use as a control agent against nematodes.
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Bioactivity of five tropical plants against *Cooperia* spp exsheathment process

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BACKGROUND: Ethnoveterinary is an ancient practice which uses plants as treatments for animals. Tropical legumes have been proposed as an alternative for anthelmintic therapy in ruminants, due to their content of bioactive compounds. Larval exsheathment is a vital transitional stage of gastrointestinal nematodes, which is necessary to succeed in the host infection. The objective of this investigation was to assess the inhibitory effect of increased concentrations (150, 300, 600, 1200 and 2400 µg/ml PBS) of *Leucaena leucocephala, Gliricidia sepium, Guazuma ulmifolia*, *Cratylia argentea* and *Azadirachta indica*, lyophilized extracts against the *Cooperia* spp exsheathment process.
METHODS: For each plant, three extraction procedures (acetone:water [AW], aqueous [AQ] and acetonic [AC]) were used. Polyethylene glycol (PEG) was used to evidence or discard polyphenols as the main bioactive compounds. A linear regression analysis was used to determine the difference in the mean percentage of exsheathment rates between the control and the treatment groups across time. General linear model was used to assess the dose-dependent response. The Kruskal-Wallis test was used to evaluate the AH effect amongst extraction procedures and PEG addition.
RESULTS: All fifteen extracts showed a dose-dependent response ($P<0.05$). *Leucaena leucocephala* (AW and AQ) inhibited 100% of larval exsheathment even at the lowest concentration. Fourteen extracts fully inhibited the exsheathment process at the highest concentration, except for *A. Indica*-AQ which inhibited 96.63±1.2%. At the highest concentration, no differences were found amongst plants and extraction procedures ($P>0.05$). The best-fit LC50 values were: 270.73 µg/ml (*G. sepium*-AW), 250.48 µg/ml (*G. sepium*-AQ) and 86.766 µg/ml (A. *indica*-AC). Polyethylene glycol addition exhibited polyphenols as the main bioactive compound.
CONCLUSIONS: Results suggest that all the three extraction procedures have similar bioactivity, and the five plants could be considered for further *in vivo* investigations, to corroborate their ability to reduce larval establishment within the host.

Effect of five bioactive plants against *Cooperia* spp egg hatching

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BACKGROUND: The emergence of anthelmintic resistance compels novel approaches to be developed and implemented, in order to improve the control of gastrointestinal nematodes (GIN) in grazing cattle. Nematode eggs, are an important biological stage of GIN, whose relatively thick tri-layered shell provides protection towards adverse environmental conditions, increasing their chances for survival. The objective of this investigation was to assess the ovicidal activity of organic extracts of *Leucaena leucocephala* (LL)*, Gliricidia sepium* (GS)*, Guazuma ulmifolia* (GU), *Cratylia argentea* and *Azadirachta indica*, against *Cooperia* spp.
METHODS: Egg hatching inhibition (EHI) assay was used to evaluate the ovicidal activity of each plant, with different extraction procedures: acetone:water (AW), aqueous (AQ) and acetonic (AC); and increasing concentrations of 600, 1200, 2400, 4800 and 9600 µg/ml. The role of polyphenols was assessed using Polyethylene glycol (PEG). A General Linear Model was used to assess the dose-dependent response of each extract. Kruskal-Wallis test was used to evaluate the AH effect amongst concentrations, extraction procedures and PEG addition.
RESULTS: The fifteen plant extracts inhibited the egg hatching in a dose-dependent manner. Best-fit LC50 values were 8670, 7920 and 1110 µmg/mL for GU, LL and GS, respectively. Differences were found for the AH activity amongst extraction procedures ($P<0.05$). At the highest concentration, LL_AQ inhibited over a 50% of *Cooperia* hatching; followed by GU_AW (45.42±1.3%). The AC extract of GS fully inhibited *the egg* hatching; conversely, the lowest activity was observed with GS both AW and AQ extracts (19.59±0.9% and 15.40±0.8%, respectively). Levamisole showed a low EHI, suggesting a high degree of resistance from *Cooperia*. PEG addition evinced the role of polyphenols in the bioactivity of most plants; however, for the GS_AC were discarded as the main bioactive compound.
CONCLUSIONS: Inhibition rates suggest that LL and GS could have an application for reducing larval density in pastures.

Treatment of Chagas disease. Up to date

Werner Apt B.

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Today there is consensus that human Chagas disease (ChD) must be treated in the acute period as well as the initial and middle indeterminate chronic period and in the determinate chronic period cardiopathy and digestive forms. The only exceptions from the etiological treatment are for those patients with chronic infection with Core Bovis and terminal cardiac insufficiency. The indication to apply specific therapy in chronic cases is the demonstration of parasites by PCR when they are not detected by optical microscopy. Currently it is accepted that a precocious treatment is able to modify the natural evolution of the disease. This is why, due to the number of patients in countries where infection is prevalent, its treatment is a public health problem. Today ChD is a transnational problem that affects 4 continents due to the migration of patients from endemic areas to countries where the disease doesn't exist. There are several drugs which act in vitro on *Trypanosoma cruzi* in cultures of epimastigotes and trypomastigotes, tissue cultures of amastigotes forms and in vivo in different species of infected animals with different strains (subpopulations) of the parasite. Nevertheless the above, the only drugs accepted universally to treat human cases due its efficiency and ethical consideration are: Nifurtimox (Lampit Bayer®) NF, and benznidazol (Rochagan Roche ®) BNZ, both drugs were developed in the decade 1960-1970 and produce side effects in 30-50% of the adults. In the acute cases NF is prescribed at day dose of 8 mg/kg/weight during 60 days in adults and 10-15mg/kg/weight in children during 60 days. The BZN dose is 5mg/kg/weight during 60 days in adults and 5-10 mg/kg/weight (average 7.5mg) daily during 60 days for children. In accidental infection by *T.cruzi* the some dose of both drugs is applied but only for 15 days. In reactivated chronic cases due to immunosuppression or depression of the immune system therapy should extender until there is an immunological response, lymphocytes CD4 are equal or higher there 200 per ml (usually 5 or more months) them the therapy can be given every other day.

Different heterocyclic nitrogenous compounds that inhibit ergosterol synthesis of *T.cruzi* have been used in chagasic patients with distinct results. Itraconazole (ITRA) and Ravuconazole (RA) are good tolerate with very few side effects, ITRA cure 31% of chronic cases and avoid hearth damage (cardiopathy) in patients with the indeterminate form. The drug has lower efficiency in acute cases (30%) than NF or BNZ. With Posaconazole (POS) some clinical trials are been conducting, the drugs produce few side effects, and patient adherence to it has been good, in relations to its efficiency the results will be ready in 2015. In relation to the investigations of BNZ in chronic Chagas cardiopaths (Proyect Benefit) results of its efficiency will be at the end of 2014.

At present we need a drug with low cost, high efficiency and with scarce secondary effects. The most likely future therapy will be combing drugs: BNZ plus POS; POSA plus NF; ITRA plus BNZ; ITRA plus NF, etc. It has been shown in vitro and in vivo synergism between POS and BNZ.

Evaluation of synthetic peptides derived from proteins of *Cryptosporidium parvum* as vaccine candidates.

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BACKGROUD: *Cryptosporidium parvum* is a zoonotic intracellular parasite that has recently acquired importance in public health. This parasite can be transmitted through water causing severe diarrhea, and consequently high morbidity in children and immunocompromised persons. Currently, there is no treatment to effectively control this disease. This study is aimed to evaluate the antibodies produced in mouse against peptides derived from the P23, CP15 and CSL proteins as vaccine candidates.
METHODS: Antigenic regions from P23, CP15 and CSL proteins were identified and characterized using bioinformatics tools, and the sequences of seven peptides were chemically synthesized. The inoculation of peptides was performed in previously weaned Swiss albino gnotobiotic mice. Before inoculation and eight days after the last inoculation, blood samples were taken. In order to evaluate the dynamics of antibody production an indirect ELISA (Lorenzo *et al.* 1993) was performed. Mice were maintained according with the national and internationals laws of ethics in research.
RESULTS: Six of the seven peptides stimulated the immune response. The higher titers found in sera were elicited by peptides of CP15 and CSL proteins; however, the peptides that induced an antibody response in the majority of mice were from CP15 protein. Finally, no adverse effects were observed in mice inoculated with peptides without adjuvant.
CONCLUSIONS: The peptides of CP15 and CSL proteins of *C. parvum* had the ability to stimulate the immune system of mice. Additional tests are needed to evaluate the effectiveness of the antibodies produced in response to such peptides.

Effect of *Toxocara canis* inoculum size on histopathology in mice

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BACKGROUND: *Toxocara canis* is an ascarid nematode parasite of canids. Larvae infect a wide range of paratenic hosts including humans, in whom the hatched larvae migrate through different tissues. Three clinical syndromes are very common: visceral larva migrans, ocular larva migrans and covert toxocariasis. Visceral larva migrans is mainly characterized by fever, hepatosplenomegaly, pneumonitis, and high eosinophilia. Other possible associations include neuropsychiatric disturbances and reactive airway disease.
METHODS: *T. canis* eggs were collected from the uteri of female adult worms and were allowed to embryonate in sterilized distilled water at 30℃ for one week to reach the larvated stage. Mice were infected by oral administration of 100, 500, 1000, 2000 and 3000 embryonated eggs per mouse in 0.3ml sterile phosphate-buffered saline (PBS). Uninfected mice only received 0.3ml PBS. Mice were separated in six groups based on inoculum size. Mice from each group were sacrificed at 7, 14, 30 and 60 days post-infection (p.i.), respectively. The liver, lung, heart, brain and kidney were removed for histopathological study.
RESULTS: We found that *T. canis* larvae could damage the tissue of infected mice in varying degrees. The larvae also could be discovered in all tissues except heart. Surface of brain, liver and kidneys appear dot hemorrhagic lesions. Eosinophilia could be observed on histologic sections even at 60 days p.i. Moreover, lung shows obvious hemorrhage and pulmonary inflammation and they were dose-dependent.
CONCLUSIONS: Our results suggest that infection of mice with a high or lower *T. canis* inoculum results in tissue damage inordinately and chronic pulmonary inflammation.
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Editor's recommendation: Parasitology Research at Springer, Berlin, Heidelberg, New York

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Parasitology Research (formerly Zeitschrift für Parasitenkunde) is one of the eldest journals in this field and was launched already in the year 1928 by Springer Publishers at Berlin, Germany. Today the 114th volume is on the market in printed and online versions being distributed in more than 115 countries. My colleague Bill Chobotar and me edited the journal since 1981 and we accept papers from practically all fields of veterinarian, medical and zoological parasitology. However, potential authors must be aware that we have a high rejection rate of about 60-70% although about 4500-5000 printed pages are produced every year. Thus it is highly recommended that authors attach very high importance on the clear and convincing presentation of their results and that they really reflect the recent standard of knowledge worldwide and cite not only a limited look on the present knowledge. The journal accepts Short Communications, Original Papers and (especially welcomes) Reviews. In additions to material, which is presented in both printed and online version there is the chance to add "electronic supplementary material", which covers original background data.

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Complex transcriptome diversity of the mosquito Rdl receptor generates a spectrum of sensitivity to the agonist, GABA

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BACKGROUND: Cys-loop ligand gated ion channels (Cys-LGICs), including the ionotropic γ-aminobutyric acid (GABA)-gated chloride channel Rdl, are the targets of effective insecticides. Rdl ('resistance to dieldrin') was identified owing to a mutation conferring resistance to the cyclodiene insecticide dieldrin. Recent studies reported compounds acting on Rdl even with the dieldrin resistance mutation, highlighting this receptor as a potential target for novel insecticides. Rdl in the model organism, *Drosophila melanogaster*, undergoes alternative splicing and RNA editing that affects receptor sensitivity to GABA and insecticides. It has yet to be determined whether mosquito Rdl shows this level of complexity.
METHODS: Rdl receptor cDNA and genomic DNA was amplified by PCR from *Culex pipiens*, *Aedes aegypti* and *Anopheles gambiae* to identify potential RNA editing sites. To determine the impact of RNA editing on receptor function, editing variants of Rdl from *A. gambiae* were expressed in *Xenopus laevis* oocytes and their sensitivity to GABA was measured using two-electrode voltage-clamp electrophysiology.
RESULTS: Rdl from *C.pipiens*, *A. aegypti* and *A. gambiae* possess four, six and seven RNA editing sites, respectively, all of which alter amino acid residues. Only one of these editing sites has been observed in Rdl of another species, that of *D. melanogaster*. In an analysis of over 100 cDNA clones of *A. gambiae* Rdl, the seven RNA editing sites were detected in 22 different edit combinations. Electrophysiology studies on these edit variants show that RNA editing can have an influence on the potency of GABA.
CONCLUSIONS: These results highlight how RNA editing can broaden the transcriptome to generate functional diversity in a receptor between species even when they appear almost identical at the genomic level. Future studies will determine whether RNA editing influences sensitivity to insecticides.

Roles of ABC transporters in schistosome physiology, drug susceptibility, and parasite-host interactions

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BACKGROUND: Praziquantel is the drug of choice against schistosomiasis. Though an excellent drug overall, praziquantel is effectively the only treatment available for schistosomiasis, a disease afflicting hundreds of millions. It is also largely ineffective against immature schistosomes. New antischistosomal agents are urgently needed. ATP binding cassette (ABC) multidrug transporters offer attractive candidate targets for new or repurposed drugs that can act as anthelmintics on their own or as adjuncts to existing anthelmintics. ABC transporters such as P-glycoprotein mediate efflux of toxins, xenobiotics, and signaling molecules, and are associated with drug resistance in different organisms, including parasitic helminths. They exhibit broad substrate specificity and are inhibited by several drugs currently in clinical use. ABC transporters are also implicated in normal physiological activities such as excretion, maintenance of permeability barrier function, and modulation of immune responses. They transport potent signaling molecules with high affinity, including several with immunomodulatory activity.
METHODS: *S. mansoni* adults, juveniles, and eggs were exposed to drug combinations or subjected to RNAi, using methods previously described.
RESULTS: Schistosomes exposed to praziquantel increase expression of ABC transporters; worms with reduced praziquantel sensitivity show higher basal expression of these transporters. Praziquantel is both an inhibitor and likely substrate of schistosome Pgp, and disruption of transporter expression or function enhances the activity of praziquantel against adult and juvenile worms. Schistosome ABC transporters also appear to be required for parasite egg production. We are currently using molecular and pharmacological tools to understand the mechanism by which these transporters might be altering schistosome susceptibility to praziquantel, and to assess the role of schistosome ABC transporters in the parasite's modulation of host immune responses.
CONCLUSIONS: These experiments could lend important insights into the function of ABC transporters in schistosome physiology, praziquantel susceptibility, and parasite-host interactions, and possibly provide targets for novel antischistosomals.

The role of IFN-gamma in CD301+ cells differentiation experimental subcutaneous cysticercosis

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BACKGROUND: Cysticercosis is an infection caused by the metacestode larval stage of *Taenia* parasites in tissues and elicits a host-parasite reaction in which the immune response may be decisive in the disease development. the aim of this study was to evaluate the role of IFN-gamma from Th1 immune response in the experimental model of subcutaneous infection with *T. crassiceps* cysticerci using IFN-gamma knockout mice.

METHODS: Male C57BL/6 and C57BL/6 KO IFN-gamma mice 8-12 weeks of age were inoculated with *Taenia crassiceps* cysticerci into the subcutaneous tissue of the dorsum. At 7 and 30 (acute phase), 60 and 90 (chronic phase) days post infection, animals from each group had their blood the subcutaneous tissues were collected for the serologic and pathology studies. IFN-gamma and IL-4 were dosed and the histopathological analysis was performed. Also the presence of CD301+ cells was detected through immunohistochemical analysis.

RESULTS: In the presence of IFN-gamma there was the control of the parasitary growth determined by the establishment of a mixed Th1/Th2 systemic immune profile. This profile also locally induced the granuloma formation constituted by cells that played important roles in the parasitary destruction and by CD301+ cells in the beginning of the infection that were associated to the Th1 axis of mixed immune response. On the other hand, the absence of IFN-gamma favored the parasitary growth and de development of a systemic Th2 immune response.

CONCLUSIONS: This profile influenced the granuloma formation with CD301+ macrophages which were important in the collagen synthesis which impair the parasitary growth.

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Nematodiasis in school aged children in the flood regions of Sapele, Delta State, Nigeria.

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BACKGROUND: The Niger Delta Area is endowed with high concentration of dendritic system of waterways which provide excellent transmission pathway for parasitic infections, especially soil transmitted nematodes, due to periodic flooding experienced in the region. Gastrointestinal nematodes such as *A. lumbricoides*, *T. trichura*, Hookworm and *S. stercoralis* are very common in children between the ages of 0-12 years worldwide. There is a great need to evaluate the status of gastrointestinal helminths amongst school aged children especially in the flood zones of the Niger Delta as the information from such surveys would afford the Government, International Agencies and Non-governmental Organizations in Nigeria to establish sustainable public health programmes with a view to reducing the prevalence of gastro intestinal helminths in the region.

METHODS: Two hundred faecal samples were collected from school-aged children in randomly selected primary schools in Sapele metropolis of Delta State, Nigeria, using standard parasitological techniques and were analysed using the formal-ether concentration technique.

RESULTS: Data revealed that 62% of total samples were positive for various helminths. Sex related worm burden recorded 45.1% and 54.8%, female and male infections respectively ($p<0.05$). However, there was greatly variability in age related infection; 6-8 years (29.2%); (40.3%) and (15.6%) for age groups 4-6 years and >11 years. Specific nematodiasis revealed four worms; *A. lumbricoides*; Hookworm; *T. trichura* and *S. stercoralis*. The study also recorded great variability in overall specific nematode burden; *A. lumbricoides* (38.7%); Hookworm (40.%); *T. Trichura* (12%) and *S. stercoralis* (8%). Polyparasitism varied greatly within the age groups.

CONCLUSIONS: The study revealed that the relatively high gastrointestinal worm burden in the sampled population is unconnected with the periodic flooding experienced in the study area. Regular anthelminthic administration to school aged pupils and improved environmental sanitation are recommended control measures.

Prospects for antiparasitic vaccines for aquacultured fish

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BACKGROUND. Parasitic infections in aquacultured fish are responsible for major economic losses related to mortality, morbidity and reduced physiological performance leading to inferior growth and quality. Antiparasitic drugs have showed some effects during specified time periods but appearance of drug resistance following continuous treatment has been demonstrated. Due to the high success demonstrated by antibacterial vaccines it may therefore be worthwhile to investigate possibilities for development of antiparasitic vaccines as well. Infections caused by metazoan parasites have all been demonstrated to elicit significant host reactions. In most cases these confer mainly lowgrade protection towards reinfection possibly due to immune evading. A better protection against reinfection has been demonstrated in fish following protozoan infections. Several examples of flagellates and ciliates inducing protective immunity in fish following infection or experimental vaccination are known and our primary candidates for successful antiparasitic vaccines should be searched for among protozoans.

METHODS. Vaccines based on *Philasterides dicentrarchi, Cryptobia salmositica* and *Ichthyophthirius multifiliis* have been investigated in some detail due to their highly protective effects. Novel approaches towards an Ich vaccine are involving DNA-vaccinology and recombinant vaccines. Administration of vaccines for fish may include injection, immersion or oral administration.

RESULTS. We here present a new experimental vaccine based on a bacterin composed of formalin killed recombinant *Yersinia ruckeri* (Yr) expressing the protective i-antigen. It may be used as an immersion vaccine. We are now investigating if similar fish host responses are being produced towards the recombinant Yr and its foreign parasitic antigen.

CONCLUSIONS. Based on these experiences and principles we may expect that a new generation of vaccines towards metazoan infections may arise.

Artemisia extracts inhibit hemozoin

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Since 7 years the association IFBV-BELHERB from Luxembourg has established a working relationship with African and South American universities, in close cooperation with other European research institutions. Several of these partners have run clinical trials with *Artemisia annua* tea [i],[ii],[iii],[iv],[v],[vi],[vii],[viii],[ix]. In all these trials a therapeutical effect of 95 % or higher was confirmed by the use over 7 days of whole leaf infusion, capsules or tablets. One of the surprising effects noticed in these trials was that that the artemisinin content had very little impact on the results. This lead us to make an analysis as complete as possible of all the constituents, organic and inorganic, in a large series of *A. annua* samples from different origins[x]. *A. annua* from Luxembourg which had shown very promising antimalarial results, excellent bactericidal properties and a strong anti-inflammatory effect[xi] contained very little artemisinin but higher concentrations of certain essential oils. The effect of water soluble polysaccharides, phytosterols and saponins has been neglected in the past because most of the *A. annua* extracts had been obtained with organic solvents. Several papers have shown that *A. annua* ingested as powdered leaves[xii] or in conjunction with fatty food significantly increases the artemisinin concentration in the blood. It is well documented in the literature that *A. afra* or *sieberi* which contain little or no artemisinin are extensively used as antimalarials[xiii]. They contain at least 5 molecules of the same antimalarial efficacy as artemisinin[xiv]. At Leiden[xv] it was found that the anti-HIV activity of *A. afra* is even higher than for *A. annua.* More recent research from the Al Quds University[xvi] has shown that aqueous infusions of several Artemisia species strongly inhibit beta-hematin, like chloroquine did. But the most important finding in several of the clinical trials, especially in Kenya and Uganda, was that people who drink one or two cups of *A. annua* tea per week become immune against malaria. Similar strong prophylactic results have been obtained with ARTAVOL, a mixture of herbs developed by the Ministry of Health in Uganda, mixture containing Artemisia without artemisinin. Resistance in this case is not related to the killing power of one single molecule like artemisinin but to the polytherapy of the whole plant which not only eliminates the parasites but boosts the immune system, avoiding thus infection, reinfection or recrudescence.

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Emerging Parasitic Infections Among Immunocompromised Hosts

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BACKGROUND: Parasitic infections in the form of protozoa or helminths produce a substantial burden of disease worldwide. Many parasites have evolved within vertebrate's immune system and therefore have developed survival strategies that lead to chronic infections. Some can cause severe disease among immune compromised hosts.
METHODS: A PubMed Search was performed to review the literature over the last 10 years to search for emerging parasitic pathogens among hosts with immune suppression from different categories including those with HIV/AIDS, transplant, chemotherapy-cytotoxic therapy, disease-modifying agents, malnutrition, and others. Data was reviewed and selected for analysis.
RESULTS: Parasitic pathogens continue to appear in the clinical horizon with expanding clinical spectrum of manifestations among severely immunocompromised hosts, particularly, among those receiving modern interventions that modulate the immune system or among those with HIV/AIDS. Some examples include *Trypanosoma cruzi* of the central nervous system in patients with HIV/AIDS, disseminated microsporidiasis and visceral leishmaniasis and viscerotropic disease among different immunocompromised hosts or free-living amebas in transplant recipients. Interestingly, and similar to the herpesviruses modulating the immune system, some parasitic infections play a role in modulating the response to other infectious pathogens and to the immunogenicity of vaccinations.
CONCLUSIONS: Clinicians need to continue to be aware of the expanding spectrum of clinical manifestations and parasitic pathogens and their immune modulation roles.

The Role of RNA Pol II in the Recruitment of Histone Modifiers to Specific Regions of the Genome of Malaria Parasites

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BACKGROUND: *Plasmodium falciparum* parasites maintain a persistent infection by systematically altering the antigens displayed on the surface of the infected RBCs and thereby avoiding the antibody response. This results from switches in expression between individual members of the multi-copy *var* gene family. Each *var* gene encodes a different form of PfEMP1, a protein placed on the surface of the infected RBCs that mediates adhesion the endothelial surfaces of the blood vessels. Only a single *var* gene is expressed at a time, thus determining the both the antigenic phenotype of the infected cells as well as their adhesive properties. Regulation of *var* gene expression involves tightly coordinated recruitment to *var* loci of proteins required for chromatin modification and transcriptional silencing/activation.
METHODS: We recently discovered that an ortholog of the histone modifier SET2 is recruited to *var* genes through direct interactions with the C-terminal domain of RNA polymerase II. In higher eukaryotes, SET2 is recruited by RNA pol II during mRNA transcription, however the ortholog in *P. falciparum* (PfSET2) appears to be recruited by RNA pol II while it is transcribing noncoding RNAs.
RESULTS: Through the creation of a dominant-negative form of PfSET2 we confirmed its importance in *var* gene regulation and the role for RNA pol II in its recruitment to *var* loci. Over-expression of the dominant-negative resulted in rapid *var* gene switching that was not random, thus potentially revealing a previously unrecognized structure to *var* gene expression patterns. CONCLUSIONS: Together these data provide clues to deciphering the complex molecular process that coordinates *var* gene activation and silencing.

ENDOPARASITIC FAUNA OF *Chrysichthys nigrodigitatus* OF A TIDAL FRESHWATER IN THE NIGER DELTA.

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BACKGROUND: *Chrysichthys nigrodigitatus;* the silver catfish is greatly sort after in the Niger Delta, making it the dominant commercial catch of artisanal fishermen in the region. However, the omnivorous feeding habit of *C. nigrodigitatus* exposes it to a variety of parasites with their attendant implication on public health of the region. It is common knowledge that consumption of animal protein could predispose the consumer to several zoonotic infections. On this premise, it is pertinent to assess the parasitic status of *C.nigrodigtatus* in the New Calabar river, Port Harcourt, Rivers State, Nigeria.

METHODS: Fish samples were collected using the stratified random sampling technique from the common landing stations along the riverbank. Selected samples were bled through the cardiac and caudal puncture techniques and dissected to expose the internal organs. These organs were macerated to express tissue dwelling parasites and were further processed for microscopy using the formol-ether concentration method.

RESULTS: There was variability in organ related endo-parasitism ($p>0.05$). The intestine recorded the highest overall infection (40.0%); the heart and liver each had (30.0%) infections and no parasites were observed from the liver. However, the sex related infection ($p>0.05$) showed that the females had more infection 9(90.0%) than the males 1(10.0%).

CONCLUSION: The variability in sex related parasitism was attributed to host specific factors. The increase in parasitic load as age (size class) increased indicated that a repeated exposure to parasite infective stages was a major factor in infectivity of the *C.nigrodigitatus* in freshwater habitats. The overall low parasite load recorded in the study was attributed to the tidal characteristics of the water body that may have influenced effective secure of hosts by parasites infective stages.

Mechanism of attenuation in *Babesia* vaccine through genomic and transcriptomic analyses

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BACKGROUND: Virulence is a dynamic and highly plastic trait among microbial populations. It differs between strains and can be manipulated to produce live attenuated vaccines. Why such differences exist or how they develop is unknown. This is particularly true of parasites with complex life histories and multiple hosts. Such mechanism influences defining traits such as virulence of the entire strain population. A better understanding of this mechanism would help explain how phenotypic plasticity occurs.
METHODS: Using multiple virulent parental and attenuated derived *Babesia bovis*, comparative genomic and transcriptomic analyses were conducted.
RESULTS: Global genomic analysis of six virulent parental and their respective attenuated derivatives illustrated that the gain or loss of virulence is not due to shared coding differences, such as insertions, deletions or changes in allele frequency, but to a significant reduction in genome diversity and content. This suggests that gene loss during the attenuation process may in turn contribute to virulence loss. It also suggests that changes in virulence results from changes in the proportion of more or less virulent subpopulations. Comparative transcriptome profiles of two of the *B. bovis* strain pairs suggested differentially regulated transcripts existed. Differentially expressed transcripts of interest include variant erythrocyte surface antigen encoding gene (*ves*) and spherical body protein (sbp) 2 gene family members. A disproportionately greater number of *ves* were upregulated in the virulent parental strains. *Sbp*2 family members were also significantly upregulated in expression in the attenuated strains.
CONCLUSIONS: We concluded that *ves* heterodimer pair upregulation and overall higher frequency of *ves* gene expressions in the virulent strains is consistent with the involvement of this gene family in virulence. Upregulation of the *sbp*2 gene family also plays a role in the attenuated phenotype. Exactly how these two gene families contribute to the loss of virulence is currently under investigation.

Induction of protective CD8+ T cell responses by malaria sporozoites.

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It is well established that immunization with attenuated malaria sporozoites induces $CD8^+$ T cells that eliminate parasite-infected hepatocytes. After injection in the skin by mosquitoes or syringe inoculation, CD8+ T cells are primed in lymph nodes. 16-36 hours later they egress from this lymphoid organs and migrate to peripheral organs such as liver where they become long term residents, and undergo a unique differentiation program. Indeed, compared to memory cells found in spleen, liver-resident memory CD8+T cell display major differences regarding their transcriptional profile differing in several hundreds gens most of which are related to control and development of immune responses. Among these differently expressed genes, liver-resident CD8+ memory T cells display higher levels of CXCR6, a chemokine receptor that binds the chemokine CXCL16 which is abundantly present in the liver.

To determine the possible role that this chemokine–chemokine receptor interaction may have in the homing or maintenance of memory cells in the liver we studied the priming, homing, homeostatic proliferation, and functional characterization of antigen-specific CXCR6 deficient CD8+ T cells after immunization with parasites or a vaccinia virus. We found that liver memory CXCR6-deficient T cells display a normal cytokine secretion and expression of cytotoxic mediators on a per-cell basis. Although primed CXCR6-deficient CD8+ T cells migrate normally to the liver immediately after activation, a few weeks later their numbers severely decrease in this organ and as a consequence they lose their capacity to inhibit the development of parasite liver stages. These results indicate that memory cells lacking CXCR6 have a major defect that severely limits their long term homing to the liver and their capacity to inhibit parasite development.

Transgenic malaria parasites for vaccine studies

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Chimeric or transgenic parasites are new research tools that have shown great potential for the evaluation of malaria specific immune responses. These strains consist of rodent malaria parasites in which a gene of interest is fully or partially replaced by the ortholog gene from a human malaria species. The generation of rodent malaria parasites expressing vaccine candidate genes from *P. falciparum* or *P. vivax* allows the development of *in vivo* assays in mice which facilitate the evaluation of immunogenic properties of vaccine constructs and the comparison of different immunization protocols. In these assays, animals are immunized to induce immune responses against a specific human parasite antigen, and then they are challenged with a chimeric rodent parasite expressing the human parasite antigen of interest.

We have developed several strains of chimeric parasites to study basic immunological aspects of immune responses induced by *P falciparum* sporozoites and to evaluate responses to vaccine candidate targeting pre-erythrocytic stages of *P. falciparum* and *P. vivax*. The use of some of these parasites has already proven most useful to initiate the selection of vaccine candidates. This *in vivo* assay helps to determine if a candidate vaccine contains properly folded protein and if it induces protective immunity in mice. The data that can be generated should greatly help developing the rationale and experimental basis to evaluate novel vaccine candidates and define immunization protocols for further testing in NHP models and eventually human volunteers.

Global genetic diversity, population structure and virulence of *Toxoplasma gondii*

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BACKGROUND: Elucidating the *Toxoplasma gondii* population structure will enhance our understanding of the factors that have driven the proliferation of one of the most successful eukaryotic human pathogens on earth. One of the primary methods employed in the analysis of *T. gondii* population genetics is the multilocus PCR-RFLP genotyping. In the past decade, numerous papers have been published by this method and identified various genotypes of *T. gondii* in domestic and wild animals, providing significant information on genetic diversity of the parasite.
METHODS: We collected PCR-RFLP typing data from literature and performed comprehensive analysis to reveal *T. gondii* genetic diversity and population structure. In addition, we developed generic markers based on two polymorphic virulence genes, ROP5 and ROP18, to investigate their association with mouse virulence.
RESULTS: The data compiled provided us with a comprehensive picture of the global *T. gondii* genetic diversity. Overall, only a few genotypes dominate in Europe, Africa, Asia and North America. However, hundreds of genotypes coexist with none being notably dominant in South America. PCR-RFLP genotype #1 (Type II clonal), #2 (Type III), #3 (Type II variant) and #10 (Type I) were found to be distributed globally. Genotypes #1, #2 and #3 are prevalent in Europe, genotypes #2 and #3 dominate in Africa, genotypes #9 (Chinese 1) and #10 are prevalent in Asia, and genotypes #1, #2, #3, #4 and #5 dominate in North America (#4 and #5 are collectively known as Type 12). We also demonstrate that the combination of ROP5 and ROP18 alleles is predictive of *T. gondii* virulence across a broad range of global isolates.
CONCLUSIONS: *T. gondii* has low genetic diversity in most parts of the world, but high diversity in South America. Mouse virulence of the parasite is likely determined by a small number of polymorphic genes.

Cystic echinococcosis in the middle region of the Nile Delta, Egypt – Clinical and radiological characteristics

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BACKGROUND*:* Cystic echinococcosis (CE) is a parasitic infection caused by the larva of *Echinococcus granulosus*, and characterized by cystic lesions in the liver, lungs, and rarely, in other parts of the body. The diagnosis relies on clinical, laboratory, and radiological examination.

AIM: to identify the clinical, radiological, and biological features of patients with CE in the middle region of the Nile Delta, Egypt.

PATIENTS AND METHODS: This study conducted on (45) patients over a period of two years. Those patients were subjected to full clinical evaluation, radiological assessment using conventional x-ray chest, abdominal US and MDCT of the abdomen and chest, and laboratory assessment.

RESULTS: This study identified the epidemiological, clinical, laboratory, and radiological characteristics of patients with CE in the middle Delta region, Egypt. The CT may display the same findings as US in diagnosing and staging of most cases, however CT is more superior to US in the evaluation of heavily calcified cysts, small inaccessible cysts and pulmonary hydatid cysts.

CONCLUSIONS: Risk factors for CE include male gender and residence in rural areas. Biological markers seem neither sensitive nor specific, whereas the serological tests, preferably two different techniques, are useful. Ultrasound shows high accuracy rate, but CT, whenever possible, should be employed as it provides additional diagnostic value.

The spatial epidemiology of tapeworm infections in humans and pigs in rural Kenya

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BACKGROUND**:** The application of spatial analyses to epidemiological datasets has provided a wealth of information regarding environmental drivers for disease transmission and the identification of high risk areas for a range of parasitic diseases such as malaria, schistosomiasis and soil transmitted helminths. Despite previous evidence of spatial clustering in tapeworm infections (*Taenia* spp.) and the role of environmental factors (e.g. temperature and humidity) in the survival of eggs in the environment (and thus, transmission of disease), little research has thus far explored the spatial epidemiology of *Taenia* spp. infections. This research aimed to examine the spatial epidemiology of *Taenia* infections in humans and pigs and to assess the role of environmental factors in observed disease distributions.

METHODS**:** The potential spatial clustering of *Taenia* spp. infections in humans and pigs was assessed by applying exploratory spatial methods to survey data from western Kenya. These were compared for three disease outcomes: human worms; human cysts; and pig cysts. Regression methods were also applied to examine the relationships between several environmental factors (e.g. precipitation, land cover) and disease occurrence for all three disease outcomes to assess the role of the environment in disease transmission.

RESULTS: The spatial distribution of all three disease outcomes was spatially heterogeneous, with spatial clustering detected. There was some evidence of spatial overlap between the spatial clusters for the three disease outcomes, although this was not as prevalent as had been expected. Regression analysis indicated that environmental factors play a role in spatially heterogeneous disease risk.

CONCLUSIONS: These results indicate a complex spatial relationship between human and pig *Taenia* spp. infections. Further research may enable the spatial targeting of control initiatives based on disease “hot-spots” and landscape risk factors.

Distantiae transmission of _Trypanosoma cruzi:_ A new epidemiological feature of acute Chagas disease in Brazil

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BACKGROUND: The new epidemiological scenario of orally transmitted Chagas disease that is emerging in Brazil, mainly in the Amazon region, represents an epidemiological challenge. That is the case of Belém city, the capital of Pará state that displays the highest number of acute Chagas disease (ACD) cases associated with the consumption of contaminated açaí juice.
METHODS: The wild and domestic enzootic transmission cycles of *Trypanosoma cruzi* were evaluated in the two (Jurunas and Val-de Cães) that report the majority of the autochthonous cases of ACD in Belém city. Moreover, we evaluated the enzootic cycle on the three islands that provide most of the açaí fruit that is consumed in these localities. We employed parasitological and serological tests throughout to evaluate infectivity competence and infection by *T. cruzi* of domestic and wild mammals. Genotyping by mini-exon gene and PCR-RFLP (1f8/Akw21I) was performed
RESULTS: In Val-de-Cães, no wild mammal presented positive parasitological tests; low serological titers were observed in 56% of examined dogs. Three of 14 triatomines were found infected (TcI). This epidemiological picture does not explain the high number of autochthonous ACD cases. In Jurunas, the cases of ACD could not be autochthonous because of the absence of any enzootic cycle of *T. cruzi*. In contrast, in the 3 island areas from which the açaí fruit originates, 66.7% of wild mammals and two dogs displayed positive hemocultures, and 15.6% of triatomines were found to be infected by *T. cruzi*. Genotyping revealed that the mammals and triatomines from the islands harbored TcI.
CONCLUSION**:** These findings show that cases of Chagas disease in the urban area of Belém may be derived from juice unproperly prepared with infected triatomines together with the açaí fruits originated from these islands. We termed this new epidemiological feature of Chagas disease as "*Distantiae* transmission".

Prophylactic Efficacy of Three Generations of Vaccines Against *Trypanosoma cruzi* Infection and Chagas Disease

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BACKGROUND: Chagas disease is prevalent in almost all Latin American countries, and considered an emerging infectious disease in the developed countries. When considered from a global perspective, Chagas disease represents the third greatest tropical disease burden after malaria and schistosomiasis.
METHODS: We have employed a computational/bioinformatics approach for unbiased screening of the *T. cruzi* genome database and identification of 11 potential candidates. Through rigorous analysis, we considered three candidates (TcG1, TcG2, TcG4) were maximally relevant for vaccine development because these candidates were highly conserved in clinically relevant *T. cruzi* strains, expressed (mRNA/ protein) in infective trypomastigote and intracellular amastigote stages of *T. cruzi*, and recognized by IgGs and $CD8^+$T cells in multiple *T. cruzi*-infected hosts.
RESULTS: We have examined the protective efficacy of three candidates (with and without IL-12 and GM-CSF adjuvants) in mice. Our data clearly established that co-delivery of the antigens elicited additive immunity and protection from *T. cruzi* infection than was noted with individual candidates, and delivery of the three antigens with cytokine adjuvants as a DNA-prime/protein-boost vaccine proved to better elicit protective immunity than did DNA-prime/DNA-boost vaccine. Upon challenge infection, DNA/protein-immunized mice expanded the vaccine-induced, antigen-specific type 1 antibody and $CD8^+$T cell responses that provided 90% reduction in acute parasite burden. However, complexity of this vaccine inhibited our ability to move forward with large-scale vaccine design.
CONCLUSIONS: We sought to further simplify the composition and determine the long-term efficacy of a vaccine against *T.cruzi* infection. In this study, we delivered the vaccine candidates using a DNA-prime/MVA-boost approach. We also tested whether DNA-MVA approach will increase the vaccine efficacy sufficiently to omit the need for IL-12 and GM-CSF adjuvants. We discuss the function of vaccine-induced antibody and T cell responses against *T. cruzi* in providing protection from acute parasitemia, and chronic parasite persistence and immunopathology in chagasic mice.

Serum Mediated Activation of Macrophages Reflects TcVac2 Vaccine Efficacy Against Chagas Disease

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BACKGROUND: Chagas disease is endemic in Latin America and an emerging infectious disease in the US. No effective treatments are available. TcG1, TcG2 and TcG4 antigens are highly conserved in clinically-relevant *Trypanosoma cruzi* (*Tc*) isolates, and recognized by B and T cells in infected host. Delivery of these antigens as a DNA-prime/protein-boost vaccine (TcVac2) elicited lytic antibodies and type 1 $CD8^+$T cells that expanded upon challenge infection, and provided >90% control of parasite burden and myocarditis in chagasic mice and dogs. Herein, we determined if peripheral blood can be utilized to capture the TcVac2-induced protection from Chagas disease.
METHODS: We evaluated the sera levels of *Tc*kDNA/*Tc*18SrDNA and murine mitochondrial DNA (mtDNA) as indicators of parasite persistence and tissue damage; and effect of sera on macrophage phenotype.
RESULTS: Circulating *Tc*kDNA/Tc18SrDNA and mtDNA were decreased by >3-5-fold and 2-fold, respectively, in vaccinated/infected mice as compared to non-vaccinated/infected mice. Macrophages incubated with sera from vaccinated/infected mice exhibited M2 surface markers (CD16, CD32, CD200 and CD206), moderate proliferation, low oxidative/nitrosative burst, and regulatory/anti-inflammatory cytokine (IL-4+IL-10>TNF-α) response. In comparison, macrophages incubated with sera from non-vaccinated/infected mice exhibited M1 surface markers, vigorous proliferation, substantial oxidative/nitrosative burst, and proinflammatory cytokine (TNF-α>>IL-4+IL-10) response. Cardiac infiltration of macrophages and TNF-α and oxidant levels were significantly reduced in TcVac2-immunized chagasic mice.
CONCLUSION: Circulating *Tc*DNA and mtDNA levels and macrophage phenotype mediated by sera constituents reflects *in vivo* levels of parasite persistence, tissue damage and inflammatory/anti-inflammatory state; and have potential utility in evaluating disease severity and efficacy of vaccines and drug therapies.
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Cardiomyopathy of Chagas Disease: Cysteinyl-S-Nitrosylation of Key Host Proteins as Candidate Biomarkers

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BACKGROUND: Chagas disease, transmitted by injection of *Trypanosoma cruzi* through *Reduviidae* insect bites (kissing bugs), is designated as the most important emerging disease in developed countries and a “neglected emergency” by the CDC and NIH, with approximately 16-18 million cases in Latin America and 120 million (~25% of the population) more at risk of infection. In 30-40% of the infected individuals, the disease may progress to irreversible cardiomyopathy after many years, with infected individuals serving as carriers and exhibiting considerable morbidity and high risk of mortality. Therapies in use exhibit high toxicity in adults and are largely ineffective in limiting the disease progression. Accordingly, it is crucial that biomarkers are identified that permit a risk assessment of asymptomatic individuals of developing chagasic cardiomyopathy. Additionally, studies for this purpose may inform our understanding of the causative pathological processes and allow the identification of new therapies that arrest or prevent the progression of clinical disease, as well as suggest tools to assess the efficacy of new therapies. Preliminary studies of acute and chronic chagasic animals suggest that pathological processes leading to chagasic cardiomyopathy would cause characteristic changes in the concentration/nitrosation of proteins in the circulating blood cells and generate a detectable disease-specific molecular phenotype.
METHODS: We present here our results comparing normal healthy controls with chagasic patient and other cardiomyopathy patient samples, in which we investigated protein abundance and cysteinyl-S-nitrosylated (SNO) changes in isolated PBMCs using our novel, quantitative SNOFlo technology.
RESULTS: Statistical analyses—including t-statistics, multivariate adaptive regression splines —were used to select a set of proteins that create an accurate risk model for the development of cardiomyopathy.
CONCLUSION: The proteins identified are a part of biochemical signaling networks that may additionally inform our understanding of the pathology and provide potential targets for more effective therapies. Supported by NIH/NIAID Contract #HHSN272200800048C.

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MnSOD^{tg} mice control myocardial inflammatory and oxidative stress and remodeling responses elicited in chronic Chagas disease

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BACKGROUND: We utilized genetically-modified mice equipped with a variable capacity to scavenge mitochondrial and cellular reactive oxygen species (ROS) to investigate the pathological significance of oxidative stress in Chagas disease.
METHODS AND RESULTS: C57BL/6 mice (wild-type, MnSOD^{tg}, MnSOD$^{+/-}$, GPx1$^{-/-}$) were infected with *Trypanosoma cruzi* and harvested during chronic disease phase. Chronically infected mice exhibited a substantial increase in plasma levels of inflammatory markers (nitric oxide, myeloperoxidase), lactate dehydrogenase, and myocardial levels of inflammatory infiltrate and oxidative adducts (malondialdehyde, carbonyls, 3-nitrotyrosine) in the order of wild-type = MnSOD$^{+/-}$ > GPx1$^{-/-}$ > MnSOD^{tg}. Myocardial mitochondrial damage was pronounced and associated with a >50% decline in mitochondrial DNA content in chronically infected wild-type and GPx1$^{-/-}$ mice. Imaging of intact heart for cardiomyocytes and collagen by the nonlinear optical microscopy techniques of MPF/SHG showed significant increase in collagen (>10-fold) in chronically infected wild-type mice; while GPx1$^{-/-}$ mice exhibited a basal increase in collagen that did not change during chronic phase. Chronically infected MnSOD^{tg} mice exhibited a marginal decline in mitochondrial DNA content and no changes in collagen signal in the myocardium. P47$^{phox-/-}$ mice lacking phagocyte generated ROS sustained a low level of myocardial oxidative stress and mitochondrial DNA damage in response to *T. cruzi* infection. Yet, chronically infected p47$^{phox-/-}$ mice exhibited increase in myocardial inflammatory and remodeling responses, similar to that noted in chronically infected wild-type mice. CONCLUSIONS: Inhibition of oxidative burst of phagocytes was not sufficient to prevent pathological cardiac remodeling in Chagas disease. Instead, enhancing the mitochondrial ROS scavenging capacity was beneficial in controlling the inflammatory and oxidative pathology, and cardiac remodeling responses that are hallmarks of chronic Chagas disease.

IL-1β and inflammasome dependent mechanisms control non-healing forms of cutaneous leishmaniasis

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BACKGROUND: Infection of C57BL/6 mice with a low, physiologic dose of most *L. major* strains, such as *Lm* Friedlin (Fn), results in a healing lesion with minimal pathology at the site of inoculation in the skin. By contrast, low dose infection of C57BL/6 mice with the West African *Lm* Seidman strain (Sd), isolated from a patient with chronic cutaneous lesions, despite eliciting a polarized Th1 response and producing growth comparable to *Lm* Fn parasites during the first weeks of infection, results in a non-healing lesion, poor parasite clearance, and severe pathology with complete destruction of the ear dermis. RESULTS: In contrast to infection with *Lm* Fn, infection with *Lm* Sd is associated with an early and persistent recruitment of neutrophils to the site of inoculation. The pathology was preceded by an upregulated expression of multiple cytokines and chemokines, including IL-17, CXCL1, IL-27, IL-10, and IL-1β, as well as early accumulation of IL-1β producing cells in the dermis. Whereas no phenotype was observed in IL-17 or CXCL-1 deficient mice, IL-1R deficient mice, lacking the receptor for both IL-1α and IL-1β, as well as mice deficient in IL-1β, ASC and caspase-1/11, showed minimal pathology and completely healed their infections with *Lm* Sd. Following infection of bone marrow-derived macrophages in vitro, both *L. major* strains induced secretion of the active form of IL-1β that was dependent on activation of caspase-1.
CONCLUSIONS: Altogether, these data suggest that early parasite driven IL-1β secretion, dependent on inflammasome assembly and caspase-1 activation, and functioning within a Th1 inflammatory setting, is responsible for the non-cure response. Further understanding of this model may help to reveal the mechanisms underlying severe cutaneous pathology following *Leishmania* infection in humans.

Parasite biodiversity revisited: frontiers and constraints

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Although parasites are widely touted as representing a large fraction of the Earth's total biodiversity, several questions remain about the magnitude of parasite diversity, our ability to discover it all, and how it varies among host taxa or areas of the world. In this overview, I will address four topical issues about parasite diversity. First, we cannot currently estimate how many parasite species there are on Earth with any accuracy, either in relative or absolute terms. Species discovery rates show no sign of slowing down, and cryptic parasite species complicate matters further, rendering extrapolation methods useless. Further, expert opinion, which is also used as a means to estimate parasite diversity, is shown here to be prone to serious biases. Second, it seems likely that we may soon not have enough parasite taxonomists to keep up with the description of new species, as taxonomic expertise appears to be limited to few individuals in the latter stages of their career. Third, we have made great strides toward explaining variation in parasite species richness among host species, by identifying basic host properties that are universal predictors of parasite richness, whatever the type of hosts or parasites. Fourth, in a geographical context, the main driver of variation in parasite species richness across different areas is simply local host species richness; as a consequence, patterns in the spatial variation of parasite species richness tend to match those already well-documented for free-living species. The real value of getting good estimates of global parasite diversity is questionable. Instead, our efforts should be focused on ensuring we maintain sufficient taxonomic resources to keep up with species discovery, and apply what we know of the variation in parasite species richness among host species or across geographical areas to contribute to areas of concern in the ecology of health and in conservation biology.

Host population stability as a driver of parasite abundance and diversity in freshwater systems

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BACKGROUND: Resource stability is a key underlying factor determining species abundance and diversity in natural ecosystems. Through a bottom-up process, the stability of local resources should facilitate the maintenance and growth of consumer populations. In host-parasite associations, we would therefore expect that across different localities parasites achieve higher abundance where their host populations (resources) are stable, i.e. experience little spatiotemporal variation in local density. Also, we would expect that within localities parasites achieve higher abundances in suitable host species with stable populations than in other suitable hosts showing marked spatial and/or seasonal variation in local density.
METHODS: We tested these predictions using an extensive dataset obtained by sampling all fish and invertebrate hosts and their metazoan parasites across multiple sites and seasons in four New Zealand lake ecosystems. Host stability was computed for each species as the coefficient of variation in density values across all samples from a given lake. Its effect on parasite abundance was tested across all host-parasite species combinations in all four lakes using generalized linear mixed models, to account for other potentially influential factors.
RESULTS AND CONCLUSIONS: Our results provide the first quantitative test of the extent to which resource stability matters for parasite populations, and how much this varies among parasite taxa. These findings also have implications for the geographic distribution of parasite diversity, as more parasite species may persist in host populations or localities characterised by stability.

Molecular detection of *Toxoplasma gondii* in women with spontaneous abortion

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BACKGROUND: Infection with *Toxoplasma gondii* during pregnancy may cause severe damage to the fetus such as spontaneous abortion and stillbirth and neurological signs. Since isolation of *T.gondii* from human placenta strongly correlates with fetal infection and abortion, this study was conducted to detect *Toxoplasma gondii* DNA by PCR in placenta among women with spontaneous abortion and stillbirth.
METHODS: This case–control study was carried out in Tehran, Iran during 2012-2013. To ascertain a possible relationship between *T. gondii* infection and spontaneous abortion and stillbirth, one hundred and ten women with abortion and the same size women with normal delivery hospitalized in Tehran maternity clinic were selected as case and control groups. Placenta samples were taken for detection of *T.gondii* DNA by G529 primer and Polymerase Chain Reaction. All data were analyzed by SPSS version 13.5 using chi square, Fisher exact tests. OR and Confidence interval also were used.
RESULTS: In this novel study, the G529 as a fragment of DNA of *T.gondii* was amplified by PCR from 7 (6.4 %) of aborted women and stillbirth and 2 (1.8%) of control, that the difference was not significant (*PV*=0.17).
CONCLUSIONS: In spite of a higher rate of *Toxoplasma* infection in women with abortion as compared to the control group, the correlation between toxoplasmosis and spontaneous abortion and stillbirth was not significant.

Molecular Characterization of *Leishmania* Species by nested PCR from clinical specimens in Kashan, Iran 2012 - 2013

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BACKGROUND: Cutaneous Leishmaniasis is one of the health problems in Mediterranean regions including Iran. Considering the increase in the incidence of disease in Kashan, this study was designed to identify the *Leishmania* species in cutaneous leishmaniasis by nested PCR using primers of kinetoplastic DNA for treatment and appropriate measure for controlling of the disease.
METHODS: 130 patients suspected for cutaneous leishmaniasis referred to two health care centers of Kashan from 2012 to 2013 were examined. The demographic information as well as signs of the disease were recorded.
The diagnostic criteria of cutaneous leishmaniasis were based on observation of amastigotes within the smear or presence of expected bands in nested PCR. *Leishmania* species was identified following the extraction of DNA from serosity and nested PCR with variable region of the KDNA. Expected nested PCR products of *L. major* and *L. tropica* were 560 bp and 750 bp respectively. The data were analyzed by SPSS using X^2.
RESULTS: Overall of 130 specimens, 87(66.9%) cases and 96 (73.8%) cases were positive for microscopy and nested PCR methods respectively. In nested PCR assay 68 (70.8%) and 26 (27.1%) of the samples were identified as *L. tropica* and *L.major* respectively, and 2 (2.1%) cases were detected as mix. From 96 positive cases 73(76%) and 23(24%) had dry and wet wound respectively (P=0.05). The majority of positive cases 70 (72.9%) had 1 to 2 wounds, and 5(5.2%) had more than 7 wounds respectively (P=0.4).
CONCLUSIONS: Based on the results of this study, both species of *Leishmania* were present in Kashan, Therefore, it was suggested that careful preventive measure be taken in rural and urban parts.
KEY WORDS: Cutaneous Leishmaniasis، nested PCR، KDNA, Molecular Characterization

Incidence and complications of Congenital Toxoplasmosis

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BACKGROUND: Primary maternal *Toxoplasma gondii* infection during pregnancy is frequently associated with transplacental transmission to the fetus with serious sequelae. This study was conducted to assess the incidence and complications of congenital toxoplasmosis.
METHODS: In this cohort study, a total of 798 blood samples of pregnant women with gestational age more than 27 weeks admitted to Shabih-Khani Maternity Hospital of Kashan University of Medical Sciences, Iran during 2007 to 2009 were tested for anti *Toxoplasma* IgG and IgM antibodies by ELISA. Acute maternal *T. gondii* infections as well as those with chronic infection were considered as case and control groups, respectively. PCR and anti *Toxoplasma* IgM antibody were performed on four neonates in the case group and five samples in the control group. The clinical signs and complications of two groups of the infants were followed for one year. Weight and gestational age of newborns of the case (4 cases) and control groups (28 cases) were recorded. The data were analyzed by SPSS ver.16.0 using Chi Square and Fisher Exact tests.
RESULTS: Out of 798 pregnant women, 5(0.6%) were positive for IgM1/100 and IgG more than 1/400 represented as acute Toxoplasmosis (case group). PCR showed a 400 bp band in three newborns of case group, but was negative in control group (P=0.048). None of the newborns in the case and control groups were positive by ELISA/IgM. CT- scan was normal in neonates with congenital toxoplasmosis. The incidence rate of congenital toxoplasmosis was 3.7 per 1000 live births. Only one newborn with congenital toxoplasmosis had jaundice (P=0.12).
CONCLUSIONS: The incidence of congenital toxoplasmosis in Kashan, Iran, was higher than the world rate. Jaundice was the only sign in the newborn with *T. gondii* infection. By neonatal screening for congenital toxoplasmosis and early treatment may reduce the severe long-term sequelae.

Gastrointestinal nematodes resistant to the three classes of broad-spectrum anthelmintics in sheep farms from tropical Mexico

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BACKGROUND: The indiscriminate use of anthelmintic drugs (AH) for the control of gastrointestinal nematodes (GIN) derived in drug resistant worm populations. Previous evidence obtained ten years ago showed that half of sheep farms in Tabasco, Mexico, had GIN resistant to at least two classes of commercial AH. Since then, AR in Tabasco has not been assessed. The objective was to determine the frequency of sheep farms with AH resistant GIN from a group of six commercial sheep flocks in Tabasco, Mexico.
METHODS: Six commercial sheep farms were included. Feces were obtained directly from the rectum of 100 adult hair sheep from each farm. Ewes were distributed into 4 groups: a) Control, b) BZ (Albendazole sulfoxide 5 mg s.c./kg BW), c) IVER (Ivermectin 0.2 mg s.c./kg BW) and d) Lev (Levamisol 7.5mg/kg BW). The fecal egg count reduction test (FERCT) was used. The GIN genera were determined by coproculture and larvae identification. The percentage reduction (%R) of eggs per gram of feces (95% confidence interval) was determined by the RESO program.
RESULTS: The %R ranged from 0 to 48%, 29 to 82% and 1 to 88% for BZ, IVER and LEV, respectively. All farms had multidrug resistant GIN (*Haemonchus* spp and *Trichostongylus* spp. were involved)
CONCLUSIONS: All farms surveyed had multidrug resistant nematodes against the three commercial classes of AH. Resistance was reported for *Haemonchus* spp and *Trichostongylus* spp.

Blastocystis – A Parasite Associated with Gastrointestinal Health?

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BACKGROUND: *Blastocystis* is a very common parasitic micro-eukaryote of humans and a vast variety of non-human hosts. The genus comprises at least 17 subtypes (ST) identified in humans, other mammals and birds; humans are natural hosts of subtypes 1—4, although ST4 appears to have a restricted geographical distribution compared to ST1—ST3. The role of the parasite in human health and disease remains an enigma, which is complicated by the fact that no effective treatment is known. By studying the prevalence of *Blastocystis* in different human cohorts, we have sought to understand the clinical role of the parasite.
METHODS: Over the past few years independent studies identifying the prevalence and subtype distribution of *Blastocystis* in distinct cohorts have been carried out, including patients with acute diarrhoea, irritable bowel syndrome, inflammatory bowel disease (IBS), and asymptomatic individuals. In all studies, the parasite was identified by culture, PCR, or by analysis of metagenomics data.
RESULTS: While the prevalence of *Blastocystis* in the asymptomatic Danish background population is estimated to >20%, the prevalence is lower in patients with functional, inflammatory and acute bowel disease. For example, in patients with Crohn's disease, active ulcerative colitis, and acute diarrhoea, carriage is rare, <5%. In patients with symptoms compatible with IBS, the prevalence is <15%. ST4 presence was enriched in patients with acute diarrhoea in Denmark and in IBS patients in the UK, but these findings await confirmation. So far, no single subtype has been convincingly correlated with disease,
CONCLUSIONS: There is increasing evidence of *Blastocystis* being associated with a healthy gut environment, and future studies should explore *Blastocystis* as a proxy for gastrointestinal health. Research should include association studies between *Blastocystis* and gut microbiota and the ability of the parasite to modulate microbial ecology and host immunity using culture and animal models.

Molecular epidemiology of human cryptosporidiosis in developing countries

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BACKGROUND: Cryptosporidiosis has been shown in the recent Global Enteric Multicenter Study as one of the major causes of moderate to severe diarrhea in early childhood in developing countries. To improve the understanding of the epidemiology of human cryptosporidiosis in developing countries, molecular diagnostic tools have been used to characterize the transmission of *Cryptosporidium* spp. at species and subtype levels. Data from these studies have shown higher diversity of *Cryptosporidium* species *C. hominis* and *C. parvum* subtypes in humans in developing countries than in industrialized nations. Nevertheless, five *Cryptosporidium* spp., *C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis* and *C. canis* are responsible for most *Cryptosporidium* infections in both immunocompetent and immunocompromised persons, and in most areas, *C. hominis* and *C. parvum* are responsible for over 90% of human cryptosporidiosis cases. Differences have been observed among endemic areas in the proportion of infections due to *C. parvum, C. meleagridis* and other zoonotic species, probably due to differences in transmission routes. Nevertheless, results of subtyping suggest that in most areas, *C. parvum* infections in humans are largely results of anthroponotic rather than zoonotic transmission.
METHODS: Differences in clinical manifestations have been observed among *Cryptosporidium* species and *C. hominis* subtypes, with *C. hominis,* especially its Ib subtype family at the gp60 locus more virulent than others.
RESULTS: After an initial infection, some children experienced subsequent infections with homologous and heterologous *Cryptosporidium* parasites, often within a year of the first infection. Data from multilocus sequence typing and population genetic studies support the anthroponotic nature of *C. parvum* transmission in humans in developing countries, and have further identified geographic segregation in *C. hominis* subtypes. They also suggest that genetic recombination plays a major role in the emergence of virulent *C. hominis* subtypes. These findings highlight the usefulness of molecular diagnostic tools in studies of *Cryptosporidium* transmission and reveal some unique characteristics of cryptosporidiosis epidemiology in developing countries.
CONCLUSIONS: The recent development in wide use of next generation sequencing tools suggests that future comparative genomic studies will improve significantly our knowledge of human cryptosporidiosis in developing countries.

Identification and immuno molecular characterization of proteins expressed in tick stages of Babesia bigemina

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BACKGROUND: Sexual reproduction, a process involving recognition and fusion of two cells, is highly conserved among different taxa. The *hap2* gene codifies for a male specific trans-membrane protein, which has been found to participate in gamete fusion in various organisms including plants and protozoa. Antibodies to *Plasmodium berghei* HAP2 inhibit gamete fusion, reducing zygote formation, thus highlighting the importance of this antigen as a transmission-blocking vaccine candidate. In this work we report the identification of a *hap2* homologue in the genome of *Babesia bigemina*, the expression analysis in tick stages and the characterization of conserved B cell epitopes.
METHODS: A sequence with high degree of similarity to *Plasmodium bergei hap2* was found in the genome of *B. bigemina*. Alignment of HAP2 from six isolates from different parts of the world showed a 98% similarity at the amino acid level. The amino acid sequence of HAP2 from a Mexican strain was analysed and used to predict hydrophilic, antigenic peptides containing B cell epitopes in the extracellular domain by bioinformatics tools. Four peptides were selected on fully conserved predicted B cell epitopes. Each peptide was chemically synthetized as a dendrimer and used to immunize rabbits. A sample of *B. bigemina* infected erythrocytes was used to purify tick stages using a previously described protocol. Rabbit serum samples were used in an immunofluorescence assay on tick stages of *B. bigemina*.
RESULTS: Alignment of all the sequences showed a 98% similarity at the amino acid level with 100% similarity in the HAP2 domain, with few point mutations, deletions and insertions observed among isolates. Importantly, *hap2* transcripts were detected in *B. bigemina* RNA from sexual stages as well as in intra-erythrocytic stages using RT-PCR. Expression was confirmed in tick stages of *B. bigemina*. Finally, all four peptides generated antibodies, which recognized the conserved, surface-exposed B cell epitopes on native HAP2 of *B. bigemina* tick stages.
CONCLUSIONS: This is the first report of a HAP2 protein from *Babesia* spp, which is expressed in tick stages and is detected by antibodies to conserved epitopes.

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Molecular insights into stage specific expression in *Strongyloides stercoralis*

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BACKGROUND: The key molecular factors that enable *Strongyloides stercoralis* (Ss) larvae to initiate infection have not been elucidated to date, and are critical for developing new insights into the biology of this nematode and identifying novel drug and vaccine targets as well as potential diagnostic antigens.
METHODS: Transcriptomic based analysis of gene expression by infective third stage larvae before (L3i) and 72 hours after (L3+) host invasion. Using differentially labeled cDNA obtained from RNA to identify highly expressed genes in either stage based on a cutoff of 2 fold increased gene expression and a false discovery rate of 1%.
RESULTS: 96 differentially expressed genes were identified. Expression of genes putatively encoding extracellular matrix proteins, were notably increased in L3i compared to L3+ larvae ($p = 0.0003$). By contrast, a chitinase-like enzyme was significantly expressed in the L3+ stage ($p = 0.001$). Following invasion, genes putatively encoding components of the ubiquitin proteasome pathway were highly expressed as were enzymes with putative catalytic activity (such as hydrolases, ligases and transferases ; $p = 0.02$). Stage-specific differences in metabolism were found as well particularly in pathways involved in amino acid, carbohydrate and lipid metabolism.
CONCLUSIONS: These data indicate that Ss larvae downregulate expression of extracellular matrix and energy metabolism genes, and increase expression of genes encoding catalytic enzymes following host invasion. These data provide important clues about the establishment of infection following host invasion.

Parasite antigen driven bystander effects of tissue-invasive helminth infections on other infectious diseases of humans

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BACKGROUND: Examination of the effects of helminth infections other infectious diseases (e.g. HIV, tuberculosis, malaria) have prompted interest in both the mechanisms involved in the bystander regulation by helminths of ongoing non-helminth antigen-specific immune responses and in the potential secondary effect (both positive and negative) of these helminth infections on the clinical outcome of these other infectious diseases.
METHODS: An integrated approach that involves population-based epidemiologic and interventional studies of both filarial/myocobacterial or filarial/malarial co-infections coupled with ex vivo antigen-specific immunologic assessments
RESULTS: Coincident filarial infections profoundly alters qualitatively and quantitatively the mycobacteria-specific and malaria-specific T cells responses in a variety of T cell compartments. The effect of anthelmintics on these modulated responses seen in co-infections will be discussed.
CONCLUSIONS: Our data addresses some of the important underlying mechanisms involved in spillover regulation of pathogen-specific T cell response and their relatively small contribution to clinical outcome.

Diphyllobothriosis: emerging fish borne disease?

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BACKGROUND: Increased popularity of eating raw or undercooked fish has contributed to recent re-emergence of diphyllobothriosis and diplogonoporosis. Whereas the number of cases in the areas considered historically endemic has dramatically declined, most new cases are reported from non-endemic areas.
METHODS: The review is based on literary and unpublished data.
RESULTS: The most common causative agent of diphyllobothriosis seems to be *Diphyllobothrium latum* with most cases reported from alpine regions of Europe (Italy, Switzerland and France); some cases are also known from Russia, Scandinavia and North America. The most important source of infection is perch, pike, burbot or walleye. The second most important species is *D. nihonkaiense* with more than 2000 human cases diagnosed mainly in Japan. The endemic area is North Pacific – Japan, Far East of Russia and Korea, as well as the Pacific coast of North America (Alaska and Canada). However, the parasite has recently been found in non-endemic areas such as Europe (Czech Republic, Finland, France and Switzerland), USA (California), China and New Zealand. Humans acquire infection by eating Pacific salmon (mostly *Oncorhynchus gorbuscha*, *O. masou* and *O. keta*). The third species is *D. pacificum*, with more than 1000 cases reported mostly from Peru, but also from Chile, Ecuador and Japan. The first five imported cases have been reported from Spain. The sources of infection are marine fish in the Pacific Ocean. *Diphyllobothrium dendriticum* is widely distributed, but the number of human cases is rather low compared with those of the above-mentioned species. Most human cases are reported from Siberia (especially Lake Baikal), but some are known also from north Canada and Alaska. Four cases have been reported from Europe. Freshwater salmonids and coregonids serve as source of human infection.
CONCLUSIONS: Morphology-based identification of clinical samples to the species level is not possible and molecular tools (*cox*1 gene), should be used.
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Prevalence of intestinal parasites in wandering cats (*Felis catus domesticus*) in Goiânia, Goiás, Brazil

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BACKGROUND: Wandering cats are important to the transmission of zoonoses for humans, mainly parasites of heteroxenic cycle, causing important diseases such as *larva migrans*, toxoplasmosis, giardiasis and cryptosporidiosis. Therefore the intestinal parasites in felines are a risk to human public health. The objective of this study was to evaluate the prevalence of intestinal parasites in wandering cats captured by the zoonoses center in Goiânia, Goiás, Brazil during the year of 2012.

METHODS: This project was approved by the ethics committee in animal research CEUA/UFG, protocol 054/2013. 154 feces samples in the period of research were collected, these samples were processed by the techniques of centrifugation and floatation in saturated solution of glucose, sodium chloride and zinc sulphate, and by the method of spontaneous sedimentation. Each sample was analyzed by all four techniques to increase the diagnostic accuracy.

RESULTS: In this study 74.68% (115/154) of the cats were infected. There was the monoparasitism of the infected cats with hookworms with 50.43% (58/115), *Cystoisospora* sp. with 7.83% (9/115), *Toxoplasma gondii* 3.48% (4/115), *Giardia* sp. 0.87% (1/115) and *Toxocara cati* with 0.87% (1/115). In the cases of poliparasitism the combination of hookworms and *Cystoisospora* sp. represented 20% (23/115) of the samples, hookworms and *T. gondii* with 6.96% (8/115), hookworms, *T. gondii and Cystoisospora* sp. with 4.35% (5/115), *T. gondii and Cystoisospora* sp. with 3.48% (4/115), hookworms, Cystoisospora sp. and Giardia sp. 0.87% (1/115), hookworms, *Cystoisospora* sp. and *T. cati* 0.87% (1/115).

CONCLUSIONS: This study showed that wandering cats are important transmitters of zoonotic parasites that lead to environmental contamination, representing a risk to human health.

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Impacts of parasites in aquaculture beyond morbidity and mortality

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BACKGROUND. One of the biggest challenges for new aquaculture enterprises is morbidity and mortality caused by pathogens, including parasites. This must be corrected if a particular enterprise is to succeed, expand and mature. Then more mature enterprises are faced with other impacts of parasites, some of which are referred to as production diseases. One of the major impacts is on growth and food conversation, as the cost of feed is often the biggest cost of an operation. These effects have been documented for a few parasite (e.g., *Sanguinicola enermis* and *Bothriocephalus acheilognathi* in carp, *Loma salmonae* in rainbow trout), and undoubtedly many other parasites significantly reduce growth in fish and invertebrates in aquaculture. Aquaculture products are destine for human consumption, and many parasites impact product quality. With marine fishes, *Kudoa* spp.(Myxozoa) causes unsightly cysts or post-harvest myoliquefaction, resulting in “softflesh syndrome”. Many helminthes in fishes are human pathogens, and thus these infections present an additional concern when they occur in aquaculture. Another impact of parasites in aquaculture is the concern that they may be amplified and spill over to regional wild fisheries. The actual or perceived role of aquaculture in spreading parasites to wild fishes is one of the biggest impediments to expansion of aquaculture in certain regions, such as in Europe and North America. The most notable example of this is caligid copepods (sea lice) in pen-reared salmon.
CONCLUSION: As with terrestrial agriculture, parasites in aquaculture cause a significant economic impact well beyond causing morbidity and mortality.

Changes of the brain in normal and immunosuppressed mice experimentally infected with *Toxocara canis*

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BACKGROUND: Toxocariasis is a soil-transmitted helminthozoonosis due to infection of humans by larvae of *Toxocara canis* (*T. canis).* Neurotoxocariasis is being recently diagnosed with increasing frequency due to improved diagnostic tools. Moreover, many studies have confirmed positive association between zoonotic *T. canis* infection and epilepsy. Other possible associations include social, learning and behavioural abnormalities especially in children. Meanwhile, in our modern era, cases of immunocompromised status have been progressively increasing due to increased incidence of malignancy as well as increased use of immunosuppressive agents for treatment of various neoplastic, autoimmune, and allergic disorders.The present study aimed at comparing some of the pathological, immunological, and biochemical changes of the brain in normal and immunosuppressed mice experimentally infected with *Toxocara canis*.
MEHODS: 240 male Swiss albino mice were divided into four groups including normal (control) group, immunocompetent *T. canis*-infected group, immunosuppressed group (control), and immunosuppressed infected group. Infected mice were subjected to larval count in the brain, and the brains from all mice were assessed for gliosis, IL-5 mRNA expression levels, and neurotransmitter profile.
RESULTS: The results showed that under immunosuppression, there were significant increase in brain larval counts, significant enhancement of reactive gliosis, significant reduction in IL-5 mRNA expression, and significant changes in neurotransmitter profile. All these changes were maximal in the chronic stage of infection.
CONCLUSIONS: Experimental *T. canis* infection induced significant biochemical and immunopathological alterations in the brains of infected animals. These changes were progressive over time, and were exaggerated under the effect of immunosuppression. Therefore, further studies should be done to establish the immunopathogenic mechanisms of neurotoxocariasis which may open avenues for future therapeutic options.

Gene expression changes induced by *Trichinella spiralis* muscle larvae excretory/secretory products in primary myoblast cultures

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BACKGROUND: *Trichinella spiralis* is an intracellular parasite of mammalian skeletal muscles. Infection by L1 larvae triggers a series of changes in the infected muscle, which eventually transforms the infected cell into the nurse cell phenotype, a structure that allows parasite survival for long periods of time. In this process, excretory-secretory products (ESP) released by muscle stage (ML) have been suggested to be involved. In this study we developed murine myoblast primary cultures as a model to evaluate the changes induced by ML ESP during differentiation from myoblast to myotubes both at the morphological and genetic expression levels.
METHODS: The hind limb muscles were collected from 2-4 day old BALB/c mice to establish myoblast primary cultures. After 8 days under differentiation conditions, total RNA was isolated from ESP-treated and -untreated myoblast primary cultures. cDNA microarray assay was performed. Raw data were averaged and normalized using GenArise. Differential expression of genes was determined by the comparison of treated and control cultures.
RESULTS: Treatment with ESP decreased by 30% the formation of myotubes, resulting in shorter myotubes with fewer nuclei than untreated cultures. Among the 22,000 plotted genes, the expression of 1280 genes was up-regulated while 1140 genes were down-regulated after treatment with ESP. These genes were associated with different cellular processes like actin cytoskeleton regulation, cell cycle, apoptosis, cell development and the myogenesis process.
CONCLUSIONS: These results indicate that *T. spiralis* ML ESP induces a series of changes in different cellular processes which need to be further studied to understand the mechanisms that lead to nurse cell formation.

The *Plasmodium falciparum* blood-stage malaria vaccine BK-SE36 trial in Northern Uganda

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BACKGROUND: The high burden of *Plasmodium falciparum* malaria, its widespread resistance to many antimalarial drugs, the dependence of all new combinations on artemisinins and renewed global efforts to control/eradicate malaria all drive the need to develop effective and affordable vaccines. Malaria vaccine clinical development remains at the forefront but no vaccine is currently available. Proteins expressed during the blood-stage of the parasite life cycle have been proposed as good vaccine candidates. Rather than preventing infections, they are expected to reduce mortality and morbidity secondary to *P. falciparum* infection. A recombinant protein based on *P. falciparum* serine repeat antigen-5 (SERA5) formulated with aluminum hydroxyl gel (BK-SE36) was safe and immunogenic in malaria-naïve adults. We now assessed BK-SE36 in a malaria endemic area in Northern Uganda.
METHODS: An age de-escalation (age cohorts: 21-40y; 16-20y; 11-15y; 6-10y) phase 1b trial and follow-up study was conducted in Lira last April 2010-Feb 2011. Safety and antibody responses were assessed in a randomized, single-blind, uni-centre trial. Post-trial (March to November 2011), the risk of malaria episodes were compared in vaccinees and control group.
RESULTS: BK-SE36 was safe and well-tolerated. In the follow-up study, significant differences between BK-SE36 vaccinees and control were observed in terms of cumulative incidence and time-to-first episodes of high parasitemia (>5000 parasites/μL) (p=0.02) and high parasitemia + fever [axillary temperature ≥37.5°C, p=0.003]. Our longitudinal data shows promise of a blood-stage vaccine candidate that gave statistically significant level of protection up to one-year post-vaccination with a study population reporting 84% usage of bednets. Moreover, we also observed that the immune response can be boosted by natural infection.
CONCLUSION: That SERA-5 does not show antigenic variation, has limited polymorphism, and is not conformation-dependent suggests a candidate for further clinical trials in 0-5 years-old.

New lakes, old parasites – trematode communities in a reservoir system in Germany

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BACKGROUND: While trematode species are well-known pathogens of human diseases that have been plaguing people for ages, these parasites have recently gained increasing attention as integral elements of ecosystems. Against this background we set out to assess the trematode diversity and community composition in snails in the urban Ruhr River reservoir system in Germany, in order to estimate the parasites' role in this ecosystem.
METHODS: Snails were collected from five reservoirs in 2012 & 2013 and examined for trematode infections; trematode species were identified morphologically and, in cases of questionable taxonomy, based on molecular markers. A total of 6,507 snails belonging to 19 species of 8 families were examined. The most abundant lymnaeid and planorbid snails were *Gyraulus albus* (1,919), *Radix auricularia* (1,909), *Stagnicola palustris* (668), *R. peregra* (349), *Lymnaea stagnalis* (245) and *Segmentina nitida* (195).
RESULTS: These six snail species harboured a diverse trematode fauna: 32 trematode species were identified to species level and a further 2 to the generic level. Overall prevalence in these hosts ranged from 1.4 to 36.1%. Component community analyses revealed distinctive trematode community composition in different snail hosts. Molecular identification of taxonomically controversial trematode groups revealed infections in planorbid and lymnaeid snails with cryptic species of *Echinostoma*, *Diplostomum* and *Petasiger* as well as bird schistosomes.
CONCLUSIONS: Our results reveal that these reservoirs offer ideal conditions for species-rich and diverse trematode communities, showing that digenean trematodes are important and integral parts of these aquatic ecosystems and highlighting their central contribution the ecosystem's biodiversity, even at a small spatial scale.

Polarization of macrophages induced by *Toxoplasma gondii* and its impact on abnormal outcomes of pregnancy in rats

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BACKGROUND: *Toxoplasma gondii* infection is the leading cause of fetal malformation among the five pathogens during pregnancy. After primary maternal infection by *T. gondii* during gestation, the parasite may invade the fetus via the placenta. Additionally, adverse bias of physiologically Th2-dominant microenvironment in pregnancy to Th1 type response may result in abnormal outcomes, including miscarriage and stillbirth. Here we set up a pregnant a inbred SD rat model, which resembles human infection, and reported the polarized activation of macrophages induced by genotype Chinese 1 strain(Wh6) of *Toxoplasma*, and its impact on pregnancy.
METHODS: Rat peritoneal macrophages were obtained by washing the peritoneal cavity and cultured for 24h before functional experiments.
RESULTS: The results showed that peritoneal macrophages of rats in pre-gestation infection led to a high expression profile of iNOS, TNF-α, and IL-12 and production of NO, indicating the polarization to classically activated macrophages (M1), while in-gestation infection drove macrophages to notably up-regulated expression of TGF-β1, IL-10, and Arg-1, and generation of urea, showing a biased alternatively activated macrophages(M2). Histopathology of placental tissues challenged with Wh6 revealed that the inflammatory score in the pre-gestation group was the highest in all experimental animals and the proportion of fetal malformations in pre-gestation group was 15.32 %(19/124), significantly higher than those in in-gestation group of 3.01 %(4/133).
CONCLUSIONS: The results strongly suggested that *Toxoplasma* Wh6 strain infection before gestation induced a Th1 type response, which is jeopardous to fetal development and associated with abnormal pregnant outcomes due to unbalanced physiological immune microenvironment at maternal-fetal interface.

Microglia Involve in the Inflammation and Neuronal Apoptosis in Reactivated Toxoplasmic Encephalitis

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BACKGROUND: Numerous evidences show that activated microglia play a critical role in the pathogenesis of central nervous system (CNS). Toxoplasmic encephalitis (TE) is frequently seen in HIV/AIDS patients. However, the effect of activated microglia involved in TE pathogenesis remains to be clarified.
METHODS: We generated a murine model of reactivated encephalitis in latent infection with *Toxoplasma gondii* induced by cyclophosphamide and the neuronal apoptosis in CNS and the profile of proinflammatory cytokines were assayed both *in vitro* and i*n vivo*. RESULTS: Microglial cells were dramatically activated in cortex and hippocampus in brain of mice. The expression of interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and inducible nitric oxide synthase (iNOS) were up-regulated in TE mice, and neuronal apoptosis was significantly increased, which coincides with the results of *in vitro* experiments. Additionally，It was found that apoptosis of the mouse neuroblastoma Neuro2a (N2a) prominently increased when N2a was co-cultured by transwell with microglia and *Toxoplasma* tachyzoites. Minocycline (a microglial inhibitor) treatment remarkably reduced both microglial activation and neuronal apoptosis *in vivo* and *in vitro*.
CONCLUSIONS: Our results indicate that activated microglia contributes to neuronal apoptosis in TE and inhibition of microglia activation might represent a novel therapeutic strategy for TE.

A recombinant P67 vaccine against *Theileria parva* in cattle: conflicting results from experimental and field challenges

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BACKGROUND. The 67 kDa sporozoite surface antigen of *Theileria parva* was evaluated as a vaccine candidate.
METHODS. The antigen was expressed as a chimeric recombinant fusion protein with Green Fluorescent Protein (GFP-p67) in a baculo virus expression system, and formulated in a water-in-oil emulsion. A dose-effect experiment was performed in 1-5 months old Zebu-cross bred cattle that were vaccinated and experimentally challenged.
RESULTS. Results showed that relatively young animals exhibited a level of natural resistance to *T. parva* tick stabilate challenge. In the older age group, animals that had been vaccinated with 8 µg of GFP-p67 antigen were significantly protected from clinical East Coast Fever (ECF). Subsequently a field trial was performed in Zambia using 2-6 months old Angoni cattle. Cattle were vaccinated twice at a 6-week interval prior to the tick season. More than 90% of vaccinated animals had antibody against GFP-p67 after the vaccination schedule. Analysis of blood samples indicated *T. parva* challenge infection in 5-35% of the animals. There was no statistically significant effect of vaccination on mortality and morbidity resulting from *T. parva* infection.
CONCLUSION. It is concluded that this vaccine formulation and/or vaccination schedule, which induces protection against experimental challenge infections with sporozoites from tick stabilates, is not sufficient to induce protection against ECF in field circumstances.

Presence of *Cysticercus bovis* infection in carcasses from a slaughterhouse from Tocantins, Brazil

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BACKGROUND: Bovine cysticercosis is a zoonotic parasitosis with cosmopolite distribution. It is caused by the presence of *Cysticercus bovis*, the larval form of *Taenia saginata*. It has great socioeconomic importance due to economic losses in condemnation of carcasses.

METHODS: The cysticerci were collected during the post mortem examination performed in bovines slaughtered in a frigorific slaughterhouse in Tocantins State, Brazil. The cysticerci were conserved in 10% formaldehyde. The macroscopic analysis occurred in the Experimental Pathology Laboratory in Federal University of Goias. The cysticerci were divided into four macroscopic stages: vesicular stage (VS), colloidal vesicular stage (CVS), granular nodular stage (GNS) and calcified nodular stage (CNS). Also the location of the cysticerci within the carcasses was observed.

RESULTS: 87 cysticerci were removed from bovine carcasses slaughtered in a slaughterhouse from Gurupi/Tocantins in the period of October 2013 to March 2014. The postmortem examination classified the cysticerci as viable (78.16%) and degenerating (21.84%). The macroscopic classification showed that 56.32% (49) were VS, 14.94% (13) were CVS, 6.9% (6) were GNS and 21.84% (19) were CNS. The cysticerci were located at skeletal muscle (48.27%), cardiac muscle (1.14%), liver (1.14%), diaphragm (31%), masticatory muscles (18.39%). 79.31% of the cysticerci were removed from male animals and 20.69% from female ones. All animals had 36 months of age. As to the origin 78.15% of the cysticerci were from animals from the city of Arraias/Tocantins.

CONCLUSIONS: Bovine cysticercosis is an important cause of carcass and organs condemnation which leads to economic losses. Its occurrence indicates the presence of human teniasis in the population near the livestock raising area.

A regional e-text for teaching parasitology to veterinary students

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BACKGROUND: Teaching veterinary parasitology in some regions of the world is hampered by the lack of relevant regional texts. Most texts are global in their perspectives but are essentially oriented towards Europe and North America which provide the largest markets for textbooks.
METHODS: The Australasian veterinary schools (Australia and New Zealand) have recently combined their resources to produce a regional text likely to be more useful to undergraduate students than the general texts currently available. Due to the relatively small market size in Australasia, it was decided to produce the text in an electronic version only. However, this approach has significant advantages as teaching approaches differ between institutions with some adopting a systematic approach based on parasite groups, other institutions teaching on a host basis and yet others adopting a case-based approach.
RESULTS: A dual approach in a standard text results in duplication and a very large number of pages. In an electronic text, these differences can be overcome by including both systematic and host based approaches connected by hot-links; image rollovers and a decreased image density for electronic format provides additional advantages. In addition, electronic references to websites can be included if students wish to explore a particular area of parasitology. The cost to students is much lower than that of purchasing a standard text and we expect that this will result in greater usage. In addition, the regional text will be available to veterinary practitioners as well as to students.
CONCLUSIONS: The electronic text was first released to students in the first semester of 2014 (March – June) and surveys of students will be undertaken at the end of the semester to assess its utility and to determine how it can be improved, a process which can be achieved more readily in an electronic text.

Acanthamoeba keratitis in Austria – monitoring from 1993-2013

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BACKGROUND: *Acanthamoeba* keratitis (AK) is a very painful inflammation of the cornea. The first cases were reported in the early seventies and in the mid-eighties the association between AK and contact lens wear was discovered. Today, acanthamoebae, besides pseudomonads and staphylococci, are regarded as the most prevalent causative agents of microbial keratitis in contact lens wearers. Due to difficult diagnostics and complicated therapy, AK very often takes a serious progression. In Austria, *Acanthamoeba* diagnostics was established in 1993, the aim of the current work was to give an overview on AK in Austria during the past 20 years.
METHODS: All ingoing samples of patients with suspected AK or unexplained keratitis were screened for acanthamoebae by culture and/or PCR and the detected amoebae were genotyped.
RESULTS: Altogether, 154 patients were positive for *Acanthamoeba*. The age of the AK patients ranged from 8-82 years (mean age 37.8) and 58% of the patients were female. As far as background information was available, 89% of the patients were contact lens wearers, almost all cases were unilateral and 17% of the patients required a keratoplasty. Usually, also the contact lens containers and/or contact lenses were heavily contaminated. All amoebae isolated by culture were able to grow at 34°C and at 37°C. The predominant genotype was T4, other genotypes found were T3, T5, T6, T10 and T11.
CONCLUSIONS: In Austria, as in many other countries, AK is clearly linked to contact lens wear, contaminated contact lens containers being the most important risk factor. The majority of AK cases is caused by genotype T4.

Successful vaccination against Babesiosis using recombinant antigens

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BACKGROUND. GPI-anchored merozoite surface proteins of *Babesia* parasites can be found in the supernatants of in vitro cultures of the parasites (soluble parasite antigens; SPA). These antigens induce protective immunity against virulent challenge infection when formulated with saponin adjuvant in a vaccine. Research aimed at identifying the protective antigens in SPA preparations of *Babesia canis* and *B. divergens*, and developing vaccines using recombinant proteins.
METHODS. Using a variety of techniques including molecular sieving, immunoprecipitation and immunoaffinity chromatography, antigens of Mr 35-40kDa were detected in SPA preparations. Genes encoding for these antigens were discovered and cloned. Recombinant antigens were produced in *E. coli* expression systems, formulated with saponin as adjuvant and used to vaccinate gerbils (*B. divergens* model, recombinant Bd37 antigen) or dogs (*B. canis* model, recombinant CBA1 antigen).
RESULTS. Results showed that vaccination with these recombinant vaccine preparations induced protective immunity in either model. Protection correlated with the level of antibodies against the vaccine antigen and merozoites at the moment of experimental challenge infection. Vaccinated animals inhibited the proliferation of the parasite in the host, which limited the development of (immune-) pathological responses.
CONCLUSION. It is concluded that among the GPI-anchored parasite antigens that are present at the surface of *Babesia* parasites, there are certain proteins that can be used to induce protective immunity in vertebrate hosts. This can also be achieved with recombinant proteins. The fact that this was shown in two different *Babesia*-host models suggests that similar vaccines can be developed for other *Babesia* species.

Invasion biology meets parasitology: a case study of Egyptian *Fasciola gigantica* in *Pseudosuccinea columella*

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BACKGROUND: The liver fluke *Fasciola gigantica* is a trematode parasite of ruminants and humans that occurs naturally in Africa and Asia. Cases of human fascioliasis, attributable at least in part to *F. gigantica*, are significantly increasing in the last decades.
METHODS: In our study, we investigated snail communities at several sites in irrigation channels in the Fayoum governorate in Egypt and tested them for trematode infections by PCR with universal and specific primers.
RESULTS: In total 689 snails of 9 different species were collected. Among these, we found high abundance of the invasive snail *Pseudosuccinea columella* (46% of all snails). Molecular detection of trematodes in this species revealed infections with *Echinostoma caproni* (2.36%) and another undefined echinostome (7.09%). Remarkably, also the liver fluke *F. gigantica* was detected in these snails with a prevalence of 3.38%.
CONCLUSION: Both high abundance of *P. columella* in the Fayoum irrigation system and common infection with *F. gigantica* might be a case of parasite spill-back (increased prevalence in local final hosts due to highly susceptible introduced intermediate host species) from the introduced *P. columella* to the human population, explaining at least partly the observed increase of reported fascioliasis-cases in Egypt. *Eichhornia crassipes*, the invasive water hyacinth, which covers huge areas of the irrigation canals, offers safe refuges for the amphibious *P. columella* during molluscicide application. As a consequence, this snail became a dominant species and might be the cause of increasing transmission of *F. gigantica* to humans and livestock.

Cathepsin B in dendritic cells and macrophages regulates mediators of the Th1 immune response during *Leishmania major* infection

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BACKGROUND: Resistance and susceptibility to *Leishmania major* is determined by development of a Th1 or Th2 immune response, respectively, orchestrated by its host. Previous studies with the use of cathepsin B (Ctsb) and L (Ctsl) inhibitors documented a switch in the Th response mounted by resistant and susceptible mice, and although it was hypothesized that these effects were due to alterations in antigen-processing, they were not further investigated.
METHODS: We generated bone marrow-derived dendritic cells (BMDC) and macrophages (BMM) from wild-type (WT), *Ctsb*$^{-/-}$ and *Ctsl*$^{-/-}$ mice, and infected them with WT or eGFP-*L. major* promastigotes. We monitored the survival and processing of intracellular parasites by flow cytometry and fluorescence microscopy, and analyzed the expression of MHC class II, CD86, CD80, and CD40 in BMDC. In addition, we determined the concentration of IL-12, IL-6, IL-10, and TNF-α in culture supernatants by ELISA, and we determined the expression of IL-12 by RT-PCR.
RESULTS: We found that the survival of intracellular parasites was comparable among WT and cathepsin-deficient BMM, and no differences in the processing of promastigotes by BMDC. However, *Ctsb*$^{-/-}$ BMDC expressed higher levels of MHC class II molecules but not of costimulatory molecules than WT and *Ctsl-/-* BMDC in response to *L. major* promastigotes. Furthermore, both *Ctsb*$^{-/-}$ BMDC and BMM produced higher levels of IL-12 in response to the parasites than their WT- and *Ctsl*$^{-/-}$ counterparts.
CONCLUSIONS: Altogether, our results show that *Ctsb*$^{-/-}$ BMDC up-regulate two important signals for the induction of Th1-immune responses: the expression of antigen-loaded MHC class II molecules, and of IL-12. Furthermore, we propose a novel role of cathepsin B, as a regulator of cytokine expression during *L. major* infection.

Temperature stress and microsporidian infection: do they impair the amphipod host?

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BACKGROUND: Increasing temperatures can be a significant stressor for aquatic organisms. Amphipods are one of the most abundant and functionally important groups of freshwater macroinvertebrates. Therefore, we conducted a laboratory experiment with *Gammarus pulex*, naturally infected with microsporidians.
METHODS: In each group, 42 gammarids were exposed to 15°C and 25°C for 24 h. Sex of gammarids was determined and microsporidian infections were detected by PCR. To quantify stress levels of the amphipods, the 70 kDa heat shock proteins (hsp70) were analyzed by western blot.
RESULTS: More males than females were detected in the randomized population sample (ratio of females/males: 0.87). No mortality occurred at 15°C, while 42.9% of gammarids died at 25°C. Sequences of three microsporidians (M1, M2, M3) were detected in this *G. pulex* population. Prevalences were 27.0%, 37.8% and 64.9% for *Microsporidium* sp. M1, M2 and M3, respectively. Cumulative prevalence was 82.4%. Multiple infections with all three microsporidians in single gammarids were detected with a prevalence of 8.1%, and bi-infections ranged between 12.2% and 25.7%. In dead gammarids, comparatively low prevalences were noted for M1 (males and females: 11.1%) and M2 (females: 11.1%; males 0%), while prevalence of M3 was higher (females: 66.7%; males: 88.9%). No significant effect of host sex on microsporidian infection was found. Significant effects on hsp70 response were detected in the high temperature exposed individuals and those bi-infected with *Microsporidium* spp. M2 + M3.
CONCLUSION: This study shows that some microsporidian infections in amphipods can cause an increase in stress protein level, in addition to other stressors. Although more harmful effects of combined stressors can be expected, experimental evidence suggests that such an increase might possibly have a protective effect for the host against acute temperature stress.

Parasite infection of elks in natural areas of Central region of Russia

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BACKGROUND: Losiny Ostrov National Park, with its area of 12881 ha, is situated in the north-east of Moscow. Its territory is divided into forest-parks, namely Mytishchinsky, Losino-pogonny, Shchelkovsky, Alekseevsky, Losinoostrovsky and Yauzsky forest-parks. The total number of elks is from 45 to 50 specimens. The Kostroma region can boast a unique and single Russian elk farm situated on the territory of Sumarokovsky wildlife preserve of the Kologriv forest, near Sumarokovo village, 27 km away from Kostroma. The reserve has up to 40 elks, 12 of them make up the milking herd. Both the Losiny Ostrov and Kostromskaya Elk Farm belong to the Russian natural areas of preferential protection.
METHODS: Conventional methods for parasitological studies of animals (life and death) were used.
RESULTS: Elks in the Losiny Ostrov national Park and Kostrmskaya Elk Farm are infested with various parasite species in the form of polyinvasion, and common for them are *Dicrocoelium lanceatum; Moniezia benedeni; Dictyocaulus filaria; Strongyloides papillosus; Eimeria bovis* and *E. ellipsoidalis.* In the helminth fauna of elks nematodes predominate. The infection in elks reached: nematodes – 100%, trematodes – 40% and cestodes – 16%. The infection with lungs nematodes made 46,5% (*Varestrongylus capriole* – 35%, *Dictyocaulus filaria* – 11,5%). Protostrongylidae larvae were found in 4 molluscs species: *Bradybaena fruticum; Cochlicopa lubrica; Succinea putris; Zonitoides nitidus.*
CONCLUSION: Carrying out long-term observations for the state of the ecosystems in general and their single components in natural areas of preferential protection is the main direction of research. Besides, routine observations can help to determine changes in ecosystems at an early stage, when their negative consequences can still be prevented. Adult animals may be infected less seriously that youngsters, they are an important source of spreading invasion and promote epizootia.

Anthelmintic resistance in gastrointestinal nematodes in grazing cattle in Candelaria, Campeche, Mexico

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BACKGROUND: Gastrointestinal nematodes (GIN) in grazing cattle are the most important endoparasitic problems affecting the animal health, especially in humid and warm climates. The aim of this study was to determine anthelmintic resistance (AR) in GIN in grazing cattle.
METHODS: The fecal egg count reduction test (FECRT) was used to determine AR in the three main anthelmintics: Levamisole, Albendazole and Ivermectin. Fifty naturally infected calves with more than 150 eggs per gram of feces (EPG) were selected. The *in vitro* test was performed to determine inhibition of egg hatching using different doses from same anthelmintic drugs. The anthelmintic evaluation was made in Microtitre plates of 96-well. One hundred eggs of nematodes and 100 µl of each anthelmintic per well were used. The data were analyzed with Probit procedure to obtain the lethal doses ($LD_{50,99}$).
RESULTS: In the FECRT the control group had 333 EPG. Albendazol was 100% effective, with Levamisol was suspect resistance (94% of reduction) and Ivermectin show 73% of effectiveness and hence resistance. In the egg hatch test was notable that Albendazol had ovicidal effect and the lethal doses was low (4 μgml^{-1}) and the LD_{99} was 198 μgml^{-1}. $LD_{50,99}$ at 1.14 $mgml^{-1}$ and 34.26 $mgml^{-1}$ was indicated to Levamisole, and 0.24 $mgml^{-1}$ and 23.99 $mgml^{-1}$ were the $LD_{50,99}$ to Ivermectin, respectively.
CONCLUSION: The Albendazole toxicity against GIN from grazing calves still the best anthelmintic, followed by Levamisole. Ivermectin showed the lowest efficacy using the FECRT. Also, showed high LD_{50}(0.24 $mgml^{-1}$) *in vitro* assays.

The *Toxoplasma gondii* vacuolar compartment and its roles in digestion of host proteins, immune evasion and persistence

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BACKGROUND: *Toxoplasma gondii* is thought to be exiled while replicating in a non-fusogenic parasitophorous vacuole (PV) within the infected cell. Although studies have suggested *T. gondii* can endocytose exogenous material, definitive evidence is scarce and the prevailing view is that the parasite lacks endocytosis. Here we investigate the ability of *T. gondii* to ingest material from the cytosol of infected host cells and assess the roles of this pathway during infection.
METHODS: We transfected mammalian cells with expression constructs for mCherry and GFP, infected them with parasite proficient or deficient for cathepsin L in the parasite lysosome-like organelle (VAC) before viewing the parasites for acquisition of GFP. We also measured the vulnerability of cathepsin deficient parasites to innate immune clearance by immunity related GTPases (Irg's) and examined their ability to persist within tissue cysts of infected mice.
RESULTS: GFP or mCherry accumulated within the endolysosomal system of cathepsin L deficient parasites exclusively, revealing for the first time that *T. gondii* has an active endocytic system during intracellular replication and that cathepsin L digests host-derived proteins. GFP uptake was partially dependent upon the tubulomembrane intravacuolar network within the PV, implicating this enigmatic structure as a possible conduit for ingestion. Cathepsin L deficient parasites were vulnerable to killing by excess accumulation of Irg's on the PV membrane, linking the ingestion pathway to evasion of innate immunity. Accordingly, infection experiments showed that cathepsin L deficient parasites are virulence attenuated and, interestingly, produced defective cysts that were also observed upon treated WT cysts with a cathepsin L inhibitor.
CONCLUSIONS: Our results suggest that *T. gondii* ingests and digests host-derived proteins as a strategy to evade innate immunity during acute infection and persist during chronic infection. The findings have potential implications for treating chronic *T. gondii* infection.

Immune competence as a factor of treatment efficacy in *Trypanosoma cruzi* infection.

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BACKGROUND: The main drawbacks for a wider application of treatment of long-term *Trypanosoma cruzi* infections include the difficulty of assessing treatment efficacy and the potential adverse effects of these therapeutics. Conversely, drug therapy in *T. cruzi*-infected children is considered to be more effective than in adult patients. Immune exhaustion in long-term infections might be a key factor in treatment efficacy. Herein, we thought to determine the association between the immunological status prior to treatment and the rate of a positive impact after treatment with benznidazole. METHODS: Children between 5-16 years of age with confirmed serology for *T. cruzi* infection were recruited and treated with benznidazole. The magnitude and quality of T cell responses to *T. cruzi*-derived antigens were measured by ELISPOT and intracellular staining assays. *T. cruzi*-specific antibodies were measured by conventional serological tests and multiplex assays.
RESULTS: *T. cruzi*-infected children exhibit more functional parasite specific T cell responses compared to *T. cruzi* infected adults. However, *T. cruzi*-infected children have a heightened state of immune activation. Within 8 months of treatment with benznidazole, the levels of total early-differentiated memory T cells increased while the levels of fully differentiated memory and activated T cells decreased in treated children. Antibody titers decreased as assessed by conventional serology and multiplex assays.
CONCLUSIONS: The prevalence of polyfunctional T cell responses specific for *T. cruzi* in children is compatible with a more competent immune status in these subjects compared with *T. cruzi*-infected adults, supporting that very long term chronic infection eventually results in T cell exhaustion. A more functional T cell profile prior to treatment in children was associated with an early positive outcome of treatment.

In vitro lethal activity of _Pleurotus_ spp. and _Lentinula edodes_ hydroalcoholic extracts against _Haemonchus contortus_ (L_3)

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BACKGROUND: *Haemonchus contortus* (HcL_3) as one of the most pathogenic parasite in sheep and it causes severe economic loses in the industry. Edible mushrooms contain bio-compounds with medical properties and could have an impact against (HcL_3). This research was aimed to evaluate the *in vitro* lethal activity *Pleurotus* spp. and *Lentinula edodes* hydroalcoholic extracts (HE) against (HcL_3).
METHODS: HE were obtained by mycelia maceration. Assays were performed in 96 well plates. Eight treatments were established: 1) control (Distilled water); 2) control (Ivermectin 10mg/mL); 3, 4, 5 and 6) *P. eryngii* (1292 strain), *P. ostreatus* (0152 strain); *L. edodes* (401 strain) and *P. cornucopiae* (1328 strain); at 20, 40 and 60 mg/mL concentrations. 200 (HcL_3) into 20 µL of an aqueous suspension were added to each well (n=4). Lectures were recorded at 24, 48 and 72 h. Total (HcL_3) and proportions of death and alive larvae were estimated. Mean of recovered larvae were analyzed using an ANOVA test, followed by Tukey test and SAS program was used.
RESULTS: The highest mortality (95%) was recorded with *P. eryngii* (1292 strain) at 48 and 72 h; followed by *P. ostreatus* (0152 strain) that caused 87% mortality at 72 h.
CONCLUSIONS: The HE from mycelia obtained from *P. ostreatus* (0152 strain) and *P. eryngii* (1292 strain) showed a high lethal *in vitro* activity against (HcL_3) and it will be considered for further assays to evaluate their potential use in the control of the sheep haemonchosis.

In vitro activity of _Pleurotus ostreatus_ compounds against _Haemonchus contortus_ infective larvae (L_3)

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BACKGROUND: The mushrooms *Pleurotus ostreatus* (*Po*) has shown therapeutic uses, *i.e.,* antimicrobial, antiviral, antioxidant. This research has focused to study the *in vitro* effect of *Po* compounds against *Haemonchus contortus* (L_3) (HcL_3), a sheep parasite.
METHODS: Two experiments were performed. Experiment 1) the *in vitro* activity of a hydroalcoholic extract (HE) from fruiting bodies Po (1123 strain) was evaluated against HcL3. Experiment 2), two purified fractions (aqueous and butanolic) were evaluated. Two identical sub-assays evaluated the activity against sheated (ShL_3) and exsheated larvae ($EshL_3$). Ninetysix well plates were used (n=4). In the first experiment, seven treatments were established: 1) water; 2) Ivermectin 10mg/mL; 3 to 7 contained HE at 20, 30, 40, 60 and 80 mg/mL. Additionally, 200 HcL3, in 20 µL of water, were deposited in each well. In the second experiment, HE fractions (aqueous and butanolic) were evaluated against ShL_3 and $EshL_3$. In both experiments lectures were at 24, 48 and 72h. Data were analyzed by ANOVA test and Tukey test was used for mean comparison.
RESULTS: The highest mortality (49%) against $EshL_3$ was obtained with the HE highest concentration at 72 h. The highest mortality (73%) was recorded with the butanolic fraction against exsheated larvae at 40 h confrontation. The aqueous fraction recorded 60% mortality after 72 h confrontation against $EshL_3$.
CONCLUSION: Nematocidal compouds are present in *Po* HE and they could have an important impact in further experiments focused to assess their potential use in the control of sheep haemonchosis.

Identification of *Sarcocystis* sp. in a northern shoveler duck (*Anas clypeata*) from a wetland of Lerma in Mexico.

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BACKGROUND: Infections by species of *Sarcocystis* are considered common in many waterfowl species. Members of the order Anseriformes usually serve as intermediate hosts for several species of *Sarcocystis*, the asexual reproduction occurs in their muscles. Macroscopic sarcocysts have been reported from many species of waterfowl from North America. However, studies and reports of *Sarcocystis* in Mexico are limited.

METHODS: 150 migratory ducks (*Anas crecca, Anas discors, Anas americana, Anas acuta, Anas clypeata* and *Oxyura jamaicencis*) were hunter-killed during the 2014 season in a wetland of Lerma (Lake of Atarasquillo: 19º 21' 22.42" N and 99º 30' 59.98" W), Mexico. Of the carcasses, 1 cm^3 sections of breast were fixed in 10% formalin, embedded in paraffin, sectioned at 5 µm, and examined after staining with hematoxylin and eosin. It was observed in photonic microscope.

RESULTS: From the 150 samples examined, *Sarcocystis* macrocysts were found in breast muscles of a single duck (*Anas clypeata*). They were yellowish white color, with a size of 7.0 x 2.0 mm and resembled grains of rice. The microcysts were identified as belonging to *Sarcocystis* sp. (cyst type II) which morphology was presented in earlier publications (Kutkienė and Sruoga 2004, Kutkienė et al. 2008).

CONCLUSIONS: This is the first report of *Sarcocystis* sp. infection in a northern shoveler duck (*Anas clypeata*) at the wetland of Lerma (Lake of Atarasquillo), Mexico.

Relapsing fever borreliosis in domestic cats and dogs

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BACKGROUND: Relapsing fever (RF) is an acute human infectious disease caused by arthropod-borne spirochetes of the genus *Borrelia*. The disease is characterized by recurrent episodes of fever that concur with spirochetemia. The RF borrelioses include louse-borne epidemic RF caused by *B. recurrentis* and tick-borne endemic RF transmitted by Argasid soft ticks and caused by several *Borrelia* spp. including *B. crocidurae*, *B. coriaceae*, *B. duttoni*, *B. hermsii*, *B. hispanica* and *B. persica*. Human infection with *B. persica* is transmitted by the soft tick *Ornithodoros tholozani* and has been reported from Iran, Israel, Egypt, India, Central Asia, and China. *O. tholozani* feeds on warm-blooded animals and lives in caves, ruins, rock crevices and man-made shelters where livestock is housed.
METHODS: During 2003-2013, 5 cats and 5 dogs from Israel were detected with relapsing fever spirochetemia based on blood smear microscopy. Of these, infection was verified by PCR and sequencing of the 16S rRNA gene as caused by *B. persica* in 4 cats and 4 dogs.
RESULTS: The main clinical findings in cats included lethargy, anorexia and anemia in all cats (Hct. 10.9-22.3%; median 17%), and thrombocytopenia in 4/5 cats. All dogs were extremely lethargic, 4/5 were febrile, 3/5 were anorectic, 5/5 were anemic (Hct. 17-34%; median 27.1%) and 4/5 were thrombocytopenic. Three dogs were co-infected with babesiosis. The survival rate of both dogs and cats was 80% following treatment with antibiotics and disappearance of spirochetemia. One cat and a dog died one day after the initiation of antibiotic treatment.
CONCLUSIONS: This is the first report of *B. persica* infection in pet cats and dogs. Infection is associated with anemia and thrombocytopenia. Fever was recorded only in infected dogs. Dogs and cats may be involved in the transmission of infection to humans via soft ticks, or serve as sentinels for human infection.

Vaccination of sheep and cattle against haemonchosis

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BACKGROUND: The blood sucking gastrointestinal nematodes of *Haemonchus* genus are probably the most economically important parasites in ruminants raised in the warm damp climates of the tropics and sub tropics.
METHODS: A vaccine containing integral membrane glycoproteins from the intestine of *Haemonchus contortus* was evaluated in 3 groups of sheep (ewes and their lambs) and in young Nelore beef cattle, all exposed to natural infection while grazing. Vaccinates received 5 µg or 50 µg of the antigen diluted in 1mg QuilA adjuvant, while controls got adjuvant alone. Sheep received six shots 21 days apart, while calves received three shots initially three weeks apart and then four more times at six week intervals.
RESULTS: There was no evidence that 50 µg of vaccine antigen was superior to a 5 µg dose, in either sheep or cattle. Vaccination of calves induced high titres of circulating antibody and reduced *Haemonchus* egg output. It also reduced the burdens of adult *Haemonchus placei* and *Haemonchus similis* by about 60% and 30% compared with control calves. Antibody titres in vaccinated ewes were relatively low and did not confer significant protection throughout the trial. In contrast, the antibody titres of vaccinated lambs were much higher and significantly reduced overall mean *H. contortus* egg counts by 78% and of worm burdens by 56% in comparison with the controls.
CONCLUSIONS: Vaccination with intestinal membrane glycoproteins from *H. contortus* could substantially reduce the transmission of *H. placei* and *H. similis* thus providing a downstream protective benefit in cattle. The vaccine also protected lambs against *H. contortus* infection, but did not protect ewes during the periparturient period.

Detection of the protozoan *Entamoeba gingivalis* in periodontal pockets

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BACKGROUND: Periodontitis is a public health issue, being one of the most prevalent diseases worldwide. However, the aetiology of the disease is still unclear: genetics of patients cannot explain the dispersed or isolated localization of gingival pockets, while bacteria-based models are insufficient to distinguish gingivitis and periodontitis. Recent advances have underlined the possible role of protozoan parasites in the establishment of periodontitis. The aim of this project was to study a potential link between colonization of gingival pockets by the protozoan *Entamoeba gingivalis* and periodontitis.

METHODS: In seven different dental clinics in France, patients were examined and diagnosis of periodontitis was assessed on clinical parameters. In parallel, samples were taken in periodontal pockets or healthy sites, further submitted to microscopic observation and molecular identification by PCR, in order to detect *Entamoeba gingivalis*.

RESULTS: Results obtained from this blind sample analysis showed a strong sensitivity of PCR compared to microscopy (78.5%), indicating that, during periodontitis, the amoebae detected in microscopy mainly – if not exclusively – belong to the species *Entamoeba gingivalis*.

CONCLUSIONS: The positive correlation between periodontitis and presence of *Entamoeba gingivalis* suggests that this parasite is either the aetiological agent of periodontitis or an opportunistic colonizer of periodontal pockets.

Canine Monocytic Ehrlichiosis - an overview

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BACKGROUND: Canine monocytic ehrlichiosis (CME) is an import canine disease worldwide. It is caused by the rickettsial organism *Ehrlichia canis*. The latter is transmitted by the brown dog-tick *Rhipicephalus sanguineus*. After an incubation period of 8-20 days, 3 consecutive phases are acknowledged in the pathogenesis of the disease: an acute, subclinical and a chronic phase. The disease is characterized by a great variety of clinical signs of which fever, lymphadenomegaly, splenomegaly and surface bleeding are common. Thrombocytopenia, leukopenia and anemia are common hematological signs. Diagnosis of the disease is based on a combination of physical examination findings, classical laboratory methods (blood smear evaluation, serology) as well as newer molecular techniques (PCR, real-time PCR). Doxycycline is the drug of choice for CME. Duration of treatment should be considered in light of the disease phase. To date, there is no commercial vaccine and tick control is the most effective preventive measure. The prognosis of the acute and the subclinical phases of the disease is good, however grave for the chronic phase. Dogs in the latter phase will eventually die due to bone marrow hypoplasia and its outcomes: peripheral pancytopenia, sepsis and/or bleeding.
METHODS: This presentation will overview the current literature on the disease in general and on new diagnostic techniques as well as recent studies on a vaccine candidates.
CONCLUSIONS: Canine monocytic ehrlichiosis is an important canine disease. Early diagnosis of the disease increases the chances for a successful treatment. Further research is needed towards vaccine development.

Use of larval, parasitic female and egg antigens from *Strongyloides venezuelensis* to detect parasite specific IgG in feces of immunosuppressed rats experimentally infected.

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BACKGROUND: *Strongyloides venezuelensis* is a rodent parasite that has been extensively used as an experimental model to study human infection. In experimental infections, infective larvae from *S. venezuelensis* migrate to lungs before establishing in duodenal mucosa. Thus, its migration in the rodent host is similar that of *S. stercoralis* in humans. Diagnosis of this infection is still an important, but difficult task, since it relies on the detection of larvae in feces by parasitological methods. The aim of the present research was to detect levels of IgG by Enzyme linked immunosorbent assay (ELISA) using alkaline extracts of larvae, adult female worms and egg of *Strongyloides venezuelensis* as antigen.
METHODS: The microtiter plates were coated with alkaline parasite extract of larvae, adult female worms or eggs from *Strongyloides venezuelensis* for antibody detection. ELISA was conducted to detect antibody in the fecal samples of immunosuppressed rats using a secondary antibody consisting of peroxidase-labeled goat anti-rat IgG. Results were expressed as index ELISA (IE), as follows: IE=absorbance of tested sample/cut-off, where cut-off is the mean absorbance of 3 negative fecal samples plus 2 standard deviations. Values of IE>1·0 were considered positive. The statistical analyses were analyzed using Two Way ANOVA. The criterion for statistical significance was set at $p<0.05$.
RESULTS: In immunosuppressed rats, it was observed that IE > 1 on day 8 and 13 post-infection (p.i) with highest level at day 13 p.i when used extract of larvae, adult female worms or eggs as antigen. In the non-immunosuppressed rats (control animals) had only detect antibodies to larval extract, on days 8, 13, and 21 p.i, with greater reactivity on day 13 p.i.
CONCLUSIONS: In conclusion, extract of larvae, adult female worms or eggs to be an alternative for strongyloidiasis diagnosis in fecal samples of immunosuppressed rats.
Support: FAPEMIG, CAPES, CNPq ,UFU

New evidence to inform deworming in child populations

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BACKGROUND: WHO's PCT Databank for 2012 for soil-transmitted helminthiases (STH) documents a total of 55 countries providing deworming treatment to 209,133,331 school-age children (SAC) and 42 countries providing deworming to 64,302,947 preschool-age children (pre-SAC). This translates into coverages of 32% for SAC and 21% for pre-SAC. As deworming programs expand to include all at-risk children, new evidence from the field can optimize their health impact.

METHODS: Primary epidemiological research from cross-sectional studies and randomized controlled trials was conducted in the Peruvian Amazon between 2007 and 2014 to contribute to the evidence base for deworming in child populations. This research was designed to determine:1) the age of first STH infection; 2) the added benefit of a health hygiene intervention post-deworming; 3) the effect of a health hygiene intervention post-deworming on absenteeism; and 4) the relationship between STH infection and stunting, among others.

RESULTS: In a population of 370 pre-SAC, the age of first STH infection was found to be 8 months, with *Ascaris*. At 9 months of age, all three STH infections were present. By 14 months of age, prevalence had reached 37%. In a study of 1,089 SAC, the benefits of a health hygiene education intervention 4 months post-deworming included improved STH-related knowledge (aOR = 18.4; 95%CI: 12.7-26.6) and a 58% decrease in *Ascaris* re-infection intensity (aIRR = 0.42; 95%CI: 0.21-0.85). SAC infected with moderate-to-heavy *Ascaris,* and light hookworm infection, missed 2.38%, and 4.62%, more schooldays than uninfected SAC. Stunting was associated with STH infection in both pre-SAC (aOR = 1.52; 95%CI: 1.05-2.19) and SAC (aOR = 1.95; 95%CI: 1.35-2.82) populations.

CONCLUSIONS: Children as young as one year of age may benefit from deworming. The co-occurrence of STH and stunting should be considered to inform both the frequency of deworming treatment and the assessment of impact of a deworming program.

Developmental stage-induced carboxylesterase isozymes in the parasitic trematode *Fasciola hepatica*.

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BACKGROUND: Steroid hormone-like molecules exert control on developmental changes of helminthes into fully reproductive adults. All invertebrates exhibit a very delicate balance of circulating steroids and the ubiquitous enzymes carboxylesterases are necessary for hydrolyzing excess hormone levels or completely depleting it for the final transition into adulthood. The liver fluke *Fasciola hepatica* exhibit abundance of carboxylesterase activity already reported in the literature, however very little scientific information is available on this enzyme role on controlling the parasite's development.
METHODS: Closed cycle laboratory-cultured *Fasciola hepatica* anthelmintic-susceptible reference strain was used as the source of carboxylesterases. *In vitro* excysed metacercariae, parenchyma migratory larvae and adult developmental stages were analyzed by PAGE carboxylesterase zymograms.
RESULTS: A 90 kDa. band containing most of the enzyme activity (2500-6000 enzyme units/mg of protein by Gomori's assay) was found in all developmental stages under denaturing SDS-PAGE, however when the proteins were sorted by charge under non-denaturing PAGE, the 90 kDa band split into seven different isozymes each exhibiting a different electrophoretic retention factor (Rf), which combined showed a distinctive zymogram pattern for each developmental stage.
CONCLUSIONS: Our results suggest that *F. hepatica* controls its developmental molting process by the catalytic action of seven different 90 kDa carboxylesterases isozymes. Our study also suggests that controlling the action of different carboxylesterases isozymes may tamper the liver fluke's biological cycle.

Sequence analysis of putative octopamine receptor gene in Mexican amitraz-resistant cattle tick strain.

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BACKGROUND: The molecular target of amitraz is thought to be the octopamine receptor (OAR). Mutational changes in the OAR gene sequence could be responsible for the ticks becoming resistant to amitraz. In previous studies a putative Australian ticks octopamine receptor was cloned and sequenced, showing no differences in sequence between Australian susceptible and amitraz resistance strains. Similar studies in amitraz resistant strains from Brazil exhibited two amino acid substitutions that differed from susceptible and Australian strains. In this work we analyzed the sequence form Mexican susceptible and amitraz resistant strains with the objective of searching mutational changes in the OAR that may explain amitraz resistance in Mexico.
METHODS: The OAR gene from a Mexican susceptible and amitraz resistant *R. microplus* reference strains were isolated by PCR and cloned into a bacterial sequence vector, sequenced and analyzed by multiple sequence alignment algorithm Clustal-Omega. Eleven isolated clones within the sequence vector were analyzed from each strain.
RESULTS: From the amitraz resistant strain we found that 5 clones represented one allele (A) and the rest of the clones represented a different allele (B). This result demonstrate that at least two different alleles of the OAR gene were present in the Mexican amitraz resistant reference strain, Allele A shared the sequence with two susceptible strains and the allele B is only found in the Mexican resistant strain and differ from the amitraz resistant Brazilian strain.
CONCLUSIONS: The discovery of two alleles in the Mexican amitraz resistance strain opens the possibility that the amitraz resistance mechanism in this strain could be explained as an altered target site. The analysis of the presence of an amitraz resistant OAR allele could be a useful diagnostic tool for amitraz resistant cattle ticks.

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***Rhipicephalus (Boophilus) microplus* embryogenesis.**

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BACKGROUND: The cattle tick *Rhipicephalus* (*Boophilus*) *microplus* is a major problem of the tropical and subtropical cattle industry, for this reason different methods for controlling ticks are under research and the need of knowing the still un-described basic biology of the ticks have resurged, this is case of basic embryology details of the cattle ticks which remain undetermined. The objective of this work was to study and characterize the *R. microplus* embryogenesis from freshly laid eggs to hatching larvae.
METHODS: Eggs were fixated every 24 h and treated eggs were placed on a microscope glass slide and stained with 4',6-diamidino-2-phenylindole (*DAPI*).Specimens were imaged on an epifluorescence microscope.
RESULTS: Fourteen stages were identified which represent the physiological events of embryonic development until larvae hatching: Early nuclear divisions (1-3 days; nuclear mitosis without cytokinesis), blastoderm (4th day) blastopore (5th day), primary thickening (5th day), dorsal field (9th day), Germ band formation (9th day), germ band segmentation (9th day), limb differentiation (12 day), nervous system differentiation (13th day), inversion (14th day), dorsal closure (15th day), ventral (16th day) post-embryo (20th day).
CONCLUSIONS: This work provides us with a system, which allows easy recognition of the distinct tick embryo development stages using simple laboratory tools, and provides a wide vision of the tick embryology, which helps us understand the similitude with other arthropods, which are currently controlled by arresting embryonic development.

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Studies on the prevalence of malaria parasite among children with splenomegaly in Aba metropolis, Abia State, Nigeria

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BACKGROUND: Malaria is common in sub-Saharan Africa, accounting for over one million deaths yearly in the region. It is implicated in many complications including splenomegaly which has been observed in some children admitted for malaria. This study was carried out to establish a possible relationship between malaria and splenomegaly.
METHODS: 403 children with confirmed cases of splenomegaly were examined for malaria parasites using RDT. Observations on treatment option, age and gender were made by administering questionnaires.
RESULTS**:** Of the 403 sampled, 83.9% had malaria infection while 65(16.1%) did not. Malaria prevalence and splenomegaly showed significantly positive correlation. Of those infected with splenomegaly, 28.2% consulted qualified medical doctors for diagnosis and treatment while 71.7% consulted traditional healers. Concomitant malaria infection was higher in the group consulting traditional healers 74.6% than those that visited qualified doctors 25.4%. Age group 13 – 15 years recorded the highest infection rate of 100% while age group 7 – 9 years recorded the least infection, 65.6%. Age was found to be statistically insignificant. The prevalence of malaria was higher in males 84% than in female 83.7% but this was statistically insignificant.
CONCLUSIONS: The observations of 403 children with confirmed cases of splenomegaly in this study are of immense public health concern. The number of people that patronized the traditional healing centres even in the presence of modern facilities is also alarming. More education and public enlightenment are needed to expose the dangers of malaria and the need for adequate health care.