Taenia solium metacestode fasciclin-like protein is reactive with sera of inactive neurocysticercosis

YA Bae[1,2], JS Yeom[3], SH Kim[1], CS Ahn[1], JT Kim[1], HJ Yang[4], Y Kong[1]

[1]Department of Molecular Parasitology, Sungkyunkwan University School of Medicine and Center for Molecular Medicine, Samsung Biomedical Research Institute, Suwon 440-746, Korea; [2]Department of Microbiology, Graduate School of Medicine, Gachon University, Korea; [3]Department of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea; [4]Department of Parasitology, Ewha Womans University School of Medicine, Seoul, Korea

BACKGROUND: Neurocysticercosis (NC), an infection of the central nervous system with *Taenia solium* metacestodes (TsM), invokes a formidable neurological disease. A host of antigens is applicable for serodiagnosis of active cases, while they demonstrate fairly low reactivity against sear of chronic NC. Identification of sensitive biomarkers for inactive NC is critical for appropriate management of patients.
METHODS: Proteome analysis revealed several isoforms of 65- and 83-kDa TsM fasciclin-like protein (TsMFas) to be highly reactive with sera of inactive NC. A cDNA encoding one of the 83-kDa TsMFas (TsMFas1) was isolated from a cDNA library. We expressed a recombinant protein (rTsMFas1) and evaluated its diagnostic potential employing sera from inactive NC (*n* = 80), tissue-invasive cestodiases (*n* =169) and trematodiases (*n* =80), and those of normal controls (*n* = 50).
RESULTS: Secretory TsMFas1 was composed of 766 amino acid polypeptide, and harbored fasciclin and fasciclin-superfamily domains. The protein was constitutively expressed in metacestode and adult stages, with preferential locality in the scolex. Bacterially expressed rTsMFas1 exhibited 78.8% sensitivity (63/80 cases) and 93% specificity (278/299 samples) in diagnosing chronic NC. Some cross-reactivity was observed with sera of cystic echinococcosis (CE; 10/56, 17.8%) and sparganosis (4/50, 8%). Positive and negative predictive values were 75% and 95.5%, respectively.
CONCLUSIONS: TsMFas may be useful for differential diagnosis of inactive NC in clinical settings, especially where both NC and other infectious cerebral granulomatoses are prevalent. In clinical settings, simultaneous multi-antigen screening using CE specific antigens (several isoforms of antigen B) and concomitant imaging scans may further contribute to differentiate inactive NC from CE.

The *R* enantiomer of the anti-tubercular drug PA-824 as a potential oral treatment for visceral leishmaniasis

Susan Wyllie, Stephen Patterson and Alan Fairlamb
Division of Biological Chemistry and Drug Discovery, Wellcome Trust Biocentre, College of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, UK.

The novel nitroimidazopyran agent, (*S*)-PA-824, has potent antibacterial activity against *Mycobacterium tuberculosis in vitro* and *in vivo* and is currently in Phase II clinical trials for TB. In contrast to M. tuberculosis, where (*R*)-PA-824 is inactive, we report here that both enantiomers of PA-824 show potent cidal activity against *Leishmania donovani*, the causative agent of visceral leishmaniasis (VL). In leishmania-infected macrophages (*R*)-PA-824 is 6-fold more active than (*S*)-PA-824. Although the *in vitro* and *in vivo* pharmacological profiles of both enantiomers are similar, (*R*)-PA-824 is more efficacious in the murine model of VL, with >99% suppression of parasite burden when administered orally at 100 mg kg-1, twice daily for 5 days. In *M. tuberculosis* (*S*)-PA-824 is a prodrug that is activated by a deazaflavin-dependent nitroreductase (Ddn), an enzyme which is absent in *Leishmania* spp. Unlike nifurtimox and fexinidazole, transgenic parasites overexpressing the leishmania nitroreductase are not hypersensitive to either (*R*)-PA-824 or (*S*)-PA-824, indicating that this enzyme is not the primary target of these compounds. Drug combination studies *in vitro* indicate that fexinidazole and (*R*)-PA-824 are additive, whereas (*S*)-PA-824 and (*R*)-PA-824 show mild antagonistic behaviour. Thus (*R*)-PA-824 is a promising candidate for late lead optimisation for VL and may have potential for future use in combination therapy with fexinidazole, currently in Phase II clinical trials against VL. Here, we report our preliminary findings as we attempt to define the mechanism of action of this promising nitro drug in *L. donovani*.

Genetic and biochemical characterisation of *Trypanosoma cruzi N*-myristoyltransferase and *N*-myristoylome

Adam J. Roberts, Leah Torrie, Susan Wyllie and Alan H. Fairlamb

Division of Biological Chemistry and Drug Discovery, University of Dundee, Dundee, UK

Trypanosoma cruzi is the causative agent of Chagas disease, which continues to be a major health problem in Latin American countries. It is estimated that there are ~10 million infected individuals worldwide leading to a death toll in the region of 10,000 per annum. Whilst the current drugs (benznidazole and nifurtimox) are most effective against the less commonly diagnosed acute stage, their curative rates significantly decrease against the chronic stage. Combined with numerous adverse reactions patients can experience whilst taking these drugs, there is a vital need for fully validated drug targets in this parasite and for new, more effective drugs to cure this disease. One prospective drug target is the enzyme *N*-myristoyltransferase (NMT) which catalyses the co and post-translational addition of myristic acid onto the *N*-terminal glycine of specific proteins in eukaryotes. Using a combination of genetic and biochemical techniques, we have probed the requirement for *N*-myristoylation, identifying more than 10 *N*-myristoylated proteins in this parasite. Firstly, it was only possible to replace both allelic copies of *TcNMT* in the presence of an ectopically expressed copy of the gene, which is indicative that *NMT* is essential for the survival of this parasite[1]. Enzymatic studies have shown the pyrazole sulphonamide inhibitor DDD85646[2] to be a highly potent against the recombinant enzyme (K_i 12.7-22.8 nM). Against the parasite, DDD85646 displays dose dependent inhibition of *N*-myristoylation, confirming it to be an inhibitor of *Tc*NMT. In summary, *N*-myristoylation has been shown to be an important and druggable biological process in this parasite.

1. Roberts *et al., Biochem. J.* (2014) **459** (323–332)
2. Frearson *et al*., *Nature* (2010) **464**, 728-732

Distribution of *Toxoplasma gondii* tissue cysts in the organs of goats and their survival in goat meat and meat *products*

Koudela, Břetislav[1]*;Juránková Jana*[2]*; Neumayerová Helena*[2]

[1]CEITEC, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic; [2]Department of Pathology and Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

BACKGROUND: Among the various kinds of commonly consumed meat, goat meat possesses a high risk of human infection due to high susceptibility of goats to *T. gondii*. We have estimated the burden of *T. gondii* in various tissues of experimentally infected goats and determined the viability of *T. gondii* cysts in vacuum packed goat meat (VP) and after the manufacturing of dry fermented sausages (DFS).
METHODS: A magnetic capture method and qPCR was used to estimate the parasite burden in different tissues of goats experimentally infected with *T. gondii* oocysts. Muscle tissues were used for production of VP and DFS. Each sample of VP or DFS was digested and bioassayed to mice.
RESULTS: The highest concentration of *T. gondii* DNA was found in lung and dorsal muscle tissue of infected goats. Bioassays showed that samples of VP without salt addition were alive after six weeks at 4 °C. Incubation at -20 °C supported the viability after 3 h, but not after 4 h. After 7 days in 2.5% of curing salt, *T. gondii* in VP were still viable, but not after 14 days at 4 °C. All the DFS samples were not positive for infective cysts.
CONCLUSIONS: All tested goat tissues were found *T. gondii* positive and therefore thorough cooking before human consumption is highly recommended. Cyst of *T. gondii* was not viable in frozen VP and DFS, however the vacuum packing contributed to *T. gondii* cyst survival.

Synthetic single and double azascorpiand derivatives as inhibitors of Fe-SOD and TR in *Trypanosoma cruzi* and their hopeful results in murine model.

Ramírez-Macías, Inmaculada[1]; Olmo, Francisco[1]; Clares, M. Paz[2]; Marín, Clotilde[1]; González, Jorge[2]; Inclán, Mario[2]; Soriano, Concepción[3]; Tejero, Roberto[4]; Krauth-Siegel, Luise[5]; García-España, Enrique[2]; Sánchez-Moreno, Manuel[1].

[1]Departamento de Parasitología, Facultad de Ciencias, Universidad de Granada, E-18071 Granada, Spain.
[2]Instituto de Ciencia Molecular, Departamento de Química Inorgánica, Universidad de Valencia, E- 46980 Paterna (Valencia), Spain.
[3]Departamento de Química Orgánica, Universidad de Valencia, E-46100 Burjassot (Valencia), Spain.
[4]Departamento de Química Física, Universidad de Valencia, E-46100 Burjassot (Valencia), Spain.
[5]Biochemie-Zentrum der Universität Heidelberg, Im Neuenheimer Feld 504, 69120 Heidelberg, Germany.

BACKGROUND: The main drugs used to treat Chagas disease are two nitroaromatic heterocycles: the furane-based nifurtimox and the imidazole-based benznidazole. Both drugs are effective in the acute phase of the disease, however, their efficacies are very low during the chronic phase.
Research on antichagasic agents is mainly focused on biochemical metabolic key pathways or crucial parasite-specific enzymes.
We have tested the in vitro and in vivo antiparasitic activity of eleven derivatives of aza-scorpiand like macrocycles and their activity as inhibitors of iron Superoxide dismutase (Fe-SOD) and Trypanothione reductase (TR) in *Trypanosoma cruzi*.
METHODS: We studied the in vitro activity of 11 compounds against extra-and intra-cellular forms of *T. cruzi*. Toxicity values against mammalian Vero cells were also calculated. We realized in vivo experiments in murine model. Fe-SOD and TR activity was studied by biochemical assays.
RESULTS: After carrying out the preliminary studies, compounds **1, 2, 7** and **11**, were promoted to in vivo assays. All the tested compounds reduced the number of circulating parasites. **7** and **11** showed the best activity during acute phase (parasitemia reduction greater than 80%). Compound **7** was the most effective in the chronic phase. Significant inhibitory values of Fe-SOD activity were found, compounds **7** and **11** showed values close to 100% inhibition at 25-50 μM concentrations.
Compounds **1, 2, 7** and **11** proved to be effective inhibitors of TR at both inhibitor concentrations of 40 and 100 μM. The inhibition of activity varied between 14.0 and 89.1% for the different compounds
CONCLUSIONS: The activity, low toxicity, stability, low cost of starting materials and straightforward synthesis make these compounds appropriate molecules for the development of affordable anti-chagasic agents.

Anthelmintic Ethiopian medicinal plants for small ruminants: *in vitro* and *in vivo* studies

Athanasiadou, Spiridoula[1]; Debela, Etana[2]; Regessa, Alemayehu[2]; Tolera, Adugna[3]; Nurfeta, Ajebu[3]; Tolossa, Ketema[4]; Terry, Sandra[1]; Houdijk, Jos[1]

[1]Disease Systems, SRUC, West Mains Road, Edinburgh EH9 3JG, UK; [2]School of Veterinary Medicine, Hawassa University, Hawassa, P.O. Box 05, Ethiopia; [3]School of Animal and Range Science, Hawassa University, Hawassa, P.O. Box 05, Ethiopia; [4]Department of Chemistry, Hawassa University, Hawassa, P.O. Box 05, Ethiopia.

BACKGROUND: Medicinal plants play an important role for parasite control in developing countries. However, scientific evidence on plant efficacy and potential side effects is scarce. Here, we present *in vitro* and *in vivo* evidence of anthelmintic properties of Ethiopian medicinal plants.
METHODS: Thirty medicinal plants, selected from an ethno-botanical survey, were dried and extracted by three solvents (methanol, 70%-methanol and water). *Teladorsagia circumcincta* L_3 motility was recorded for 24 h prior and 24 h after addition of the 90 resulting extracts through an automated larval motility assay that quantifies anthelmintic activity. Faecal egg counts (FEC) and feed intake were measured in sheep, sham-infected or infected with 15,000 L_3 *T. circumcincta*, and drenched with water concoctions of two plants that showed strong *in vitro* efficacy.
RESULTS: Twenty-three of 25 plants, routinely used by traditional healers against endo-parasites, including *Albenzia anthelmintica*, showed 100% larval motility inhibition for at least one of the solvents tested. Furthermore, 3 of 5 plants routinely used against ecto-parasites, including *Dodonea angustifolia*, also showed strong *in vitro* anthelmintic activity. Drenching with *A. anthelmintica* and *D. angustifolia* concoctions reduced FEC by 51 and 57%, respectively ($P<0.05$). Feed intake was temporarily reduced for *A. anthelmintica* only.
CONCLUSIONS: The study shows that scientific validation of ethnoveterinary knowledge has great potential to assist informing plant-based parasite control strategies.

Funding was from the DFID/BBSRC/SG CIDLID program (BB/H009299/1)

Imidazole-containing phthalazine derivatives inhibit Fe-SOD performance in *Leishmania* species and are active in vitro against visceral and mucosal leishmaniasis

Ramírez-Macías, Inmaculada[1]; Gómez-Contreras, Fernando[2]; Navarro, Pilar[3]; Marín, Clotilde[1]; Rosales, Maria José[1]; Campayo, Lucrecia[2]; Cano, Carmen[2]; Urbanová, Kristína[1]; Sanz, Ana M.[2]; Yunta, María J. R.[2]; Sánchez-Moreno, Manuel[1]

[1]Departamento de Parasitología, Facultad de Ciencias, Universidad de Granada, E-18071 Granada, Spain.
[2]Departamento de Química Orgánica, Facultad de Química, Universidad Complutense, E-28040 Madrid, Spain.
[3]Instituto de Química Médica, Centro de Química Orgánica M. Lora-Tamayo, CSIC, E-28006 Madrid, Spain.

BACKGROUND: Assuming the need for new drugs against leishmaniasis, different parasite targets have been proposed. One of the most striking characteristics of the *Leishmania* parasites consists of its ability to survive in the presence of reactive oxygen species. Therefore, targets most investigated are enzymes involved in antioxidant defence that are either exclusive to the parasite or present significant differences with respect to their homologues in humans. Within this context, one enzyme that plays an essential role in the struggle of the parasite against oxidative stress is iron superoxide dismutase (Fe-SOD), which is present in all trypanosomatids, but not in humans.
We have tested the in vitro leishmanicidal activity of a series of imidazole-containing benzo[*g*]phthalazine derivatives.
METHODS: We studied the in vitro activity of a series of imidazole-containing benzo[*g*]phthalazine derivatives **1**-**4** on *Leishmania infantum*, *Leishmania braziliensis* and *Leishmania donovani*, and their cytotoxicity on J774.2 macrophage. Subsequently, we perform specific tests with in order to estimate the possible mechanism of action for each one of them, which include an infectivity assay, SOD enzymatic inhibition assay, metabolite excretion assay and TEM
RESULTS: All compounds showed selectivity indexes higher than the reference drug and the less bulky monoalkylamino substituted derivatives **2** and **4** were more effective than their bisalkylamino substituted counterparts **1** and **3**. Infection rate measures and ultrastructural alterations studies confirmed that 1 and 2 were highly leishmanicidal and induced extensive parasite cell damage. Modifications in the excretion products of parasites treated with **1**, **2** and **4** were also consistent with substantial cytoplasm alterations. The most active compounds **2** and **4** were potent inhibitors of Fe-SOD.
CONCLUSIONS: Molecular modelling suggested that **2** and **4** could deactivate Fe-SOD due to a sterically favored enhanced ability to interact with the H-bonding net that supports the antioxidant features of the enzyme.

Antileishmanial activity of tetradentate complex compounds: in vitro and enzymatic study

Ramírez-Macías, Inmaculada[1]; Chaves-Lemaur, Guillermo[1]; Urbanová, Kristína[1]; Cussó, Olaf[2]; Marín, Clotilde[1]; Rosales, María José[1]; Costas, Miquel[2]; Ribas, Xavi[2], Sánchez-Moreno, Manuel[1].

[1]Departamento de Parasitología, Facultad de Ciencias, Universidad de Granada, E-18071 Granada, Spain.
[2]Departament de Química, Universitat de Girona, E-17071 Girona, Catalonia, Spain.

BACKGROUND: Leishmaniasis are a group of sandfly-transmitted diseases caused by *Leishmania* species that affect mainly the developing countries. There are three predominant clinical forms, cutaneous, muco-cutaneous and visceral leishmaniasis with no successful treatment. Therefore, the need for new effective treatment molecules still remains. In our research, we try to develop molecules that inhibit selectively parasite-specific enzymes such as iron superoxide dismutase (Fe-SOD), a key enzyme in the oxidative stress defence mechanism in the trypanosomatid family.
METHODS: The initial in vitro activity assays comprise cytotoxicity tests against J774.2 macrophages and promastigote and amastigote assays that help us to determine the in vitro effectiveness of each compound. Subsequently, we perform specific tests with the most active compounds (selectivity index, SI > 20) in order to estimate the possible mechanism of action for each one of them, which include an infectivity assay, SOD enzymatic inhibition assay, metabolite excretion assay and TEM.
RESULTS: In this work, we tested the *in vitro* antiparasitic activity of 12 new tetradentate complex compounds on three *Leishmania* species (*L. braziliensis*, *L. donovani* and *L. infantum*). Comp 3, 6-9 and 11 showed extraordinary antileishmanial properties: inhibition of promastigote and amastigote growth (SI from 22 to 156); prevention of parasitic infection (decrease in the infection rate up to 88% in comparison to Glucantime®); glucose metabolism alteration and life-threating ultrastructural damage (mitochondrial swelling, lack in ribosomes and intense vacuolization). We determined that Comp 8 acts as a selective inhibitor of *L. donovani* and *L. infantum* Fe-SOD (IC50 < 0.1 μM, and < 1 μM, respectively) which is more than 5000 and 500 times superior than the IC50 of the human Cu,Zn-SOD.
CONCLUSIONS: Based on the extremely low cytotoxicity, high effectiveness against parasitic growth and the high selectivity against the leishmanial SOD, we advise to continue the work in *in vivo* studies.

Molecular detection of *Trypanosoma* (*Megatrypanum*) spp. in deer ked (*Lipoptena cervi*) in Poland

Werszko, Joanna; Karbowiak, Grzegorz; Szewczyk, Tomasz

W. Stefański Institute of Parasitology, Polish Academy of Sciences, Twarda 51/55, 00-818 Warsaw, Poland

BACKGROUND: *Megatrypanum* trypanosomes are common parasites of ruminants. According to previous studies their main vectors are flies belonging to the Hippoboscidae, Tabanidae and Muscidae families. The Hippoboscidae flies commonly attack deer, however, their role as a blood parasite vector is weakly known.
METHODS: A total of 227 deer keds (*Lipoptena cervi*) (127 females, 100 males) were collected in November 2013 and in January 2014 from red deer (*Cervus elaphus*) in the forest area near Strzałowo, north-eastern Poland (N53°46', E21°27'). DNA was isolated using a Genomic Mini AX Tissue kit (A&A Biotechnology, Gdynia). A nested PCR reaction was conducted using the universal primers for the *Trypanosoma* genus (TRY927F and TRY927R) amplified a fragment of approximately 913 bp, corresponding to the region between 767 and 1680 bp. in the 18S rRNA gene in the first round. SSU561F and SSU561R were used for the second round, these primers produced a 565bp product from the region between 880 and 1440 bp. Positive PCR samples were sequenced, consensus sequences for both strands compared to the sequences described in the GenBank database using NCBI BLAST. DNA sequence alignments were conducted using GenDoc 2.7.0.
RESULTS: The prevalence of *Lipoptena cervi* infection with *Trypanosoma* sp. was 22,47% with 100 males and 127 females. The obtained partial 18S rRNA nucleotide sequences (Lc23KG isolate) showed 100% similarity with *Trypanosoma* spp. isolated from fallow deer from Scotland (GenBank Accession No. AJ009165).
CONCLUSIONS: This is the first report of isolation of *Megatrypanum* trypanosomes from *Lipoptena cervi* in Poland, confirmed by molecular methods. *Lipoptena cervi* may be a vector for *Megatrypanum* trypanosomes.
ACKNOWLEDGMENTS: The study was supported by NCN grant 2011/01/B/NZ7/03574.

Microsporidium sp. in the *Ixodes ricinus* tick from Kyiv, Ukraine

Karbowiak, Grzegorz[1]; *Wróblewski, Piotr*[1]; *Szewczyk, Tomasz*[1]; *Didyk, Yuliya*[2]; *Ovcharenko*, *Mykola*[1,3]

[1] W. Stefański Institute of Parasitology of the Polish Academy of Sciences, Twarda 51/55, 00-818 Warsaw, Poland; [2] I.I.Schmalhausen Institute of Zoology of NASU, Khmelnytskogo 15, 01601 Kyiv, Ukraine; [3] Pomeranian Academy, Arciszewskiego 22A, 76-200 Słupsk, Poland

BACKGROUND: Microsporidia are common parasites of many invertebrates. However, hard ticks (Ixodidae) with infections with microporidia are rarely reported.
METHODS: Preliminary studies of microsporidia infections in ticks were conducted in 2013 in Kyiv, Ukraine. Ticks were collected from vegetation from a recreational area (Syretsky arboretum) in the city, using the flagging method. A total of 40 *Dermacentor reticulatus* (31 females, 9 males) and 24 *Ixodes ricinus* (7 males, 6 nymphs, 11 larvae) ticks were sampled. DNA extraction was carried out by the alkaline lysis method. PCR was performed with the primers V1f and 580r, specific for Microsporidia and amplifying the small subunit of the 16S rRNA gene. Electrophoresis was conducted in 1.5 % agarose gels. Positive PCR products were purified using a NucleoSpin Gel and PCR Clean-Up kit (Machery-Nagel GmbH & Co. KG) and sequenced using the primers V1f, 530f, 964r, 1492r, HG4f, MC3f and 580r. The 7 sequencing fragments originating from each PCR product analysed were compiled using the software Contig Express Vector NTI Advance ver. 11.0, Invitrogen Corp. The sequences were analysed using BLAST, and compared with sequences from GenBank.
RESULTS: One sequence was obtained from an *Ixodes ricinus* male, identified as *Microsporidium* sp. Loire. It presented the 97.9% similarity to a strain (accession GenBank number HM566198), occurring in *Ostrinia nubilalis* (Lepidoptera: Crambidae) in France.
CONCLUSIONS: This is the first report of detection of *Microsporidium* sp. in the tick *Ixodes ricinus* in Ukraine, that has been confirmed by molecular methods.

Changes in hard ticks ranges during the last decade in middle Europe: possible causes and potential threats

Karbowiak, Grzegorz[1]; Nebogatkin, Igor[2]; Didyk, Yuliya[2]; Szewczyk, Tomasz[1]; Stanko, Michal[3]; Pet'ko, Branislav[3]

[1]W. Stefański Institute of Parasitology of the Polish Academy of Sciences, Twarda 51/55, 00-818 Warsaw, Poland; [2]I.I.Schmalhausen Institute of Zoology of NASU, Khmelnytskogo 15, 01601 Kyiv, Ukraine; [3]Institute of Parasitology, Slovak Academy of Sciences, Hlinková 3, 040 01, Košice, Slovakia

ABSTRACT: During the last decade, changes in the occurrence area of hard ticks have been observed, both at extremes of altitude and latitude, as well as within their prior range. The documented observations concern the species *Dermacentor reticulatus*, *Haemaphysalis punctata* and *Ixodes ricinus*. New populations of *D. reticulatus* appeared in western Poland and the Carpathian region of Slovakia, with similar situations observed in Hungary, Romania and Ukraine, in areas were this tick was absent or rare, new foci of their occurrence appeared. Moreover, *D. reticulatus* is a species adapted to dwelling in urbanized areas. An expansion for *Ixodes ricinus* was observed in southern and middle Europe, to Scandinavia in the north and the Ural to the East. There is evidence of expansions in the southern part of Ukraine and the altitudinal ranges in Bosnia & Herzegovina, Czech Republic and Slovakia. *Haemaphysalis punctata* is a tick species occurring in southern and middle Europe; it is documented that new foci appeared in northern Ukraine. Some of the primary reasons for the expansions to the ranges of these species are climatic changes, moving of their hosts occurrence areas, the ability to live in anthropogenic landscapes, and international tourism and trade. The main potential threats of increased numbers of hard ticks are greater numbers of cases of piroplasmosis, tularaemia, new zoonotic foci of West Nile virus, tick-borne encephalitis virus, boutonneuse fever, Lyme borreliosis and other infections.

Epidemiological patterns of soil-transmitted helminthiases-related mortality in Brazil

Martins-Melo, Francisco Rogerlândio[1,2]; Lima, Mauricélia da Silveira[1]; Ramos Jr, Alberto Novaes[1]; Alencar, Carlos Henrique[1]; Heukelbach, Jorg[1,3]

[1]Department of Community Health, School of Medicine, Federal University of Ceará, Fortaleza, Brazil; [2]Federal Institute of Education, Science and Technology of Ceará, Caucaia, Brazil; [3]Anton Breinl Centre for Public Health and Tropical Medicine, School of Public Health, Tropical Medicine and Rehabilitation Sciences, James Cook University, Townsville, Australia
Corresponding: Francisco Rogerlândio Martins-Melo; E-mail: rogerlandio@bol.com.br

BACKGROUND: Soil-transmitted helminth (STH) infections are widely distributed in tropical and subtropical areas. They are the main cause of disease burden in school-age children in developing countries, often resulting in anaemia, reduced school performance, growth stunting, malnutrition and death. We analyzed the epidemiological patterns of STH-related mortality in Brazil, from 2000 to 2011.
METHODS: We performed a nationwide study using mortality data obtained from the Mortality Information System of the Brazilian Ministry of Health. We included all deaths in the country between 2000 and 2011, in which STH (ascariasis [ICD-10: B77], hookworm diseases [ICD-10: B76], and trichuriasis [ICD-10: B79]) were mentioned on the death certificates as underlying or associated causes of death (multiple causes of death). We calculated crude mortality rates (per 1,000,000 inhabitants) by sex, age group, race/color, and region of residence. Trends over the 12-year period were assessed using Joinpoint regression models.
RESULTS: During the study period, 12,491,280 deaths were recorded. STH was identified in 853 deaths, with 97.0% (827) caused by ascariasis, 2.9% (25) by hookworm infections, and 0.1% (1) by trichuriasis. Average annual crude mortality rate was 0.38 deaths per 1,000,000 inhabitants. Females (0.39 deaths/1,000,000 inhabitants/year), age group <10 year-olds (1.43 deaths/1,000,000 inhabitants/year), indigenous race/color (0.41 deaths/1,000,000 inhabitants/year), and residents in the Northeast region (0.56 deaths/1,000,000 inhabitants) had the highest rates. Mortality decreased significantly over the entire period (Annual Percent Change [APC]: -6.9%; 95% confidence intervals [CI]: -8.1 to -5.6), with differences between regions: a decrease in the Southeast (APC: -8.9%; 95% CI: -13.2 to -4.3) and South (APC: -15.4%; 95% CI: -20.3 to -10.1) regions, and stability in the North, Northeast and Central-West regions.
CONCLUSIONS: Despite the mortality decreasing in Brazil, STH remain a neglected health problem, with marked regional differences, affecting mainly children and low-income populations. This is the first nationwide population-based study on STH-related mortality in Brazil, using multiple causes of death.

Metagenomic analysis of the gastric microbiota culturable from a patient with gastritis concomitant with Barrett's esophagus

Andrés Julián Gutiérrez-Escobar[1], Martín Bayona-Rojas[1], Carlos Barragan-Vidal[1], Sebastián Rojas-Lara[1] y Ricardo Oliveros[1].

[1] Investigador Grupo de Investigación en Ciencias Biomédicas y Genética Humana Aplicada GIBGA. Universidad de Ciencias Aplicadas y Ambientales U.D.C.A Bogotá, Colombia.

BACKGROUND: Barrett's esophagus is a distal metaplasia characterized by the transformation of squamous mucosa into columnar mucosa. This esophageal phenotype is a product not only of the chronic reflux of gastric acids, but also by microorganisms that colonize the oral cavity and stomach. Two classes of microbiota can be identified in Barrett's esophagus; microbiota type I is associated with the normal esophagus and type II with an inflamed esophagus. The present study describes the gastric microbiota of a patient with antral gastritis concomitant with Barrett's esophagus absent infection with *Helicobacter pylori*.
METHODS: Gastric biopsies were obtained following the protocol of Sydney and following ethical practices. The isolates were cultivated under microaerophilic conditions on Columbia Agar supplemented with IsoVitaleX™ and 7% sterile blood. Extracted DNA was sequenced using 454-GS and the results analyzed on the MG-RAST server.
RESULTS: Gram negative isolates were found and bacteria resistant to levofloxacin, amoxicillin, tetracycline, erythromycin, and clarithromycin. The phyla *Bacteroidetes, Firmicutes, Fusobacteria* and *Proteobacteria,* the genus *Bacteroides* and the species group *Bacteroides fragilis* were most abundant. Functionally, the metabolism of carbohydrates, amino acids, and to a lesser extent, the metabolism of cofactors and vitamins were most dominant, and of which the enzymes β-glucosidase (EC 3.2.1.21), β-galactosidase (EC 3.2.1.23) and β-N-acetylhexosaminidase (EC 3.2.1.52) were most dominant.
CONCLUSIONS: The findings of this study suggest a possible pathogenic role, previously undescribed for *Bacteroides fragilis,* associated with human gastritis when concomitant esophageal pathology exists.

Molecular evolutionary analysis of adherence: evidence of positive selection operating AlpAB locus and horB adhesins of *Helicobacter pylori*

Andrés Julián Gutiérrez-Escobar[1]

[1]Grupo de Ciencias Básicas y Genética Humana Aplicada (GIBGA). Universidad de Ciencias Aplicadas y Ambientales-U.D.C.A, Bogotá, Colombia.

BACKGROUND: The adherence to the epithelial gastric cell is a key step in the physiopathology of *Helicobacter pylori* infection. There are several outer membrane proteins involved in the adherence that are expressed in a sequential fashion, meanwhile the attachment to the host cell takes place. Currently, the adhesins AlpA, AlpB and HorB have attracted the attention because of i) its role in the adherence, ii) The differential activation of intracellular pathways in the gastric host epithelial cell between Asian and European strains and iii) in its role in the fitness according to gene deletion studies. It is largely known that *H. pylori* have a global distribution with regional divergent populations that have evolved following host conditions.
METHODS: The software Muscle was use to align the DNA and protein sequences. The alignment was evaluated using Jmodeltest to detect evolutionary models that fix it. Then, we apply the tests MKT, permutation (FsT) and recombination from DNAsp5.0 to determine populational features of the adhesins. Finally, the software PhyML 3.0.1 was used for the phylogenetic reconstruction using 1000 boosttrap iterations and the package codeml from PAML 4.5 was use to detect positive selected sites.
RESULTS: it was identified that the evolutionary history of alpA, alpB and HorB adhesins followed a birth and death evolution model under purifying selection in which the deleterious mutations have been actively purged from the population allowing the fixation of beneficial changes for the fitness. It was detected based on permutation tests that *H. pylori´*s populations from America-Europe (FsT0.51395.) and Asia-Europe (FsT 0.46030) for alpA and the populations from America-Europe (FsT 0.43711) for alpB present a significant genetic differentiation mediated by positive selection as suggest by the MKT test results. Finally, it was found using PAML that a 9.51% of alpA, a 8.5% of alpB and a 5% of HorB proteins evolved under strong specific positive selection following linage specific geographic patterns.
CONCLUSIONS: It is possible that the adherence to the host cell mediated by AlpA/B and HorB adhesins can become in a new virulence marker for *Helicobacter pylori* infection.

Histone deacetylation inhibition and activation affects *Trypanosoma cruzi* replication, differentiation, infectivity and gene expression

Vanina A. Campo[1]

[1]Instituto de Investigaciones Biotecnológicas “Dr. Rodolfo Ugalde” (IIB-INTECH). Universidad Nacional San Martín (UNSAM), Campus Miguelete, Av. 25 de Mayo y Francia, San Martín, Buenos Aires, Argentina.

Background: Histones post-translational modification, mediated by histone acetyl transferases (HATs) and Histone deacetylases (HDACs) enzymes, is one of the most studied factors affecting gene expression. However, whether these enzymes affect gene expression in the human parasite *T. cruzi* has not been yet explored. In this study, four HDACs inhibitors and one activator for sirtuin deacetylases were used in order to assess their effect on the biology of the CL Brener strain from *T. cruzi*.
Methodology: HDACs inhibitors (HDACis) Trichostatin A, Apicidin, Sirtinol and Nicotinamide and the activator Resveratrol were used for treatment of infective and proliferative forms of the parasite at different concentrations. Western blot analysis using anti-acetylated H4 antibodies was performed to show changes in the amount of acetylated histones produced by these drugs. Changes on the abundance of specific transcripts involved in cell cycle, differentiation, chromatin association and histone acetylation were analyzed by real time PCR.
Results: All HDACis produced enrichment on acetylated histones while the opposite effect was observed with the activator Resveratrol on both proliferative and infective forms. Real time PCR analysis showed changes of more than two fold on trypomastigotes transcript abundance by treatment with Apicidin and Sirtinol. Also, this study describes for the first time the effect of Resveratrol on *T. cruzi* biology. This drug killed proliferative forms of the parasite, reduced infectivity of trypomastigotes and inhibited differentiation and/or replication of proliferative intracellular amastigotes.
Conclusions: The data presented showed that HDACis can differentially affect transcripts levels. HDACis anti-parasitic activity showed stage specific effects, being inhibition of differentiation to infective forms by Apicidin and Trichostatin A the most important. Also, sirtuins activation by Resveratrol caused stronger anti-parasitic effects than the inhibition with Sirtinol or Nicotinamide, making this drug an interesting candidate for further studies and sirtuins promising targets for development of therapeutic drugs.

Cruzipain expression in virulent and avirulent forms of *Trypanosoma cruzi* clone H510

San Francisco, Juan; Muñoz, Christian; Astudillo, Constanza; Gutiérrez, Bessy; González, Jorge[1].

[1]Molecular Parasitology Unit, Faculty of Health Sciences, University of Antofagasta, Antofagasta, CHILE.

BACKGROUND: *Trypanosoma cruzi* is the causative agent of Chagas disease. The parasite virulence depends among others on the strains or clone and several virulence factors have already described. Cruzipain, the major *T.cruzi* cisteine proteinase have been reported as a virulence factor involved on cell invasion and host immune resistance.

METHODS: To demostrate the role of cruzipain on virulence, we use the *T.cruzi* H510 clone. An alicuot of the H510 clone have been cultured on mice during 25 years, selecting a virulent forms of the clone named H510***vir***. The other, was cultured by 25 years on axenic mediun selecting an avirulent form (H510***avir***). Then, we evaluated the in vitro cell invasion of H510***vir*** and H510***avir*** in presence or abscence of anti-cruzipain antibodies or E 64d inhibitor. In the same way, cruzipain expression on H510***vir*** and H510***avir*** forms were evaluated by western blot and flow cytometry analyses. The expression of mRNA was evaluated by real time PCR. Finally the secretome of H510***vir*** and H510***avir*** was evaluated by immunoblot.

RESULTS: H510***vir*** was three times more infective to Vero Cells than H510***avir***. However, cell invasion of H510***vir*** was reduced by 60% when parasites were incubated in presence of anti cruzipain antibodies or inhibitor E64d. Western blot and flow cytometry analyses revealed that cruzipain was more expressed on H510***vir*** than H510***avir***. Cruzipain was secreted in higher ammount by H510***vir*** trypomastigotes. Finally, the expression of cruzipain mRNA was also higher in H510***vir*** than in H510***avir***.

CONCLUSION: Cruzipain is strongly associated with the *T.cruzi* virulence. However, chemical or immunological inhibition do not abolish cell infectivity.

Support: FONDECYT 1131007 (J.G)

Prevalence of surra among camels in Cholistan, Pakistan

Sonia Tehseen[ab], Muhammad Fiaz Qamar[c], Nusrat Jahan[a], Stijn Deborggraeve[d], Phillippe Buscher[d]

[a]. Government College University Lahore, Pakistan
[b] Higher Education Commission of Pakistan
[c] University of Veterinary and Animal Sciences, Lahore Pakistan
[d] Institute of Tropical Medicine, Antwerp, Belgium

BACKGROUND: Camel trypanosomosis (surra) adversely affects the health, productivity and working capacity of camels. *Trypanosoma evansi*, the causative agent, is principally transmitted by hematophagous flies (*Tabanus*, *Stomoxys*, *Chrysops*, *Haematopota*, *Lyperosia* and *Atylotus*).The Cholistan desert is an extension of the Great Indian Desert and stretches over an area of 26,330 Km^2. Out of a total population of 1.2 million camels in Pakistan, 80,000 live in the Cholistan desert with Berella and Mareecha as the two common camel breeds.

METHODS: A cross sectional study was carried out in three districts of the Cholistan desert, Rahimyar Khan, Bahawalpur and Bahawalnagar, to estimate the prevalence of *T. evansi* and to compare the diagnostic performance of the following tests: Giemsa stained thin smear, formol gel test, card agglutination test (CATT/*T.evansi*), immune trypanolysis (TL), ELISA/*T.evansi* and polymerase chain reaction (PCR) for *T. evansi* type A and type B. Packed cell volume (PCV) was also recorded.

RESULTS: From the 950 visited animals, 7 (0.7%) were positive in Giemsa stained thin smear, 42% were positive in the formol gel test. 400 specimens that were all positive in the formol gel tests were shipped to the Institute of Tropical Medicine (ITM) in Antwerp to be analysed in the TL, ELISA and PCR. With PCR as surrogate for parasitological testing, a molecular prevalence of 30% was recorded. None were positive in *T. evansi* type B. 48% were positive in immune trypanolysis. CATT was applied on 314 samples with 54% seropositives.

CONCLUSIONS: Based on the immune trypanolysis as reference test for *T. evansi* specific antibodies, Mareecha breed was found to be at a significantly ($p \leq 0.05$) higher risk for surra than Berella. No significant differences in seroprevalence were observed between females and males and between adults and non-adults.

More specimens will be shipped to ITM for further analysis, including ELISA.

Molecular characterization of two Colombian nematophagous fungi strains of *Arthrobotrys* genus and evaluation of their *in vitro* predatory activity against cattle gastrointestinal parasitic nematode infective larvae

Márquez-Lara, Dildo[1]; Cubides- Cardenas, Jaime Andrés[1], Patiño-Burbano, Rocío Esperanza[1]; Díaz, Diego[2]; Gómez-Sánchez, Yonattan[1](ŧŧ); Montero-Acevedo. Eva Karina; García-Castro, Fredy Enrique[1] Zuñiga- Lopez, Aldemar[1;] Mendoza de Gives, Pedro[3]

[1]Programa de Salud Animal CORPOICA, Centro de Investigación en Salud Animal –Ceisa– Tibaitatá, Km 14 vía Mosquera. Colombia[2] Practica veterinaria privada; [3]Laboratorio de Helmintología, CENID- Parasitologia Veterinaria, INIFAP, Paseo Cuaunahuac 8534, Jiutepec, Morelos, México

BACKGROUND: The resistance of ruminant gastrointestinal nematodes to chemical anthelmintic drugs has encouraged the development of sustainable non-chemical control strategies against these endoparasites. The use of nematophagous fungi is currently considered the most promising alternative tool over conventional control methods. It is important to identify the potential of native strains of nematophagous fungi in different regions worldwide. The aim of this study was genotyping two isolates of *Arthrobotrys* genus by PCR and also to evaluate their *in vitro* predatory activity against infective larvae of gastrointestinal nematodes (GIN) in cattle.
METHODS: Nematophagous fungi isolates were obtained from soil samples or feces of cattle and sheep. DNA extraction was performed by fractioning using heat shock and phenol chloroform. A simple PCR using selected species - specific primers, was performed followed by the ITS sequences. The *in vitro* predatory activity of fungal strains was performed by the interaction of 200 GIN larvae with 1 x 10^6 *Arthrobotrys oligospora* or *A. musiformis* conidia or chlamydospores; respectively into water agar plates.
RESULTS: Seventeen isolates belonging to *Arthrobotrys* genus were obtained; from which 13 strains were identified as *A. oligospora* and four strains as *A. musiformis*. The *in vitro* evaluation reveled 97.5%(± 2.9 SD) and 89.2% (±5 SD) predation average values; respectively. The ITS1 from 5.8S rDNA gene region allowed molecularly identifying these isolates.
CONCLUSIONS: This is the first record about autochthonous nematophagous fungi from Colombia and it is part of a national project focused to establish the potential use of these microorganisms for controlling cattle GIN in Colombia.

Spatial and temporal genetic variations of *Zeuxapta seriolae* on the yellowtail *Seriola lalandi* in the southeastern Pacific: implication for aquaculture

Sepúlveda, Fabiola [1,2]*; González, M. Teresa*[2]*.*

[1]Programa de Doctorado en Ciencias Aplicadas, mención Sistemas Marinos Costeros, Universidad de Antofagasta, Antofagasta, Chile, email: fabiolasepu@gmail.com.
[2]Laboratorio de Ecología Parasitaria y Epidemiología Marina LEPyEM, Instituto de Ciencias Naturales Alexander von Humboldt, Universidad de Antofagasta, Chile, email: teresa.gonzalez@uantof.cl.

BACKGROUND: *Zeuxapta seriolae* is a monogenean with worldwide geographical distribution, which is considered a serious pathogen in cultured species of *Seriola.* Along the southern Pacific (SEP), individuals of *S. lalandi* annually approaches at summer time in the Northern Chilean coast showing 70% of prevalence of this parasite. Because the origin of these fish and of the parasite infestation are unknown, we evaluate whether *Z. seriolae* present spatial and/or temporal genetic variations.
METHODS: Fragments of 758 bp of the mtDNA cytochrome c oxidase subunit I gene were sequenced for 148 individuals collected from three localities along the northern coast of Chile (23° to 30°S) and one from Juan Fernandez Island (JF, 33°S) between 2012 and 2014.
RESULTS: A total 39 haplotypes were detected. Of these, 28 were unique haplotypes. The haplotype diversity was 0.877 and nucleotide diversity was 0.0062. There were genetic differentiation between parasites from coast and JF (AMOVA, Fst= 0.119, p< 0.05; Fsc= 0.098, p< 0.05), but not among years (Fct= -0.0229, p= 0.605). Neutrality tests and mismatch distribution analyses indicated stable populations of *Z. seriolae*.
CONCLUSIONS: Two populations of *Z. seriolae* were detected in the SEP. Oceanographic barriers and short generational time of parasites limiting egg and larvae dispersal can explain the genetic heterogeneity of this species. Our results demand to study the population parameters for the different parasite populations in order to understand their infestation dynamics.
Funded by FONDECYT 1130629

Antihelmintic resistance in bovine gastrointestinal nematodes from Cundinamarca and Boyaca Municipalities (Colombia)

Márquez-Lara, Dildo[1]; García-Castro, Fredy Enrique[1]; Jiménez-Pallares, Gabriel Virgilio[2], Cubides-Cárdenas, Jaime Andrés[1], Zuñiga- Lopez, Aldemar[1].

[1]Programa de Salud Animal CORPOICA, Centro de Investigación en Salud Animal – Ceisa– Tibaitatá,Km 14 vía Mosquera.[2] Practica veterinaria privada.

BACKGROUNDS: The faecal egg count reduction test (FECRT) is the main method of detection of anthelmintic resistance (AR) in nematodes of veterinary importance. Guidelines of how to conduct a FECRT were published by the World Association for the Advancement of Veterinary Parasitology (WAAVP) and it is considered as standard method worldwide. This study was conducted to determine AR in gastrointestinal nematodes (GIN) of cattle in Colombia.
METHODS: Nematode anthelmintic resistance was determined in 40 calves, three to 12 months of age, allotted to four groups of ten animals each: 1) control (untreated); 2) Bovex® 25% (5 mg/kg); 3) Ivomec® 1% (0.2 mg/kg) and 4) Ripercol® 18.8% (1 mg/kg). The faecal egg count reduction test (McMaster technique) was used to detect the presence of resistance. Resistance was determined when the FERCT was below 95% and the lowest 95% confidence interval limit was 90. FECRT = 100 [1 – (T2 / T1 x C1 / C2)].
RESULT AND DISCUSION: Anthelmintic resistance was found in 25% of the farms: Bovex® 25% and Ivomec® 1% resistance was detected in 17% and 8% of the farms, respectively. Anthelmintic resistance against the two assessed compounds was identified in *Cooperia spp.* The mean reductions in the egg fecal counts (epg) after treatments were recorded as follows: Ripercol® 1%,=97.89% (± 6%, range 66% - 100%); Bovex® 25%=95.9% (± 9.3%, range 51% - 100%) and Ripercol® 18.8%l,= 99.4% (± 0.92%, range 97.6 - 100%). No Levamisol resistance was recorded.
CONCLUSIONS: This is the first record about AR in GIN in Colombia using an *in vivo* assay in Cattle. This study conducted in 36 cattle farms reveled that the use of chemical anthelmintic drugs is the unique method of control used in Cundinamarca and Boyaca municipalities of Colombia.

Dissecting the transcriptional regulation of Plasmodium development

Manuel Llinás

W126 Millennium Science Complex, Department of Biochemistry and Molecular Biology and the Center for Malaria Research, The Pennsylvania State University, State College, PA, USA 16802

Until recently, the study of transcriptional regulation in the malaria parasite *Plasmodium falciparum* has advanced slowly due to a lack of recognizable transcription factors. With the identification of the Apicomplexan AP2 protein family (ApiAP2) of DNA binding proteins, there has been a resurgence in activity in this area. My laboratory has been using a variety of whole-genome approaches coupled with biochemistry, to characterize the role of the ApiAP2 family of transcriptional regulators during parasite development. Our results have identified genome-wide DNA binding targets for specific members of the ApiAP2 protein family, which we are using to guide efforts to assign regulatory roles to these proteins. In recent work we have characterized several of these factors in detail and have identified a master regulator of sexual stage commitment which demonstrates the first transcriptional-driven developmental switch in protozoan parasites. In this talk I will describe how we are integrating genome-wide gene expression data with specific studies focused on individual ApiAP2 proteins to better understand transcriptional regulatory networks in the Plasmodium parasite.

Insights from Plasmodium metabolomics

Manuel Llinás

W126 Millennium Science Complex, Department of Biochemistry and Molecular Biology and the Center for Malaria Research, The Pennsylvania State University, State College, PA, USA 16802

The genome of *Plasmodium falciparum* indicates that the metabolic pathways utilized by this organism are highly unique. Recent efforts to comprehensively examine the biology of *P. falciparum* have focused on transcriptome and proteome analysis to gain insight into *Plasmodium*-specific pathways. The third crucial component that remains to be established is the metabolome: the complement of small-molecule metabolites and their relative levels. Our lab has begun to characterize various aspects of parasite metabolism using high accuracy mass-spectrometry to simultaneously measure metabolites from complex cellular extracts from parasite-infected cells. The approaches we are using allow us to assay various aspects of the *P. falciparum* metabolome. One approach has been to examine the interaction of *Plasmodium* with the host red blood cell using targeted measurements of specific metabolites shared with the host erythrocyte, and we are using ^{13}C and ^{15}N isotopic labeling experiments to directly trace flux through known biochemical pathways. We are also using metabolite measurements to map genetic control of metabolism by assaying global metabolite patterns in the parents and progeny of a *Plasmodium falciparum* genetic cross. Finally, we have begun to profile the metabolic fingerprint of hundreds of current and future antimalarial compounds, which originate from several recent massive drug screens, to characterize their mode of action. Results from these studies are beginning to unravel the divergence of metabolism in *P. falciparum* and promise to provide unique avenues for future drug intervention strategies.

Fasciola hepatica antigens in the inhibition of inflammatory responses

Cervi L. Clinical Biochemistry Department, CIBICI, Faculty of Chemical Science, National University of Córdoba, Córdoba, Argentina.

Dendritic cells (DC) represent a highly specialized antigen presenting cells, whose main role is to sense infections in tissues or dangers signals and to contact with T lymphocytes in lymph organ, to develope effector or tolerogenic immune responses.
It has been shown that both immature and semi-mature DC have been associated with tolerance induction through the generation, among others, of regulatory T cells. Products from helminth parasites, after being recognized by innate immune cells, are capable of inducing several changes, such as the down-regulation of pro-inflammatory cytokines in TLR ligand maturated-DC.
In this work, we tested a cell-based preclinical strategy for collagen induced arthritis (CIA) using DC stimulated with helminth antigens plus a TLR ligand. Among different TLR ligands used in combination with a total extract of *F. hepatica* (TE), CpG was the most efficient stimulus to induce tolerogenic properties in DC. DC treated with TE plus CpG (T/C-DC) displayed an activation phenotype with a moderate production of pro-inflammatory cytokines, high levels of anti-inflammatory cytokines and IDO expression. The treatment of DBA/1J mice with collagen II pulsed T/C-DC reduced the severity and incidence on the CIA symptoms, and also prevented joint damage by a mechanism depending on Foxp3 cells.
Among the proteins present in TE, we found that a low molecular weight fraction (lower than 10 kDa), was able to decrease the TLR-initiated mice and human DC maturation, diminishing the capacity of these cells to initiate allogenic responses. More importantly, we established a protease inhibitor Kunitz type as the principal molecule responsible for suppressed pro-inflammatory cytokine production in LPS-activated DC, printing tolerogenic features on DC to reduce inflammatory responses. Collectively, we allocate a modulatory role for this protein, which could be involved in immune evasion mechanisms by the parasite.

Relationships of genetic diversity with geographical spread in *Panstrongylus megistus* (Hemiptera: Triatominae), the main vector of Chagas' disease in Brazil

Bargues, María Dolores[1]; Klisiowicz, Debora[2]; Mateo, Lucia[1]; Chagas, Vivian[1]; Artigas, Patricio[1]; Cavassin, Francelisse Bridi[2]; Mas-Coma, Santiago[1]

[1]Departamento de Parasitología, Facultad de Farmacia, Universidad de Valencia, Av. Vicente Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain
[2]Departamento de Patologia Básica, Setor de Ciências Biológicas, Universidade Federal do Paraná, Centro Politécnico, Curitiba, Paraná, Brazil

BACKGROUND: In Chagas' disease, the study of the genetic characteristics of triatomine vectors is the main tool for epidemiological control, because of the absence of effective drugs. Studies were made on populations of the triatomine species *Panstrongylus megistus*, the most important vector of Chagas' disease in Brazil since *Triatoma infestans* eradication.
METHODS: The nuclear ribosomal DNA intergenic region, comprising the complete sequences of the internal transcribed spacers ITS-1 and ITS-2 plus the 5.8S gene, were studied. Specimens came from 26 localities of different states of Brazil.
RESULTS: The whole intergenic region was a mean of 1,516.9 bp in length, providing 26 combined haplotypes, 21 of ITS-1 and 12 of ITS-2, showing ITS-1 (3.00%) more nucleotide differences than ITS-2 (1.33%). The microsatellites found were useful to study the interpopulation exchange and potential recolonizations after elimination by control implementation. Network and phylogenetic analyses shows the old origin of *P. megistus*, suggesting that the State of São Paulo may be considered one of the spreading centres. Results suggest that the diversification is still ongoing today by geographical isolation of populations of this species.
CONCLUSIONS: There is a relationship of the genetic diversity with the geographical spread characterizing this vector. This explains its ability to colonize distant areas and different ecotopes, including dwelling reinvasion phenomena, after insecticide control action, in human habitats, and consequently its importance in Chagas' disease epidemiology.

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

Detection of medically and veterinary importance filarial parasite *Brugia malayi* in dogs in Sri Lanka

Kuruppu-Sarath[1], *Wickramasinghe-Susiji*[2], *Samarasekara-Dilhani* [3], *Rajapakse-R.P.V.J.* [4]

[1]Public Health Veterinary Service, Ministry of Health, Sri Lanka; [2]Department of Parasitology, Faculty of Medicine, University of Peradeniya.; [3]Anti Filariasis Campaign, Ministry of Health, Sri Lanka.; [4]Department of Veterinary Pathobiology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya.

BACKGROUND: Lymphatic filaraisis (LF) is a parasitic disease that causing long term and permanent disability in affected humans. *Wuchararia bancrofti* and *Brugia malayi* are mainly responsible for LF in the world. Brancoftian filariasis is the most predominant form present in Sri Lanka. Recent investigations by Anti Filariasis Campaign, Ministry of Health, Sri Lanka revealed that the occurrence of many Brugian filariasis cases. Dogs and cats may be acting as reservoir animals for *B. malayi* in Sri Lanka and this was suggested by Dissanaike in 1979.

METHODS: The survey was carried out to test dogs for microfilaria (mf) in Western Province in Sri Lanka. In addition, we have identified the microfilarial species present in dogs and also determined the rate of mf infection. 570 blood samples were collected from 400 domestic dogs and 170 stray and community dogs in the Western Province. First, all the samples were screened for mf in wet film. Then, Giemsa staining was performed for mf positive samples. Then we performed multiplex PCR and real time PCR for the selected samples using species specific primers to determine the species.

RESULTS: Within 570 samples, 39% of dogs had mf (25% unsheathed and 14% sheathed) in their blood. Furthermore, 44% and 23% of stray and community dogs and domestic dogs had sheathed mf respectively. Morphologically, those sheathed mf were similar to *B. malayi*. Interestingly, molecular investigation clearly showed that some of the dogs enrolled in this study had a *B. malayi* infection.
CONCLUSIONS: Therefore, the present study indicated that the possible role of dogs as a reservoir animal for *B. malayi* in Sri Lanka for the first time. Thus, general public living in the western province are at high risk of getting LF filariasis.

ICOPA 2014 - Abstract

The Leishmaniases: update on the Global epidemiological overview

Argaw-Dagne, Daniel[1], *Ruiz-Postigo, José Antonio*[1], *Jannin, Jean*[1]

1Department of Control of Neglected Tropical Diseases, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland.

BACKGROUND: Leishmaniasis is a vector-borne disease caused by a protozoan parasite transmitted by the bite of infected sandflies. The disease is endemic in over 98 countries in one of the three major forms; cutaneous, mucocutaneous or visceral.

METHODS: As part of updating the Global leishmaniasis epidemiological information for WHO Global Health Observatory, structured questionnaires filled by the National Programmes, surveillance data reported to WHO and the WHO publication(2012) on the global incidence estimates were reviewed.

RESULTS: The annual incidence of visceral leishmaniasis (VL) worldwide is estimated at 300,000 versus the reported 58,000; whereas cutaneous Leishmaniasis is 1million cases versus the reported 220,000. Six countries, Bangladesh, Brazil, Ethiopia, India, south Sudan and Sudan account for over 90% of the global VL burden. Cutaneous leishmaniasis is much more widely distributed in the Americas, eastern Mediterranean region, middle east extending to western and central Asia.

Mortality data are scant and largely derived from health institution data. The reported case-fatality rate for VL in 2011 was in Brazil(8.4%), Ethiopia(6%), south Sudan (5%).

Epidemics of VL has also affected many endemic countries in the last 10 years. South Sudan was the most afflicted in the period 2009 to 2012 where over 28,000 cases and 850 deaths were reported.

Increasing trend of HIV/VL coinfection have been reported from Brazil and Ethiopia. In Brazil, the reported *coinfection* rate increased from 2.5% (2005) to 6.6% (2011). In Ethiopia, it increased from 19% during 1998/1999 to 34% during 2006/2007. However, the introduction and scaled up implementation of antiretroviral treatment has helped to reduce incidence of VL and increase survival of coinfected patients in many countries.

CONCLUSIONS: The WHO/NTD roadmap has clearly stipulated the leishmaniasis control or elimination strategic milestones for the different epidemiological zones of the WHO regions. It is envisaged to reduce the incidence of VL to less than one case per 10,000 population per year at sub-district level in South East Asia by 2020. In the other regions targets have been set up to control all forms of the disease. In order to achieve the targets, a concerted effort is required, accessing remote endemic areas by scaling-up service delivery, enhancing the surveillance system and ensuring national ownership.

Molecular diagnosis and characterization of Giardia duodenalis in Colombian children suggest the predominance of Assemblage B

Ramírez, Juan David[1]; Heredia, Ruben Darío[2]; Hernández, Carolina[1]; Moncada, Ligia Inés[2]; Reyes, Patricia[2]; López, Myriam Consuelo[2]; Pinilla, Análida Elizabeth[3]

[1] Facultad de Ciencias Naturales y Matemáticas, Universidad del Rosario, Bogotá – Colombia; [2] Departamento de Salud Pública, Facultad de Medicina, Universidad Nacional de Colombia, Bogotá – Colombia.; [3] Facultad de Medicina, Universidad Nacional de Colombia, Bogotá – Colombia.

BACKGROUND: Giardiasis is a parasitic infection that affects around 200 million people worldwide. This parasite presents a remarkable genetic variability observing 8 genetic clusters named as ´assemblages´ (A-H). These assemblages are host restricted and maybe zoonotic observing that A and B infect humans and animals around the globe. The knowledge of the molecular epidemiology of human giardiasis in South-America is scarce and also the usefulness of the molecular diagnosis of this pathogen in fecal samples remains controversial. The aim of this study was to conduct a cross-sectional study to compare the molecular targets employed for the molecular diagnosis of Giardia DNA and to discriminate the parasite assemblages circulating in the population.
METHODS: We analyzed 181 fecal samples from Children at La Virgen, Cundinamarca, Colombia that were DNA-extracted and analyzed by SSU rDNA, tpi and gdh loci.
RESULTS: We observed prevalence by microscopy of 13% and by PCR around 76-80% depending on the molecular marker. Additionally, a lack of statistical concordance between microscopy and PCR was detected. Regarding the genetic assemblages, we observed assemblage A (3%), assemblage B (90%) and mixed infections assemblages A+B (7%). Hence, the sub-assemblages were typed observing AI, AII and BIV across the population.
CONCLUSIONS: This study represents a reliable attempt to understand the molecular epidemiology of giardiasis in Colombia and the use of PCR to detect cryptic infections. The epidemiological implications are herein discussed.

Insecticidal Effect of Oil extracts from African Basil leaf (*Ocimum gratissimum*) and Neem seeds *(Azadirachta indica)* on *Aedesaegypti* mosquito in South East in Nigeria.

Nwaorgu– Obioma[1]; Chukwuzoba – Obinna Anthony[1]

Department of Parasitology and Entomology Nnamdi Azikiwe University, Awka, Anambra State- Nigeria.

BACKGROUND**.** Mosquito borne diseases which causes thedeath of least 2 million people annually is a serious public health problem especially in Sub-Sahara Africa. Measures targeted at their control have relied on synthetic pesticides which have resulted to development of resistance, high levels of pesticides residues, environment pollution among others, hence the need for effective biodegradable pesticides.Recently, use of environmental friendly, biodegradable, natural, user friendly, effective and inexpensive botanical insecticides is gaining ground. In this study, the mosquitocidal action/repellent effect of various solvent concentrations of oil extracts from *Azadirachta indica* seeds and *Ocimum gratissimum* leaves on *Aedes**a**egypti* were assessed

METHODS**.** Acetone was used for extraction of oil from samples of *Ocimum* leaves and neem seeds. This was made into formulations of different concentrations (10%, 5%, 2.5% and 1.25%). Repellency and mortality tests were carried out with adult *Aedes* mosquitoes reared from pupae stage using the concentrations of these formulations. Resultswere read at minutes and hourly intervals for repellency and mortality respectively.

RESULTS**.** Results showed that at concentration of 100µl/ml, neem and *Ocimum* extracts induced 100% and 65% mortality after 24 hours respectively whilethe lowest concentration (12.5µl/ml) recorded 25% and 70% mortality for *Ocimum* and neem respectively. With regard to repulsion, 100% and 80% were recorded for neem and *Ocimum* respectively at 100µl/ml concentration after 15 minutes of exposure while the lowest conc. (12.5µl/ml) recorded 45% and 55% repulsion for *Ocimum* and neem respectively. Probit analysis indicates that smaller dosage of neem seed oil would be preferable when compared with *Ocimum* leaf oil for mosquitocidal and repulsive actions against *Aedes aegypti* respectively.

CONCLUSIONS**.** Study therefore confirms that locally grown *Ocimum* and neem have repulsive and mosquito cidalpotentials for use in control of nuisance and adverse health impact of mosquito bite.

Deciphering the biological properties of an enigmatic *Trypanosoma cruzi* I domestic genotype (TcI_{DOM})

Cruz, Lissa[1]; Vivas, Angie[1]; Montilla, Marleny[2]; Hernández, Diana Carolina[3]; Flórez, Carolina[2]; Parra, Edgar[4]; Ramírez, Juan David[1]

[1]Universidad del Rosario, Bogotá, Colombia.

[2]Grupo de Parasitología, Instituto Nacional de Salud (INS), Bogotá, Colombia.

[3]Red Chagas Colombia, Instituto Nacional de Salud (INS), Bogotá, Colombia.

[4]Grupo de patología, Instituto Nacional de Salud (INS), Bogotá, Colombia.

BACKGROUND: Chagas disease is an endemic zoonosis in Latin America and caused by the parasite *Trypanosoma cruzi*. *T. cruzi* I presents the broadest geographical distribution in the continent. Recently, a particular genotype associated to human infections has been reported and named as TcI_{DOM}. Hence, the aim of this study was to untangle the biological features of these pathotypes in murine models.

METHODS: We infected ICR-CD1 mice with five TcI strains (two domestic, two sylvatic and one natural mixture) and determined the course of infection during 91 days (acute and chronic phase of the disease) in terms of parasitemia, qualitative Real Time PCR, immune response (IgG titers) and tissue invasion by means of histopathology studies.

RESULTS: Statistically significant differences were observed in terms of parasitemia curves and prepatent period between domestic (TcI_{DOM}) and sylvatic strains. There were no differences in terms of IgG antibodies response across the mice infected with the five strains. Regarding the histopathology, in the acute phase, all the mice presented myocarditis, myositis and amastigotes infiltration. One mouse infected with the sylvatic strain presented encephalitis and myoenteritis. Our results indicate that domestic strains present higher parasitemias and low levels of histopathological damage

CONCLUSIONS: These results highlight the sympatric and behavioural differences of domestic and sylvatic TcI strains and provide a light for the understanding of the pathogenesis and virulence features of TcI strains as responsible of severe forms of cardiomyopathies in South-America.

Blastocystis subtype 3 (Allele 34) is associated with urticaria in Argentinean patients

Casero, Daniel Rodolfo[1]; Mongi, Florencia[1]; Ramírez, Juan David[2]

[1]Departamento de Parasitología del Hospital Nacional de Clínicas, Facultad de Ciencias Médicas, Universidad Nacional de Cordoba, Argentina; [2]Unidad Clínico-Molecular de Enfermedades Infecciosas (UCMEI), Facultad de Ciencias Naturales y Matemáticas, Universidad del Rosario, Bogotá-Colombia.

BACKGROUND: Blastocystis is an enteric protist linked to gastrointestinal and skin disorders. This protozoan exhibitsremarkable genetic diversity and multiple subtypes (ST). However, the knowledge of the distribution of STs in South-America is scarce. Therefore, we aimed to determine the *Blastocystis* subtypes circulating in Argentinean patients with urticaria.
METHODS: To determine the Blastocystis STs association with pathologies, fecal samples were submitted to DNA extraction, PCR, sequencing and ST identification according to DNA barcoding as previously reported.
RESULTS: ST analyses, revealed ST1 (11%), ST2 (8%), ST3 (78%) and ST6 (2%). No association between Blastocystis ST and clinical symptomatology was detected (p=0.5). Blastocystis 18S database retrieved the following alleles: 2 and 4 (ST1), 9 (ST2) , 122 (ST6), 134(ST3) for patients with non-like urticaria symptoms and 34 (ST3) only detected in a cohort of Argentinean patients presenting urticaria.
CONCLUSIONS: This represents the first report of Blastocystis populations/subtypes and urticaria in South-America.

13th INTERNATIONAL CONGRESS OF PARASITOLOGY
AUGUST 10th - 15th, 2014
ICOPA XIII MÉXICO

Auricular specific tropism of wild genetically distant *T. cruzi* isolates is associated with ECG alterations during acute infection

Mijares A[1], Alvarado A[2], Colmenárez V[2], Noriega J[2], Marques J[3], Carrasco HJ[3], Mendoza I[3], Rodríguez-Angulo H[1]*

1 Instituto Venezolano de Investigaciones Científicas, Caracas 1020A, Venezuela. **2** Universidad Centroccidental "Lisandro Alvarado", Facultad de Veterinaria, Cabudare 3023, Venezuela. **3** Universidad Central de Venezuela, Instituto de Medicina Tropical, Caracas 1040, Venezuela.

Background: Chagas disease is characterized by myocarditis and arrhythmias. The clinical outcome in acute outbreaks is highly variable and the causes are largely unknown. The aim of this work was to analyze association among cardiac tropism from strains belonging to DTUs TcI and TcIV with ECG and serological alterations. **Methods and Results**: Balb/c mice were infected with *T. cruzi* isolates (P1 and P2) obtained from wild specimens of *Panstrongylus geniculatus*. Parasitemia and survival were determined during the first 21 days post-infection and ECG, blood, skeletal muscle and heart samples were taken. P1 isolate showed a higher parasitemia and mortality than P2. An increment of PR and QTc interval, ectopic beats, retrograde conduction and increasing of serological markers of cardiac damage was observed in P1 infected mice. Analysis of heart and muscle sections showed a higher inflammation for P1 isolate, especially in skeletal muscle. Interestingly, myocarditis was principally located at auricles and AV region, a fact that can be associated with the ECG alterations observed. **Conclusions**: Our results suggest for first time that the strain-specific auricular pattern of cardiac invasion and inflammation may be involved in the ECG variations observed, giving insights about the mechanisms of arrhythmias in Chagas disease.

Antigenicity and immunogenicity of a novel chimeric protein antigen based on the *Fasciola hepatica*

Hernández-Guzmán, Karina[1], *Vallecillo, Antonio Javier*[2]; *Sahagún-Ruiz, Alfredo*[3]; *Cruz-Mendoza, Irene*[1]; *Quiroz-Romero, Héctor*[1]

[1]Departamento de Parasitología, Facultad de Medicina Veterinaria y Zootecnia, [2]Departamento de Inmunología, Instituto de Investigaciones Biomédicas, [3]Departamento de Microbiología e Inmunología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, C.P. 04510, México D.F., México.

BACKGROUND: Leucine aminopeptidase (LAP) is an exopeptidase located in epithelial cells in the digestive tract of *Fasciola hepatica* and helps catabolise the peptides generated by protein degradation, whereas cathepsin L1 (CL1) is an endoprotease involved in the evasion of the immune system and helps parasites migrate and obtain food.
METHODS: We developed a chimeric protein (rFhLAP-CL1) that includes the most antigenic regions of LAP and CL1 and part of the catalytic domain of both enzymes; the sequences were joined by overlapping PCR, and the product was subcloned into the pET15b/Rosetta system to express a chimeric protein of ~25 kDa. Antigenicity of rFhLAP-CL1 was tested using cattle sera and its immunogenicity was confirmed in a rabbit model.
RESULTS: Chimeric protein has three consensus epitopes: DGRVVHLKY at the 54-62 position, VTGYYTVHSGSEVELKNLV at position 119-137, and YQSQTCLPF at positions 161-169. Antigenicity of the chimeric rFhLAP-CL1 protein was demonstrated by western blot assays, as the rFhLAP-CL1 was recognised by antibodies from cattle naturally infected by the trematode (100%); the chimeric protein formulated with incomplete Freund adjuvant induced stronger antibody responses on rabbits, produced specific antibodies with a final titre of 1:10,000.
CONCLUSIONS: The chimeric protein rFhLAP-CL1 was recognised when tested against naturally induced cattle antibodies and was able to induce significant immunogenicity in rabbits. Further evaluation of this chimeric protein in fascioliasis diagnosis as well as protective efficacy against parasite challenge of the vaccine candidate must be conducted.

Anthelmintic potential of *Calotropis procera, Azadirachta indica* and *Punica granatum* against *Gastrothylax indicus*

Aggarwal, Rama[1]; Bagai, Upma[1]

[1]Department of Zoology, Panjab University Chandigarh, 160014, India

BACKGROUND: Because of increasing development of anthelmintic resistance and limited availability of commercial drugs, there is a growing interest in the ethno-veterinary approach to examine traditionally used plants by local farmers. An assessment of cytotoxic potential is necessary to ensure a relatively safe use of medicinal plants.
METHODS: Ethanolic and aqueous extracts of *Calotropis procera, Azadirachta indica* and *Punica granatum* were prepared and were subjected to phytochemical screening. Anthelmintic activity of extracts in comparison with albendazole was evaluated through *in vitro* studies using worm motility inhibition assay on *Gastrothylax indicus*. LC 50, percent worm motility inhibition (%WMI) and mean mortality index (MI) were determined for all the plant extracts along with albendazole. Various concentrations (5 - 5000µg/ml) of all the plant extracts and albendazole were used to detect their cytotoxic effects against Hela cell line to determine CC 50 by MTT assay.
RESULTS: Phytochemical analysis revealed the presence of phenols, alkaloids, saponins, tannins, flavonoids, steroids and triterpenoids. Significant anthelmintic effects ($p<0.0005$) were observed on live *G. indicus* worm. LC 50 values were determined to be 12.05 µg/ml ±3.24 and 23.52 µg/ml ±6.4 for *C. procera*, 24.37 µg/ml ± 4.11 and 21.02 µg/ml±4.6 for *A.indica*, 18.92 µg/ml ±4.54 and 24.43 µg/ml ±6.96 for *P. granatum* ethanolic and aqueous extracts respectively, whereas it was 29.23 µg/ml ±4.51 for albendazole.MI ranged from 0.73 to 1.0 and %WMI was observed to be between 70% and 100% for various extracts.CC 50 values, of all the plants extracts were determined to be >1000µg/ml and for albendazole it was found to be > 10 µM.
CONCLUSIONS: All the three plants can be potential source for novel anthelmintics and were found to be safe for medicinal use.

Detection of anti-*Trypanosoma cruzi* antibodies at a blood bank in the Yucatan Peninsula

Concha-Valdez, Fanny[1,2]*; Cañas-Ruíz, Rocío*[1]*; Sosa- Matú, María Cecilia*[3]*; Gómez-Herrera, David*[1]*; Marín, Clotilde*[1]*; Escobedo-Ortegón, Javier*[2]*; Barrera-Pérez, Mario*[2]*; Sánchez-Moreno Manuel*[1]

[1] Departamento de Parasitología. Facultad de Ciencias. Universidad de Granada. España.

[2] Centro de Investigaciones Regionales "Dr. Hideyo Noguchi". Universidad Autónoma de Yucatán. México

[3] Banco de Sangre del Hospital General Regional No. 17 del Instituto Mexicano del Seguro Social de Cancún Q. Roo, México.

Background. *Trypanosoma cruzi*, the causal agent of Chagas disease in humans, is a widely spread protozoan in Latin America. It is endemic in more than 21 countries of the continent. Chronic infected people are asymptomatic during an indeterminate stage but can represent a significant risk of transmission due to blood donations and organ transplantation. Blood transfusion is recognized as the second most important way for the transmission of Chagas disease; on the other hand, it can be recognized as the main important route in industrialized countries. The aim of the present work was to detect anti-*T. cruzi* antibodies in blood donors in the Yucatan Peninsula.

Methods. We implemented 5 serological diagnostics in 969 individuals; two in-house ELISA were used: a parasite lysate (ELISA-H) as was the semi-purified superoxide dismutase excreted by *T. cruzi* (ELISA-SODe), Western blot against the same antigen (WB-SODe), Indirect Immunofluorescence (IIF), and one commercial test.

Results. The serological tests results showed a seroprevalence range of low to high from 5 (0.51%) donors by the commercial ELISA (Chagas ELISA IgG+IgM) and 19(1.96%) by IIF, 39(4.02%) detected by ELISA-H; 114 (11.76%) by WB-SODe and 148 (15.27%) as the highest seroprevalence by ELISA-SODe. The evaluation of the reliability of the ELISA SODe in the diagnosis of Chagas disease showed a sensitivity of 100% and specificity of 86.42%.

Conclusions. The excellent sensitivity and good specificity of SODe antigen for the detection of anti-*T. cruzi* antibodies in donors lead us to confirm that the serological test performed with this biomarker would be a helpful test for screening as confirmatory test for Chagas disease.

Phylogenetic analysis of eleven species of monogenean parasites (Dactylogyriidae) from marine cat fishes (Siluriformes: Ariidae) using 28S r DNA, off Visakhapatnam Coast, Bay of Bengal

Ummey Shameem; Illa Krishnaveni

Department of Zoology, Andhra University, Visakhapatnam, 530 003, A.P., INDIA

BACKGROUND: Monogenean parasites, one of the largest group of ectoparasites found on fishes are highly host specific and exhibit species diversity with morphological similarity. Because of their close similarities in morphological characters, studies involving molecular characterization is essential to through light on their proper identification and in assigning phylogenies to understand the processes of parasite speciation and diversification.
METHODS: A total of 818 fishes (*A. jella* - 702 and *A. dussumieri* – 116) were examined with a sample collection of 40 – 50 fish per month and all the monogenean parasites were isolated from gill washings of host under a stereo zoom microscope. They were preserved in 100% ethanol and stored at -20°C until further use for molecular studies. They were identified based on detailed study of morphological characteristics. All species were processed for molecular studies involving DNA isolation, PCR amplification, and sequence analysis. After quantification and purification, the DNA was amplified by PCR using the Primer F (5′ - ACCCGCTGAATTTAAGCAT- 3′) and R (5′- TCCGTGTTTCAAGACGG- 3′). PCR amplification was performed by following protocol in a final volume of 20µl PCR reaction.
RESULTS: Altogether 11 species were identified distributed under three genera with 5 species under Hamatopeduncularia, 4 species under Chuhanellus (Dactylogyriidae) and one species each under Neocalceostoma and Thysanatohaptor (Neocalceostomatidae). Four species, two each under Hamatopeduncularia and Chuhanellus were considered as new. Genus *Thysanatohaptor* was erected and reported for the first time as species *Thysanatohaptor rex.* Phylogenetic studies using 28S rDNA and the BLAST analysis revealed the closely related nature of these parasites, as they formed a single cluster in the phylogenetic tree in comparison with other species of monogeneans parasites, in lieu with the findings obtained through morphological studies. The Bayesian inference analyses with the Transition Model revealed same tree topology as obtained by Maximum Parsimony, Maximum Likelihood methods. All the sequences were deposited in Gene Bank and accession numbers were assigned.
CONCLUSIONS: The evolutionary relationship of families Dactylogyriidae and Neocalceostomatidae was resolved properly by Maximum Parsimony, Maximum Likelihood and. Neighbour-joining methods. The present study yielded a well resolved phylogenetic relationship between monogenean species within each family and is in congruence with morphological classification of Boeger and Kritsky (2001).

Development of a high-throughput sequencing method to identify Anisakidae species in fish

Seesao, Yuwalee[1-2]; Audebert, Christophe[3-4]; Gay, Mélanie[1]; Thébault, Anne[5]; Verrez-Bagnis, Véronique[6]; Sophie, Merlin[3-4]; Odile Bourgau[1]; Marc, Jérôme[6]; Isabelle, Cos[7]; Cuzzucoli Diane[5]; Le Fur, Bruno[7]; Viscogliosi, Eric[2]; Dupouy-Camet, Jean[8]; Dei-Cas, Eduardo[2]; Aliouat-Denis, Cécile-Marie[2].

[1]ANSES-Agency for Food, Environmental and Occupational Health and Safety, Boulogne-sur-Mer, France, Yuwalee.SEESAO@anses.fr; [2]IPL-CIIL-BDPEE, Univ Lille Nord de France, France; [3]Gènes Diffusion, Douai, France; [4]PEGASE-Biosciences, Institut Pasteur de Lille, France; [5]ANSES-DER-UMA-MSA, Maisons-Alfort, France; [6]IFREMER-BRM-EM[3]B, Nantes, France; [7]Innovation platform Nouvelles Vagues, Boulogne-sur-Mer, France; [8]Cochin Hospital, Paris Descartes University Paris, France;

BACKGROUND: To better define the impact of fish parasites of the Anisakidae family on consumers' health as well as to improve the safety of fish products, we set up the French national Fish-Parasites network (ANR-10-ALIA-004) to sample fish, collect Anisakid nematodes and analyze prevalence data.
METHODS: A total of 290 fish (11 species) were sampled (Channel, North sea, Mediterranean sea, Bay of Biscay and Ireland sea). Nematodes were collected from corporal cavity, liver and fish fillets and gDNA was extracted in pool. A consensus primer pair was designed to target the *COX2* gene of *Anisakis*, *Contracaecum*, *Pseudoterranova* and *Hysterothylacium* genera. Parasites were identified using high-throughput sequencing (PGM Ion Torrent, Life Technologies). An automated analysis workflow (Galaxy) compared the *COX2* sequences with those of a reference database.
RESULTS: High-throughput sequencing allowed the identification of 190,451 nematodes. Six runs were necessary to get all the *COX2* sequences. The automated sequence identification placed the *Anisakis* genus as the most prevalent (99%). The species *A. simplex* represented 95%. Other *Anisakis* species (*A. pegreffii*, *A. paggiae*, *etc.*) added up to 2.7% and 1.3% corresponded to undefined species. The other genera were also identified (below 1%).
CONCLUSIONS: A high-throughput sequencing technology was successfully applied to quickly and reliably establish the Anisakid species proportion in fish.

Assessesment of the antiparasitic efficacy of curcumin in rabbits

Cervantes-Valencia, María Eugenia[1]; Alcalá-Canto, Yazmín[1], Ducoing-Watty, Adriana Margarita[2]

[1] Department of Parasitology, FMVZ-UNAM, 04510. México City
[2] Department of Genetics and Biostatistics, FMVZ-UNAM, 04510. México City

BACKGROUND: Coccidiosis in domestic rabbits (Oryctolagus cuniculus) is a major cause of morbility and mortality. It mainly affects young animals. Currently in the treatment of coccidiosis sulfonamides, ionophores and quinolones are used, which can become toxic for infants and pregnant females. It is important to find alternatives for the treatment of coccidiosis, one of which is the use of curcumin (Curcuma longa).
METHODS: Twenty-four New Zealand white rabbits eight months of age with an average weight of 3 kg was administered orally an aqueous extract of Curcuma longa (aeCl). 4 groups of 6 rabbits each were formed. Control group, group 2, 3 y 4 received 10, 25 and 40 mg/kg of aeCl. During the experiment the weight of each rabbit was recorded and stool samples were obtained.
RESULTS**:** At 28 days, statistically significant differences ($p < 0.05$) in the log average number of oocysts per gram of feces in rabbits, between the control group and the groups receiving 25 and 40 mg/kg of aeCl, and between these two groups. At 35 days it was observed that there is statistically significant difference ($p < 0.05$) between the control group compared to the groups receiving 25 and 40 mg/kg of aeCl. At 42 days is observed that the 3 treatment groups are significantly different than the control group.
CONCLUSIONS: The aqueous extract of Curcuma longa is a good alternative to reduce the parasite load in rabbits infected with Eimeria spp at a dose of 40 mg/kg.

Diagnostic needs for the Global Programme for Elimination of Lymphatic Filariasis

Hoti S.L.

Regional Medical Research Centre, Nehru Nagar, Belgaum, 590010, India

BACKGROUND: Lymphatic filariasis (LF) is endemic in 82 countries and is a major public health problem in India. It is a vector borne parasitic disease causing long lasting physical disability. Mass annual single dose drug administration (MDA) with DEC, co-administrated with albendazole has been carried out for 6-8 rounds in different regions of India. Currently the programme is now facing major challenges of diagnostics and the types of diagnostics available may not be adequate. Hence, we developed xenomonitoring tools for monitoring infection and infectivity in vectors.
METHODS: A simple and inexpensive method for extraction of DNA useful in PCR assay was developed for determining the infection in vectors. In order to develop an assay for determining infectivity, genes upregulated in infective (L3) stage of *Wuchereria bancrofti* were identified and used developing a RT-PCR for detecting L3 stages in vectors. The RT-PCR assay developed was tested for specificity and sensitivity and validated in a multicentric trial.
RESULTS: The simple DNA extraction method was found to be useful for detecting *W. bancrofti* infection in a pool of 25 *Culex quinquefasciatus* by Ssp I PCR assay with high sensitivity and specificity. The RT-PCR assay was found to be highly stage specific and sensitive in detecting a single infective stage of *W. bancrofti* in a pool of 25 *Cx. quinquefasciatus.* Multicentric validation of the RT-PCR assay indicated that the assay has potential application for monitoring the transmission in the on-going LF elimination programme
CONCLUSIONS: A simple and inexpensive method for extracting DNA, and a infective stage specific RT-PCR assay have been developed, which have potential in the assessment of transmission in the LF elimination programme.

Molecular identification and phylogeny of selected polyopisthocotylean monogeneans infecting carangids (Carangidae, Teleostei) using nuclear and mitochondrial markers

Tambireddy Neeraja[1]*; Gayatri Tripathi*[2]*; Annam Pavan Kumar*[2]

[1]Department of Aquatic Animal Health Management, College of Fishery Science, Sri Venkateswara Veterinary University, Muthukur – 524 344, Andhra Pradesh (State), India; [2]Central Institute of Fisheries Education (ICAR), Panch Marg, Off Yari Road, Versova, Andheri (W), Mumbai - 400 061, India.

BACKGROUND: In the class Monogenea, fifty three families were recognized in the most recent morphological phylogenetic analysis, but at least ten other 'families' were omitted because of uncertainties about origins and/or validity. There is a need to extend molecular taxonomic methods in solving these taxonomic discrepancies and also in the analysis of phylogenetic relationships among different taxonomic groups of monogeneans. Molecular methods are mostly being applied for identification and evaluation of phylogenetic relationships of monopisthocotylean monogeneans of freshwater fishes and such studies pertaining to marine fish monogeneans are limited. Further such studies have not been extended on the monogeneans infecting a specific taxonomic group of fishes. The present study was carried out to identify the monogenean parasites on marine fishes of the family carangidae by using nuclear 28S rRNA and mitochondrial cytochrome *c* oxidase I (COI) genes and to study the evolutionary relationships among the identified polyopisthocotylean monogeneans.
METHODS: Total genomic DNA was extracted from parasites using DNeasy tissue kit. Nuclear partial 28S rDNA was amplified using universal primers (C1 and D2) and Mitochondrial partial cytochrome *c* oxidase I gene (COI) was amplified using the primers ASmit1 and ASmit2. Full length sequences of the 28S rRNA and the COI genes obtained by using Gene Runner V 3.0 software were edited and aligned using ClustalW program with default settings implemented in MEGA V 5.0 software. Partial nuclear 28S rRNA gene was used to reconstruct the evolutionary relationship among the eight species of polyopisthocotylean monogeneans (Order: Mazocraeidea) using Neighbor-joining, Maximum Parsimony, Maximum Likelihood and Bayesian Inference methods. The genetic distance values at COI locus across species was estimated using K2P model.
RESULTS: The molecular phylogenic analysis of polyopisthocotylean monogeneans using partial nuclear 28S rRNA gene are consistent with the conclusions drawn from traditional morphological methods. The short barcode sequences (~230bp) of COI gene clearly demarcated the species with high distance values between species.
CONCLUSIONS: Our results revealed the evolutionary relationship among eight species of polyopisthocotylean monogeneans of the order Mazocraeidea by using partial nuclear 28S rRNA gene. The COI gene sequences clearly discriminated all the species with sufficient increment of distance values from within species to between species.

Effect of *Toxoplasma gondi*i infection in the uterine natural killer (uNK) cell population

Besné-Mérida A[1], Cedillo-Peláez C[1], Rico-Torres CP[1], Vargas-Villavicencio JA[1], García-Vázquez FJ[2], Farfán-Morales JE[2], Correa D[1]*

[1]Laboratorio de Inmunología Experimental; [2]Departamento de Anatomía Patológica. Instituto Nacional de Pediatría, SSa
*Correspondencia: kamsoluzar@gmail.com

BACKGROUND: In mice placenta, uterine natural killer cells (uNK) promote vascular remodeling and provide conditions for maintenance of pregnancy producing INFγ, VEGFA and IL-18 among other products. We evaluated the effect of *T. gondii* infection in the population of uNK cells, their products and damage in maternal-fetal interface, as well as other cytokines (IL-6, IL-10, IL-12 and TGF-β) related with infection.
METHODS**:** Female mice were inoculated i.v. with four doses of Me49 strain tachyzoites of *T. gondii* at 10 days of gestation and euthanized 72 hours later; tissues were collected to determine parasite load by qPCR, to assess damage in maternal-fetal interface by histopathology and to analyze the products of uNK cells by immunohistochemistry.
RESULTS**:** Inflammatory cells were found in placenta, increasing according to the infecting dose, however in the fetus, it was only found at the highest dose; necrosis was observed in the decidua and the chorionic labyrinth, but only in some placentas at higher doses.
The number of uNK cells increased according to the infective dose but being significant only at the highest dose; interestingly uNK cells were not producing INFγ at this pregnancy time and independently on infection status; however, local neutrophils at the placenta were found positive for this cytokine. IHC evaluation of IL-6 and IL-18 showed no significant differences among groups or the control group.
CONCLUSION**:** Murine uNK cells present at the middle of gestation do not produce INFγ, regardless on *T. gondii* infection status but do. IL-18 and IL-6 are not related to infection by *T. gondii* at the level. The cytokines produced by local uNKs remain to be demonstrated.

SEASONAL PARASITISM PREVALENCE AMONG *VARROA DESTRUCTOR* AND *APIS MELLIFERA* ON TWO COUNTIES OF RIO DE JANEIRO, BRAZIL

Souza, Roberta Coelho Pereira de [1]; *Serra-Freire, Nicolau Maués* [2]

[1] master's degree student (robertavm@gmail.com) [2] head researcher of the national reference laboratory on vectors of rickettsial diseases, FIOCRUZ, Brazil.

BACKGROUND: Although bees have major ecological and agricultural importance, nowadays, with the focus on climate change, there is huge concern about the health of these insects. Parasitic mites are a real threat to survival of these insects, emphasis on *Varroa destructor* Anderson & Trueman, 2000 as an important biological vector of several pathogens. This study aimed to report the prevalence of parasitism between *Varroa destructor* and *Apis mellifera*, in different seasons, in two counties of the State of Rio de Janeiro.
METHODS: Ten Langstroth beehives of africanized honey bees (*Apis mellifera*) were monthly investigated during a year study (Aug/12-Sep/2013) on each county. Each bee had it body inspected under a stereomicroscope and the contents resulted from the collection bottles were held in fine mesh sieve, where the mites were separated, counted and preserved in an Ependorf type container filled with isopropanol. Finally the prevalence rate of parasitism was then calculated as the number of mites divided by the number of bees, multiplied by 100.
RESULTS: *V. destructor* were found parasitizing all beehives examined. In the county of Barra do Piraí the prevalence rates were identified as 2.67% (spring), 4.75% (summer), 5.28% (autumn) and 4.14% (winter). On Guapimirim county, spring (3.63%), summer (0.93%), autumn (1.56%), winter (3.21%). The results corroborate the literature that, in Brazil, the degree of infestation of varroatose is low, around 2-5%, without causing the death of affected hives (Moretto & Leonidas, 2003).
CONCLUSIONS: Symbiosis between *V. destructor* and *A. mellifera* occurs, and the prevalence rate of this parasitism remains low.

Inspection, identification and evaluation of blackfly and aquatic wildlife and other tributaries Huallaga in the city of Huanuco Peru – 2013

Diestro-Diestro, Alicia[1]; Solis-Acosta, Hilda[2]; Gárate-Camacho, Inés[3]; Jimenez-Vinchez, Ricardo[1]; Mendoza-Flores, Paula[1]

[1]Laboratorio de Entomología Médica y Veterinaria FCB-UNMSM, Avenida Venezuela S/N Lima City, Peru; [2]Laboratorio de Serología y Bioquímica Parasitaria, Instituto de Medicina Tropical Daniel A. Carrión-UNMSM, Avenida Amezaga S/N Lima City, Peru; [3]Laboratorio de Parasitología Humana y Animal FCB-UNMSM, Avenida Venezuela S/N Lima City, Peru.

BACKGROUND: Huanuco is located at an altitude of 1989 meters, seated on both banks of the Huallaga River and tributaries.
Since 2004 it has been designing strategies and activities in order to control the population of Simulides coming seriously affecting the health of people, presenting pictures of fever, headache, nausea ,dermatitis , among others.
In our setting, the Simuliidae, are not yet listed as vectors, but in other South American countries have been alerted to the potential risk of disease vectors such as onchocerciasis.
The aim of this study was to identify and evaluate the samples collected in the Huallaga River and tributaries (Higueras, Garbanzo and Huancachupa), to strengthen prevention and control of blackflies.
METHODS: The blackflies and other aquatic fauna collected by the field staff of the Regional Health Authority-DIRESA Huanuco, were delivered to the Medical Entomology Laboratory and Veterinary FCB - San Marcos, where he identified and evaluated a total of 17 150 specimens.
RESULTS: Of the specimens identified, 13 050 (76 %) are Simuliidae (730 eggs, 11 910 larvae and 390 pupae); 1080 (6.3 %) are Ephemeroptera (1070 larvae and 10 pupae in three families); 2190 (12.8%) to Chironomidae (2070 larvae and pupae of two Sub 120 families) and 830 (5%) correspond to larval Trichoptera in two families.
CONCLUSIONS: From the results it can be concluded that the percentages of Simuliidae outweigh the aquatic fauna that would be acting as drivers of eggs and larvae of Simuliidae populations; so integrated, secure and monthly cleaning of the basins and application program in the basins with BTI greater larval density control is recommended.

A new trichomonad from the oropharynx of necrophagous birds of prey

Martínez-Herrero, María del Carmen[1]; *Rodríguez-Arce, Irene*[3]; *Ponce-Gordo, Francisco*[2]; *Martínez-Díaz, Rafael Alberto*[3]; *González, Fernando*[4]; *Molina, Rafael*[5]; *Gómez-Muñoz, María Teresa*[6]

[1]Universidad CEU Cardenal Herrera, Instituto de Ciencias Biomédicas, Facultad de Veterinaria, Departamento de Producción y Sanidad Animal, Salud Pública Veterinaria y Ciencia y Tecnología de los Alimentos, Calle Tirant Lo Blanch 7, 46115 Alfara del Patriarca, Valencia, Spain; [2]Universidad Complutense de Madrid, Departamento de Parasitología, Plaza de Ramón y Cajal, 28040 Madrid, Spain; [3]Universidad Autónoma de Madrid, Departamento de Medicina Preventiva, Salud Pública y Microbiología, Facultad de Medicina, Calle Arzobispo Morcillo 4, 28029 Madrid, Spain; [4]GREFA, Grupo de Rehabilitación de la Fauna Autóctona y su Hábitat, Monte del Pilar s/n, 28220 Madrid, Spain; [5]Centre de Fauna Saltvage de Torreferrussa, Catalan Wildlife-Service-Forestal Catalana, Santa Perpètua de la Mogoda, 08130 Barcelona, Spain; [6]Universidad Complutense de Madrid, Facultad de Veterinaria, Departamento de Sanidad Animal, Avenida Puerta de Hierro s/n, 28040 Madrid, Spain

BACKGROUND: *Trichomonas gallinae* is an emergent parasite for wild birds that induces necrotic lesions at the upper digestive tract leading to death by starvation. A decade ago, only one species was recognized, but genetic analysis of different isolates all over the world has shown a wider scenario. In 2014, a new species has been described (*T. stableri*) in band-tailed pigeons (*Patagioenas fasciata monilis*). Here, we describe a new variant isolated from asymptomatic necrophagous birds (Egyptian vultures-*Neophron percnopterus* and black vultures-*Aegypius monachus*) from Spain. Genetic (ribosomal genes ITS1/5.8S/ITS2 and 18S), morphological (optical and scanning electron microscopy) as well as host range evidences support our findings.
METHODS: Necrophagous birds (n=17) were sampled from Wildlife Recovery Centers from different provinces of Spain. Swabs moistened in TYM (Trypticase-Yeast-Maltose) medium were used to isolate the parasite from the oropharyngeal cavity and incubated at 37ºC. Growth was monitored during 15 days by inverted microscopy. DNA extraction was performed using a commercial kit. PCR of the ribosomal region ITS1/5.8S/ITS2 and 18S was performed from all the isolates. BLAST analysis and phylogenetic trees by Neighbour-joining and Maximum Likelihood methods were used to classify the sequences. Morphological analysis were done by scanning electron and optical microscopy using Motic Images Plus 2.0 ML software and comparison with other *T. gallinae* isolates.
RESULTS: All the isolates from vultures displayed the same nucleotide sequence for both ribosomal targets and different to *T. gallinae* isolates. Phylogenetic analysis showed that maximum homology was present with a *T. vaginalis* strain. The morphological study revealed significant differences with *T. gallinae* in some measures: length of trophozoite body, costa, free flagella, recurrent flagellum and axostyle.
CONCLUSIONS: Genetic and morphological results as well as host range indicate that a new species should be considered. More research is required to understand the complete diversity of oropharyngeal bird trichomonads.

Surprising interactions between schistosomes and amphistomes in Kenyan *Biomphalaria pfeifferi*: amphistome dependence on and dominance of schistosome infections, with some implications for schistosome control

Laidemitt, M. R.[1], Mutuku, M.W. [2], Dweni, C.K. [2], Zhang, S.-M. [1], Mkoji, G.M. [2] and Loker, E.S. [1]

1. Center for Evolutionary and Theoretical Immunology, Department of Biology, Parasitology Division, Museum of Southwestern Biology, University of New Mexico, Albuquerque, NM87131

2. Centre for Biotechnology Research and Development, Kenya Medical Research Institute (KEMRI), Kenya.

BACKGROUND: *Schistosoma mansoni* is commonly transmitted by *Biomphalaria pfeifferi* in streams in western Kenya. Also transmitted by *B. pfeifferi* in these streams are amphistomes, trematodes that mature as adults in domestic ruminants. Because amphistomes are common (some of our collections retrieve 25% or more snails shedding amphistomes), and because their intramolluscan stages may have predatory effects on schistosome sporocysts, amphistomes may have an unappreciated detrimental effect on schistosome transmission in such streams.
METHODS: We have examined amphistome-schistosome interactions to learn if amphistomes are dominant when present in co-infections, and if amphistomes can be manipulated to achieve an even greater effect on schistosome transmission.
RESULTS: Thus far we have identified 19 distinct lineages of amphistomes in Kenya, at least four of which rely on *B. pfeifferi* as their snail host. Field-collected *B. pfeifferi* with patent amphistome infections can rarely be superinfected with *S. mansoni*. Attempts to establish experimental infections with amphistomes in *B. pfeifferi* have been unsuccessful, but further study has revealed that if snails are first exposed to amphistomes, and later exposed to *S. mansoni* infection, that the snails subsequently shed amphistome cercariae, or in some cases, amphistomes and schistosomes. We have since confirmed that field-derived snails not shedding any kind of cercariae when exposed to *S. mansoni* surprisingly often shed amphistome cercariae. We interpret the results to mean that amphistome miracidia may frequently penetrate *B. pfeifferi*, but then require a later facilitating effect from *S. mansoni* to achieve a patent infection. Once facilitated though, they largely dominate the schistosome infection.
CONCLUSIONS: Widespread exposure of snails to amphistomes, at least with the isolates we are working with now, may have two distinct effects: 1) preventing subsequent production of *S. mansoni* cercariae by these snails; and 2) only snails exposed to *S. mansoni* (or possibly other trematodes) would shed amphistome cercariae.
Supported by NIH R01 A1101438 and a Gates Grand Challenges Award.

Identification and characterization of *Aeromonas sobria* from *Labeo rohita* (IMC) using Vitek-2 system

Gowhar Hamid Dar[1]; M. Z Chishti[2]; Azra N. Kamili[1]; Fayaz Ahmad[3]; Touwsief Ahmad Tantry[2] and Shoaib Ali Dar[4]

[1]Department of Environmental Science, University of Kashmir, Srinagar-190006, India; [2]Parasitology laboratory, CORD, University of Kashmir, Srinagar-190006, India; [3]Department of Zoology, University of Kashmir-190006, India; [4]Department of Zoology, Punjabi University, Patiala, India

BACKGROUND: *Aeromonas* infections are probably the most common bacterial diseases diagnosed in cultured warm water fish. The common occurrence of this disease relates to stress conditions such as poor water quality, overcrowding (because of polyculture), or rough handling and *Aeromonas* septicemia is a fatal infectious disease of fish.
METHODS: Isolation of fish pathogenic bacteria was carried out by culture dependent approach. Dilution or spread plating technique, incorporating media and incubation conditions often of dubious relevance were used. To isolate bacteria from the skin, the surface of the fish was swabbed over an indeterminate area, and the inoculum was spread over the surface of nutrient-rich medium, such as tryptone soya agar (TSA), with incubation at 15–25^{o}C for 7– 14 days. Biochemical characterization was carried out using the VITEK- 2 system.
RESULTS: In this study the *Aeromonas* infection was found only in the *Labeo rohita*, one of the Indian major carps, found in the fish pond at District Poonch of Jammu and Kashmir State. Strain was observed under microscope for cell shape and it was found bacillus in shape and it gave grams -ve reaction upon Gram staining. Molecular approach was employed to identify the strain
CONCLUSIONS: Our results suggest that the presence of *Aeromonas* infection in the fish may be due to the fact that polyculture technique was employed in the pond. The presence of this type of infection is a concern as this can cause serious damage to our fish industry.

TIMING THE ORIGIN OF PRIMATE MALARIAS.

Escalante, Ananias A[1]; Pacheco, M. Andreina[1]

[1]Center for Evolutionary Medicine and Informatics, The Biodesign Institute, Arizona State University, Tempe, Arizona, USA

BACKGROUND: There have been several phylogeographic and phylogenetic studies involving *Plasmodium* species that incorporate some form of molecular clock analyses. In the case of primate malarias the discovery of new species in apes, as well as molecular data emerging from malarial parasites from lemurs, have enriched the discussion regarding the evolutionary events leading to the extant diversity of primate malarias. Nowadays, it is accepted that such diversity is the result of complex evolutionary processes where host switches have been common. Here, we explore the challenges of providing a time-frame for such events by revising different molecular dating approaches.

METHODS: We applied molecular dating and phylogenetic analyses using complete mitochondrial genomes from a broad spectrum of primate malarial parasites and other haemosporidia. We investigate the effect of different calibration points and explore their biogeographical implications on individual loci or using the complete mitochondrial genome as a unit.

RESULTS: We reject the assumption that the *Plasmodium* mitochondrial genes, using the complete genome as a unit or each locus separately, evolve at a constant rate.

CONCLUSIONS: The use of fossils from the host as absolute calibration and the assumption of a strict clock likely underestimate time when performing molecular dating analyses on malarial parasites. By exploring different calibration points, we found that the time for the radiation of primate parasites may have taken place in the Eocene, a time consistent with the radiation of African anthropoids. Overall, the radiation of primate malarias seems to be determined by the evolutionary dynamic of their hosts, such dynamics includes biogeographical events that could involve multiple hosts. The importance of exploring alternative scenarios in molecular dating studies is highlighted.

Schistosoma mansoni: Identification of oxamniquine drug resistant genes, determination of mode of action and drug redesign

[1]Valentim, Claudia; [1]Stahl, Stacey; [2]Chevalier, Frederic; [1]Taylor, Alexander B; [1]Cao, Xiaohang; [1]Holloway, Stephen P; [3]McHardy, Stanton; [1]Hart, P. John; [2]Anderson, Timothy J.C; [1]LoVerde, Philip T.

[1]Department of Biochemistry, University of Texas Health Science Center, San Antonio, Texas
[2]Department of Genetics, Texas Biomedical Research Center, San Antonio, Texas, USA
[3]Center for Innovative Drug Discovery, University of Texas, San Antonio, Texas, USA

BACKGROUND: Oxamniquine (OXA) kills *Schistosoma mansoni* but not *S. haematobium* or *S. japonicum*. High level resistance to oxamniquine evolved in the human blood fluke *(Schistosoma mansoni)* in Brazil in the 1970s and has been selected in laboratory populations. We exploited the genome sequence and genetic map to identify the mutations underlying this trait, to determine the mode of drug action and the basis for species-specific drug action.
METHODS: To do this, we staged a cross between parental parasites that differed by ~500 fold in OXA resistance, determined drug sensitivity in clonally-derived F2 parasites, and used genotyping to identify a Quantitative Trait Locus (QTL). Biochemical and molecular tests were used to validate the identified genes responsible for drug resistance.
RESULTS: A single QTL (LOD=31) on chromosome 6 contained the causative gene (a sulfotransferase, SmSULT). SmSULT was validated using both RNAi knockdown and biochemical complementation assays. By identifying the gene for drug resistance, we demonstrated the mechanism of action. OXA is taken up by adult schistosomes. SmSULT sulfonates oxamniquine by catalyzing the transfer of a sulfuryl group (SO3) from the active sulfate donor such as 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to OXA to activate the drug to bind to schistosome macromolecules like DNA to interfere with DNA synthesis and transcription and killing the adult worms. In the laboratory-selected resistant parasite an amino acid deletion close to the active site interferes with drug binding, while in the field derived resistant isolate a C→R mutation disrupts enzyme tertiary structure.
CONCLUSIONS: These results demonstrate the utility of linkage mapping in schistosmes. Ongoing crystallography studies of protein-drug interactions demonstrate the structural relationship between the sulfate donor (PAPS), the sulfotransferase (SmSULT) and the drug OXA, while phylogenetic studies revealed close homologues of SmSULT in *S. haematobium* and *S. japonicum*. These studies pave the way for rational design of second-generation OXA derivatives that can kill all human-infective schistosome species.

Autophagy regulates survival through transcription dependent mechanisms in *Echinococcus granulosus*

Loos Julia A.[1]*, *Nicolao M. Celeste*[1]*, *Cumino Andrea C*[1].
[1]Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Funes 3350, MDP, Argentina. CONICET. *Authors contributed equally

BACKGROUND: Autophagy participates in cell survival and intervenes actively in the larval development in helminths. TORC1 pharmacological inhibition, through rapamycin or metformin (agonist drug of AMP activated protein kinase -AMPK-), induce autophagy. AMPK, an eukaryotic metabolism master regulator, is activated when intracellular ATP levels fall, regulating growth, development and autophagy. In this work, we studied how the autophagy that occurs in *Echinococcus granulosus* larval stages is induced by AMPK activation and different Ca^{2+} mobilizing agents.
METHODS: Protoscoleces from hydatid cysts of infected cattle and metacestodes from CF1 mice with secondary hydatidosis were maintained in *in vitro* culture. SEM and TEM were used to investigate autophagosome progress. Expression of autophagy (*atg*1-*atg1*8), ER stress and FoxO genes were analyzed by RT-PCR/qPCR from drug-treated parasites (metformin, cyclosporine-A, TFP, BAPTA and bortezomib). *In toto* immunofluorescence and immunoassays to determine the Eg-Atg8/LC3 expression and localization were performed. Eg-AMPK-α (total and AMPK-P^{Thr176}) and Eg-calcineurin-A expression by confocal microscope were evaluated. Finally, a single CREB homologue was identified. Data were compared using the student's t test.
RESULTS: Eg-AMPK is activated in basal condition and in metformin treatment, inducing autophagy in the cestode. Metformin treated parasites demonstrated autophagic structures from ultrastructural images, upregulated Eg-Atg8/LC3II expression and Eg-*atg*6-8-12-16-18 transcription, reduced glycogen level and Eg-*pepck* and Eg-*g6pase* (genes coordinated by CREB via) in both larval stages. Eg-Atg8/LC3, detected in the tegument, excretory system and calcareous corpuscles, co-localized with Eg-AMPK-α in these calcium-stored cells.
CONCLUSIONS: *Echinococcus* autophagy could be regulated by non-transcriptional inhibition through TOR, transcription-dependent up-regulation via FoxO and AMPK and calcineurin via CREB to promote survival in *Echinococcus* lifespan.

Plasmodium knowlesi: expression; characterization, and immunogencitity testing of Pichia pastoris-expressed recombinant apical membrane antigen 1 (AMA1)

Muzamil Mahdi Abdel Hamid[1,2], *Alan W. Thomas*[2], *Bart W. Faber*[2]

[1]Institute of Endemic Diseases, University of Khartoum, Sudan;
[2]Biomedical Primate Research Centre, The Netherlands

BACKGROUND: To provide a platform to investigate critical issues surrounding AMA1 vaccine development, such as adjuvant selection, dose finding, longevity of the response, and eventually establishing correlates of protection, we are using the P. knowlesi/rhesus macaque model system. P. knowlesi is a simian malaria parasite that recently has been established as the fifth human parasite and causes life-threatening disease. In the present study P. knowlesi (H-strain) apical membrane antigen 1 (PkAMA1) domain I-II-III and PkAMA1 domain I-II were heterologously expressed in Pichia pastoris using comparable methodologies to the production of clinical grade P. falciparum AMA1 (PfAMA1).
RESULTS: PkAMA1 I-II-III was produced with low yield that could not be overcome by the use of a *Pichia pastoris* codon optimized gene, while PkAMA1 domain I-II was produced with high yield using the codon-optimized gene. SDS-PAGE under non-reducing conditions showed that the both proteins were highly pure, while SDS-PAGE under reducing conditions revealed a single proteolytic cleavage site that resembles the site found in PfAMA1. Both proteins bound quantitatively to the monoclonal antibody R3/1C2, indicative for proper conformation of the proteins. Rabbit immunisations were performed with the novel adjuvant CoVaccine HT. This resulted in very high antibody titers and high in vitro growth inhibition levels reaching over 95% at 6 mg/mL for PkAMA1 DI-II-III. Separate immunisation studies with Montanide ISA51 and Montanide ISA720 showed two fold less antibody levels that translated to lower growth inhibition levels. Comparison of the immune response to PkAMA1 domain I-II-III with PkAMA1 domain I-II showed that domain III may be an important target for immune responses.
CONCLUSIONS: These results open the route to set-up the *P. knowlesi* rhesus macaque model system to test vaccination strategies that can be used for the design of human vaccination studies

The mechanistic target of rapamycin homolog in *Giardia duodenalis* (gTOR): bioinformatics- and inhibitors-based insights

Argüello-García, Raúl; Cervantes-Tovar, Cleotilde Aurora; Ortega-Pierres, Guadalupe

Departamento de Genética y Biología Molecular, CINVESTAV-IPN, 07360 Mexico City, Mexico.

BACKGROUND: *Giardia duodenalis* is an early divergent protozoan causing giardiasis, a common parasitic diarrheal disease worldwide. The complete Giardia genome database (GiardiaDB) provides a platform to study multiple processes including response to environmental stresses as nutrient deprivation and encystation that lead to autophagy and cell differentiation respectively. In these, the *Giardia* homolog of mechanistic target of rapamycin (gTOR) may have a regulatory role, as occurs in other eukaryotes, through two complexes: mTORC1 related to protein translation, lipid biosynthesis and autophagy and mTORC2 related to growth factor signaling, cell cycle arrest and cytoskeleton organization. It is unknown if these complexes are functionally expressed in *G. duodenalis.*
METHODS: The structure and domains of gTOR were raised from GiardiaDB and I-Tasser platforms. The kinase, rapamycin- and ATP-binding domains were analyzed and compared by ExPaSy and ClustalW tools with other counterparts along to homologs involved in possible gTORC1/gTORC2 complexes. Encystation and autophagy of *G. duodenalis* trophozoites were induced *in vitro* and the effect of three inhibitors was tested: LY94,002 as general PI3K inhibitor and rapamycin or AZD8055 as allosteric or ATP-competitive gTOR inhibitors respectively.
RESULTS: The gTOR sequence displayed a larger but typical mTOR-like structure with conserved kinase and ATP-binding domains and the reported absence of residues involved in rapamycin-FKBP12 binding was confirmed. Accordingly, encystation was inhibited in a concentration-dependent manner by LY94,002 and AZD8055 and autophagy was induced by AZD8055 and inhibited by LY94,002 while rapamycin had no effect. In GiardiaDB, homologs of possible gTOR partners as mLST8 (gTORC1/gTORC2) and Raptor (gTORC1) were identified.
CONCLUSIONS: These data suggest the likely presence of a gTORC1 complex with a minimal machinery to promote encystation, possibly through activation of a giardial SREBP-1 that is processed during cyst formation, and autophagy through direct inhibition of gTOR. Further biochemical studies to support these notions are on the way.

The M32 family of metallocarboxypeptidases in *Trypanosoma brucei*.

Frasch, Alejandra Paula; Bouvier, León Alberto; Cazzulo, Juan José; Niemirowicz, Gabriela Teresa

Instituto de Investigaciones Biotecnológicas "Dr. Rodolfo A. Ugalde", IIB-INTECH/ UNSAM-CONICET. Av. 25 de Mayo y Francia, B1650KNA, San Martín, Buenos Aires, Argentina.

BACKGROUND: Metallocarboxypeptidases (MCP) of the M32 family of peptidases have been identified in a number of prokaryotic organisms but they are absent from eukaryotic genomes with the remarkable exception of those of trypanosomatids. The genome of *Trypanosoma brucei*, the causative agent of Sleeping Sickness, encodes one such MCP which displays 72% identity to the characterized *Tc*MCP-1 from *Trypanosoma cruzi*. As its orthologue, *Tc*MCP-1, *T. brucei* MCP is a cytosolic enzyme expressed in both major stages of the parasite.
METHODS: In order to understand the role of the M32 family of MCPs in trypanosomatids, both alleles of *TbMCP-1* were interrupted in the procyclic stage of the parasite by homologous recombination. Phenotype of double knock out cells was analyzed through direct stained of the nucleus and kinetoplast with DAPI to determine the nuclei/kinetoplast (N/K) configurations. Analysis of basal bodies and flagella by indirect immunofluorescence was performed in order to explain the delayed in the kinetoplast division.
RESULTS: These knock out parasites show slower growth rates and there are abnormal forms of the parasites *in vitro*, which have a higher number of nuclei than of kinetoplasts. Analysis of the phenotype showed a delay in the replication of the basal body and all the organelles which division is directly linked to this structure (kinetoplast, flagellum and Golgi apparatus).
CONSULSIONS: Our results indicates that M32 family of MCPs is linked to the cell cycle. Therefore these proteins could be potential candidates as drug targets.

13th INTERNATIONAL CONGRESS OF PARASITOLOGY
AUGUST 10th - 15th, 2014
ICOPA XIII MÉXICO

Effect of EhCP112 overexpression in *Entamoeba histolytica* virulence

García-Rivera[1] Guillermina[1], Cuellar-Silva Patricia[1], Javier-Reyna Rosario[1], Betanzos Abigail[1], Orozco Esther[1]

[1]Department of Infectomics and Molecular Pathogenesis, Center for Research and Advanced Studies. Av. IPN N. 2508, México DF

BACKGROUND: *Entamoeba histolytica* is the protozoan responsible of amoebiasis. Through several virulence mechanisms, this parasite invades and destroys host tissues. EhCPADH, formed by the adhesin EhADH112 and the cysteine proteinase EhCP112, is a parasite molecule involved in some of these events. This complex is localized in the surface and in the cytoplasm of trophozoites. Although, *E. histolytica* contains around 50 cysteine proteinases genes, only a small subset is expressed and few have been described in the surface. The aim of this work is to determine the importance of EhCP112 in the *in vitro* virulence properties of *E. histolytica.*

METHODS: *Ehcp112* complete gene was amplified from genomic DNA and cloned in the pNeoExEh vector. Trophozoites were transfected with this construction and stable cells were selected with G-418. EhCP112 overexpression was determined by WB and RT-PCR assays. In these transfectant trophozoites, we evaluated their adherence efficiency, their rate of erythrophagocytosis and their cytopathic and cytotoxic effect on MDCK cells.

RESULTS: Trophozoites were transfected with the pNeo or pNeo-Ehcp112 vectors and selected with G-418 (40 µg/ml). The EhCP112 overexpression was revealed by RT-PCR and WB assays, using specific primers and a specific antibody against-EhCP112 that we produced, respectively. These EhCP112 overexpressing trophozoites erythrophagocyted more than control trophozoites transfected with pNeo vector. Cytopathic and cytotoxic assays showed that trophozoites with more EhCP112 destroyed faster and in a greater amount the MDCK monolayer in comparison with control parasites.

CONCLUSIONS: The overexpression of EhCP112 enhanced virulence properties of *E. histolytica*, therefore EhCP112 could be considered an important virulence factor involved in the pathogenicity of this parasite.

An antioxidant response is involved in resistance of *Giardia duodenalis* to albendazole

Argüello-García, Raúl[1], Cruz-Soto, Maricela[2], González-Trejo, Rolando[1], Paz-Maldonado, Luz María T[3], Martínez-Espinoza, Rodrigo[1], Bazán-Tejeda, María Luisa[1], Mendoza-Hernández, Guillermo[4], Ortega-Pierres, María Guadalupe[1]

[1]Departamento de Genética y Biología Molecular, Centro de Investigación y de Estudios Avanzados-IPN, 07360 Mexico City, Mexico; [2]Probiomed, Cruce de carreteras Acatzingo-Zumpahuacán, Tenancingo, 03020 Estado de Mexico, Mexico; [3]Laboratorio de Biofarmacéuticos Recombinantes, Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí, 78210 San Luis Potosí, Mexico; [4]Departmento de Bioquímica, Facultad de Medicina-UNAM, 04580 Mexico City, Mexico.

BACKGROUND: Albendazole (ABZ) is a therapeutic benzimidazole for giardiasis that usually targets β-tubulin. ABZ-resistance in *Giardia duodenalis* has been studied however the molecular basis of this process are not completely understood because typical mutations at the β-tubulin gene are absent in ABZ-resistant *Giardia*. In previous work ABZ-resistant (1.35, 8 and 250 μM) and ABZ-susceptible clones were compared and some representative enzymes of energy metabolism, cytoskeleton-associated proteins and one antioxidant enzyme (NADH oxidase, NADHox) were found to be differentially expressed. Since ABZ is normally converted into sulphoxide (ABZ-SO) and sulphone (ABZ-SOO) metabolites, in this work the presence of these metabolites and the levels of antioxidant molecules in the aforementioned *Giardia* ABZ-susceptible and -resistant clones were analyzed.
METHODS: Production of reactive Oxygen species (ROS) and levels of ABZ-SO/ABZ-SOO were determined after exposure of *Giardia* trophozoites to ABZ by dichlorodihydrofluorescein diacetate-based fluorescence and liquid chromatography respectively. The mRNA and protein levels of other antioxidant enzymes in *Giardia* resistant clones (thioredoxin peroxidase TRP, flavodiiron protein FDP) were determined by RT-PCR and proteomics. The intracellular sulfhydryl (R-SH) pool was quantified using dinitrobenzoic acid and growth of ABZ-susceptible clones pre-treated with cysteine and then exposed to ABZ was determined.
RESULTS: The three ABZ species induced ROS formation. The accumulation of ABZ-SO and ABZ-SOO was lower in all ABZ-resistant clones. Consistent with NADHox over-expression, TRP and FDP enzymes were up-regulated as determined by proteomics and mRNA levels in ABZ-resistant clones. Likewise R-SH pool was increased according to the degree of ABZ-resistance and cysteine (1 to 2 mM) supported growth/survival of ABZ-exposed trophozoites.
CONCLUSIONS: These results showed that ABZ metabolites are produced by *G. duodenalis* and all have a pro-oxidant action. In ABZ-resistant parasites, an antioxidant response involving ROS-metabolizing enzymes and low molecular-weight thiols is a contributing effector mechanism that participates to overcome the pro-oxidant cytotoxicity of ABZ.

MOLECULAR CHARACTERIZATION OF STRAINS *Trypanosoma cruzi* NATIVE OF PERU.

Solís Acosta Hilda María[1]; Diestro Diestro Alicia[2]; Calderón Marisela[1]; Garate Camacho Inés Miriam[2]; Huamán Reyes Ana María[1]; Ferrer Cruz Alejandro[1]; Ávila Arosemena Julia Graciela[1]; Esteban Arias[1]; Borja Santa Cruz Nelly[1]; Fernández Giusti Alicia[1]; Sáez Flores Gloria[2]; Tarqui Terrones Kathia[3]; Carlos Nancy[2]; Espinoza Quinteros Sofía[1]; Valverde Ticlia Fanny[3]; Fajardo Narda[2]; Cayo Irene[1].

[1]Facultad de Medicina, Universidad Nacional Mayor de San Marcos, Instituto de Medicina Tropical Daniel A. Carrión. José Santos Chocano 199 Bellavista city Perú.
[2] Facultad de Ciencias Biológicas Universidad Nacional Mayor de San Marcos, Ciudad Universitaria Lima Perú. [3]Instituto Nacional de Salud INS. Lima city- Peru.

BACKGROUND: The present study aims to identify *Trypanosoma cruzi* strains circulating in our country; it is known that the complex *T. cruzi* is a heterogeneous population of histoparasitos, which have a domestic life cycle, intermediate and wildlife, including reservoirs humans, vectors, domestic and wild animals. Was characterized 7 isolates (strains) of *Trypanosoma cruzi* from different regions of Peru, by technical of molecular biology PCR –RFLP.
METHODS: Descriptive transversal Study . Activation and Maintenance of 7 strains of *T. cruzi* were isolated from BALB " C" first mice intraperitoneally and then seeded in artificial culture media modified BHI (epimastigotes) maintained at room temperature from different geographical regions of Peru (Nazca , Arequipa , Moquegua , Ucayali and Bagua) .
RESULTS: Subsequently the DNA was extracted epimastigotes. DNA extraction from epimastigotes cultured strains was obtained by the method QIAamp Mini Kit PCR amplification. After 7 *T.cruzi* isolated were maintained in culture (epimastigote) PCR to be performed. It was used: sequence analysis of the ribosomal DNA ITS region. The PCR was carried out in a final volume of 25 ul reaction containing 2 ul of DNA template, 12.5 Mix Mirakel ul, 1 ul Forward first , first reverse 1 ul , 8.5 ul vand . And DNA electrophoresis was made on agarose gels. The amplified products were analyzed on agarose gels 1. 5 %. Analysis of results. Results Obtained data were processed with the programs: EditSeq , Seqman , MegAling , for phylogenetic trees. They were sent to Germany and sequences of some strains of *Trypanosoma cruzi* (GenBank) were used for the respective comparisons.
CONCLUSIONS: Conclude that 5 of the isolates (strains) we belong to the lineage TCI and 2 lineage TCIIa circulating in our environment.

Comparative analysis of *L. guyanensis* strains showing distinct profiles of susceptibility to antimony

Sousa, Cíntia[1]; Pinheiro, Liliane[2]; Torres-Santos, Eduardo Caio[2]; Cupolillo, Elisa[1]; Porrozzi, Renato[1]

[1]Laboratóio de Pesquisa em Leishmaniose, FIOCRUZ, Rio de Janeiro, Brasil
[2]Laboratório de Bioquímica de Tripanosomatídeos, FIOCRUZ, Rio de Janeiro, Brasil.

BACKGROUND: Pentavalent antimony is the main drug used against American cutaneous leishmaniasis, however, occurrence of treatment failure with this drug has been reported. The treatment failure phenomenon is complex and the acquirement of the parasite drug resistance phenotype is one of factors envolved. Therefore, it is critical to understand the mechanisms that lead to resistance.
METHODS: We compared the biological profile of two strains of *L. guyanensis*, selected according to the therapeutic profile. We analyzed the susceptibility to SBIII of the two strains (Therapeutic success - TS; therapeutic failure - TF) and then in two lineages with *in vitro* induced-resistance coming from the TS strain (with drug pressure – TS^{W}; without the drug – $TS^{W/O}$) through cytotoxicity assays, growth curve and cell cycle analysis.
RESULTS: The TS strain was more sensitive to the drug *in vitro* (IC50 24.2 µM), while samples TF, TS^{W} and $TS^{W/O}$ were less sensitive (IC50 82µM, 84µM and 110µM). In the growth curves and cell cycle analysis TS showed a lower growth rate and lower percentage of parasites in the G2 phase of the cell cycle (9.6% of the parasites in the stationary phase G2), while TF, TS^{W} and $TS^{W/O}$ achieved higher population density and a large percentage of parasites in G2 phase in the stationary phase (18.5%, 23% and 17%).
CONCLUSIONS: The changes in the cell cycle may be a phenomenon caused by the drug resistance induction leading to an increase in parasites proliferation. Higher population growth can cause changes in the microenviroment inducing metacyclogenesis and/or others phenotypes related with parasite persistence.

Prevalence of antibodies against *Trypanosoma cruzi* en blood donors in Tabasco, México.

Pacheco Gil L.[1]., Beltran F.[2], Ortiz J.[3], Mirabal CG[1]

1. Universidad Juárez Autonoma de Tabasco 2. Centro Estatal de Hemoterapia Tabasco.
3. Hospital “Gustavo A. Rovirosa”

Background: American tripanosomiasis is an endemic disease in Mexico. Parasitemia follows a bite by an infected kissing bug in the acute phase fever is the most common clinical manifestation of disease, although 20% of those infected are asymptomatic. There is a global concern about the risk of disease from blood transfusion and there is no data available from the south of Mexico.

Objective: Aim of this study was to assess the prevalence of antibodies against *T cruzi* in blood donors in Tabasco, Mexico.

Method: A retrospective study of 28301 blood donors from the biggest blood bank in the city and a General Hospital was done I. Samples were collected in a two year period (2012-2013) and were tested for the presence of antibodies against T. cruzi with ELISA as usual in transfusion practice. Epidemiological characteristics of donors were collected from bank and hospital database.

Results: The presence of antibodies show a prevalence of 1 % without significant difference between places and was higher in men between 26 and 55 years old. Almost 90% of them live in a no rural place
Conclusions: The prevalence in general population where previously reported as 5.3% however we found in this study a lower prevalence in apparently healthy people. Tabasco is considered a hot zone for this parasitosis and Chagas infection in blood donors captures only a segment of the population infected with Chagas Disease.

Analysis of the presence of carbohydrates in *Taenia solium* calreticulin

González-González, Ulises [1], Cruz-Rivera, Mayra [1], Flisser, Ana [1], Mendlovic Fela[1,2]

[1]Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad Nacional Autónoma de México. Av. Universidad 3000, Copilco Universidad, México DF 04510.
[2]Facultad de Ciencias de la Salud, Universidad Anáhuac del Norte, México DF.

BACKGROUND: *Taenia solium* is a helminth whose intermediate host is the pig, which becomes infected by ingesting feces with *T. solium* eggs, producing porcine cysticercosis. Humans are the definitive host and become infected after ingestion of pork meat with live cysticerci. These cysts evaginate and attach to the intestinal mucosa where they differentiate into tapeworms producing taeniosis. The parasite relies on cellular regulating proteins including glycoproteins that are described to be important in the host-parasite interaction; one of them is *T. solium* calreticulin (TsCRT). This protein is differentially expressed during each stage of development and presents two potential sites of glycosylation.
METHODS: Mice were immunized with recombinant TsCRT (rTsCRT) to produce specific anti-rTsCRT antibodies. Western blot of the crude extracts of the different stages of the parasite subjected to deglycosylation with PNGase F, O-Glycosidase and Sialidase-A were performed and the anti-rTsCRT antibodies were used to identify the native TsCRT. We also carried out a Lectin blot of the extracts to analyze the carbohydrates present in the native TsCRT.
RESULTS: Invaginated and recently evaginated cysticerci show predominantly one band when probed with the anti-rTSCRT antibodies whereas in tapeworm crude extract and excretion/secretion products a second heavier predominant isoform appears. When the extracts were PNGase-F deglycosylated, expression of the high molecular weight band disappeared, suggesting that TsCRT is *N*-linked glycosylated. The main carbohydrate composition of TsCRT was: Galβ1-3GalNAc, α-LFuc, Gal, GalNAc and to a lesser extent α-Gal and Galβ1-4GlcNAc as revealed by Lectin blot analysis.
CONCLUSION: TsCRT is glycosylated during the transformation from cysticerci to tapeworm. The specific function of TsCRT at the different stages could be regulated by the presence or absence of N-linked carbohydrates. The appearance of the glycosylated form implies a role of this protein during parasite differentiation. TsCRT glycosylation is supported by the presence of two N-glycosylation sites in the amino acid sequence of the protein.

Pregnancy and childbirth features of women infected with Giardiasis

Azamova Zarrina Sh., Kuropatenko Maria V.

Institute of Experimental Medicine, FSBI "RIEM" NWB RAMS, Saint-Petersburg, Russia

BACKGROUND: There are some modern studies devoted to Giardiasis influence on pregnant woman's and newborn's health, showing that mother who has Lamblia can also have deterioration of her health and that one of her future child. These studies concern clinical forms of Giardiasis only, but there are no any other findings devoted to influence of asymptomatic and subclinical Giardiasis forms on pregnancy. The goal of the study was to investigate pregnancy features and their termination among women with asymptomatic and subclinical Giardiasis forms.
METHODS: Some criteria of pregnant women with asymptomatic and subclinical Giardiasis forms (Giardiasis carriers) (1gr.-n=28) and with clinical infection forms (2gr.-n=25) were investigated. The control group included pregnant women without Giardiasis (3gr.-n=81).
RESULTS**:** A high frequency of early toxicosis was detected both in the first and in the second group (1gr.-57,1±1,6%; 2gr.-44,0±2,0%; 3gr.-21,0±2,1%). Pruritus gravidarius was found 4-5 times more frequently in the infected women groups than in the control one (21,4±1,8%; 24,0±1,9%; 4,9±1,1%; $p<0,05$). According to the obstetric anamnesis, the previous pregnancies resulted in spontaneous abortion at the group of Giardiasis carriers in 1,5 times more often (25,0 ± 1,4%) than in the second group (16,0±1,5%), and in 3 times more often than among women of the control group (7,4±1,0%; $p<0,01$). During observed pregnancy Giardiasis carriage was accompanied by a risk of spontaneous abortion in early terms and premature birth in 1,5 times more often, while the clinical Giardiasis infection was observed in 2,5 times more often than the absence of Giardiasis at pregnant women.
CONCLUSIONS: The obtained results suggest that the presence of Giardiasis in organism of pregnant women is a risk factor of obstetric and perinatal pathology. It is necessary that parasitological screening and specific treatment of women should be made before pregnancy for timely preventing the negative effects of Giardiasis.

Expression, enzyme activity and *in-silico* modeling of a phospholipase A2 of *Toxoplasma gondii*

Moncada-Giraldo, Diego[1]; *Arenas-Soto, Aylan*[1]; *Molina-Lara, Diego*[1]; *Murillo-Leon, Mateo*[1]; *Cardona, Nestor*[1]; *Gomez-Marin, Jorge*[1]

[1]Grupo de Investigación GEPAMOL, Centro de Investigaciones Biomédicas, Universidad del Quindío, Armenia-Quindío, Colombia

BACKGROUND: Secretory phospholipases A2 (sPLA2) are enzymes widely distributed in all organisms. There are evidences that phospholipase activity is involved in the invasion process of the intracellular parasite *Toxoplasma gondii.* In this work we identified that the recombinant protein with accession code in ToxoDB TGGT1_273620 has a sPLA2 activity.
METHODS: We used the expression vector *Escherichia coli* BL21-DE3 for protein production. The structural model was obtained from QUARK (http://zhanglab.ccmb.med.umich.edu/QUARK) and the stereochemical quality was evaluated with PROCHECK (http://www.ebi.ac.uk/thornton-srv/software/PROCHECK) and QMEAN (http://swissmodel.expasy.org/qmean/cgi /index.cgi). Assay for PLA2 activity was performed with *sPLA2 assay kit from CAYMAN chemical.* Invasion assays were performed using flow cytometry in VERO cells with RH tachyzoites tagged with GFP.
RESULTS: We obtained a ~20 kDa recombinant product with sPLA2 activity. This product has a Vmax= 0.2992 µmol/min/ml and a Km=0.441. The structural model shows an active site with a propitious chemical environment for sPLA2 activity. The catalytic dyad has a distance of 1.185Å between H175 and D125; there are amino acids with positive charge around the active site. Concentrations of 8 and 4 µg/ml of recombinant PLA2s decreased the *Toxoplasma gondii* invasion to VERO cells.
CONCLUSIONS: We described the first *Toxoplasma* secretory PLA2 with a histidine active site. Identification of this protein is the initial step for a specific biological, biochemical and structural understanding of the molecular mechanism in which these enzymes participate in the parasite biology.

Prevalence and viability of *Toxocara canis* in public parks in the city of Culiacán, Sinaloa.

Vargas-Nava, Alexis Israel[1]; Castro del Campo, Nohemí[1]; Enríquez-Verdugo, Idalia[1]; Portillo-Loera, Jesús José[1]; Borbolla-Ibarra, Eleazar[1]; Gaxiola-Camacho, Soila Maribel[1].

[1]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Sinaloa.

BACKGROUND: Public parks are points of infection of parasitic diseases that are transmitted primarily through feces of animals and the land of the parks, because in these places both animals and people use them as meeting places, this makes it a public health problem. The Toxocariosis whose causal agent is *Toxocara canis* and *Toxocara cati* are cosmopolitan parasites affecting mainly dogs, cats and infants. In this study the prevalence and viability of *Toxocara canis* eggs in public parks in Culiacan, Sinaloa was determined.
METHODS: A total of 1,180 soil samples were collected from 236 public parks. The processing of these samples was performed by the technique of flotation with zinc sulfate (Santarém *et al*., 2009). The identification of parasite eggs was performed by observing the morphology by the optical microscopic double blind. To determine the viability of the eggs of *Toxocara canis* the sample was stained with trypan blue 0.1% and observed under optic microscope.
RESULTS: In this study it was found that of the 236 sampled public parks, 26 has tested positive for the presence of parasites (11.02 %), with 25 of them viable (95.15 %), only 18 parks were positive for *Toxocara canis* (7.63 %) presenting a viability of 94.4 %, with a rate of 95% confidence .
CONCLUSIONS: The results show that *Toxocara canis* has a prevalence of 7.63% in public parks in Culiacan, Sinaloa, and high viability, confirms that this parasite may be a risk factor infecting dogs and humans in the city.

Molecular characterization of *Giardia duodenalis* in Piauí State, Brazil

Melo, Ana Carolina Fonseca Lindoso[1]; *Brito, Vanessa Meneses*[1]; *Durigan, Maurício*[2]; *Franco, Regina Maura Bueno*[2]; *Souza, Anete Pereira de* [2]; *Fernandes, Nelciane de Sousa*[1], *Santos, Augusto Cesar Cardoso dos*[1], *Ferreira, Hygor Fernandes*[1], *Trindade, Reginaldo Almeida da*[3]

[1]Universidade Federal do Piauí, Parnaíba, Brasil; [2]Universidade Estadual de Campinas, Campinas, Brasil; [3]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil.

Abstract

BACKGROUND: *Giardia duodenalis* is a protozoan that infects humans and a wide variety of animals. Studies of *G. duodenalis* genotyping showed that this species is a complex composed of eight assemblages A-H. The assemblages A and B infect humans and many species of animals, therefore are considered potentially zoonotic. This study aimed to characterize the assemblages of *G. duodenalis* infecting individuals from Parnaíba city in the state of Piaui, Brazil.

METHODS: A total of 145 faecal samples were collected from different parts of the studied city. The extraction of parasitic DNA was performed using the ZR Fungal/Bacterial DNA miniprep™ (kit from Zymo Research®). The samples amplified by PCR were further purified using the β-Giardin gene (*bg*) as marker. For the DNA sequencing reaction and analysis, BigDye Terminator v3.1 Cycle Sequencing kit and automatic sequencer ABI Prism™ Model 3700 DNA Sequencers (Applied Biosystems®) was used, respectively. The sequence chromatograms obtained were aligned by BioEdit Sequence Alignment Editor Version 7.0 software, comparing with homologous sequences available in GenBank. Phylogenetic analysis was performed using neighbor joining method by the software MEGA 5. Values of "bootstrap" were calculated by analysis of 100 replicates.

RESULTS: The *bg* gene amplification was performed in 35 faecal samples. From these, 26 samples were possible to sequence the amplified product. According to the molecular analysis, three genetic groups were identified, and assemblage A was observed in 24 isolates, all of them belonging to sub-assemblage AII.

CONCLUSIONS: This work showed that the assemblage A was the most prevalent genotype found in *G. duadenalis* infecting humans in Parnaíba city, with total predominance of the subtype AII as revealed by the subtyping analysis. These results suggest that antropozoonotic transmission may be the most common mode of giardiasis infection in the studied city. Financial support: CNPq (Processo 476396/2011-5)

Key-words: Parasitology, protozoa, *Giardia duodenalis.*

The effect of current developmental projects around Agulu Lake on the status of urinary schistosomiasis in Agulu community, Anambra State, Nigeria

Ekwunife, Chinyelu[1]*; Nwaorgu, Obioma*[1]*; Eneanya, Obiora*[1]*; Ukaga, Chinyere*[2]*; Ezeunala, Mercy*[3]

[1]Nnamdi Azikiwe University, Parasitology and Entomology, Awka, Nigeria, [2]Imo State University, Animal and Enviromental Biology, Owerri, Nigeria, [3]National Institute of Pharmaceutical Research and Development, Microbiology and Biotechnology, Abuja, Nigeria

BACKGROUND: Investigation to reveal the present status of *Schistosoma heamatobuim* in Agulu community around Agulu Lake, Anambra State, Nigeria was made. This lake is implicated with the transmission of the disease. The study showed a remarkable decline in the level of infection. The question then is, what could have led to this decline?
METHODS: Centrifugal examination of 600 urine samples from primary school pupils from two primary schools situated very close to the lake was done. The prevalence rate recorded was compared with rates recorded in the community between 2007 and 1990. Questionnaire distributed to 300 respondents and personal observations were used to determine the reason for the decline in prevalence.
RESULTS: Current infection level in 2014 showed an overall prevalence of 8(1.3%). Earlier records showed high prevalence rates of 54% in 1990, 55.2% in 2004 and low prevalence rates of 28.4% and11.4% in 1992 and 2007 respectively. Some respondents (60%) attributed the decline in infection to massive road construction around the lake. Others (40%) attributed the decline to the health education campaigns which has been on in churches and schools in the community over the past 20years. Personal observations showed that massive constructions around the lake prevented people from getting in contact with the water.
CONCLUSIONS: Except for the high prevalence rate in in 2004, the distribution of the disease is variable and the rates lowered as the years progressed. The decline in rate of infection could be attributed to the reduction and even non-contact with the water body. Health education campaigns mounted by researchers over the years as well as observed bore holes sunk by many families may have been contributing to the decline in infection. It is therefore suggested that measures like barring people from visiting infected water bodies, providing alternative good water supply and embarking on massive health education campaigns should be implemented in other infected communities. This will go a long way in the reduction and possible eradication of the disease in afflicted areas of the world as observed in this Agulu lake community.

Identification of a ubiquitin - calmodulin conjugate in the cytosolic fraction of *Ascaris suum.*

Suárez de Mata, Zadila; Guilarte Del Valle; García, Mildred; Monzón, María

Departamento de Biología Celular, Universidad Simón Bolívar, Apartado 89000, Venezuela.

BACKGROUND: The main function of ubiquitin is the labeling of intracellular proteins for degradation by the proteasome, a multienzymatic complex. Also, by attaching to proteins ubiquitin can modify their function without degradation. In a previous study ubiquitin and calmodulin were isolated and purified from the cytosolic fraction of *Ascaris suum* muscle. Antibodies anti-ubiquitin and anti-calmodulin were found to label a 31 kDa band when Ascaris cytosolic fraction was subject to immuno-blot. Trypsin incubation of tissues is widely used to prove the existence of internal ubiquitin conjugates. Here we use trypsin´s splitting of conjugates to identify ubiquitin-calmodulin conjugates. Trypsin cleaves the last two aminoacids of ubiquitin and/or ubiquitin-cojugates, releasing a 74 residue peptide which is indistinguishable from intact ubiquitin by SDS-PAGE.
METHODS: To detect and favor the formation of ubiquitin-calmodulin conjugates, 400 ug of the cytosolic fraction proteins were incubated for 30 min at 37 C, with and without trypsin (130 ug), in the presence of 100 uM calcium and 100 uM ATP at pH 8.5. Subsequently, 5% TCA was added, and the cooled incubation mixture was centrifuged at 16,000 g for 5 min. The pellet was resuspended in buffer sample before SDS-PAGE.
RESULTS: The trypsin incubation of *Ascaris* cytosolic fraction caused both the degradation of 31 kDa band present in this fraction and a paralell increase in the intensity of the ubiquitin band in SDS-PAGE. In addition, the 31 kDa band was only present in preparations of *Ascaris* muscle that contained the ubiquitin and calmodulin bands.
CONCLUSIONS: Our results confirm 31 kDa protein band of Ascaris muscle cytosolic fraction as a conjugate of ubiquitin with calmodulin.

Factors involved in transmission and development of congenital Chagas disease: from the laboratory to Public Health

Carlier, Yves

Laboratoire de Parasitologie, Faculté de Médecine, Université Libre de Bruxelles (ULB), Bruxelles, Belgique ; Department of Tropical Medicine, SPHTM, Tulane University, New Orleans, LA, USA

BACKGROUND AND METHODS: At least 2 million women in fertile age are estimated to be chronically infected with *T. cruzi* in Latin America, with a maternal-fetal average rate of parasite transmission by 5%. This presentation will discuss the role of the 4 main actors (parasite, mother, placenta, fetus) involved in the transmission and/or development of congenital Chagas disease.
RESULTS: i) Parasite: various genotypes of *T. cruzi* can induce human congenital infection; if asymptomatic congenital cases generally display less than 100 p/mL in blood, 100 to 1000 p/mL can be found in blood of symptomatic cases, and the rare lethal clinical forms can reach up to 55000 p/mL. ii) Mothers transmitting parasites to their fetuses display higher parasitemias associated with lower capacities to mount parasite-specific immune responses (IFN-g). iii) Placenta: parasites are rarely found in the villous trophoblast, indicating that the latter likely contributes to limit the infection; by contrast, parasites are present in the chorion and chorionic plate, to finally enter within embedded fetal vessels. iv) Infected fetuses/neonates are able to overcome their immunological immaturity to mount parasite-specific immune responses (T CD8+ cytotoxic cells producing FN-g) able to control parasite multiplication.
CONCLUSIONS: Congenital infection with *T. cruzi* results from: i) a weak maternal adaptative type 1-immune response inducing a high parasitic charge in retroplacental blood; ii) an hematogenous route of parasite transmission through placental areas deprived of trophoblast; iii) an insufficient fetal/neonatal type 1-parasite-specific immune response to control parasite multiplication. More investigations are needed to specify the role of parasite genotypes in such mechanisms.

Spontaneous serological negativization in patients with chronic chagas´ disease in placebo arm of randomized clinical trial TRAENA

Prado, Nilda Graciela; Hernandez Yolanda Maria; Tomas Juan Gonzalo; Fernandez Marisa Liliana; Velazquez Elsa Beatriz; Esteva Monica Ines; De Rissio Ana Maria; Ruiz Andres Mariano; Riarte Adelina Rosa

Instituto Nacional de Parasitología Dr. M Fatala Chaben. ANLIS-MALBRAN. Buenos Aires. Argentina

BACKGROUND: Since 1988 there have been reports of spontaneous serological negativization (SSN) without previous trypanocidal therapy in patients chronically infected with *Trypanosoma cruzi*. Their epidemiological, parasitological and clinical significance remains unknown.

METHODS: Demographic, serological and clinical characteristics of the placebo group were compared between those with SSN by conventional ELISA (cELISA), by ELISA with recombinant antigen F29 (F29) or both, and those of the same group with persistence positive serologies (PPS) for 11 years.

RESULTS: Fifty out of 323 (15.47%) placebo patients presented SSN; 27 of them by F29, 8 by cELISA and 15 by both techniques. Fifty four percent of SSN group were females vs 58% in the PPS (p=ns).The average age of SSN group was 38.43 ± 9.53 years vs 39.25 ± 10.15 of the PPS (p=ns). There were no differences between groups in duration of follow-up. SSN by both tests were more frequent in natives of Santiago del Estero (p <0.01). Baseline titers of cELISA were 0.293 ± 0.069 OD in SSN vs 0.352 ± 0.066 in the PPS (p<0.0001; 95% IC 0.0370-0.0822). Baseline F29 titers were 0.301 ± 0.129 SSN vs 0.412 ± 0.176 OD in PPS (p<0.0001 (95% IC 0.0672-0.1551). Baseline clinical stages (according to Kuschnir) and clinical changes during follow-up were not statically different, 1/50 primary events were detected in SSN group vs 18/273 in the PPS (p=ns) and there was no mortality in SSN vs 11/273 in the PPS (p=ns)

CONCLUSIONS: The highest frequency of SSN by both techniques in patients from Santiago del Estero could be related to *T.cruzi* strains circulating in this area. Patients with SSN during follow-up had lower titers of cELISA and F29 at baseline than SSP. Although there were absolute differences in deaths and primary events, the SSN group showed no statistical difference in disease progression

Two women presenting worsening cutaneous ulcers during pregnancy: diagnosis, immune response and follow up.

Conceição-Silva, Fátima [1*], *Morgado, Fernanda N.*[1], *Pimentel, Maria Inês F.*[2], *Vasconcellos, Erica CF*[2], *Schubach, Armando O.* [2], *Valete-Rosalino, Cláudia M.* [2], *Kropf, Pascale* [3], *Müller, Ingrid* [3*]

[1]Laboratório de Imunoparasitologia – IOC – Fiocruz, Brazil
[2]Laboratório VigiLeish– IPEC– Fiocruz, Brasil
[3]Imperial College London, Faculty of Medicine, Section of Immunology, United Kingdom
*fconcei@ioc.fiocruz.br

BACKGROUND: Changes in innate and adaptive maternal immunity during pregnancy are tightly linked to gestation, normalize post partum and can have beneficial or detrimental consequences. Pregnancy has been associated with a shift in the balance
of T helper cell subsets and reduced cell mediated immunity. However, our knowledge
of the impact of parasitic diseases on both mothers and infants is still very limited. In this context, exacerbation of American tegumentary leishmaniasis (ATL) during pregnancy has been reported; however, the underlying immunological mechanisms are
not clear.
METHODOLOGY: We analysed parasite burden, local (in situ immunohistochemistry) and systemic (cytokines, arginase) immune responses of 2 ATL patients during pregnancy and after delivery as well as 6 nonpregnant ATL patients and 10 healthy volunteers matched by sex and age.
RESULTS: Our results show that exacerbation of lesions during pregnancy was accompanied by lower levels of in situ IFN-gamma and inducible nitric oxide synthase (NOS2), and reduced frequencies of antigen-specific IFN-gamma production. After delivery, the healing of lesions correlated with reduced parasite load, increased in situ expression of NOS2 and increased systemic and in situ IFN-gamma.
CONCLUSIONS: Our results suggest that exacerbation of lesions in the two pregnant ATL patients coincides with a transient impairment of maternal immune responses that favours parasite growth. A better understanding of the nature of maternal immune modulation and the clinical evolution of leishmaniasis during pregnancy can help to improve management of pregnant patients.

Treatment of infections with intestinal coccidia and microsporidia

Romero-Cabello R

For the management of intestinal coccidiosis general treatment of disorders caused by protozoa and against the respective specific parasite is recommended. The goal of treatment is to relieve symptoms and disappearance of the parasite, if possible.
General management includes fluid and electrolytes replacement, proper diet, lactase deficiency correction, regulation of intestinal transit, decreased intestinal inflammatory process, restoration of the immune response, identification and correction of diseases of the biliary tract and try to reverse the muscle loss and correct weight loss. For patients who present with HIV infection, the handling of highly active antiretroviral therapy (HAART), to increase the CD4 cell count to above 100 cells, ideally achieve or maintain CD4 above 200, you can also prevent re-infection with these intestinal parasites.
The treatment for these organisms is: Cryptosporidiosis: the Nitazoxanide is used in doses of 500 to 1,000 mg twice a day for 14 days; Paromomycin at dose of 1500 mg to 2000 mg per day; macrolides such as spiramycin, azithromycin, roxithromycin, and clarithromycin. The elimination of the parasites is also better in patients with CD4> 50 cells. The Cyclosporiasis can be treated with trimethoprim-sulfamethoxazole for 7-10 days, in HIV-infected individuals and higher long-term doses are recommended; ciprofloxacin 500 mg every 12 hours for 7 days. The Isosporiasis is treated with trimethoprim-sulfamethoxazole at doses of 160/800 mg for two to four weeks; sulfadoxine 500 mg and pyrimethamine 75 mg / day + folinic acid 10 to 25 mg / day; ciprofloxacin 500 mg every 12 hours for 7 days.
For microsporidiosis there are no effective treatment, the best is an effective anti-HIV treatment to achieve CD4 cell counts above 100 cells, there are no medications approved by the FDA and have been used in research: oral fumagillin 20 mg three times a day, and albendazole 400mg twice a day for 2-4 weeks or albendazole 400mg twice a day plus itraconazole 400mg per day.
In summary for handling these parasitic diseases should make a full assessment of the patient to provide corrective antiparasitic and managing the consequences of parasitosis and immunosuppression status if applicable.

Plasmodium vivax subtelomeric variant proteins and cytoadherence to the human spleen

Fernandez-Becerra Carmen[1], Bernabeu Maria[1], Ferrer Mireia[1], Lopes Stefanie C.P[2], Thomson Richard[1], Martin-Jaular Lorena[1], Costa Fabio T.M[2], Lacerda Marcus V.G.[3,4], del Portillo Hernando A.[1,5].

1 Barcelona Centre for International Health Research, Barcelona, Spain
2 Instituto de Biologia, Universidade Estadual de Campinas, Campinas, Brazil
3 Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Manaus, Amazonas, Brazil
4 Universidade do Estado do Amazonas, Manaus, Amazonas, Brazil
5 Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain

BACKGROUND: The lack of a continuous *P. vivax in vitro* culture for blood stages has prevented progress in understanding the molecular basis of pathology in this species. Recent observations have challenged the dogma that *P. vivax*- infected reticulocytes do not sequestrate in the deep vasculature of internal organs.
METHODS: To identify *P. vivax* genes possibly involved in pathology, we generated *P. falciparum* transgenic lines expressing members of vivax multigene families. These transgenic lines were used in adhesion experiments on human spleen fibroblasts and spleen cryosections. In parallel, we also performed adhesion experiments using *P. vivax* isolates from the Brazilian Amazon basin.
RESULTS: We show that a transgenic line expressing a VIR protein but not another transgenic line expressing a member of a distinct sub-telomeric family (Pv-FAM-D) mediated adherence to human spleen fibroblasts. Spleen-adherence specificity was shown as neither transgenic line bound to human lung fibroblasts. To extrapolate these results to natural infections, adhesion experiments on human spleen fibroblasts and spleen cryosections were performed using *P. vivax* field isolates. Results demonstrated adherence, albeit variable, among different isolates. Moreover, this adherence was partially inhibited by anti-VIR antibodies.
CONCLUSIONS: These results together with recent literature observations challenge the dogma that *P. vivax*-infected reticulocytes do not cytoadhere in the microvasculature of internal organs. The understanding of the evolutionary advantages of *P. vivax* spleen adhesion as well as the identification of receptors involved in this adherence may suggest new control and elimination strategies against this species.

Predicting maps of triatomines distribution in Oaxaca, Mexico.

Hernández-Osorio, Luis Alberto[1,2]; Rivera-García, Raúl[3]; Jiménez-Solís, Gerardo Enrique[4]; Guzmán-Bracho, Carmen[5]; Calvillo-Martínez, Santiago[6]; Manning-Cela Rebeca Georgina[1].

[1]Departamento de Biomedicina Molecular, CINVESTAV-IPN, 07360, México D.F., México. [2]Facultad de Ciencias Químicas, Universidad Autónoma Benito Juárez de Oaxaca, 68120, Oaxaca City, México. [3]Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional-IPN, Oaxaca, 68120, Oaxaca, México. [4]Servicios de Salud de Oaxaca, 68120, Oaxaca, México. [5] Instituto de Diagnóstico y Referencia Epidemiológicos, 01480 México D.F., México. [6] Unidad de Investigación en Biomedicina, Facultad de Estudios Superiores Iztacala, 54076, Estado de México, México.

BACKGROUND: American trypanosomiasis is a highly disabling and often deadly disease that is endemic in Latin America. The etiological agent, *Trypanosoma cruzi*, is transmitted by hematophageous triatomines from the Reduviidae family. In the absence of an efficient treatment and an effective vaccine, the interruption of parasite transmission to human is based on vector control. Although Mexico is an endemic area, specific programs for vector control have not been implemented. Therefore, the identification of domestic area with infected triatomines with *T. cruzi* are needed to initiate the rational design of vector control strategies.
METHODS: In this work, we used Geographic Information Systems (GIS), remote sensing techniques and analysis of maximum entropy (Maxent), to study the geographic distribution of the triatomine species present in Oaxaca, Mexico, collected for 9 years. Maps of domestic abundance of triatomines and *T. cruzi* infection rates in the vectors were obtained, as well as predictive maps of the presence/absence of triatomines.
RESULTS: The geographic distribution map of the seven triatomine species found in this study, showed a specie-specific pattern of distribution associated to bioclimatic factors. The analyses of vector abundance and *T. cruzi* infection rates in the triatomines studied, showed a high risk of Chagas disease transmission in domestic areas of Oaxaca. Efficient maps of the presence/absence prediction were obtained for *T. barberi, T. phyllosoma and T. mazzotti*.
CONCLUSIONS: The Maxent analysis shows the generation of a robust hypothetical model of distribution that could be a useful tool for the design and implementation of triatomine control programs in Oaxaca.

Screening of Marine Derived Natural Products against Chagas Disease

Siqueira-Neto, Jair[1,2]; James, Robert[3]; Kellar, Danielle[1]; Linington, Roger[3]; McKerrow, James[1,2].

1. University of California San Francisco
2. University of California San Diego
3. University of California Santa Cruz

BACKGROUND: Chagas' disease caused by the *Trypanoma cruzi* protozoan parasite was is a main cause of death due to heart failure in Latin America. There are only two available drugs, Benznidazole and Nifurtimox and both have limited or unproven efficacy in the chronic phase of the disease. Recent failure in the Clinical Trials with the promising ergosterol biosynthesis inhibitors strengthened the urgent need for new and better chemotherapy.

METHODS: Historically, the majority of drugs are derived from natural products. Tractability and limited availability are common drawbacks. An operation was streamlined to build a library of marine natural products at the University of California Santa Cruz able to generate sufficient amount of compounds from reliable sources. The library composed by 2,500 pre-fractions was screened against *T. cruzi* in image-based high-content screening.

RESULTS: We have identified 102 “hits” from the primary screening showing more than 80% parasite elimination and no obvious sign of host cell cytotoxicity. The natural product “hits” were further fractionated into individual compounds, and then tested in dose-response to determine the anti-parasitic potency (EC_{50}) and host cell toxicity (CC_{50}). One of the selected compounds demonstrated sub-micromolar EC_{50} against intracellular parasite, no cytotoxicity up to 20 µM and is now being tested for *in vivo* efficacy in an acute Chagas mouse model.

CONCLUSIONS: Preliminary results confirmed the great potential of marine natural products as anti-parasitic agents. An acute Chagas mouse model is being used to evaluate the *in vivo* efficacy of a purified compound.

13th INTERNATIONAL CONGRESS OF PARASITOLOGY
AUGUST 10th - 15th, 2014
ICOPA XIII
MÉXICO

Comparison of *Leishmania* DNA extraction yield and quality using kits or in-house systems and different tissues aiming the diagnosis of canine visceral leishmaniasis by PCR

Araújo, Kamila[1]*; Batista, Maria das Graças*[2]*; Antas-Cordeiro, Aline*[3]*; Nóbrega, Gilzane*[4]*; Camboim, Expedito*[5]*; Andrade, Paulo*[6]*; Melo, Marcia*[7]

[1]Veterinarian, Patos,PB , Brasil; [2] Veterinarian, Caicó, RN, Brasil; [3]Veterinarian, City Sanitary Surveillance Agency, Patos, PB, Brasil; [4]Veterinarian, Universidade Federal da Paraíba, Areia, PB, Brasil; [5]Vaterinarian, EMATER, Patos, PB, Brasil; [6]Professor, Departament of Genetics, Universidade Federal de Pernambuco, Recife, PE, Brasil; [7]Professor, Academic Unit of Veterinary Medicine, Universidade Federal de Campina Grande, Patos, PB, Brasil.

BACKGROUND: The dog has a central role as a reservoir in visceral leishmaniasis in many countries. Most dogs have subclinical infections, making laboratory diagnosis obligatory. Serology is the usual method, but polymerase chain reaction (PCR) is sometimes used in routine diagnosis, being moreover the most important tool for evidencing the presence of *Leishmania infantum* in research laboratories. Despite its frequent use, there has been no systematic comparison of tissues as source of DNA and extraction methods, and the consequent results in terms of DNA quantity and purity and of applicability in PCR.
METHODS: In this paper three classic DNA extraction methods and six tissues as a source of DNA were assessed: whole blood, conjunctival swab, bone marrow, liver, spleen and popliteal lymph node, from 16 asymptomatic animals. DNA extraction methods were the isopropanol technique (Ausubel et al. 2002), silica extraction (as described by the Australian Commonwealth Scientific and Industrial Research Organisation - CSIRO) and a commercial kit (DNeasy Blood & Tissue Kit Qiagen®). The DNA was quantitated, its purity measured by photometry (260 nm/280 nm) and amplified by PCR (Lambson et al. 1999).
RESULTS: The commercial kit performed better than the other methods for all tissues (organs) tested, both in terms of quantity of DNA extracted and purity. The isopropanol technique had an intermediate performance and the silica was unsatisfactory. Spleen, liver, and popliteal node biopsies were the best DNA source. PCR using DNA with the commercial kit from popliteal lymph node, spleen and liver had mean positivities of 72.2, 66.7 and 66.7 %, respectively. There was no positive result in PCR from blood, bone marrow and conjunctival swab in any three techniques assessed.
CONCLUSIONS: Together, our results indicate that DNA extraction by a suitable commercial kit is more efficient than traditional in house methods, and that spleen, liver or popliteal lymph node biopsies should be preferred to other cells sources for *Leishmania infantum* DNA extraction.

A new *Leishmania infantum* gene codes for an antigen recognized by sera from naturally infected dogs

Melo, Marcia[1]*; Andrade, Paulo*[2]

[1]Professor, Academic Unit of Veterinary Medicine, Universidade Federal de Campina Grande, Patos, PB, Brasil; [2]Professor, Departament of Genetics, Universidade Federal de Pernambuco, Recife, PE, Brasil;

BACKGROUND: This work describes the likely occurrence of a gene on *Leishmania infantum* chromosome 28 not previously annotated by the Artemis platform from the Sanger Institute. Its product is an antigen recognized by canine visceral leishmaniasis sera.
METHODS: Gene isolation was done by immunoscreening of a cDNA *Leishmania chagasi* library using canine visceral leishmaniasis sera. The similarity between the gene sequence obtained from one reactive clone (in pSportII plasmid) and chromosome 28, as deposited in the TriTryp database, was stated by the NCBI version of Basic Local Alignment Search Tool (BLAST). The sequences of the putative gene products as indicated by ORFinder/blastp were also analyzed by InterProScan in order to identify possible domains.
RESULTS: The contig obtained from the forward and reverse sequences of the reactive clone were similar to a syntenic region of chromosome 28 of *L. infantum* JPCM5, *L.* donovani BPK282A1 and *L. major* Friedlin (99%, 99% and 90% identity, respectively). The clone sequence has 295 bp and is identical to *L. infantum* homologous region except for the deletion of five bases. The putative new gene is located between genes LinJ.28.380 (Katanin protein) and LinJ.28.390 (hypothetical protein), a region encompassing about 5000 bp (from base #132117 to base #137587) and is translated in the same direction as its neighbors. Another clone obtained from the same cDNA library also maps in this region. At least three ORFS are candidates for the gene, but none of the putative proteins were similar to previously described polypeptides in GenBank nor had domains or signatures recognized by InterPro.
CONCLUSIONS: Together, our results suggest that the automatic annotation of all trypanosomatid genomes missed at least one gene, possible due to its size or lack of other gene characteristics. Other genes may be brought to light when more gene sequences coding for antigens and other cell proteins are independently studied.

13th INTERNATIONAL CONGRESS OF PARASITOLOGY
AUGUST 10th - 15th, 2014
ICOPA XIII MÉXICO

Trypanocidal activity of coumarins from *Calophyllum brasiliense* on Mexican strains of *Trypanosoma cruzi.*

Rodríguez-Hernández, Karla[1]; Martínez-Martínez, Ignacio, Agredano-Moreno, Lourdes[3], Jiménez-García, Luis Felipe[3], Reyes-Chilpa, Ricardo[2]; Espinoza-Gutiérrez Bertha[1]

[1]Departamento de Inmunología, Instituto de Investigaciones Biomédicas, UNAM, 04510, México City, México.
[2] Departamento de Productos Naturales, Instituto de Química, Universidad Nacional Autónoma de México, México City, Distrito Federal, México.
[3] Laboratorio de Biología Celular, Facultad de Ciencias, UNAM, 04510, México City, México.

BACKGROUND: The currently available treatments for Chagas disease caused by the protozoan *Trypanosoma cruzi* show limited therapeutic potential, and are associated with serious side effects. Therefore, exists a great need for development of new compounds with greater activity against the parasite and lower toxicity to the host. The aim of this study was to determine the trypanocidal activity of the mammea-type coumarins isolated from the leaves of the tropical tree *Calophyllum brasiliense* on epimastigotes and tripomastigotes of three Mexican strains of *Trypanosoma cruzi*.
METHODS: A mixture of coumarins mammea A/BA+ A/BB+ A/BD (6:3:1) was isolated from the organic extract of the leaves, and its effects on *T. cruzi* epimastigotes and trypomastigotes were evaluated. Mobility, growth recovery, morphology, and ultrastructural changes of the parasites after being exposed to these compounds were analysed.
RESULTS: The mixture of coumarins showed tripanocidal activity against epimastigotes y trypomastigotes of three Mexican strains of *T. cruzi* (IC_{50}= 6.92-14.3 µg/mL). By using transmission electron microscopy, we observed that the compounds caused severe ultraestructural alterations in the parasites such as reduction in the citoplasmic density, abnormal chromatin condensation, intense cytoplasmic vacuolization, alterations of nuclear envelope, and plasma membrane, light mitochondrial swelling and the appearance of autophagic vacuoles leading to death of the parasite.
CONCLUSIONS: Our results confirm and extend the knowledge about mammea type coumarins as an important resource of tripanocidal drugs, and indicate that these compounds could be a promising therapeutic option, since these showed greater pharmacological potency than Benznidazole (100µg/mL), and it has been previously reported to have low mammalian toxicity (LD_{50} > 3000 mg/kg, mice) *in vivo* and *in vitro* and (LC_{50} = 124mM, human lymphocytes).
ACKNOWLEDGEMENTS: This work was supported by Grant IG20513 from PAPIIT-DGAPA-UNAM to Dr. Ricardo Reyes Chilpa, IN206512 DGAPA-UNAM and grant from Fundación Miguel Alemán to Dr. Bertha Espinoza.

Genotoxicity induced by *Taenia solium* and its reduction by immunization with calreticulin in a hamster model of taeniasis.

Oscar Chávez-Talavera[1], Ana María Salazar[1], Fela Mendlovic[2], Mayra Cruz-Rivera[2], Montserrat Sordo[1], Guillermina Ávila[2], Ana Flisser[2], Patricia Ostrosky-Wegman[1].

[1]Genomic Medicine and Environmental Toxicology Department, Biomedical Research Institute, National Autonomous University of Mexico (UNAM), Mexico City, Mexico. [2]Microbiology and Parasitology Department, Faculty of Medicine, UNAM, Mexico City, Mexico.

BACKGROUND: Genotoxicity induced by neurocysticercosis has been demonstrated in vitro and in vivo in humans. The adult stage of *Taenia solium* lodges in the small intestine and is the main risk factor to acquire neurocysticercosis, nevertheless its carcinogenic potential has not been evaluated.
METHODS: In this study, we determined the genotoxic effect of T. solium infection in the hamster model of taeniosis. In addition, we assessed the effect of oral immunization with recombinant T. solium calreticulin (rTsCRT) plus cholera toxin as adjuvant on micronuclei induction. Blood samples were collected from the orbital venous plexus of noninfected and infected hamsters at different days postinfection, as well as from orally immunized animals, to evaluate the frequency of micronucleated reticulocytes as a measure of genotoxicity induced by parasite exposure and rTsCRT vaccination.
RESULTS: The infected hamsters presented an increased frequency of micronucleated RETs. Oral administration of CT has no effect on DNA damage reduction but inclusion of rTsCRT in the vaccination protocol, either alone or given together with the adjuvant, presented a lower genotoxic effect.
CONCLUSIONS: Our results indicate that infection with T. solium caused time-dependent DNA damage in vivo and that rTsCRT immunization could have reduced the genotoxic damage induced by the presence of the tapeworms.

In situ expression of cytokines in abomasal wall associated with induced protection against ovine experimental haemonchosis.

Buendía-Jiménez, Javier; Cuenca-Verde, César; Martínez-Labat, Pablo; Perez-Luna, Ivan; Muñoz-Guzmán, Marco; Cuéllar-Ordaz, Alfredo; Alba-Hurtado, Fernando

Departamento de Ciencias Biológicas, FES-Cuautitlán, Universidad Nacional Autónoma de México, Cuautitlán Izcalli Edo. de México, México.

BACKGROUND: Application of a *Taenia hydatigena* larvae vesicular concentrate (ThLVC) in lambs induce protection against *Haemonchus contortus* associated to increase of eosinophils, CD4 lymphocytes and γδ lymphocytes in abomasal wall (AW). The cytokines involved in this protection are unknown. In this study we correlated the expression of some cytokines in AW with the protection induced by the ThLVC in lambs.
METHODS: Six groups (G) of lambs (n=5) were inoculated with ThLVC or vehicle /infected or non-infected with L3 of *H. contortus* and /euthanized at different days. G1: vehicle/non-infected/day49; G2: ThLVC/non-infected/day0; G3: ThLVC/infected/day3; G4: ThLVC/non-infected/day49; G5 ThLVC/infected/day49 and G6 vehicle/infected/day49. Fecal egg count (FEC) and blood eosinophils (BE) were periodically measured. The adult phases (FA) in abomasum were counted and the numbers of eosinophils, mast cells, and cells expressing IL-2, IL-4, IL-6, IL-10 and IFNγ in AW were determined.
RESULTS: Lambs of G6 had FEC´s and number of FA higher ($p<0.05$) than lambs of G5. The lambs of G4 and G5 had higher ($p<0.001$) BE average than lambs of G1 and G6. Lambs of G5 had a higher number ($p<0.001$) of eosinophils, mast cells, cells expressing IL-4, IL-6, IL-10, IFNγ and IL-2 in the AW than other groups. The worm burden was negatively correlated with the number of eosinophils, mast cells, and the expression of IL-2, IL-4, IL-6 and IFNγ in different regions of the AW.
CONCLUSIONS: Results suggest that the protection induced by the ThLVC is associated with cytokine expression of both Th1 and Th2 response in the AW.

B-cell epitopes of antigenic proteins in *Leishmania infantum*: an in silico analysis

Assis, Luciana[1,2]; Sousa, João[3]; Pinto, Nedja[4]; Silva, Abrão[5]; Vaz, Antônio[6]; Andrade, Paulo[7]; Carvalho, Edgar[8,9]; Melo, Marcia[6].

[1]Graduate Program in Medicine and Health, Universidade Federal da Bahia-UFBA, Salvador, Brazil; [2]Academic Nursing Unit, Universidade Federal de Campina Grande-UFCG, Cajazeiras, Brazil, [3]Faculdade Santa Maria-FSM, Cajazeiras, Brazil; [4]Graduate Program in Veterinary Medicine, Universidade Federal de Campina Grande-UFCG, Patos, Brazil, [5]Academic Unit of Exact and Natural Sciences, Universidade Federal de Campina Grande-UFCG, Cajazeiras, Brazil, [6]Academic Unit of Veterinary Medicine, Universidade Federal de Campina Grande-UFCG, Patos, Brazil, [7]Department of Genetics, Universidade Federal de Pernambuco-UFPE, Recife, Brazil, [8]Graduate Program in Medicine and Health, Immunology Service, Universidade Federal da Bahia-UFBA, Salvador, Brazil, [9]Instituto Nacional de Ciências e Tecnologia de Doenças Tropicais - INCT DT(CNPq/MCT), Salvador, Brazil.

BACKGROUND: Serodiagnosis of visceral leishmaniasis is often hindered by cross-reactions to other parasitic diseases. Identifying specific B-cell epitopes in proteins is therefore important for immunodiagnostics, as well as for disease control by vaccines. This study aimed to identify linear and conformational B-cell epitopes and to evaluate the secondary structure of antigen proteins in *Leishmania infantum* using in silico analysis.
METHODS: Linear epitopes were predicted using the Immune Epitope Database and Analysis Resource (IEDB), BepiPred and BcePred programs. The conformational B-cell epitopes were identified using the CBTOPE server.
RESULTS: The combination of the predictions using IEDB, BepiPred and BcePred generated 148 linear epitopes from the calpain-like cysteine peptidase (CP), thiol-dependent reductase 1 (TDR1) and HSP70 proteins. In total, 164 conformational epitopes were predicted, mostly located in the linear epitope region. The predicted epitopes are located in a helix and random coil regions in the thiol-dependent reductase 1 and HSP70 proteins.
CONCLUSIONS: New linear and conformational B-cell epitopes of *L. infantum* proteins were identified in silico, and the prediction using various programs ensures greater accuracy of the results, as suggested by confirmation of previously identified HSP70 epitopes.

Diagnosis and tools after specific treatment of human Chagas disease

Luquetti Alejandro O., Anis Rassi & Anis Rassi Júnior
Hospital das Clínicas, Universidade Federal de Goiás, Brazil

Serological diagnosis of *Trypanosoma cruzi* infection is necessary and nowadays may be performed with a high degree of specificity and sensitivity, even with the traditional tools, ELISA, indirect immunofluorescence and indirect hemagglutination, if used properly (reagents and laboratory practice of good quality (GLP). Inconclusive results account for less than 2%. The combination with new tools (several recombinant antigens and/or synthetic peptides), increases accuracy. The problem is to apply them after antitrypanosomal treatment in order to assess cure. Congenital newborns have negative seroconversion in all cases several months after therapy. Those in the acute phase of the disease and children under 12 years of age (recent chronic phase) cure in a lower proportion (i.e. around 60%) if infected with TcII or TcV. The proportion of cure in those that harbour TcI seems to be much higher and of faster documentation (Colombia and Central America). Negative serology is obtained after 2-5 years of follow up. After treatment of patients in the chronic late phase of the disease, approximately 20%) have positive parasitological tests, indicating treatment failure. Another group of nearly 30% have negativization of serological tests after variable periods of time. The remaining patients follow a pattern of decreasing IgG titers and are still under active follow up. Parasitological tests remain negative. For this group, other markers are necessary. Perhaps a better strategy is to look for changes in proteomics, instead of searching for antibodies that depend on a long lasting immune response. In this sense, *T. cruzi* has enzymes that cleave human proteins. The destruction of them will show non-cleaved enzymes that are investigated by proteomics. Some recent studies have addressed this issue and results are promising.

Mucosal delivery of ACNPV baculovirus driving expression of the Gal-lectin LC3 fragment confers protection against amoebic liver abscess in hamster

Carrero, Julio César[1]; Meneses, María[1]; Aguilar, Hugo[1]; Hernández, Joselín[2]; Garduño, Alejandra[3]; Vaca, Luís[4]; Laclette Juan Pedro[1].

[1]Depto. de Inmunología, Instituto de Investigaciones Biomédicas; [2]Unidad de Medicina Experimental, Hospital General de México; [3]Bioquímica Diagnóstica, Fes Cuautitán and [4]Depto de Biología Celular, Instituto de Fisiología Celular. Universidad Nacional Autónoma de México, 04510, México D.F., México.

Background: Mucosal vaccination against amoebiasis using the Gal-lectin of *E. histolytica* has been proposed as one of the leading strategies for controlling this human disease. However, most mucosal adjuvants used are toxic and the identification of safe delivery systems is necessary. **Methods:** Here, we evaluate the potential of a recombinant *Autographa californica* baculovirus driving the ex-pression of the LC3 fragment of the Gal-lectin to confer protection against amoebic liver abscess (ALA) in hamsters following oral, nasal or intramuscular immunization. **Results:** Hamsters immunized by oral and intramuscular routes showed complete absence (58% and 55%, respectively) or partial development (21% and 20%, respectively) of ALA, resulting in some protection in 79% or 75% of animals when compared with the wild type baculovirus and sham control groups. In contrast, nasal immunization conferred only 21% of protection efficacy. Levels of ALA protection in all groups showed lineal correlation with the development of an anti-amoebic cellular immune response evaluated in spleens, and release of INFγ and IL-4 in the liver. Protection in the group immunized by the intramuscular route, but not the mucosal routes, was also associated with development of seric IgG an-ti-amoeba antibodies. **Conclusion:** These results suggest that baculovirus driving the expression of *E. histolytica* vaccine candidate antigens are useful for inducing protective cellular and humoral immune responses against extraintestinal amoebiasis following oral and intramuscular immunization, and therefore they could be used as a strategy for mucosal or systemic delivery of an anti-amoebic vaccine.

Project supported by grants CONACyT 167788 and DGAPA IN213611.

Dendritic cell subtypes and effector T cell in human mucocutaneous leishmaniasis

Rodríguez, Orquídea L., Lugo, Dennis A., Cabrera, Maira, Zerpa, Olga, Tapia, Felix J.

Instituto de Biomedicina, UCV, MPPS, Apartado 4043, Caracas 1010A, Venezuela

BACKGROUND: Mucocutaneous leishmaniasis (MCL) is characterized by an exacerbated inflammatory response and parasites spread from a primary lesion to distant sites, resulting in destructive secondary lesions. Dendritic cells (DCs), myeloid and plasmacytoid (mDCs and pDCs) are involved in the activation of naive T cells. The aim was to correlate activation of DCs with effector T cell functions measured by the expression of specific T cell transcription factors in peripheral blood by flow cytometry.
METHODS: Characterization was done using: mDCs (CD11c +), pDCs (CD123 +), activated DCs (CD83), mature DCs (CD86 +), Th1 (T-bet), Th2 (GATA-3), Th17 (ROR-gamma t) and Treg (FOXP-3). Groups were MCL (n=13); Endemic (EC, n=8) and non-endemic (HC, n=7) control individuals.
RESULTS: 69.2% and 38.5% of MCL patients expressed CD83 above CS values for mDCs and pDCs, respectively. None and 7.7% MCL patients expressed CD86 in m-DCs and p-DCs, respectively. In MCL with activated mDCs their T cell expressed ROR-gamma t = 88%, FOXP-3 = 80%, GATA-3 = 67% and T-bet = 50%, whereas MCL with non-activated-mDCs T cells were different ($p < 0.05$) showing ROR-gamma t = 75%, FOXP-3 = 100%, GATA-3 = 50% and T-bet = 0%. Comparison of activated vs non-activated pDCs in MCL, gave differences ($p < 0.01$) on ROR-gamma t = 80% vs 100% and T-bet = 20% vs 38%. Multiple variance analyses showed that mDCs CD83+ in MCL are associated with ROR-gamma t, FOXP-3 and GATA-3, whereas pDCs CD86+ was associated with T-bet.
CONCLUSIONS: Results suggest a higher activation of mDCs than pDCs in MCL patients, associated with Th1/T-bet response, whereas, activation of pDCs is associated with Th17- ROR-gamma t. Financed by CDCH UCV PG-09-7429-2008.

Bovine lactoferrin resolves infection in a murine model of intestinal amoebiasis

Carrero, Julio César[1]; León-Sicairos, Nidia[2,3]; Martínez-Pardo, Leonardo[1]; Bobes, Raúl[1]; Laclette, Juan Pedro[1]; de la Garza, Mireya[4].

[1]Departamento de Inmunología, Instituto de Investigaciones Biomédicas, UNAM, México, D.F. 04510, México. [2]Unidad de Investigación, Facultad de Medicina, Universidad Autónoma de Sinaloa, Culiacán Sinaloa. 80246, México. [3]Departamento de Investigación del Hospital Pediátrico de Sinaloa. Culiacán Sinaloa. 80200 México. [4]Departamento de Biología Celular, Centro de Investigación y de Estudios Avanzados-IPN, Apdo. Postal 14-740, México, D.F., México.

Background: Mucosal immunity plays a major role in defense against pathogens to which it is exposed. The protein lactoferrin (Lf), part of the innate immune system, has a broad spectrum of action against virus, bacteria, fungi and protozoa. We have reported previously that bovine Lf (bLf) is able to kill trophozoites of *Entamoeba histolytica* in *in vitro* cultures. The aim of this study was to evaluate the therapeutic effect of oral administration of bLf in the resolution of cecal amoebiasis of susceptible mice. **Methods:** Mice C3H/HeJ strain were intracecally infected with 1 x10^6 trophozoites, and after 15 days, orally treated with 20 mg/kg bLf administered each day during a week. After sacrifice, ceca of mice were exscinded and processed for histological analysis. **Results:** Treatment with bLf was able to eradicate the infection in 63% of mice, since neither trophozoites nor evidence of epithelial damage and/or swelling was found in the tissue sections. The rest of the treated animals (37%) showed a decrease in the number of trophozoites and in the amount of mucus secreted to the lumen, compared with untreated and infected mice ($P < 0.05$). Analysis of the cytokine secretion profile by immunohistochemistry revealed that infected animals but untreated showed a Th1 profile (INFγ, TNFα, IL-6), whereas the ceca of cured mice showed a mixed Th2/regulatory profile (IL-10 and TGFβ) which was associated with the little inflammation observed in the cecum of these animals. **Conclusions:** These results suggest that oral administration of Lf can control intestinal amoebic infection, killing amoebas or favoring their removal re-establishing the anti-inflammatory intestinal environment and homeostasis (immunomodulation). Finally, this study leaves open the possibility of using lactoferrin, a natural component of innate immunity, as a potent anti-amoebic.

Project supported by grants CONACyT 167788, DGAPA IN213611 and CECYT (2006, 2008; Sinaloa, México).

13th INTERNATIONAL CONGRESS OF PARASITOLOGY
AUGUST 10th - 15th, 2014
ICOPA XIII MÉXICO

Analysis of pteridine pathway and antioxidant enzymes of a nitric oxide natural resistant and susceptible strains of *Leishmania braziliensis*.

Souza, Nathalia.P.[1]; *Menna-Barreto, Rubem.F.S.*[2]; *Cupolillo, Elisa*[1] ; *Cuervo, Patrícia*[1]

[1]Laboratório de Pesquisa em Leishmaniose, Instituto Oswaldo Cruz – Fiocruz
[2] Laboratório de Biologia Celular, Instituto Oswaldo Cruz - Fiocruz

BACKGROUND: *Leishmania (Viannia) braziliensis*, one of the main etiological agents of American Tegumentary Leishmaniasis (ATL) in the American continent, is associated with various clinical outcomes including self-healing localized cutaneous lesions, multiple disseminated lesions and metastasis to oropharyngeal mucosa. Both the infecting parasite and the host immune response contribute for the clinical presentation. The production of cytokines, reactive oxygen species (ROS) and nitric oxide (NO) normally leads to the destruction of phagocytosed microorganisms. The *L. (V.) braziliensis* strains used in this study were previously characterized as being naturally resistant or susceptible to NO. In addition, the resistant strain was also related to higher number of lesions and severe injuries than the susceptible strain, as well as to resistance to pentavalent antimony, the first-line drug for ATL. However, the molecules and/or metabolic pathways of the parasite that contribute to such resistance are unknown. The objective of this work is to identify molecules associated with NO resistance that ultimately may be related to the different clinical manifestations caused by these polar strains. METHODS: The susceptibility of the strains to H_2O_2 and Menadione was analysed by calculating IC_{50}. In addition, the expression profile of the genes of the pteridine pathway: arginase 1, pteridine reductase and biopterin transporter as well as the antioxidant enzymes like tryparedoxin peroxidase was measured by qPCR. RESULTS: Our preliminary results pointed to similar susceptibility of both strains to exogenous H_2O_2 and Menadione. In addition, qPCR analysis showed similar levels of the genes but their levels of expression varies according to the growth stage. CONCLUSIONS: This suggest that we can exclude ROS as part of the molecular mechanism involved in the observed phenotype and these gene expressions are not directly related to the NO resistance differences in promastigotes.

Resistance management in sheep: a New Zealand perspective

Sutherland, Ian; Leathwick, Dave

AgResearch, Grasslands Research Centre, Private Bag 11008, Palmerston North, New Zealand.

Strategies for managing anthelmintic resistance, which do not compromise effective worm control, are crucial for the sustainability of profitable extensive livestock farming. In New Zealand, these strategies rely on 1) The identification and mitigation of high-risk practices, 2) Using effective drenches and 3) Maintaining an adequate refuge of unselected parasites. Each of these recommendations has been tested experimentally, and regularly updated to adapt to changing circumstances and new knowledge. Examples are: 1) a recognised high-risk factor is the use of persistent anthelmintics, particularly in ewes pre-lambing. Despite this, the use of controlled-release capsules is still common, due to perceived economic benefits. A recent study has established, however, that these benefits are not guaranteed. 2. The introduction of two new active families provided new options for sheep farmers. Modelling studies have recommended the tactical use of these products in order to minimise selection for resistance. It is noted, however, that resistance to monepantel has already occurred on a number of goat farms in New Zealand. 3. Farmer acceptance of refugia is high in New Zealand, with numerous strategies employed to maintain an adequate pool of unselected parasites. Most encouraging for sheep farmers is the demonstration on a small number of farms that a carefully-planned and properly implemented strategy for resistance management, utilising these three recommendations, can effectively mitigate the issue without negatively impacting on worm control. The challenge now for researchers and advisers is to ensure the adoption of these strategies across the wider industry.

The salient participation of the protein tyrosine phosphatase of regenerating liver, PRL in *Entamoeba histolytica's* migration

Ramírez-Tapia, Ana Lilia; Espíritu-Gordillo, Patricia; Baylón-Pacheco, Lidia; Rosales-Encina, José Luis

Department of Infectomic and Molecular Pathogenesis, CINVESTAV-IPN, 07360 Mexico City, Mexico.

BACKGROUND: Nowadays, evidence has accumulated suggesting that PRLs may play a major role in tumorigenesis and metastasis. Enhanced migration is a fundamental characteristic of tumor cells as well as *Entamoeba histolytica,* which is a highly mobile and invading parasite that has a single gene encoding for PRL compared with the 3 isoforms identified in humans. In this study we show the EhPRL participation in *E. histolytica's* migration and pathogenesis.
METHODS: We carried out RT-PCR assays from axenic trophozoites and those recovered from amebic liver abscess (ALA) to evaluate mRNA expression of EhPRL. To analyze the EhPRL participation in migration, EhPRL with N or C-terminal HSV-tag were obtained into EhpN*eo*-CAT and transfected into trophozoites; Chemotaxis Index (CI) was obtained by transwell assays with fibronectin or adult bovine serum (ABS) as chemoattractant. Wound-healing assays, immunofluorescences and cellular fractionation also were done.
RESULTS: The mRNA expression of EhPRL was higher in trophozoites from ALA than axenic trophozoites. By Transwell and wound healing assays we demonstrated that overexpression of EhPRL is modulating migration's amoeba in response both ABS and fibronectin. Trophozoites overexpressing HSV-EhPRL (N-terminal) were more active migrating than either trofozoites overexpressing EhPRL-HSV (C-terminal) or trophozoites transfected with the empty vector. The protein HSV-EhPRL (N-terminal) is located in plasma membrane and cytoplasm while in trofozoites overexpressing EhPRL-HSV (C-terminal), the protein is mainly in cytoplasmic fraction.
CONCLUSIONS: Our results suggest EhPRL participation in virulence and development of ALA, because EhPRL is involve in migration of *E. histolytica* and its participation may be mediated by the EhPLR post-translational modification in its CAAX box.

Peptide mimics of CarLA – a viable alternative?

Pernthaner, Tony; Deng, Qing; Umair, Saleh; Roberts, Joanna; Shaw, Richard; Sutherland, Ian.

AgResearch, The Hopkirk Research Institute, Palmerston North, New Zealand.

BACKGROUND: The glycan molecule CarLA (carbohydrate larval antigen) is expressed in the cuticle of numerous parasites L3 capable of infecting livestock. Immunologically, CarLA is a protective antigen in sheep and potentially in other livestock species. The mucosal antibody response to the molecule consists predominantly of IgA and IgG and prevents establishment of ingested larvae. However, the structure and composition of CarLA have not been defined and chemical synthesis of epitopes or the entire molecule is unlikely. The aim of this study, therefore, was to identify peptide mimics of a CarLA epitope which shares conformational and immunological properties of the parent molecule, and which could serve as a functional substitute.
METHODS: A disulphide-constrained heptapeptide phage display library was used to select phage clones with specific affinity for the anti-CarLA mAb PAB1. Clones were then tested for their ability to bind PAB1 by ELISA. To assess if any of the synthetic peptides exhibited reactivity with antibodies in biological fluid, a pool of saliva samples from immunised mice was assayed.
RESULTS: Biopanning resulted in the identification of 12 phage clones that bind to PAB1. Four of these clones were recognised by salivary IgA from sheep in an ELISA, suggesting biological activity. Two of these clones, when used to immunise mice, resulted in a serum antibody response which recognised native CarLA in an ELISA.
CONCLUSIONS: The results demonstrate that the selected peptide mimotopes are of biological relevance, and are the first to mimic the PAB1 epitope of CarLA, a defined larval glycan epitope which is conserved between many nematode species. The utility of the clones in vaccination of livestock and in diagnostics is continuing.

Protein phosphatase 2C of *Leishmania* regulates the apoptosis of the parasite

Marco David Ornelas-Cruces[1], Alma Reyna Escalona-Montaño[1], Norma Salaiza-Suazo[1], Laila Gutiérrez-Kobeh[1], Patricia García-López[2], Magdalena Aguirre-García[1].

[1]Departamento de Medicina Experimental, Facultad de Medicina, UNAM, Dr. Balmis 148, Col. Doctores, México 06726, D. F., México. Tel.: 56232657, E-mail: maguirre@unam.mx México D.F., México. [2]Laboratorio de Farmacología, Subdirección de Investigación Básica, Instituto Nacional de Cancerología, México D.F., México.

BACKGROUND: Apoptosis is a normal component of the development and health of multicellular organisms. There is now increasing experimental evidence that a similar form of cell death is operative in unicellular eukaryotes, including trypanosomatids of the genus *Leishmania*. In these parasites the apoptosis is induced by different stimuli such as heat shock, reactive oxygen species (ROS), starvation and antiparasitic drugs. Once apoptosis is triggered, a cascade of events common to mammalian apoptosis takes place such as production of ROS and lipid peroxidation, increase in cytosolic Ca^{2+} levels, changes in mitochondrial membrane potential ($\Delta\Psi m$), exposition of phosphatidylserine in the outer leaflet of the plasma membrane, release of cytochrome c, and induction of proteases. One protein that has been related to cell death is the protein phosphatase 2C (PP2C). PP2C dephosphorylates a number of intracellular substrates like cyclin-dependent kinase, mitogen-activated kinase (MAPK) and Bad. Sanguinarine is an alkaloid obtained from the bloodroot plant *Sanguinaria canadensis* which can inhibits the enzymatic activity of PP2C, induces apoptosis and inhibits tumor formation and growth of human cancer cell and the mechanism still unknown. It has been proposed a novel biological activity of sanguinarine in the apoptosis mechanism, through its PP2C inhibition activity following the phosphorylation of p38MAPK. Our group has cloned and characterized the PP2C of *Leishmania major* and *Leishmania mexicana* and these proteins have similar biochemical properties to the PP2C of other eukaryotes. Therefore, the aim of this study was to determinate whether PP2C of both species of *Leishmania* promastigotes is involved in the modulation of apoptosis through phosphorylation of p38MAPK.
METHODS: The promastigotes of *L. major* and *L. mexicana* were incubated with different concentrations of sanguinarine for three days. After this time we analyze the phosphatase enzyme activity and we observed the apoptosis with TUNEL, flow cytometry and Western blot. Finally we identified the phosphorylation of p38MAPK and the presence of Bcl-2 and Bax proteins.
RESULTS: Sanguinarine inhibited in a dose-dependent growth of parasites and phosphatase activity in extracts of both species of *Leishmania*. Also TUNEL demonstrate that the higher the concentrations of sanguinarine were larger percentage of parasites in apoptosis and flow cytometry demonstrate a significant expression of phosphatidylserine. Western blot assays showed that there is a differential p38MAPK phosphorylation in both species of *Leishmania*.
CONCLUSIONS: Our results suggest that the inhibition of the PP2C with sanguinarine increases the phosphorylation of p38MAPK and induces the apoptosis of the parasite.

Enzootic ***Trypanosoma cruzi*** **from the south coast of Jalisco, México**

Ramírez-Martínez, María Magdalena[1]; López-Castellano, Nancy[1]; Moreno-Castellón, Tania[1]; Ibarra-López, Martha Pilar[1]; Iñiguez-Dávalos, Luis Ignacio[1]; Lopez-Cancino, Sury Antonio[2]; Izeta-Alberdi, Amaia[2]; Ramsey, Janine M.[2].
1 Departamento de Ecología y Recursos Naturales, Centro Universitario de la Costa Sur, Universidad de Guadalajara, México. maleni.ramirezm@gmail.com
2 Centro Regional de Investigación en Salud Pública, Instituto Nacional de Salud Pública, Tapachula, Chiapas.

BACKGROUND. The southern coast of Jalisco has high ecosystems diversity with consequential high species richness due to its location. Eight of 18 recognized vector species of *Trypanosoma cruzi* reported in Mexico are present in Jalisco, with an overall infection prevalence of ~53%. Unfortunately, little is known regarding the enzootic transmission of *T. cruzi* infection in wild mammal communities.

METHODS. Systematic collections of rodents and bats were conducted in July, 2012, in six counties of the south coast of Jalisco: Autlán de Navarro , Casimiro Castillo , La Huerta , Cihuatlán Villa Cuautitlan, and Villa Purification. Animals were anesthetized and heart tissue excised with immediate preservation in ethanol; animal specimens are stored in the Zoology Collection of the CUCS. A PCR (S34/S67 and mini-exon) to amplify and typify *T. cruzi* DNA was used on tissues from 50 rats and 50 bats; positives were confirmed by DNA sequencing.

RESULTS . 29 species of bats and 21 species of rodents were collected. 49 cardiac samples of *Liomys pictus* from all six counties, and 50 samples from 12 species of bats from Autlán de Navarro were tested. Only five *L. pictus* from Autlán, Cihuatlán, Cuautitlán and Villa Purificación and three bat specimens were confirmed positive by sequencing: *Glossophaga soricina, Desmodus rotundus* and *Sturnira ludovici*.

CONCLUSIONS . This is the first report of *T. cruzi* infection in *L. pictus*, an abundant species inhabiting anthropogenic environments which can be found in close contact with humans. All three bat species have been reported infected in Mexico from Morelos, Campeche or Chiapas states, except for *D. rotundus*; the present report confirms and extends the enzootic interaction higher latitudes and coastal areas in Mexico. It is noteworthy that the confirmation of infected individuals using DNA sequencing is essential to avoid false positive tests.

Simvastatin and benznidazole reduces E-selectin, ICAM-1 and VCAM-1 expression in *Trypanosoma cruzi*-infection.

Campos-Estrada, Carolina[1], *López-Muñoz, Rodrigo*[1], *Kemmerling, Ulrike*[2], *Leiva, Mario*[1], *Maya-Arango, Juan*[1]

[1]Department of Clinical and Molecular Pharmacology, ICBM, Faculty of Medicine, University of Chile, Santiago, Chile. [2]Department of Anatomy and Developmental Biology, ICBM, Faculty of Medicine, University of Chile, Santiago, Chile.
Email: ccampos@ciq.uchile.cl

BACKGROUND: Chagas disease is caused by *Trypanosoma cruzi*. This parasite triggers an inflammatory response to control host's infection. As the inflammatory response persists, the patients develop Chronic Chagas Cardiomyopathy (CCC). Important pathophysiological mechanisms involved in the CCC includes microvascular alterations, due to endothelial dysfunction. This process is characterized by increased expression of vascular Cell Adhesion Molecules (ICAM-1, VCAM, and E-selectin), allowing inflammatory cell recruitment. Benznidazole is the current treatment of Chagas disease, but it has no proven efficacy in the chronic phase. However, simvastatin may improve the therapeutic efficacy of benznidazole, due to its pleiotropic roles in modulating inflammatory responses in the *T. cruzi* infected endothelium in CCC.
METHODS: The effect of both drugs on ECAMs expression in *T. cruzi* infected endothelial cells (EA.hy926) was determined by flow cytometry, immunofluorescence and western blot. Leukocyte adhesion to EA.hy926 cells was also assayed by cell adhesion assay. Finally, the effect of simvastatin and benznidazole pre-treatment upon NF-κB activation was determined by confocal microscopy.

RESULTS: After 16 hours of infection, the peak of ECAMs expression is reached in *T. cruzi* infected EA.hy926 cells. This effect was sustained for further 48 hours. Simvastatin and benznidazol treatment, during 24 hrs before infection, decreased ECAMs expression and cell adhesion, without affecting the cell viability and cytoskeleton. Thus, the effect is independent of their trypanocidal activity. Furthermore, both drugs blocked NF-κB activation. In conclusion, both drugs modulated the *T. cruzi*-induced endothelial activation. As the inflammation has a key role in the pathogenesis of Chagas disease, simvastatin and benznidazole may contribute to the treatment of inflammation in Chagas disease.

Acknowledgements: Grants From FONDECYT 1130189, 11110182 and 1120230.

Lipid levels in patients with invasive amoebiasis

Flores, María S.[1]; Obregón-Cárdenas, Adriana[1]; González-Vázquez, Mayra[1]; Tamez, Eva[2]; Rodríguez, Elba[2]; Quintero, Isela[1]; Maldonado, Guadalupe[1]; Arévalo, Katiushka[1]; Galán, Luis[1]; Bosques, Francisco [2].

[1]Instituto de Biotecnología, Facultad de Ciencias Biológicas; [2]Facultad de Medicina, Universidad Autónoma de Nuevo León, Nuevo León, México.

BACKGROUND: World Health Organization includes amoebiasis in its Special Programme for Research and Training in Tropical Diseases, to establish strategies for prophylaxis and therapies to control this disease among poor population. The *Entamoeba histolytica* infection causes 70,000 deaths yearly worldwide. Many parasites induce changes in lipid profiles in patients having active infections. The aim of this study was to search the levels of cholesterol in patients with amoebic liver abscess (ALA) and find the correlation between cholesterol and other clinical features.
METHODS: This study included 108 patients with diagnosis of ALA and 140 clinically healthy volunteers inhabiting amoebiasis endemic area. Cholesterol and triglycerides were determined in sera by enzymatic-spectrophotometric test. From the clinical records were took the number of punctures, the size of the abscess, the stay at the hospital, hematology and standard liver function test data.
RESULTS: The ALA patients studied presented hypocholesterolemia in 93% of cases. We did not find correlation between the hypocholesterolemia and the size of the abscess, nor their number or location. Hypocholesterolemia does not correlate with the hospital stay required by the patients. Hypocholesterolemia does not correlate with clinical laboratory tests. Triglycerides levels were within normal levels. The differences in triglyceride levels among patients with ALA and the healthy group were not significant. Patients, whose cholesterol levels continued to decrease despite receiving anti amoebic treatment and hospital care, presented the most severe cases of ALA or even death.
CONCLUSIONS: Patients with invasive amoebiasis had hypocholesterolemia but normal levels of triglycerides. Hypocholesterolemia does not correlate to any of the clinical and laboratory features analyzed. Hypocholesterolemia correlates with the severity of hepatic amoebiasis. We suggest survey cholesterol levels to predict the outcome of patients with invasive amoebiasis.

Instituto de Biotecnología, Facultad de Ciencias Biológicas; Facultad de Medicina, Universidad Autónoma de Nuevo León, Nuevo León, México

User ID 2553
Name : PhD Maria Flores

Prevalence of Haemoparasites of Cattle from three abattoirs in Ibadan Metropolis, Oyo State, Nigeria

Okorafor, Ugochukwu U. P. [1] *and Nzeako Sidney* [2]

[1]Federal College of Animal Health and Production Technology, Moor Plantation, P. M. B. 5029, Ibadan, Oyo State, Nigeria.[2]University of Port-Harcourt, Port-Harcourt, Rivers State, Nigeria.
[1]Department of Animal and Environmental Biology, Faculty of Biological Sciences, College of Natural and Applied Sciences, University of Port Harcourt.[2]Department of Microbiology Technology, School of Science Laboratory Technology, College of Natural and Applied Sciences, University of Port Harcourt.

BACKGROUND: It is estimated that approximately half of cattle raised in Nigeria belong to the communal and commercial farmers who are resource-poor and can only afford communal graze lands which is characterized by poor management of cattle and low productivity. This practice predispose the animals to a range of haemoparasites such as the trypanosomes (*Trypanosoma vivax, T. congolense* and *T. brucei*), Babesia (*Babesia bigemina, B.bovis*) Anaplasma and Ehrlichia (Cowdria), and to a less extent Theileria (*Theileria parva* and *T.veilifera*). These parasites transmitted by ticks and blood-sucking flies cause substantial losses in cattle production, in terms of diseases, reduced productivity, infertility and often death.
METHODS: 180 venule blood samples were obtained from apparently healthy cattle of both sexes consisting of three breeds; 94 White Fulani, also called 'Bugani' (78 males and 16 females), 42 Sokoto Gudali (16 males and 26 females) and 44 Red Bororo (34 males and 10 females). Haemoparasites were detected using the techniques of wet mount, stained thin blood smear and buffy coat as described by Cheesbrough.
RESULTS: A total of 12 (6.67%)of were positive for different haemoparasites out of the 180 examined animals. Abattoir-specific prevalence varied across abattoirs with Bodija abattoir recording 6 (3.33%) infections; Akinyele abattoir 4(2.22%) while 2(1.11%) infections were recorded at Olorunsogo abattoir. Three species of haemoparasites were identified, namely; *Trypanosome* spp; *Theileria* spp and *Babesis* spp. *Trypanosoma brucei* had the highest prevalence of 3.81%; *Theileria parva,* 0.56% and *Babesia bigemina,* 2.22%. *T. brucei* showed a prevalence of 2.22%, 1.11% and 0.56% at Bodija, Akinyele and Olorunsogo abattoirs respectively. *Theileria parva* showed a prevalence of 0.56% at Bodija abattoir and 0.0% prevalence at both Akinyele and Olorunsogo abattoirs. *Babesia bigemina* showed prevalence of 0.56%, 1.11% and 0.56% in Bodija, Akinyele and Olorunsogo abattoirs respectively. Sex related haemoparasitemia did not vary significantly ($X^2 = 8.7551$, df = 1, P>0.05) in the study, however, parasitemia was higher in cows (4.45%) than in bulls (2.22%). Breed-specific parasitemia showed that Sokoto Gudali had the highest parasite load (2.78%) followed by the Red Bororo (2.22%) and White Fulani (Bujani) having the lowest parasite load (1.67%). The study also showed that breed specific parasitemia varied significantly ($X^2 = 5.3860$, df = 2, P<0.05).
CONCLUSIONS: The present report confirms the presence of carrier populations of haemoparasites-infected cattle which both serve as a reservoir of infection for tick-vectors, susceptible livestock and humans.

Humoral immune response of *Didelphis virginiana* against *Gnathostoma turgidum*

Zazueta-Moreno José M[1], *Torres-Montoya Edith H*[2], *Rendón-Maldonado José G*[1], *López-Moreno Héctor S*[1], *Osuna Ramírez Ignacio*[1], *Osuna-Martínez Lorenzo U*[1], *Domínguez-Molina Yessica R*[1], *Elvic Noguera-Corona*[3] *and Díaz-Camacho Sylvia Paz*[1]

[1]Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Av. de las Américas y Blvd. Universitarios S/N, Culiacán, Sinaloa 80010, México
[2]Escuela de Biología-UAS, Av. de las Américas y Blvd. Universitarios S/N, Culiacán, Sinaloa 80010, México
[3]Laboratorio de Patología, Facultad de Medicina-UAS, Av Eustaquio Buelna S/N, Tierra Blanca, Culiacán, Sinaloa 80030, México
e-mail: marcialzazueta_moreno@hotmail.com

BACKGROUND: Parasites of the genus *Gnathostoma* have a complex life cycle where several wild and domestic animals are involved and they are considered as first, second, intermediate, paratenic or definitive host. It has been reported that Virginian opossum *Didelphis virginiana* is the definitive host of *G. turgidum* and spontaneous expulsion of live worms is common, suggesting seasonality of the infection; however, the immunological mechanisms of worm expulsion are unknown. In that sense, there is lack of information about opossum's immune response against *G. turgidum* and its role during deworming.
METHODS: In this work an analysis of the humoral immune response was performed for determine of anti-*Gnathostoma* antibodies in sera of naturally infected opossums by ELISA. Also, natural infection was monitored periodically by coproparasitoscopic and abdominal ultrasonography.
RESULTS: Anti-*Gnathostoma* antibodies level was higher in infected opossum compared with non-infected animals used as controls. The titles of antibodies in sera of infected animals were from 1.26 to 1.6 OD (492 nm) in the beginning of the study and IgG levels decrease from 1.2 to 0.85 OD (492 nm) at the end of the infection. Ultrasonograpic images of the abdomen showed slight liver damage during April and May. Liver lesions gradually increased in size and number up to August. Similarly, granulomatous lesions in the stomach appeared during April and persisted until September. However, the ultrasonographic abnormalities of the liver and stomach were resolved around November or December.
CONCLUSIONS: Data suggest that *G. turgidum* adult worms could be expelled from opossums as a result of the host immunological mechanisms or as part of the parasite life span.

Expression of GP 14, 24 and 50 in metacestodes and adults of *Taenia solium.*

Cruz-Rivera Mayra[1], Mendlovic Fela[1,2],Torres Jose[3], Carrillo-Farga Jorge[3], Wilkins Patricia[4], Flisser Ana[1].

[1] Facultad de Medicina, UNAM, [2] Escuela de Ciencias de la Salud. Universidad Anáhuac, México Norte, [3] Instituto de Hematopatología, [4] Centers for Diseases Control and Prevention, GA, USA.

BACKGROUND. *Taenia solium* is a helminth parasite that causes two different diseases in humans; cysticercosis and taeniosis. Neurocysticercosis is caused by the establishment of *Taenia solium* metacestodes in the central nervous system. Immunodiagnosis of neurocysticercosis use a western blott with an enriched fraction of glycoproteins (LL-GP), which has been used extensively for diagnosis and epidemiologic surveys. Seven antigenic glycoproteins are contained in the LL-GP fraction (GP50, 39-42, 24, 21, 18, 14 and 13 kDa), some have been used as a target for cloning, expression and synthesis of peptides in order to improve assay performance. However, there is no information on the localization and its possible function. METHODS. Cysticerci and tapeworms were processed for immunohistochemistry. Tissue sections (1µm) were incubated with biotynilated anti-GPs antibodies and streptavidine-HRP, the reaction was developed with 3-3 diaminobenzidine; counterstaining was performed with hematoxilin. RESULTS. GP14 is expressed in the parenchyma and muscle cytons as well as suckers and rostellum of the scolex of cysticerci, this glycoprotein has a low expression in mature proglottids. GP24 was identified in calcareous corpuscles and the excretory system of the vesicular membrane in cysticerci; in mature proglottids this protein was localized in most of the reproductive structures like spermatozoa, vas deferens, ovarian lobules, uterine epithelium and vaginal duct. In cysticerci, GP50 was localized in the glycocalix, parenchyma, calcareous corpuscles and subtegumentary cytons; in mature proglottids this glycoprotein was found in the cirrus sac and spermatozoa. Interestingly, GP14 and GP24 are not present in oncospheres, while GP50, shows an intense staining in the latter. CONCLUSION. These findings are of interest since these glycoproteins are specific for the diagnosis of neurocysticercosis, suggesting its expression and important participation in both stages of *T. solium* development.

In vitro mode of action studies of compounds identified with activity against *Trypanosoma cruzi*, utilising whole-cell image based techniques

Sykes, M.L and Avery, V.A.

Discovery Biology, Eskitis Institute for Drug Discovery, Griffith University, Nathan, Queensland 4111, Australia.

Chagas disease, endemic to South and Central America, is estimated to effect 10-12 million people and cause over 15 000 deaths per year [1]. The disease comprises an acute and chronic stage and the latter can go undetected for up to 30 years before heart related complications can result in sudden cardiac death. The acute stage is successfully treated with the nitroheterocyclic drugs benznidazole and nifurtimox, however there are doubts about the efficacy of these drugs in the chronic stage [2]. Also, treatment causes side effects in many patients and can result in cessation of therapy. The introduction of new, effective and non-toxic compounds into the drug discovery process for Chagas disease is an ongoing challenge. To discover active compounds against the parasite, we have developed a whole-cell, image-based assay to enumerate *T. cruzi* intracellular amastigotes following exposure of *T. cruzi* infected 3T3 fibroblasts to compounds [3]. A separate fluorescence-based assay has also been developed to determine compound activity against the motile, extracellular trypomastigote. Utilising these technologies, a proprietary compound library containing both FDA approved compounds and compounds with previously reported biological activity against other cell lines or disease indications was screened against the parasite. The *T. cruzi* selectively active hits, the antihistamine clemastine fumarate (CF) and the antifungal ciclopirox olamine (CPX), have not previously been identified with activity against the parasite. Utilising these assay platforms, we have demonstrated that supplementation with Fe^{3+} suggests iron chelation to be paramount to the activity of CPX against *T. cruzi*. Currently, histamine receptors have not been identified in *T. cruzi* and therefore the target of CF is unknown. We have probed 3T3 infected cells with fluorescently labelled histamine to determine if there are potential receptors in *T. cruzi*, and supplemented

histamine *in vitro* to estimate if CF activity is decreased. As CF has demonstrated activity against the human cytochrome P450, CYP 2D6 [4], this compound has also been profiled *in vitro* against *T. cruzi* CYP51 and the results will be discussed.

References

1. Clayton J (2010) Chagas disease 101. Nature 465: S4-5.
2. Mady C, Ianni BM, de Souza JL (2008) Benznidazole and Chagas disease: can an old drug be the answer to an old problem? Expert Opinion on Investigational Drugs 17: 1427-1433.
3. Sykes ML, Avery V (2013) Approaches to Protozoan Drug Discovery: Phenotypic Screening. Journal of Medicinal Chemistry in press.
4. Hamelin BA, Bouayad A, Drolet B, Gravel A, Turgeon J (1998) In vitro characterization of cytochrome P450 2D6 inhibition by classic histamine H1 receptor antagonists. Drug Metab Dispos 26: 536-539.
5. ClinicalTrials.gov (2013) A Study of the Use of Oral Posaconazole (POS) in the Treatment of Asymptomatic Chronic Chagas Disease.

Monitoring response to treatment of visceral leishmaniasis by measuring antigen-specific serum antibody responses

Duthie, Malcolm S[1]; Vallur, Aarthy C[1]; Liang, Hong[1]; Reinhart, Caroline[1]; Reed, Steven G[2];

[1]Infectious Disease Research Institute (IDRI), Seattle, USA

BACKGROUND: Visceral leishmaniasis (VL) is the disease manifestation of infection with either *Leishmania donovani* or *L. infantum*. Although fatal if left untreated, various treatment regimen are available. To date, however, determining the optimal regimen for use in humans has been largely empiric because simple objective and quantitative indicators have been lacking.

METHODS: We recruited clinically-confirmed VL cases (resulting from either *L. donovani* or *L. infantum* infection). Patients were then treated with either the currently recommended ambisome or sodium stibogluconate regimen. Blood was collected and sera prepared at the time of diagnosis, then again at times throughout and beyond the completion the treatment. Serum antigen-specific IgG antibody responses were measured by ELISA.

RESULTS: Analyses of responses to the antigens rk39 and rk28 supported their use for confirming diagnosis but also indicated that the magnitude of response was unaltered by treatment. Responses to antigen rk26 and Li73 were also elevated at time of diagnosis but, in contrast, declined upon and were markedly reduced for an extended period of time after treatment. Similar observations were made for VL patients from Bangladesh and Ethiopia (both *L. donovani* infection) and Brazil (*L. infantum* infection).

CONCLUSIONS: Our data identify antigen-specific antibody responses that decline during VL treatment regardless of infecting parasite species and geographic setting. Monitoring these responses could potentially help patient management by identifying treatment complications. These antigens could also be used as a simple, quantitative assessment of efficacy during trials of new intervention or treatment regimen for VL.

Ocular toxoplasmosis, the most common etiology of pediatric uveitis in Colombia

Lonngi-Ardila M[1], Aguilar-Sierra MC[1]; Aristizabal-Duque C[2]; Ríos HA[1]; Chaparro-Zuñiga SY[2]; Sánchez-León HA[1]; Rodríguez-Alvira F[1]; de –la-Torre A[1,2]

[1] Fundación Oftalmológica Nacional/Servicio de Inmunología, Universidad del Rosario, Carrera 24 No 63 C 69, Bogotá, Colombia
[2] Grupo GEPAMOL, Centro de investigaciones Biomédicas, AV Bolívar 12 Norte, Armenia, Quindío, Colombia

BACKGROUND: It has been reported that infectious uveitis is the second leading cause of blindness in the pediatric population in Colombia; nevertheless, there are no studies to determine the clinical characteristics of uveitis in children.
PURPOSE: To describe the Clinical features of uveitis in pediatric patients treated in two ophthalmology centers in Bogotá Colombia.
METHODS: The clinical charts of children with diagnosis of uveitis between 200 and 2013 were retrospectively reviewed. Data was analyzed and compared to previous reports.
RESULTS: Uveitis was found in 312 children. Mean age of presentation was 10.9 years old. Posterior uveitis was the most common type, of insidious onset and chronic course. The most common etiology was toxoplasmosis, followed in by idiopathic uveitis and toxocariasis.
CONCLUSIONS: This is the first study of the clinical features of uveitis in children in Colombia. Toxoplasmosis is the most frequent cause of uveitis in this country. It will enhance awareness and knowledge of pediatric uveitis in developing countries and it should assist in the creation of public health policies for outcome improvements.

13th INTERNATIONAL CONGRESS OF PARASITOLOGY
AUGUST 10th - 15th, 2014
ICOPA XIII MÉXICO

Drug search for leishmaniasis by using virtual screening and grid computing

Rodrigo Ochoa[1], Viktors Berstis[2], Andrés Flórez[3], Stanley Watowich[4] and Carlos Muskus[1]

[1]Programa de Estudio y Control de Enfermedades Tropicales, PECET, Universidad de Antioquia, Medellín, Colombia.

[2]World Community Grid, IBM, Armonk New York, United States.

[3]German Cancer Research Center (DKFZ), Division Theoretical Systems Biology, Heidelberg, Germany.

4. University of Texas Medical Branch, Galveston, USA.

BACKGROUND: Leishmaniasis is a worldwide disease present in 98 countries and caused by protozoan parasites of the genus Leishmania. Currently the number of officially approved drugs to treat the disease are few and all are associated with adverse effects from mild to severe, including death. Moreover, the emergence of strains resistant to these drugs complicates the therapeutic landscape. This emphasizes the importance of finding new drugs that are effective and safe for the management of the disease. In this project, massive docking simulations were done by using 583 *Leishmania* structures and 600.000 molecules or compounds.

METHODS: From PDB (Protein Data Bank) database, 53 *Leishmania* protein structures, mainly obtained by X-ray crystallography, were collected. Each structure was subjected to molecular dynamics simulations of 10 ns, in order to model slight changes in the orientation of their atoms and bonds under simulated biological conditions. From trajectories, 530 different structures were generated and along with the original 53 proteins, were subjected to molecular docking using the software AutoDock Vina against 600.000 test compounds previously obtained and filtered from the zinc database. Due to the exhaustive nature of the process, the docking was run in the World Community Grid, where more than 2 million and worldwide associates computers, processed the data.

RESULTS: After two years of computation over a billion files with the results were received. Four repetition, for each protein-compound complex were done. Compounds were organized by the affinity score, calculated by the AutoDockVina software, in order to prioritize the best compounds, for experimental validation *in vitro* and *in vivo*. Based on these results, three molecular targets, dehydrogenase, dihydroorotate phosphodiesterase and tyrosyl tRNA synthetase were associated with higher affinity. Regarding the chemical compounds, all of them reported favorable physico-chemical properties to become a drug. Beside that, some compounds have substructures commonly used for *Leishmania* treatment, as quinolines. Additionally, compounds reporting high affinity scores during molecular dynamics trajectories for each of their receptors, were taken into account in order to evaluate the influence of the flexibility of the active or binding site chosen in the interaction.

CONCLUSIONS: Several chemical compounds were predicted to target three *Leishmania* proteins with high affinity. In addition these results suggest that computational approaches combined with *in vitro* and *in vivo* test, may accelerate the path to get new anti-Leishmanial compounds.

Plasmodium tRNA synthetases as antimalarial drug targets

Jackson, Katherine E.; Pham, James S.; Lim, Erin; Pasaje, Charisse Flerida A.; Yeoh, Lee M. Dawson, Karen L.; Turner, Kelsey E. Ralph, Stuart A.

University of Melbourne, Parkville, VIC, 3010, Australia

BACKGROUND: Malaria is caused by intracellular parasites of the genus *Plasmodium*. Protein translation occurs in three compartments within *Plasmodium* parasites: the cytosol, the mitochondrion and a relic plastid called the apicoplast. Aminoacyl tRNA synthetases charge tRNA for each of these compartments but *Plasmodium* encodes too few tRNA synthetases to allow a unique enzyme for each amino acid in all compartments. The tRNA synthetases for Ala, Cys, Gly, and Thr are found only once in the genome and we show that each of these enzymes is dual targeted to the cytosol and the apicoplast. Because of the dependence of *Plasmodium* parasites on efficient translation, this process is a promising drug target.
METHODS: We have taken two approaches to drug discovery against *Plasmodium* tRNA synthetases – one is to inhibit the bacterial-like aaRSs that service the apicoplast based on existing inhibitors, the other is to pursue novel and existing inhibitors of the dual targeted aaRSs that serve functions in both the cytosol and the apicoplast.
RESULTS: We show that inhibitors of apicoplast aaRSs lead to delayed death phenotypes and apicoplast defects, while inhibitors of the dual targeted aaRSs lead to immediate death and arrest of nascent protein synthesis. We have isolated stable parasite lines that are resistant to several of these aaRS inhibitors and demonstrate amplifications of the tRNA synthetase target genes through Illumina sequencing.
CONCLUSIONS: Inhibitors of dual targeted tRNA synthetases have a rapid mode of action that arrests protein translation and blocks parasite proliferation, as well as mopping up surviving parasites by leading to loss of apicoplast.

Comparison of tissue-fluid ELISA and PCR methods for *Toxoplasma gondii* in pork

Lee, Ji-Yun[1], Kim, Tae Im[1], Dai, Fuhong[1], Nam, Ho-Woo[2], Kang, Seung-Won[3], Kim,Young-ha[3] ,Yoo,Mi-sun[3] , Youn, Hee-Jeong[4], Hong, Sung-Jong[1]

[1]Department of Medical Environmental Biology, Chung-Ang University College of Medicine, Seoul 156-756, Korea; [2]Department of Parasitology, Catholic University College of Medicine, Seoul 137-701, Korea; [3]Parasitology and Insect Disease Research Laboratory, Animal and Plant Quarantine Agency, Anyang city 480-757, Korea; [4]College of Veterinary Medicine, Seoul National University 151-742, Korea

BACKGROUND: Pork is regarded as a major source of human toxoplasmoisis. Survey report on *Toxoplasma gondii* infection from commercial pork products is scarce in South Korea yet. To establish fast, easy and accurate diagnosis methods, PCR and ELISA diagnostic are evaluated in this study.
METHODS: To obtain *Toxoplasma gondii*-infected tissue samples, 13 piglets, of one farrow, 4-week-old, were divided into 4 groups. Piglets of Group 1 were injected intravenously with 10^3 or 10^4 tachyzoites of RH strain. Group 2 and 3 were fed orally with 500 or 1,000 mouse brain cysts of ME49 strain. Serum samples were obtained from all piglets every week for 7 weeks and stored at −20°C until used. The piglets were reared with a restricted feeding regime. After 7 weeks, the piglets were killed by electric shock and tissue samples were taken from diaphragm, abdomen, shoulder, back muscle, heart, liver, tongue and spleen. Sera and tissue fluid were tested for specific antibody by ELISA using crude antigen of RH tachyzoites.
RESULTS: Piglets in all experimental groups infected by *T. gondii* became sero-positive from 2 weeks post-infection. Group 2 piglets showed highest IgG antibody levels in sera during infection period and in tissue fluids. This group was adopted as *Toxoplasma gondii*-infected positives. To standardize PCR method, RH strain tachyzoites were inoculated in 0.3 g tender loin of healthy safe pork and total genomic DNAs were extracted.
CONCLUSIONS: A conventional PCR employing a specific primer set of the 529-bp gene detected as low as 1 tachyzoite/0.3 g pork.