

EP001 - Understanding the effects of the Chagas disease control program in Venezuela after 50 years using eco-epidemiological modelling

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The most important path to fight Chagas disease is the interruption of vector transmission by controlling vector populations. Nevertheless, after 50 years of the Chagas disease control program (CDCP) implementation in Venezuela, the infection persists, and the processes leading to such persistence are not fully understood. The aim of this work is to broaden the understanding on the effect of the CDCP on the seroprevalence trend of anti-*Trypanosoma cruzi* antibodies within the Venezuelan population. We developed and parameterized an epidemiological model applying pattern oriented modelling paradigms, which allowed us to identify relevant parameters but also overcome incomplete knowledge of given processes in the system. Moreover, a comprehensive analysis of the seroprevalence trend as well as of the infection persistence is provided. We quantify the probability of vector infection for the period 1958-1968 in two ways; constant and age-dependent. We show how the observed diminishing on age-dependent seroprevalence is related to the decreasing on such probability and we also quantified the time horizon for the extinction of seroprevalence from the system without any way of transmission or when the only possible path of transmission is the congenital. This study represents one of the first attempts to quantify probability of infection, as well as a deeper understanding on the effects of the CDCP on age-dependent seroprevalence in terms of the reduction of the infection probability.

We conclude that the CDCP, as it was conceived, constitutes a robust starting point above which new strategies should be built since it allowed such a dramatic decrease in seroprevalence. However, improved control measurements are still necessary to avoid a new increasing on Chagas disease seroprevalence in Venezuela. **Supported by:** German Academic Exchange Service (DAAD) And Helmholtz-Centre for Environmental Research (UFZ)

EP002 - Seroepidemiological survey and attempt to isolation *Toxoplasma gondii* in dogs from the Zoonosis Control Center of Vitória, Espírito Santo, Brazil

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Toxoplasma gondii, protozoan that causes toxoplasmosis, is able to infecting a wide variety of warm-blooded animals and penetrates into different cells. Dogs have been found naturally infected with *T. gondii*, with prevalence ranging from 17.3 to 94% in Brazil. The rate of canine infection is an indication of urban contamination by *T. gondii*, a risk to humans, since both are exposed to common sources of infection, such as environment and diet. The contact of dogs with cat feces can lead to contamination of the animal's coat, and therefore, the household environment, exposing the owner to infection. The most used tests to detection of anti-*T. gondii* antibodies in dogs are the indirect immunofluorescence assay (IFA), indirect hemagglutination, the modified agglutination test (MAT) and enzyme linked immunosorbent assay (ELISA). Brazil has a diversity of strains, however, few seroepidemiological surveys and no genetic or molecular survey of *T. gondii* are reported in Espírito Santo. The aim of this study was to investigate antibodies IgG anti-*T. gondii* in sera of dogs by ELISA and attempt to isolate the parasite in tissue samples of dogs from the Zoonosis Control Center of Vitória, Espírito Santo. 110 animals were evaluated by clinics aspects and toxoplasmosis serology. Our results demonstrated that 49 (44.54%) samples were positive by ELISA. 97 animals had not show any clinics aspects and 13 animals presented some sintomatology related to the disease. We observed that 44 (45.36%) and 5 (38.43%) presented positive serology to toxoplasmosis, respectively. The organs of 19 seropositive dogs were submitted to peptic digestion and inoculated in female Swiss mice. No cysts were found in the brains of mice inoculated. Our results demonstrate high prevalence of toxoplasmosis in dogs in Vitória City. **Supported by:** CNPq

EP003 - ISOLATION AND CHARACTERIZATION OF *TRYPANOSOMA CRUZI* STRAINS FROM WILD TRIATOMINES CAPTURE IN SÃO PAULO STATE.

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Recent studies accomplished during 1990 to 2006 in 645 cities in the state of São Paulo showed an increase of triatomine infestations of 1.2% in 1990 to 2.9% in 2006, becoming important the wild Triatominae search in this State to know the real situation of *T. cruzi* cycle. Thus, after notification and capture of two fourth instar nymphs of *Panstrongylus megistus* positive for Trypanosomatidae in the municipality of Santo Antônio do Jardim, in the mesoregion of Campinas, the border with the State of Minas Gerais, a wild strain of *T. cruzi* was isolated in Mogi Guaçu Chagas laboratory. The study of parasitemia in Wistar rats showed an acute phase of approximately 30 days, with a prepatent period of 8 days and parasitemic peak around day 20 post-infection. Histopathologic analysis performed on serial sections of heart, skeletal muscle, liver and colon showed inflammatory infiltrate in the heart, skeletal muscle and colon in 10th days post-infection. On the 20th day observed an increase in the intensity of the inflammatory process with the presence of amastigote nests in heart, skeletal muscle, and colon. Biological characterization of Mogi strain shows that this presents low parasitemia and virulence to Wistar rats with skeletal and cardiac muscle tropism in the acute phase of infection, which may be classified in biodema III, according to Andrade (1974). The molecular characterization showed compatible with TcI. **Supported by:** FAPESP

EP004 - Importance of PCR in routine diagnosis of tegumentary *Leishmaniasis* in Instituto de Infectologia Emílio Ribas.

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Mucosal *Leishmaniasis* (ML), which main causative agent in Brazil is *Leishmania* (V.) *braziliensis*, is the severe form of tegumentary *Leishmaniasis*. Initial clinical manifestation of ML is often misdiagnosis with cutaneous *Leishmaniasis* (CL) and other dermatologic diseases, leading to inadequate treatment. Thus, early diagnosis with species identification is fundamental for correct prognostic, drug administration and avoid resurgence of lesions and progress for mucosal form. Unfortunately, traditional diagnosis methods are unable to identifying of parasite's species, which now is possible with molecular methods. The technique of polymerase chain reaction (PCR) with analysis of restriction fragment length polymorphism (RFLP) has been reported as a useful tool for species-specific *Leishmaniasis* diagnosis. Here we present an application of the technique PCR-RFLP for identification of patients infected with *L. (V.) braziliensis*. The sensibility of the method was compared to traditional diagnosis methods as: direct investigation (DI), Montenegro Skin Test (MST) and in vitro culturing. The study was enrolled with 128 DNA samples from patients attended in IIER: 69 suspected of CL and 59 suspected of ML. PCR was able to detect the parasite's DNA in 87.5% of the samples while DI was positive in 61.8% samples, MST 62.8% and in vitro culturing 19.3%. *L. (V.) braziliensis* electrophoresis pattern was observed in 96 of 112 positive samples: 45 from ML suspected patients and 51 from CL ones. This data shows that 73.9% (51/69) of the patients clinically diagnosis as CL were infected with *L. (V.) braziliensis* and may develop mucosal lesions if not treat adequately. Thus, due to the higher sensibility of the technique and the high frequency of patients infected with *L. (V.) braziliensis* we recommend the use of this technique as routine in public Brazilian hospitals of endemic areas. **Supported by:** CAPES, FAPESP, LIM 48- FMUSP

EP005 - CANINE VISCERAL *LEISHMANIASIS* IN THE TOURIST TOWN OF EMBU DAS ARTES, SP - EVALUATION OF PCR TECHNIQUE INVOLVING DIFFERENT TISSUES OF SEROPOSITIVE DOGS

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We evaluated the efficacy of kDNA-PCR reaction, analyzing different tissues samples from dogs with CVL identified and sacrificed in an seroepidemiological survey conducted in Embu das Artes. This survey also demonstrated the absence of both, human infection, and the presence of the classic vector of human disease (*L. longipalpis*), suggesting that a different pattern of transmission may be occurring for CVL in this city. We investigate some aspects involved in the transmission and epidemiology of CVL in this touristic town, comparing them with the results obtained from PCR and classical tests, as: direct parasitology tests and in vitro culture of the isolated parasites. A specific protocol for collection and storage of the samples was carried out with the dogs euthanized. Thus, tissues samples from: spleen, lymphnode, skin with and without lesion, and blood of 26 dogs euthanized after positive serology for *Leishmaniasis* were individually evaluated by the two classical tests and by kDNA-PCR, the latter done in triplicate. From the 26 dogs, 22 (84.6%) were positive by direct parasitological test, at least in one of the four different tissues examined. Similar results were observed for in vitro culture, where 21 samples (80.77%) were positive, indicating the importance of the specific care at the moment of sample collection. PCR reaction presented higher levels of positivity in most of the tissues analysed when compared with other methodologies. We verified PCR amplification in 24/26 (92.30%) of samples from lesion and in 23/26 (88.46%) of samples from spleen, indicating that both invasive and non-invasive samples can be used by the PCR technique. PCR-RFLP restriction analysis with *HaeIII* were also performed with amplified products of each infected dog and none of the samples tested were digested, indicating a pattern not suggestive of *L. braziliensis*, which is in agreement with a visceral infection which is usually caused in our country by *L. chagasi*. **Supported by:** FAPESP, CNPq, FMUSP-LIM48

EP006 - RISK FACTORS AND SEROPREVALENCE OF *Toxoplasma gondii* INFECTION IN PREGNANT WOMEN IN JATAÍ MUNICIPALITY, STATE OF GOIAS

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Toxoplasmosis is a worldwide zoonosis caused by the *Toxoplasma gondii*, an Apicomplexa obligate intracellular parasite that infects birds and mammals, including humans. Congenital toxoplasmosis occurs due to vertical transmission of *T. gondii* via placenta when mothers acquire primary infection during gestation, sometimes leading to abortion or fetal abnormalities. This study aimed to determine the prevalence of IgG antibodies to *T. gondii* in serum samples from pregnant women and the main risk factors involved in the transmission of toxoplasmosis in Jataí, Goiás, Brazil. A total of 139 serum samples from pregnant women were analyzed by indirect enzyme linked immunosorbent assay (ELISA) to detect IgG antibodies and by capture ELISA to detect IgM and IgA antibodies anti-*T. gondii*, and all participants answered a structured questionnaire. The age ranged from 13 to 43 years, and the prevalence of IgG antibodies to *T. gondii* was 73%. On the other hand, 12 (8.7%) and 5 samples (3.6%) showed positive serology to IgM and IgA anti-*T. gondii*, respectively, suggesting that some pregnant women were acutely infected, at risk of congenital transmission. There was no association between the infection and age, education or family income. The variables concerning the number of pregnancies, consumption of pork or beef, ingestion of raw/undercooked meat or sausage and poor washing of fruits and vegetables showed no significant association with the presence of IgG to *T. gondii*. However, the consumption of untreated water (OR=1.435; $p < 0.0001$), the presence and contact of cats (OR=2.667; $p < 0.0001$) and the contact with soil (OR=3.361; $p < 0.0001$) were associated with seropositivity to toxoplasmosis. The high prevalence of positive serology for toxoplasmosis found in this region, associated with the risk factors reinforces the need to create a local program of prevention, diagnosis and treatment of pregnant women to avoid congenital toxoplasmosis. **Supported by:** CNPq e FAPEG

EP007 - IMMUNE DIAGNOSIS OF CANINE VISCERAL *LEISHMANIASIS* IN A NON-ENDEMIC AREA OF MINAS GERAIS STATE, BRAZIL.

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Introduction: Human visceral *Leishmaniasis* (HVL) constitutes a public health problem that affects millions of people throughout the world. This disease represents one of the major emergent parasitological diseases, caused by the species *Leishmania infantum* in Brazil. Dogs play an important role in the maintenance of this disease in the human environment serving as reservoirs for this infection. Therefore, the diagnosis of Canine Visceral *Leishmaniasis* (CVL) represents an important step for control of HVL. Enzyme-linked immunosorbent assay (ELISA) has been one of the methods most often employed in the diagnosis of CVL since they present satisfactory indices of sensitivity and specificity. The aim of the present study was to evaluate the occurrence of CVL in the municipality Juiz de Fora, Minas Gerais, considered as non-endemic area for this disease. Materials and methods: Blood of 210 dogs from a public kennel of this municipality were used for this study. The ELISA was carried out using the EIE-leishmaniose-visceral-canina-Bio-Manguinhos kit, produced by Bio-Manguinhos/FIOCRUZ, Rio de Janeiro, Brazil. All the procedures were performed according to the manufacturer's instructions. In parallel, questionnaire with animal clinical aspects was filled out by a veterinary physician. Dogs were scored for several symptoms, including those attributed for CVL as onychogryphosis, lymphadenopathy, cutaneous alterations and conjunctivitis. Animals with one symptom were arbitrarily considered as asymptomatic and two or more symptoms were classified as symptomatic. Results and Conclusion: Of the 210 dogs examined, 1.43% were positive by EIA-LVC Bio-Manguinhos, of these 66.67% were considered asymptomatic and 33.33% were classified as symptomatic with two characteristic clinical signs of CVL. These results show the importance of diagnosing CVL in non-endemic area and reinforce the necessity of epidemiological survey in Juiz de Fora. **Supported by:**FAPEMIG, CNPq and UFJF

EP008 - OCCURRENCE OF CANINE VISCERAL *LEISHMANIASIS* DETECTED BY THE CHROMATOGRAPHIC IMMUNOASSAY BASED ON THE DUAL-PATH PLATFORM (TR DPP®), IN JUIZ DE FORA, MINAS GERAIS, BRAZIL.

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Introduction: In Brazil, visceral *Leishmaniasis* (VL) is an endemic zoonotic disease caused by *L. infantum*. This disease is a serious public health problem that affects an estimated 4,000 new human cases each year. In general, VL is a rural disease, but nowadays this disease has spread to the urban centers. Domestic dogs are established reservoir hosts of zoonotic VL and there is a clear correlation between human and canine infection rates. This study aimed to carry out a serological survey on visceral canine *Leishmaniasis* (VCL) at Juiz de Fora, Minas Gerais. Methods: Blood was collected from 200 dogs from a public kennel of Juiz de Fora municipality. Diagnosis was performed using the TR DPP® kit, produced by Fiocruz (Bio-Manguinhos, RJ, Brazil). This kit is a rapid test for detection of antibodies in serum of infected dogs utilizing recombinant antigen of *L. infantum*. Serum of animals (5 µl) was used in each test and the results were visually after 15'. Despite visual evaluation, the results were considered as "positive" only after a lecture by using a DPP optical reader device provided by the manufacturer. True positive and negative controls were used. In parallel, questionnaire with animal clinical aspects was filled out by a veterinary physician. Dogs were scored for several symptoms, including those attributed for VCL as onychogryphosis, lymphadenopathy, cutaneous alterations and conjunctivitis. Animals with one symptom were arbitrarily considered as asymptomatic and two or more these symptoms were classified as symptomatic. Results and Conclusion: From 200 dogs examined, 3.5% showed positive by TR DPP® kit, of these 85.7% were asymptomatic and only one animal had two or more characteristic clinical signs of VCL. This study indicates that the TR DPP® kit may be use for serological survey and these results show the need to diagnose this disease in non-endemic regions. **Supported by:**FAPEMIG, CNPq and UFJF

EP009 - PREVALENCE OF TOXOPLASMOSIS IN RURAL AREA OF SANTA TERESA, ESPIRITO SANTO

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Toxoplasma gondii is one of the most common zoonotic infectious agents worldwide. Epidemiological data shows an increase number of individuals with toxoplasmosis and the prevalence of the disease can vary from different regions in Brazil. In Espírito Santo, investigations were directed to specific cohorts (congenital and ocular toxoplasmosis) and do not represent the reality in the state. The present study demonstrated the frequency and the related factors with toxoplasmosis in Santa Teresa city, located in Espírito Santo state. We evaluated 304 samples by ELISA from 78 individuals and these samples were from a previous study of malaria, considering that the infectious agents belong to the same phylum: Apicomplexa. Demographic and socioeconomic factors associated with toxoplasmosis were collected using questionnaires. Our results demonstrated that the mean age of the participants was 46; of whom, 40 were male (51.30%) and 38 females (48.70%) and no pregnant woman was evaluated in this study. Out of 78 selected individuals, only three showed knowledge about toxoplasmosis infection (3.85%). In addition, 54 (69.20%) persons reported activities related to rural environment. Besides, 38 (48.70%) have at least one animal at home; 26 of them did not know where these animals defecate. Only five (6.41%) admitted consumption of meat from wild animals and 60 (76.93%) of the individuals eat pork beef / sausage at least once a week. 18 (23.07%) eat occasionally raw meat. This study suggests that the inhabitants of rural communities in Espírito Santo are extremely exposed to the parasite. Therefore, a preventive action must be reinforced in specific regions aimed at early diagnosis to minimize the risk of the toxoplasmosis. **Supported by:** FAPES

EP010 - Evaluation of seroreactivity to *Toxoplasma gondii* in alligatorsFERREIRA, F.B.¹; SANTIAGO, F.M.¹; MACÊDO JÚNIOR, A.G.¹; SILVA, M.V.¹; JÚNIOR, Á.F.¹;VITALIANO, S.N.²; GENNARI, S.M.²; OLIVEIRA SILVA, D.A.¹; MINEO, J.R.¹; SANTOS,A.L.Q.¹; MINEO, T.W.P.¹

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Toxoplasmosis, induced by the etiologic agent *Toxoplasma gondii*, is an infection with worldwide distribution that affects a large variety of warm-blooded hosts. This parasite has the felines as definitive hosts and virtually all other homeothermous animals have been described as intermediate hosts. However, infections in cold-blooded animals are yet to be described. In this sense, the present study aimed to search for specific antibodies to *T. gondii* in serum samples of two species of alligators from the Brazilian fauna - *Melanosuchus Niger* and *Caiman crocodilus*. We obtained 104 serum samples, collected from animals sampled in the middle Araguaia region, which were submitted to four serological tests: modified agglutination test (MAT), indirect hemagglutination test (IHA), indirect ELISA and *Western blotting*. Due to the lack of specific reagents, we used heterologous secondary antibodies against chicken IgY antibodies, which are phylogenetically related to IgY antibody from alligators. In MAT, 8 (7.7%) of the samples were considered positive, while 96 (92.3%) were considered non-reactive. In IHA, we found 28 (26.9%) reactive samples, while 76 (73%) serum samples were negative in the assay. By ELISA, 26 (25%) of the tested samples were shown as positive for *T. gondii*, while 98 (75%) were negative by the test. Serum samples with concordant results were submitted to *Western blotting*, which revealed antigenic recognition of proteins with high molecular weights. In this sense, according to the different methods applied, we conclude that there is serological evidence of *T. gondii* infection in alligators, leading to a possible break in a long lasting paradigm – that the parasite is only able to infect warm-blooded hosts. In order to confirm this hypothesis, further studies should be performed in order to search for specific antibodies and parasite isolation from reptiles. **Supported by:** CAPES, CNPq, FAPEMIG, FINEP

EP011 - Risk factors and seroprevalence of Toxoplasmosis in horses of Bauru southern Brazil

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Toxoplasma gondii is an intracellular parasite of the Apicomplexa group, occurring in most terrestrial vertebrates, with felids as definitive hosts. This infection is present in 60% of the adult population of São Paulo. Toxoplasmosis can be acquired by ingesting food/water contaminated with oocysts present in cat feces or undercooked meat containing cysts. Horses are infected only by oocysts in water and pasture, serving as sentinels of environmental transmission. Most studies reported 20-35% seroprevalence of this infection, as in Europe, due to the use of horse meat for human consumption or in a few studies in Brazil. Here, we analyzed 729 serum samples from horses with sex, race and age defined, from breeders of São Paulo state in Southern Brazil. ELISA IgG was performed, with 25.7% (187/729) positivity. All positives sera were also confirmed by the modification agglutination test (MAT), with only 2,9% discordant samples. Female horses presented a 27,7% higher seroprevalence as compared to 20,3% of their male, but this result was also associated to higher age of this group. There are close relationships between infection and the type of maintenance as free living pasture animals without defined race (34,7%) or confined animals with defined race (21,8%). Our results also demonstrate that infection of animals is progressively higher according to age, showing a constant exposure in lifetime. This prevalence, as corrected for age groups, is quite similar to reported results in bovines from the same region. Horse data may indirectly demonstrate the environmental contamination in their living habitats, which is similar to bovines which meat is also used for human consumption. The breeding and maintenance of those large animals correlated to the prevalence of toxoplasmosis in these animals. These data serves as reports of environmental risk of *Toxoplasma* infection in meat producing animals, allowing their use in intervention measures for adequate meat production. **Supported by::**HCMUSP & IMTSP