Epidemiologia- Epidemiology

EP01 - THE SPATIAL EPIDEMIOLOGY OF AMERICAN VISCERAL LEISHMANIASIS: THE OCCURRENCE OF HOT SPOTS IN THE RISK TRANSMISSION RELATED TO A DOG POPULATION IN RIO DE JANEIRO.

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The epidemiology of Visceral Leishmaniasis is highly influenced by ecological factors related to the interplay among the habitats, parasite, vectors and hosts. Geographic Information Systems (GIS) have been proved to be an important tool to map and analyze environmental factors and the temporal distribution of both hosts and vectors. Based on that methodology, a model was developed for mapping the distribution of serological titers of a dog population, followed up during one year, from an endemic area of American visceral Leishmaniasis in Rio de Janeiro, Brazil. Two places for sand fly capture, and the occurrence of chicken houses were also mapped using Garmin GPS receivers to permit the calculations of latitude and longitude. For the GIS analysis, ArcGis software was used to merge the location database with the study group data in relation to the micro environmental characteristics. Based on the maps that were generated, It was observed in this endemic area the occurrence of "hot spots" where the higher serological titers were clustered and the parasites' transmission looks to be very high when compared with the neighboring areas. Our results suggested that the occurrence of chicken houses could have some role on the reduction of the risk of transmission and forming points of low transmission.
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EP02 - VALIDATING THE TAXONOMIC STATUS OF *LEISHMANIA (VIANNIA)* SPECIES

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Among the 14 *Leishmania* species circulating in Brazil 6 belong to the *L. (Viannia)* subgenus. The genetic distance observed among these species can be higher than the observed distance between some species in this subgenus. In fact, there is no appropriate concept to define species in the genus *Leishmania;* some of them were described only considering eco-epidemiologic features, but not all are supported by biochemical and/or molecular characteristics. The high homology among *L. (Viannia)* species, the sympatric occurrence and the observation of natural hybrids between some *L. (Viannia)* indicate that genetic flow might be occurring in this group. In this study biological, biochemical and molecular methods were employed to characterize *Leishmania* species. Eighteen *Leishmania* strains representing the 5 *L. (Viannia)* described species associated with human CL in Brazil, were selected. All of them were analyzed by growth curve and were typed by isoenzymes, PCR-RFLP/sequencing hsp70 and ITSrDNA. So far, the biological analyzes indicate that strains/species shared patterns indicating that biological behavior are limited for taxonomical purpose, but corroborate the close relationship among *L. (Viannia*) species. The samples were classified in 12 zymodemes and they were clustered according to their specific classification. Analyzing reference strains by hsp70 it was observed good species discrimination. However, when the other isolates, representing each species were included in the study, only *L. braziliensis* strains classified in the same zymodeme of the reference strain showed a profile compatible to this species and the others were similar to *L. naiffi*. It was also observed distinct profiles among the *L. guyanensis* strains. The results show the difficulty of defining criteria to name *Leishmania* species, the definition of an appropriated method to characterize the described species and pointed to the need of revising the *Leishmania* taxonomy.

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EP03 - *TRYPANOSOMA CRUZI* LINEAGES ASSOCIATED WITH REACTIVATION IN IMMUNOSSUPRESSED CHAGASIC PATIENTS

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The factors involved in Chagas disease reactivation are not clear. They may be related to selective host immune depletion and/or to specific parasite populations. *T. cruz*i major lineages (TCI and TCII subgroups or DTUs) were evaluated in HIV(+) and HIV(-) chagasic patients with and without reactivation from Minas Gerais - Brazil. The amplification of the D7 domain of the 24Sα and 18S rDNA genes and the mini-exon intergenic region were performed. The characterization of nuclear DNA was performed in 39 chagasic/HIV(+) patients and demonstrated the presence of TCI in 5.1%(2/39); TCII in 94.9% (37/39) corresponding to 8.1% (3/37) TCIId and 91.9% (34/37) IIb. *T. cruzi* characterization performed in 50 chagasic patients HIV(-), revealed TCI and TCII in 4% (2/50) and 96% (48/50) patients respectively, TCIIb/e (95.8%) and IId (4.2%) was also identified in the last group. Among the 29 HIV co-infected patients not reactivated, the TCI was detected in 6.9% (2/29); TCII in 93.1% (27/29) corresponding to TCIIb/e and IID in 92.6%(25/27) and 7.4% (2/27) respectively. *T. cruzi* reactivation was detected in 10 patients HIV(+) and two HIV(-) immunossupressed (neoplasies or transplants), TCII major lineage was detected in all samples from blood and/or central nervous system-CNS, corresponding in 91.7% (11/12) of patients to TCIIb/e and 8.3% (1/12) to TCIId (HIV+). The same *T. cruzi* II DTUs were detected in blood and CNS fluid. There was no statistical difference in the *T. cruzi* genotypes distribution among HIV(+) and HIV(-) chagasic patients, or between reactivated and not reactivated patients or associated with blood and/or CNS invasion in reactived patients. Besides, 33,3% (1/3) of TCIId patients showed reactivation, no specific *T. cruzi* lineages or DTUs were associated to reactivation or to CNS invasion. Both the TCIIb/TCIId had potential for reactivation.
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EP04 - Follow up of toxoplasmosis's seroprevalence in pregnant women assisted in a public health center in Rio de Janeiro, Brazil.

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Toxoplasma gondii (*T.gondii*), an obligate intracellular parasite found in many species throughout the world, causes a variety of clinical syndromes in human and animals. Toxoplasmosis during pregnancy can cause congenital infection and manifest as mental retardation and blindness in the infant.The infection is acquired by contact with cats, which excrete living oocysts into the environment and cause environmental contamination. Thus, the accidental ingestion of oocysts through contact with soil or the cleaning up of cat faeces could be sources of T. gondii infection, especially for pregnant women. In addition, consumption of raw meat containing tissue cysts of T. gondii is also an important route for the infection. The aim of this work was to know the seroprevalence of toxoplasmosis in pregnant women assisted in Centro de Saude Escola Germano Sinval Faria (ENSP/FIOCRUZ), besides supplying data that can contribute to the formulation of politcs of maternal-infantile health. For that, A retrospective study of the results of Serological tests (IgG and IgM) was accomplished in 504 pregnant woman, during the entire year of 2008. Preliminary results showed that 58.9% were IgG-positive and, of these 2.4% were also IgM-positive. Whereas we identified 195 pregnant women as susceptible to toxoplasmosis (IgG and IgM negative), wich represents 38,7% from the pregnant woman studied. This study demonstrate the prevalence of toxoplasmosis in the population assisted by CSEGSF and showed that control measure should be adopted in order to reduce the incidence and complications of that infection in pregnant women.

EP05 - Identification of *Leishmania infantum* in the semen and prepucial secretion of naturally infected dogs

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The presence of *Leishmania* sp. in the semen of infected dogs contraindicates the use of these animals in reproduction for the possibility of transmission of venereal disease. However, it is not yet certain that the transmission occurs from the contact of the infected semen, with the prepuce of the dog. The parasite was identified in penis and prepuce of the dog. Thus, this work looked for to identify to presence *L. infantum* in the semen and prepucial secretion of infectede dogs, in order to verify the transmission potential of venereal disease for the contact between prepuce/penis and vulva/vagina, independently of having semen deposition in the vagina of the bitche.
Seven adult dogs naturally infected with *L. infantum* had been evaluated. The animals had been submitted to the prepucial secretion collection. Afterwards was made the collection of semen. The samples had been analyzed by PCR using itself a pair of specific starters MC1/MC2 for *L. infantum*. As negative controll had been used prepucial secretion and semen of a negative dog (negative sorology; cytology, PCR and negative culture of bone marrow), and as positive control one sample of *L. chagasi* (MHOM/BR/1967/BH46). *L.Infantum* in 85,7% (6/7) of the semen samples was disclosed to positivity for Diniz et al. (2005) they had gotten positivity of semen in 36,4% (8/22) of infected dogs. The prepuce was positive in 71,4% (5/7) of the dogs. The dogs with positive semen had also disclosed positivity in the prepucial secretion in 83,3% (5/6) of the cases. The negative dog for semen was also negative for prepuce. The presence of *L. infantum* in the prepucial secretion of dogs, being it potential source of infection for dogs during the coitus, independently of the infectious condition of the semen; as well as during its collection for artificial insemination.
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EP06 - *Leishmania infantum* in the semen and secretion of dogs treated with Allopurinol and Amphotericin B

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Dogs infected with *Leishmania* sp. they should not be used for reproduction for the possibility of transmission of venereal disease. However, it is not known if this form of transmission would occur when the dogs are in treatment. The treatment can be based on the use of Aluporinol and Amphotericin B, disclosing expressive reduction of the parasitic load in the dog. This work looked for to identify if the treatment with Alopurinol and Amphotericin B would be capable to reduce the transmission risk of venereal disease, Six adult dogs naturally infected with *L. infantum* submitted to the treatment suggested. The animals had been submitted to the prepucial secretion collection, followed of semen collection. The samples had been collected before and at 60 and 150 days of treatment, and analyzed for PCR usinga pair of specific starters MC1/MC2 for *L. infantum* (Cortes et al., 2004). As means of negative controlled had been used prepucial secretion and semen of a negative dog (negative sorology, PCR and negative culture of bone marrow), and as positive control one sample of L. chagasi (MHOM/BR/1967/BH46). The test of Wilcoxon for comparison of the results was used. At 60 days all the dogs that had positive semen become negatives (5/5; P=0,06), thus remaining until 150 days (3/3; P=0,25). In the same way it happened with the prepucial secretion at 60 days (4/4; P=0,25), but not at 150 days (3/4; P=0,25). A dog with negative semen became positive at 60 days; e a dog with negative prepucial secretion remain negative until the 150 days. For the first time was evaluated the effectiveness of the treatment with Alopurinol and Amphotericin B in eliminating the *L infantum* of the semen and prepucial secretion. However, these results need to be confirmed with a bigger number of observations.
Supported by PUC Minas

EP07 - *Pneumocystis* in autopsied lungs of general population dying from accidents and other causes in the community.

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Documentation of pulmonary infections by *Pneumocystis jirovecii* after the primary infection in adult population in the community would provide important information on the incidence of this fungal pathogen in the community and on the relevance of immunocompetent adult human hosts in the transmission and circulation of this fungus. Previous research to document this infection in the lungs of immunocompetent adults has been unsuccessful because the methods of detection used lacked adequate sensitivity to detect asymptomatic infections. Lungs of 77 individuals median age 44 years (5.1 to 88 years) with legally required autopsies in Santiago were examined for *Pneumocystis* using nested-deoxyribonucleic acid (DNA) amplification of the large subunit mitochondrial ribosomal ribonucleic acid *P. jirovecii* specific gene and immunofluorescence microscopy. Causes of death were violent [automobile accidents (n. 22), homicides (n.10), and suicides (n.20)], and non-violent [diseases causing a rapid demise in the street (n. 19), and undetermined (n. 3)]. Lung tissue concentration techniques were needed to reach the sensitivity of the nested-DNA amplification and fluorescence microscopy assays. *Pneumocystis* forms compatible with cysts and trophozoites plus *P. jirovecii*-DNA were identified in 33 (60.0%) of 55 people dying from violences, and *P. jirovecii*-DNA in 15 (78.9%) of 19 with rapid demise from non-violent causes. Microscopy and DNA amplification techniques gave consistent results. The study documents that a mild and active pulmonary *P. jirovecii* infection, compatible with the concept of colonization, is prevalent in over 60% of the general adult population in Santiago and strengthen the concept that adults participate in the circulation of *P. jirovecii* as an infective reservoir. *P. jirovecii*-negative results suggest that *P. jirovecii* is cleared from the human lung after the primary infection and that the positive cases correspond to frequent self-limited re-infections. Further research to identify whether this mild *P. jirovecii* lung infection poses a pathogenic role as a co-factor in pulmonary diseases of immunocompetent individuals is warranted.
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EP08 - The use of a ribosomal RNA based PCR coupled to sequencing to identify *Leishmania* in paraffin-embedded skin biopsies of American Cutaneous Leishmaniasis patients from Pará State, Brazil.

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Paraffin-embedded tissues are commonly stored by educational and research institution consisting a rich bank of samples that can be used in follow-up or epidemiological studies. However, the paraffin inclusion process involves the use of substances that can cause DNA degradation. This DNA damage constitutes a major challenge in the application of DNA-based techniques in such samples. In this study we verified the possibility of applying a PCR-based protocol to identify strains of *Leishmania* in 33 paraffin-embedded skin biopsies of patients with American Cutaneous Leishmaniasis (ACL) from Evandro Chagas Institute sample bank (Pará State, Brazil). After treatment of each sample with xylene and several washes with PBS, DNA was obtained by phenol-chloroform protocol and then used in PCR or nested PCR based on the nucleotide sequence coding the small subunit ribosomal RNA (SSUrDNA) (Uliana et al., 1994). The obtained amplicons were cloned and sequenced by chain termination method, to determine the SNPs that distinguish between different *Leishmania* species or groups. From the 33 samples, ten (30%) resulted positive in a simple PCR; other nineteen (58%) were positive after the application of a nested-PCR and four (12%) samples remained negative. Among the 29 PCR products obtained, 10 were sequenced to differentiate *L.(L.) amazonensis* from *L. (Viannia)* species, the etiological agents of ACL at the studied geographical region. In six samples we identified *L. (L.) amazonensis* and the other four samples contain organisms belonging to *L. (Viannia)* sub-genus. The results indicated that the protocol used in this study allowed the detection of the pathogen in 88% of the paraffin-embedded samples as well as the identification of the organisms by sequencing the PCR product. This opens a huge opportunity to study stored samples and promote relevant contributions to epidemiological studies. Supported by FAPESP and CAPES.

EP09 - TISSULAR PARASITISM OF *DIDELPHIS MARSUPIALIS* NATURALLY INFECTED WITH *LEISHMANIA INFANTUM CHAGASI* IN RIO DE JANEIRO, BRAZIL.

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In peri-urban and even urban in areas, where sylvatic canids are not found, the *Didelphis* is the most important sylvatic reservoir of American Visceral Leishmaniasis. It was depicted as the first non-canid wild animal naturally infected with *Leishmania infantum chagasi* with a high seroprevalence in endemic areas. In spite of the notorious importance of the opossums as reservoirs of AVL, there is no report on the presence of amastigotes in tissues of these naturally infected animals. The investigation of natural infection**s** was restricted to serological, molecular diagnostics and tissular parasitism was only detected by indirect methods such as: culture and hamster inoculation. In the present study, we have observed in imprints from spleen and lymph nodes from three opossums, a scarce parasitism with a small number of macrophages parasitized by few amastigotes. The infection with *L. (i) chagasi* was confirmed in the spleen samples from one animal by positive PCR and Dot Blot results. The animals presented no clinical sign of leishmaniasis. Our results reports for the first time, the presence of amastigotes in tissues from *D. marsupialis* naturally infected with *Leishmania i. chagasi* in an endemic area of visceral leishmaniasis in Rio de Janeiro.
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EP10 - RELEVANCE OF ECO-EPIDEMIOLOGIC CHARACTERISTICS AND POPULAR KNOWLEDGE FOR THE DEVELOPMENT OF INTEGRAL EDUCATIONAL PROGRAM TO PREVENT INTESTINAL PARASITES DISEASES.

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The goal of this work is to test the impact of health education on the prevalence of intestinal parasites among school children (0 to 14 years of age) from a semirural area of Salsipuedes (Córdoba, Argentina). In order to evaluate the initial parasitic burden, coproparasitological examinations were determined in a cohort of inhabitants of the sampled households. The prevalence of intestinal parasitosis was about 28,12% being *Enterobius vermicularis*, *Giardia lamblia*, and the commensal *Blastocystis hominis* the main parasites found. There were no cases of polyparasitism. Socio-cultural and sanitary information were simultaneously retrieved. Questionnaires, drawing analyses and interviews were used to generate quantitative and qualitative data focusing on knowledge, practices and perceptions of intestinal parasitosis in mothers and children of this community. Mother knowledge about prevention of parasite transmission, epidemiology, symptoms and treatment was generally good, although high contradictions were found. For example, half of the persons consider that parasitosis is not an illness and about 60% that they cannot be prevented. Although recognising sources of infection, people (86%) claim that parasites appear because of sugar and candies ingestion. Mothers and children identify parasites mainly as macroscopic worms and do not recognize protozoan parasites or infectant forms like cysts and eggs. Furthermore, there is no clear association between ways of transmission and prevention practices. Popular medicine and consultation to “witch doctors” are usual practices. Our research highlights the importance of gathering information on perceptions and behaviour for the design and implementation of a community-based intestinal parasite educational programme. In this sense, we developed cartoon-based health educational material for primary school-aged children and teachers, focusing on information about infectant and microscopic forms of the parasites as well as the transmission of parasitic diseases associated with an improvement of hygienic behaviour as major keys in preventing the spread of intestinal parasitic infections.
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EP11 - Immunoenzimatic evaluation of TSSA-II recombinant antigen of *Trypanosoma cruzi* in sera from naturally infected dogs in an endemic area for Chagas disease, Chaco, Argentina.

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TSSA (Trypomastigote Small Surface Antigen) protein is found in the surface of the parasite and allows differentiating the two lineages of *T. cruzi*, Tc I (TSSA I) and Tc II (TSSA II). GST-TSSA II recombinant antigen was evaluated through ELISA technique (0.1µg/well, sera dilution 1/500) using 101 sera from dogs. The following sera groups (**G**) were formed, **G1**: 14 positive control sera, corresponding to naturally infected dogs, from which isolates were characterized by MLEE as TcIIe; **G2**: 60 negative control sera (NC), corresponding to dogs of an endemic area, all negative for xenodiagnostic (Xe), serology and PCR; **G3**: 18 NC sera, corresponding to dogs of Salta city, not endemic area for *T. cruzi*, all with negative serology for *T. cruzi*; **G4**: 2 dogs sera from which the isolates were characterized by MLEE as TcI; **G5**: 9 sera with positive serology for *T. cruzi* and negative Xe, all from the same endemic area. The calculated sensitivity was 85.71% (12/14) with a CI (95%) of 63.81-100; specificity was of 100% (0/78) with a CI of (95%) 99.36-100. Observed concordance was 0.97, Kappa index was 0.91 (CI-95%- 0.78-1.00). The calculated ROC area was of 0.97, EE 0.02; CI (95%) 0.925-1.02 Delong. From **G4,** 100% was negative. From **G5,** 66.66% (6/9) were positive and 33.33% (3/9) negative. The concordance between typing test (MLEE) and TSSA-II was almost perfect according to Landis and Koch scale for the interpretation of Kappa index. According to ROC analysis, the test has a high performance for the diagnostic, in the conditions in which it is normatized. The diagnostic capacity of TSSA-II antigen in a sera panel from naturally infected dogs was very good, being an important tool for epidemiological studies of seroprevalence and/or association with Chagas disease.
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EP12 - CLUSTER DETECTION OF CUTANEOUS LEISHMANIASIS ON MULTIPLE VECTOR CONTACT LOCATION MODEL FROM HYPOTHETICAL CASE-CONTROL DATA

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The Cutaneous Leishmaniasis (CL) is endemic in nine province of Argentina. One of the most important factors that avoid the construction of risk map of CL is the unknown knowledge of the location of vector contact. The objective of this work is generate a statistic model for detect clusters of CL cases, in despite of the unknown location of vector contact of patients, using hypothetical data. From the cartography of Oran Department, we generate a grid and took the coordinate centroids points. Patients and controls information was collected taking into account all the sites that these persons had been frequent. Each point corresponds at x_i-y_j data and a position matrix for each patient was generated. The sum of de matrixes for de cases and controls has generated two data sets, and the subtraction of these sets can be used either analysis through of scan statistic on poisson model or calculate the mean for windows that include at least five centroids (using ANAVA tool). These additions generate scores equal to zero for each pair x_i-y_j as a minimum. The population data for scan statistic analysis was obtained from fraction areas values (unit of census used for INDEC). The geographic representation for the result from subtraction between sets of cases and controls, was made thought of kernel density using the ArcGIS 9.3 software. The areas with high intensity nucleuses, the primary cluster (Scan Statistic) and the means scores observed were consistent with the expected hypothetical data proposed in the model ($p<0,05$).
The unknown knowledge of the location of vector contact can be solve using case-control data set approach in Geographic Information System support and the multiple site concurrence of these persons.
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