

# Visceral Leishmaniosis: An Old Disease with Continuous Impact on Public Health

Marcella Zampoli Troncarelli,  
Deolinda Maria Vieira Filha Carneiro and Helio Langoni  
*University Estadual Paulista, School of Veterinary Medicine and Animal Science  
Brazil*

## 1. Introduction

Visceral Leishmaniasis (VL) is an important Zoonosis, caused by *Leishmania* spp. protozoa. VL is commonly present in tropical countries, due its complex epidemiological characteristics that involve environmental and climatic conditioning factors; phlebotomine vectors and several species of domestic and wild animals. These aspects determine high difficulty for the disease control.

VL is considered the second most important protozoosis and one of the six main infectious-parasitary diseases in the world (WHO). However, VL is a neglected disease that occurs in 80% of poor or miserable populations witch survive with less than two dollars per day (Desjeux, 2004). VL is considered an emergent/re-emergent Zoonosis with high mortality levels, with continuous and serious impact on Public Health.

## 2. Epidemiology

### 2.1 Definition and etiology

Visceral leishmaniasis (VL) is a worldwide zoonotic disease caused by flagellar protozoa (family Trypanosomatidae, order Kinetoplastida, genus *Leishmania* (*L. donovani* complex) (Farrel et al., 2002). *L. chagasi* is frequently reported in Americas (New World), genetically similar to *L. infantum* that is found in some countries of Mediterranean and Asia. *L. infantum* / *L. donovani* are the more prevalent species in Europe, Asia and Africa (Old World).

### 2.2 Distribution and classification

The VL origin in the New World is controversial. *L. chagasi* may be carried out by wild dogs some millions years ago, but some researches appoint that *L. infantum* may be disseminated after the European colonization. In a study of evolutive and geographical history of *L. donovani* complex, using specific molecular tests, it was possible to infer that *L. (L.) chagasi* and *L. (L.) infantum* are, in fact, different denominations of the same parasite specie, considering their molecular and biochemical similarity (Luke et al., 2007). It was possible to suggest that the primordial lineage of *Leishmania* spp. could be originated in South America.

VL was initially described in Greece, in 1835, and was called “ponos” or “hapoplinakon”. The denomination “calazar” is proceeding from India, which means “black skin” due the clinical characteristic presented in severe cases of infection (Lainson et al, 1987).

VL is classified according to its clinical characteristics and epidemiology in five types: Indian, African, Mediterranean, Chinese and American. The Indian type is considered an antrozoonosis caused by *L. donovani*, mainly identified in children, adolescents and adults determining frequent outbreaks with high mortality levels. This VL type occurs in Afghanistan, Iran, Iraq, Jordan, Israel, Lebanon, Oman, Saudi Arabia, Syrian, Yemen and Bengala. The African type, similar to the Indian one, considering the susceptibility and clinical signs, is caused by *L. donovani* or *L. infantum*. The transmission areas of African type are next to forests. The Mediterranean type is a zooantroponosis mainly transmitted in the near-house place. This type is frequently identified in children with five year of age or less, and dogs. *L. infantum* is involved and also participates on the wild cycle infecting foxes and coyotes. It occurs in Mediterranean, the Middle East and the South of Russia. The Chinese type is another one antrozoonosis caused by *L. infantum* that complete its biological cycle in dogs, raccoons, coatis, and in children of China. The American type is an antrozoonosis in urbanization process. *L. chagasi* is enrolled predominantly in children of 15 years of age or less, being transmitted by *Lutzomyia longipalpis*. In Colombia, *Lutzomyia evansi* is considered a secondary vector. In Latin America, VL is present in 12 countries with 90% of cases occurring in Brazil. Great urban centers and capitals are being invaded by VL, including Belo Horizonte, Teresina, São Luís, Fortaleza and Rio de Janeiro, where autochthon cases are registered.

First VL human case in Brazil was reported in 1913, when Migone identified amastigotes forms in necropsy materials collected from a man from Boa Esperança, Mato Grosso state (Brasil, 2006). Until the 90's, the main reported VL cases in human were from Northeast states. However, the recent and fast VL expansion to the Southeast, Midwest and North states has being verified. Northeast states presented the greatest number of VL cases in 2001 (81.7%), followed by North regions (8.8%), Southeast (7.6%) and Midwest (1.9%). However, in 2003, the disease was reported in 58%; 15%; 7% and 19% of the Northeast; North; Midwest and Southeast states, respectively, demonstrating VL urbanization in Brazil (Silva et al., 2001b; Lindoso & Goto, 2006).

Canine visceral leishmaniasis (CVL) prevalence is also high in Brazil, ranging 1% to 36% according to Brazilian region (Silva et al., 2001b). Araçatuba was the first city in Sao Paulo state where CVL was diagnosed (Luvizotto et al., 1999). Forty one cities in Sao Paulo state present the CVL transmission, and human VL transmission was reported in 28 cities, with 10% to 15% prevalence levels (Camargo-Neves, 2005).

### 2.3 Vectors

The vectors related with *Leishmania* dispersion are phlebotomines (mainly *Lutzomyia longipalpis*). Fleas and ticks are considered other possible leishmaniasis vectors. Reservoirs are infected by phlebotomine females biting during its blood feeding in the skin or the peripheral blood of the reservoirs (mainly in dogs).

In Brazil, the main country of VL transmission in Latin America, the vectors *L. longipalpis* or *L. cruzi* are enrolled on disease transmission.

Phlebotomines are small size insects (3-5 mm length) and present a straw coloration. They multiply on organic materials like humid soil, leaves, manure, etc. The action area of phlebotomines is of 150-300 meters (300-600m diameter). They usually fly in small salts and when the females are blood feeding, they keep their flies erect.

## 2.4 Reservoirs

The direct transmission (person-to-person) of VL is rare. In regions of the New World, where *L. chagasi* is endemic, the infection dispersion/maintenance in human beings had been also attributed to the canine reservoirs. They live closely to human and attract the vector to human houses. Dogs present elevate subcutaneous parasitism levels with high infectivity to the VL vector. The infectivity levels for phlebotomines are similar in asymptomatic and symptomatic dogs (Zivicnjak et al., 2005).

On the other hand, *Leishmania* can be also identified in wild animals as foxes (mainly *Lycalopex vetulus* and *Cerdocyon thous*) (De Lima et al., 2006), and opossums (*Didelphis albiventris*) (Santiago et al., 2007). Equines, cats and rodents have been also identified as reservoirs.

Cats are susceptible to both VL and integumentary leishmaniasis. Xenodiagnosis studies with experimental *Leishmania* infections in cats demonstrated the evidence of feline's infectivity to the vector *L. longipalpis*. Skin lesions and protozoan distribution in organs were verified in infected cats, but the disease was self-limited after few months post-infection. Cats' eclectic habit as well as the vectors adaptation to different animal species would be favorable factors for VL transmission (Dantas-Torres et al., 2006).

VL in cats may be also associated with immunosuppressive diseases such as leukemia and feline immunodeficiency. Recent studies are been developed in order to investigate the real feline role on VL epidemiology: if they are important reservoirs or only accidental hosts.

## 2.5 *Leishmania* life cycle

Infected vertebrate hosts (reservoirs) are bitten by phlebotomine vectors during the blood feeding. Vectors acquire macrophages containing *Leishmania* amastigotes forms which multiply by binary division. Amastigotes differentiate to promastigote (flagellar) forms which colonize vectors' pharynges and esophagus and stay adhered to epithelium by their flagella. Then, these promastigotes forms differentiate to metacyclical promastigotes (infecting) forms.

Biological cycle is completed when vectors bite new vertebrate hosts and the infecting *Leishmania* forms are inoculated in the hosts' epidermis and phagocyted by macrophages. After phagocytosis, promastigotes change to amastigotes which intensively multiply by binary division. Macrophages become devitalized and break and release the amastigotes which are phagocyted by new macrophages in a continuous process. The haematogenic and lymphatic dissemination of protozoa for other tissues and organs occurs especially in mononuclear phagocytical system cells (Ikeda-Garcia & Marcondes, 2007).

## 2.6 Characteristics and risk factors

VL has been considered as a re-emergent disease in some countries and has assumed a new profile due economic and social changes in the two last decades. Initially, VL was

considered a typical and sporadically agricultural disease (Nunes et al., 2001). It reached dogs and human beings that lived in close contact with forest regions (Gontijo & Melo, 2004). However, VL has been pointed currently as a re-emergent zoonosis, characterizing a clear process of epidemiological transition. LV has become an urban endemic disease that represents serious problems for Public Health due its zoonotic potential. The frequent deforestation has reduced the availability of original wild animals to vector. Then, dogs and human actually represents alternative sources for vector's feeding (Desjeaux, 2004).

This zoonosis has demonstrated an increased prevalence in recent years, not only considering the number of registered cases, but also regarding its geographic distribution. The important social, political and environmental damages (Fig. 1), added to continuous population migrations for urban centers without sanitary structure (Fig. 2) and lack of educative programs, associated to the easy vector adaptability to new environments and to the high population of dogs which are potential reservoirs, had contributed to establish favorable conditions to the VL urbanization (Camargo-Neves, 2005). Reduction of the disease ecological space is also verified, leading to important outbreaks.



Fig. 1. Environmental damage caused by houses building in Botucatu, Sao Paulo State, Brazil. The human changes in natural areas, the vector adaptation and the high number of possible reservoirs species represent a serious VL transmission risk to human and animals.



Fig. 2. Organic material accumulation near to a house in Sao Manuel, Sao Paulo State, Brazil. This situation may represent a risk for vector multiplication and VL transmission to both human and animals, especially in tropical countries.

### 2.7 Susceptibility

People of both sexes and all ages are susceptible to VL. However, the incidence is highlighted in children with nine years of age or less, and in immunosuppressed individuals. On the other hand, in recent endemic areas, a new situation regarding VL susceptibility has been observed, and the number of VL cases in children and adults is practically the same. This fact could be related to the protozoa's pathogeny/pathogenicity variations and also to immunosuppressor influences which adult persons are continuously exposed, like stress.

Approximately 12 million of people in the world are infected by *Leishmania* and about 1.5-2 million new cases (25-50% as VL type) are notified by year. However, the official data does not represent the real number of cases, due to passive detections, under-diagnosed cases, asymptomatic cases and low number of countries with obligatory notification (only 32 of 88 endemic countries demand obligatory cases registration). In accordance with the Regional Program of Leishmaniasis, all American countries registered more than 5,000 VL cases in 2006.

### 3. Clinical signs in human and treatment

The incubation period of VL in human is variable, ranging from 10 days to 24 months, with an average of two to six months. Clinical manifestations of the disease in humans are presented gradually, starting with apathy, weight loss, anemia, fever and lymphadenopathy, and liver and spleen enlargement. Pneumonia and other severe manifestations due to secondary

bacterial infections are also related. VL can be lethal in approximately 95% of cases without treatment (Murray et al., 2005).

Treatment of human cases also is recommended as VL control method. The pentavalent antimonial substances (sodium estibogluconate and N-metil glucamine antimoniate) are the election drugs for use in humans, since 70 years ago. In India, where 50% of the VL cases occur, the miltefosin is being used - since March of 2002 - administered by oral route, also indicated in oncology. This drug presents relatively efficient safety and allows up to 98% of cure. It is indicated in refractory cases to the therapy with conventional antimoniates. However, the patients can develop gastric, intestinal and theratogenic collateral effects. Amphotericin B is another therapeutic option that also determines collateral effects and is considered a high cost drug.

#### 4. HIV-VL co-infection

More than 30 million people in the world are infected by HIV, and at least one third of this population lives in leishmaniasis endemic areas (WHO). The overlapping of geographic areas where leishmaniasis and HIV occur is due to the process of urbanization.

In Europe, 70% of LV cases in adults are associated with HIV, and more than 9% of the individuals with AIDS suffer a just acquired or reactivated leishmaniasis. Users of intravenous drugs are considered of high risk for the co-infection, by sharing needles. From 80's on, this co-infection started to be described in the Europe, particularly in Spain, Italy and South France. In people infected by HIV, leishmaniasis presents severe clinical manifestations and determines lethal immunosuppressive synergism with HIV with stimulation of the viral response. However, the opportunistic behavior of *Leishmania* on HIV-VL co-infection is still not clear.

The HIV-VL co-infection is considered a world priority by WHO. In this context, it was established a worldwide network of further notification of cases.

#### 5. Clinical signs in dogs

Canine Visceral Leishmaniasis (CVL) determines a chronic systemic disease with several clinical manifestations, with difficult diagnosis. CVL incubation period is variable. Clinical signs can be verified in dogs between three months to various years after infection (mean three to seven months) (Brasil, 2006).

Lymphadenopathy; abdominal distension due liver and spleen enlargement; skin lesions and/or alopecia (Fig. 3); emaciation; apathy; ocular lesions (Fig. 4); onicogryphosis; haemorrhages; diarrhoea and pneumonia are the main clinical signs reported. Locomotors and neurological alterations are occasionally related (Slappendel & Ferrer, 1998).

Clinical signs in dogs may have different presentations, depending on the region (Cruz, 2006; Duprey et al., 2006; Albuquerque et al., 2007). In a recent study, the main clinical manifestations observed in 100 examined dogs from an endemic VL area in Brazil were: emaciation (60%); spleen enlargement (57%); alopecia (51%); ocular lesions (46%); skin ulcers (43%); liver enlargement (38%); onicogryphosis (37%) and lymphadenopathy (21%) (Troncarelli et al., 2008).

Symptomatic CVL is frequently reported, but asymptomatic cases represent about 60% of infected dogs. Asymptomatic parasited dogs with or without antibodies have been identified in natural and experimental infections (Farrel, 2002). The infected asymptomatic animals represent a serious problem for public health because they are important unidentified reservoirs. On the other hand, when an infected asymptomatic dog is diagnosed, control measures may be difficult because the euthanasia is usually not authorized by dog's owner.



Fig. 3. Symptomatic VL seropositive dogs in the Zoonosis Control Center (ZCC) in Bauru – an important VL endemic area of Sao Paulo State, Brazil. A: alopecia, emaciation, skin lesions, onicogriphosis and apathy. B: lymphadenopathy (popliteal), emaciation, dehydration.



Fig. 4. Symptomatic VL seropositive dogs from Bauru, Sao Paulo State, Brazil. A: severe periocular, nasal and auricular skin lesions (humid dermatitis, alopecia, crusts and secondary bacterial infection). B: ocular lesions, jaundice.

## 6. Diagnosis

### 6.1 Dogs

Considering that VL is a further notification zoonosis and reinforcing the risks to public health represented by *Leishmania* infected dogs, CVL diagnosis must be the more accurate than possible. It is important that the technical groups involved on CVL diagnosis specifically know about the available tests, as its limitations and clinical interpretation (Luz et al., 2001; Gradoni, 2002; Ikeda-Garcia & Marcondes, 2007).

The diagnosis result must not be individually analyzed. There are various dependent factors that must be evaluated, like epidemiological issues, clinical signs, laboratory diagnosis results, etc. A positive result, if punctually analyzed, can condemn a non-infected dog to the euthanasia and a negative result if not correctly evaluated can contribute to the maintenance of an infected dog as a *Leishmania* reservoir (Machado, 2004; Zivicnjak et al., 2005).

#### 6.1.1 Serology

Detection of circulating antibodies anti-*Leishmania* spp. in dogs by serological tests is an essential tool for CVL diagnosis. Serum samples or eluted blood samples can be examined by serological tests (Fig. 5).

Dogs generally present seroconversion after three months post-infection. Antibodies titles remain high for at least two years after *Leishmania* infection (Ikeda-Garcia & Marcondes, 2007). CVL clinical signs and antibodies titers are not necessarily dependent factors (Reis, 2001; Ferreira et al., 2007).

Available serological tests commonly used for CVL diagnosis are Indirect Haemagglutination (IHA); Agglutination in Latex (AL); Direct Agglutination (DA); Immunoelctrophoresis; Indirect Immunofluorescence Antibody Test (IFAT) (Fig. 5); Enzyme-linked Immunosorbent Assay (ELISA); Complement Fixation; Immunoprecipitation in gel and Western blot. Brazilian Health Ministry (Brasil, 2006) determined that the official labs must apply ELISA as a selection diagnosis and IFAT as a confirmatory test, in CVL surveillances.

IFAT usually shows high sensitivity and specificity values, ranging from 68 to 100% and 74 to 100%, respectively (Grosjean et al., 2003; Alves & Bevilacqua, 2004). ELISA` sensitivity and specificity values range from 71 to 100% and 85 to 100%, respectively. These tests are feasible, fast and inexpensively executed (Ikeda-Garcia & Marcondes, 2006). However, a positive result may not represent an active disease and also infected dogs may have negative results by serological tests. Two tests with 21-30 days interval are recommended.

Due to the phylogenetic similarity between *Leishmania* genus and *Trypanosoma cruzi* (*T. cruzi*), serological cross-reactions (Fig. 6) and false-positive results are quite common (Zanette et al., 2006). There are endemic areas, especially in the Americas, where *Leishmania* spp. and *T. cruzi* incidences are superposed and co-infection with the two parasites may occur both in dogs and in human beings (Savani et al., 2005; Madeira et al., 2006). In fact, the results from serological tests may show *Leishmania* spp. and *T. cruzi* antibodies, signifying the occurrence of infection by the two parasites, rather than cross-reactions (Grosjean et al., 2003; Rosypal et al., 2007). By this way, the improvement of diagnosis methods for the correct identification of canine infection is necessary to better comprehend the disease's status in dogs and also to contribute to its control.





Fig. 5. Blood sampling for CVL serological diagnosis at Zoonosis Control Center (ZCC) of Bauru, Sao Paulo State, Brazil.

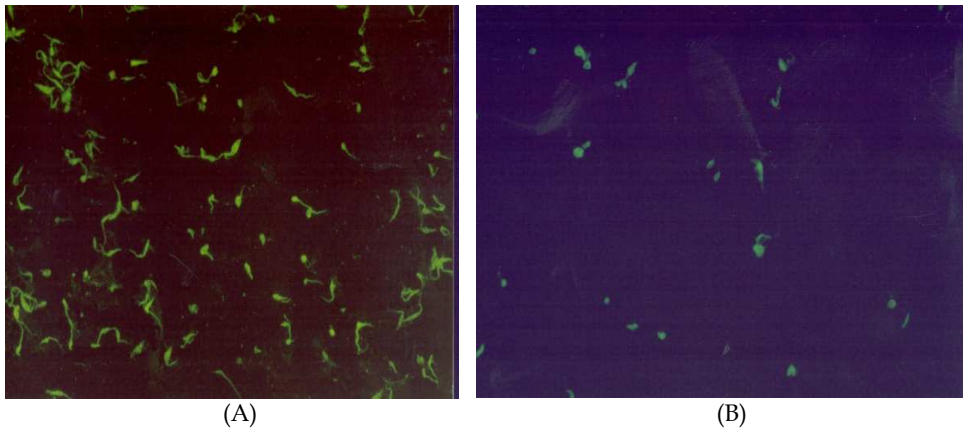


Fig. 6. Positive results for *Leishmania* spp. and *Trypanosoma cruzi* by indirect immunofluorescence tests in serum samples collected from a dog in an VL endemic area in Brazil. Note the morphologic similarity between the protozoan parasites. A: promastigotes forms of *L. major*. B: trypomastigotes forms of *T. cruzi* "Y" strain. Pictures credits: Zoonosis Research Nucleus (NUPEZO), Univ Estadual Paulista (UNESP), Botucatu, Sao Paulo, Brazil.

A 16.5% (33/200) cross-reaction between *Leishmania* spp. and *T. cruzi* in blood samples collected from dogs in an endemic VL area in Brazil was verified. Twenty-six (78.8%) of 33 dogs that showed anti-*Leishmania* spp. and anti-*T. cruzi* antibodies also tested positive by direct parasitological examination and PCR for *Leishmania* spp., which indicates that these dogs presented leishmaniasis. No liver or spleen sample from the 200 dogs analyzed showed a positive PCR result for *T. cruzi*. These findings support the occurrence of cross-reactions between *Leishmania* spp. and *T. cruzi* in IFAT; they also corroborate the need for simultaneous PCR and/or parasitological examination to establish canine leishmaniasis (CL) diagnosis.

On the other hand, it is important to reinforce that cross-reactions between *Leishmania* and other tripanosomatids or different microorganisms (including *Ehrlichia canis*; *Babesia canis*; *Toxoplasma gondii* or *Dirofilaria immitis*) can occur, producing false-positive results (Rosario, 2002; Luvizotto, 2003; Alvar et al., 2004). The use of recombinant or purified antigens (like gp63, gp72 and gp70; rK39, rK9 and rK26) improves the sensitivity and specificity of serological tests (Rosário, 2002; Zijlstra et al., 2001).

### 6.1.2 Culture

Blood samples and lymph node, bone marrow and spleen aspirates can be used for VL diagnosis by culture in special medium like Liver Infusion Tryptosis (LIT); NNN and RPMI-1640 (Sundar & Rai, 2002).

*Leishmania* amastigotes forms presented in original biological samples collected from vertebrate hosts modify to promastigotes (flagellar) forms in culture medium and can be visualized by optical microscope (Fig. 7).

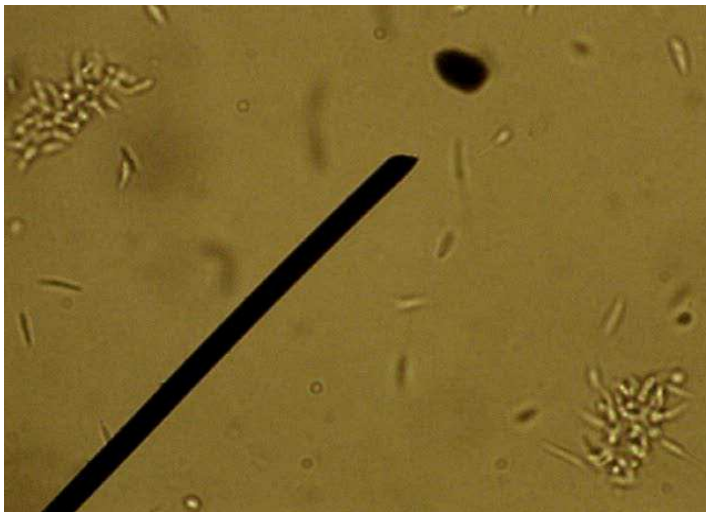


Fig. 7. *Leishmania* promastigotes forms isolated in Liver Infusion Tryptosis (LIT) medium after four weeks incubation at 27°C. Optical microscopy 1000X.

Culture diagnosis is commonly used for researches purposes because it is a time and material-consuming method. Results are lately obtained because *Leishmania* presents a slow multiplication in culture media. A positive result can be verified until four months post-incubation, especially in case of low parasite load presented in biological collected samples.

### 6.1.3 Parasitological identification

#### 6.1.3.1 Citology

*Leishmania* amastigote forms can be visualized in lymph nodes squashes (or aspirate); bone marrow; spleen aspirate; skin biopsy; skin nodules aspirate; liver biopsy and blood

squashes. Giemsa, Leishman, Wright and Panotico are the most common dyes used (Sundar & Rai, 2002). Amastigotes forms have 2-5  $\mu\text{m}$  size. For the correct diagnosis, it is necessary to observe three parasites' structures: cytoplasm, nucleous and kinetoplast (Fig. 8).

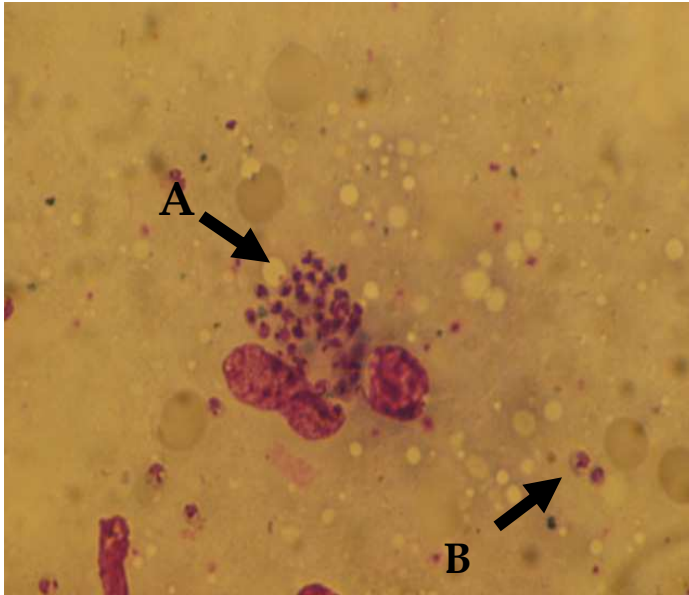


Fig. 8. Dog's liver *imprint* showing the protozoa's amastigote structures. Giemsa dye, 1000X. A: amastigotes forms in a macrophage cytoplasm. B: individual protozoa's amastigote form presenting cytoplasm, nucleus and kinetoplast.

Aspirative cytology is generally used in vet clinics because it is a relatively easy and low invasive procedure. Cytology sensitive values are directly related to parasite load, biological material collected, technical experience and time for slide examination (Ikeda-Garcia & Feitosa, 2006). This test presents sensitivity levels of 58%, 70% and 96%, for lymph node, bone marrow and spleen aspirates, respectively (Zijlstra et al., 2001). The 100% specificity allows the indication of cytology as a gold standard method. However, negative results are not uncommon, especially in chronic and/or asymptomatic CVL cases.

### 6.1.3.2 Immunohistochemistry

Skin, liver and lymphoid tissues are the election materials for VL diagnosis by immunohistochemistry/immunocytochemistry (IHC). Blood squashes; cytological slides; histological and frozen tissues can also be examined (Ikeda-Garcia & Feitosa, 2006). IHC allows retrospective studies and can be indicated for skilful exams.

Immunoglobulins conjugated to enzymes are used to identify *Leishmania* amastigotes forms in tissues. High sensitivity and specificity values of this method allow an accurate and fast diagnosis despite the eventual low parasites load on samples (Ikeda-Garcia & Marcondes, 2007). Amastigotes presents a hazel-brown coloration, easily visible by conventional optical microscopy.

### 6.1.4 Molecular diagnosis

Polymerase Chain Reaction (PCR) allows the parasite's DNA detection and does not depend on the dog's immunological and/or clinical status (Soares et al., 2005; Reithinger & Dujardin, 2007). PCR can be used in cases of inconclusive reactions, anergy or cross reactions in serological tests, and shows sensitivity and specificity values near 100% (Lachaud et al., 2002; Gomes et al., 2007). Blood samples (Silva et al., 2001a); lymph node aspirates; bone marrow aspirates; biopsy fragments (skin, liver, spleen, etc.) and any kind of biological material can be tested by PCR for *Leishmania*'s DNA search.

Different PCR protocols have been studied for leishmaniasis diagnosis in animals (Strauss-Ayali et al., 2004), human and vectors (Aransay et al., 2000). Primers used are usually *Leishmania* genus-specific (Fig. 9.) or specie-specific (Solano-Gallego et al., 2001). Detection of more than one *Leishmania* species can be obtained by multiplex PCR (mPCR) protocols (Lachaud et al., 2002a,b). Parasite load can be evaluated by real time PCR (qPCR) techniques (Nicolas et al., 2002). The association of molecular methods with conventional diagnosis tests for VL diagnosis allows an accurate evaluation and also contributes to epidemiological and genetic studies (Singh & Sivakumar, 2003; Moreira et al., 2007).

PCR also contributes with diagnosis elucidation, especially in case of cross reactions results by serological tests. 93.9% sensitivity and 85.8% specificity levels were obtained by PCR for leishmaniasis diagnosis in spleen and liver fragments collected from dogs in Bauru, an endemic CVL area in Brazil (Troncarelli et al., 2009). Using the LINR4 and LIN19 (Ikonomopoulos et al., 2006) primers it was possible to identify 30 of 33 *Leishmania* infected dogs that were both positive for *Leishmania* and *T. cruzi* by IFAT.

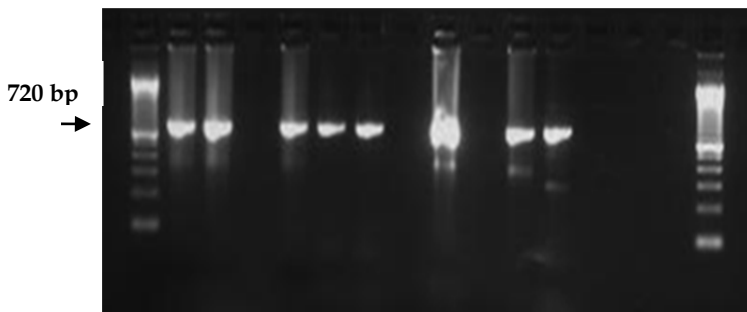


Fig. 9. *Leishmania* spp. kDNA amplification by Polimerase Chain Reaction. Samples: spleen fragments collected from euthanized dogs from Bauru (a Visceral Leishmaniasis endemic area in Sao Paulo State, Brazil). Primers LIN19 and LINR4. Agarosis gel 2%. bp - base pairs; LD - DNA ladder; C+ positive control (*L. major*); 1,3,4,5,9,10 - positive samples; 7 - positive sample with high DNA concentration; 2,6,8,11 - negative samples; 12 - negative control (milliQ water); 13 - PCR negative control (MIX-PCR).

### 6.2 Human

VL human diagnosis is difficult, because it is generally based on clinical signs that are lately identified. VL is a chronic disease, with a long incubation period with gradual and slow clinical manifestations, in the main number of cases. Infected people just call for a doctor when clinical signs are evident like abdominal distension - due liver and spleen

enlargement, hypoproteinemia, anemia - besides progressive emaciation; pain; lymphadenopathy or other severe disorders. In these cases, the diagnosis is done, but the prognostic is reserved to bad, especially in case of young and/or immunosuppressed patients (Brasil, 2006).

Serology tests can be done, but results are questionable and normally do not depend on patient infection and/or clinical status. The intra-dermal reaction (Montenegro's test) is usually negative in VL human cases during disease's clinical course, but can be positive in asymptomatic individuals or after the clinical cure (Ikeda-Garcia & Marcondes, 2007).

Culture of blood marrow aspirates and direct parasitological exam of blood marrow squashes are the main sensitive tests for human VL diagnosis, but sampling method is invasive and painful. Liver and spleen biopsy are not recommended, especially in chronic cases, due haemorrhages risks due organs enlargement caused by *Leishmania* infection.

## 7. Epidemiological vigilance, prophylaxis and control

Considering the difficulties of VL control and monitoring, actions are centered on the better definition of risk transmission areas. The new target is to reinforce vigilance measures in States and counties where no LV human or canine cases are related, in order to avoid or to minimize the disease transmission in these non endemic areas. In the VL endemic areas, after the epidemiological stratification, the control measures generally are individually evaluated and implemented. However, it is important that integrated measures are adopted for an effective control.

VL incidence especially in Americas is very high, but control strategies must be improved. Surveillance systems are usually inadequate and there are no sufficient human resources for diagnosis, treatment and control methods implementation. The Regional Program supported by the Global Program on leishmaniasis prevention and control prepared an action plan to be started in 2007. This plan includes a collective work for diagnosis tests standardization; strengthening of human resources; decentralization of public health programs of VL prevention and control; surveillance system improvement; strategic partnerships development and communities' involvement.

### 7.1 Vector control

The control strategy of this zoonosis includes: the early identification/treatment of human cases, the residual insecticides spraying in domiciles and near-house areas and add physical barriers in doors and windows in order to prevent the exposition to the vector. Photo-stables pyrethroids are usually sprayed in the human houses' walls, and animals' installations, like hen and swine houses, stables, etc; considering that these animals and its organic substances attract phlebotomines. It is important to reinforce the adequate destination of garbage, the removal of rubbish and organic substances in the near-house areas.

### 7.2 Control in dogs

#### 7.2.1 Euthanasia

The identification of serologically positive dogs, followed by euthanasia (Fig. 10) is one of control measures adopted in endemic areas (Brasil, 2006). VL coexists in dogs and humans but generally the disease in dogs precedes the occurrence of human cases. This reinforces

the importance of fast diagnosis in dogs, especially in endemic areas. Dogs are considered competent and abundant reservoirs, and cohabit with humans.



Fig. 10. Euthanasia of a symptomatic VL seropositive dog in Zoonosis Control Center (ZCC) in Bauru, Sao Paulo State, Brazil. Approximately 20-30 dogs are euthanized at the ZCC daily.

The impact of the control by positive dogs' elimination is very conflicting, because it is showing laborious, with doubtful effectiveness and for the veterinarians and owner's point of view. The euthanasia of seropositive dogs is frequently discussed. In recent studies, the evolution of seropositivity levels in humans, in two areas - one with elimination and another one without elimination of the seropositive dogs - were evaluated and there was no significant difference after one year of serological monitoring. However, the risk to the domestic man and to other animals - considering the importance of dog as a reservoir, and the low efficiency of therapeutical protocols for dogs - justifies, until the moment, the recommendation for euthanasia of infected dogs, as complementary method of control and prophylaxis of VL.

### 7.2.2 Collars with repellent substances

Collars impregnated with deltamethrin or other repellent substance can be associated with other control measures for VL prevention in dogs. This measure avoids dogs to be bitten by the vector.

A study done in Andradina (Camargo Neves, 2005), an endemic VL area in Sao Paulo State, Brazil, demonstrated that both CVL and human VL prevalences had decreased when a prevention program was initiated with deltamethrin collars putting in dogs. It is important to reinforce that collars were replaced each three to four months interval, in order to maintain the residual action of the repellent.

### 7.2.3 Vaccination

Vaccination is considered another control method of CVL. There are several studies regarding dogs' immunization, and the main researches' objective is to develop a safe, low

cost and highly immunogenic vaccine against VL in dogs. In experimental studies, filtered proteins from *Leishmania* promastigotes culture stimulated high immunity in different hosts, and conferred good protection in BALB/c mice infected with *L. major*. In other research, it was demonstrated that the vaccine produced from promastigotes antigens of *L. infantum* allowed 92% of protection in dogs from endemic areas.

The Fucose Manose Ligand (FML) was the first antigenic glycoprotein approved for dogs's immunization in Brazil. The main question regarding this vaccine was the probable difficulties to differentiate naturally infected from vaccinated animals by conventional serological tests.

A new recombinant vaccine using the A2 *Leishmania* antigen fragment was recently approved by Brazilian Ministry of Agriculture.

National researches institutes in Brazil are also involved on CVL vaccines development. The main effort is to produce a recombinant CVL vaccine associated with Rabies virus, to be used in national vaccination programs.

### **7.2.4 Treatment**

Treatment of dogs with drugs indicated for human application is not recommended in Brazil (Brasil, 2006). Despite several studies have been conducted demonstrating that clinical signals are minimized or eliminated after treatment, there are some controversies about the parasitological cure versus the apparent clinical cure. Some researches reinforce that dogs could remain as *Leishmania* reservoirs representing a public health risk.

On the other hand, CVL treatment is allowed in Spain and in other endemic countries. Glucantime is sold for veterinary use, and the doses volume is adjusted for use in small animals. Moreover, there are some medical pet foods with low protein levels indicated for dogs with leishmaniasis, considering that CVL commonly determines hepatic and renal lesions by immune-complexes deposition.

## **7.3 Control difficulties**

### **7.3.1 General factors**

One of the biggest challenges for the strategies control improvement is the identification of each element of the epidemiological chain and its impact on VL transmission. The high prevalence of VL in endemic areas, especially in tropical countries are due environmental and climatic changes; low financial support on Health and Education programs; discontinuing of control measures; vector's adaptation to environments modified by human; vectors genetic variances; co-infection with immune-suppressor diseases like AIDS, or chemotherapy treatment; urban problems like under-nutrition, house lacking and sanitary deficiencies.

### **7.3.2 Diagnosis fails**

The delay on human and canine VL diagnosis usually determines under-notification of cases and treatment fails. This may be determined by lack of information on VL by population and eventually by some health professionals contributing to VL severity increase, high mortality levels and dissemination risks. The time between sampling, diagnosis and control

measures is too long (more than 80 days), determining the maintenance of infected dogs as reservoirs during several months. The strategy of seropositive dogs culling after 80 days blood sampling and diagnosis reduces in 9% the VL prevalence. If the euthanasia of seropositive dogs is done after 7 days of sampling and diagnosis, there is a 27% reduction in seroprevalence.

#### **7.3.4 Vectors` characteristics**

Phlebotomines are highly adaptable insects. As they multiply in organic materials, its control is very hard. The use of insecticides must be done by official agents with extremely sense, due the insecticides` residues that can prejudice the ecosystem and the environment.

It is also important to consider the potential of other species of insects and mites as VL transmitters.

#### **7.3.5 Reservoirs` characteristics**

Dog is generally considered a family member. Its close contact with human may represent a serious public health risk considering the VL transmission potential in endemic areas. In some situations, when an infected dog is identified by diagnosis methods, it is sent to another place by its owners, in order to avoid the dog`s euthanasia. As there is no control about dogs` transit from endemic to non endemic areas, the risk of introduction and dissemination of VL in different regions is very high.

It is also important to consider the reservoirs potential of wild animals as and the role of some domestic animals like cats on VL transmission.

### **8. Continued education**

Continued education programs on VL are essential for population orientation and to establish directing global actions involving community and public health organs for the disease prevention and control. The educative actions must be implemented in schools and neighbor associations in order to provide information regarding the main VL clinical signs and the prevention methods. Responsible ownership must be emphasized because dogs` replacement levels after infected dogs` removal for euthanasia are extremely high, especially in VL endemic areas.

### **9. Conclusion**

VL represents a serious Public Health challenge in the world. The high disease expansion both in number of cases and in geographic areas reinforces the necessity of effective VL monitoring and control strategies. The complexity of causal agent, the variability of reservoirs species and the vectors adaptability to different ecosystems demand systematic and integrated actions in different points of the epidemiological chain. These factors explain why leishmaniasis is an old disease with continuous impact on public health.

The improvement of diagnosis methods, the surveillance studies, the vaccine immunoprophylaxy, the systematic control of vectors, the researches for effective antimicrobials and therapeutical protocols for dogs and humans, the continued education regarding VL and responsible ownership are some measures for the VL control success.



## 10. Acknowledgments

We thanks to Zoonosis Control Center (ZCC) of Bauru, Sao Paulo State, Brazil to allow the CVL research studies; to Zoonosis Research Nucleous (NUPEZO) - School of Veterinary Medicine and Animal Science - Univ Estadual Paulista (UNESP) Botucatu, Sao Paulo, Brazil, for lab support; to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the financial support to VL studies; and to all students/researches on VL that contribute with leishmaniasis prevention and control in the world.

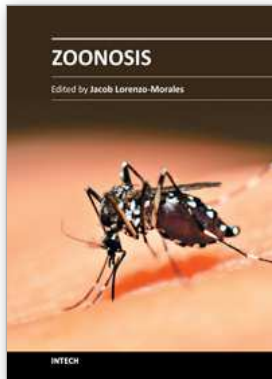
## 11. References

- Albuquerque, A.R.; Aragão, F.R.; Faustino, M.A.G.; Gomes, Y.M.; Lira, R.A.; Nakasawa, M. & Alves, L.C. (2007). Aspectos clínicos de cães naturalmente infectados por *Leishmania (Leishmania) chagasi* na região metropolitana do Recife. *Clínica Veterinária*, No.71, pp.78-80, ISSN 1413-571X.
- Alves, W.A. & Bevilacqua, P.D. (2004). Reflexões sobre a qualidade do diagnóstico da leishmaniose visceral canina em inquéritos epidemiológicos: o caso da epidemia de Belo Horizonte, Minas Gerais, Brasil, 1993-1997. *Cadernos de Saúde Pública*, Vol.20, No.1, pp.259-265.
- Aransay, A.M.; Scoulica, E. & Tselentis, Y. (2000). Detection and Identification of *Leishmania* DNA within Naturally Infected Sand Flies by Seminested PCR on Minicircle Kinetoplastic DNA. *Applied Environmental Microbiology*, Vol.66, pp.1933-1938.
- Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. (2006). *Manual de vigilância e controle da leishmaniose visceral*. Brasília, Brazil, pp.9-18.
- Camargo-Neves, V.L.F. *Leishmaniose visceral americana: doença emergente no estado de São Paulo*. São Paulo: Superintendência de Controle de Endemias - SUCEN, Coordenadoria de Controle de Doenças, Secretaria de Estado de Saúde de São Paulo, 2005. Available in: <<http://www.comciencia.br/reportagens/2005/06/17.shtml>>. Accessed in September 2007.
- Camargo-Neves, V.L.F. Utilização de coleiras impregnadas com deltametrina a 4% para o controle da leishmaniose visceral americana. Resultados preliminares de um estudo conduzido no Estado de São Paulo, Brasil. Consulta de Expertos OPS/OMS sobre Leishmaniasis Visceral en Las Américas Brasília, 2005 p99.
- Cruz, B. (2006). *Cães apresentam alta incidência de leishmaniose em município pernambucano*. Rio de Janeiro: Agência Fiocruz de Notícias. Available in: <<http://www.fiocruz.br/ccs/cgi/cgilua.exe/sys/start.htm?infoid=776&sid=9>>. Accessed in September 2007.
- Dantas-Torres, F.; Simões-Matos, L.; Brito, F.L.C.; Figueiredo, L.A. & Faustino, M.A.G. (2006). Leishmaniose felina: revisão de literatura. *Clínica Veterinária*, ano XI, Vol.61, pp. 32-40, ISSN 1413-571X.
- De Lima, H.; Carrero, J.; Rodriguez, A.; De Guglielmo, Z. & Rodriguez, N. (2006). Trypanosomatidae de importância en salud pública en animales silvestres y sinantrópicos en un área rural del municipio Tovar del estado Mérida. *Venez. Biomédica*, Vol.26, pp.42-50.
- Desjeux, P. (2004). Leishmaniasis: current situation and new perspectives. *Comparative Immunology. Microbiological Infectious Diseases*, Vol.27, pp.305-318.

- Duprey, Z.H.; Steurer, F.J.; Rooney, J.A.; Kirchhoff, L.V.; Jackson, J.E.; Rowton, E.D. & Schantz, P.M. (2006). Canine visceral leishmaniasis, United States and Canada, 2000-2003. *Emergent Infectious Diseases*, Vol.12, No.3, p.440-446.
- Farrell, J.P. (2002). *Leishmania*. World class Parasites: Vol.4. London: Kluwer Academic Publishers, pp.45-57.
- Ferreira, E.C.; Lana, M.; Carneiro, M.; Reis, A.B.; Paes, D.V.; Silva, E.S.; Schallig, H. & Gontijo, C.M.F. (2007). Comparison of serological assays for the diagnosis of canine visceral leishmaniasis in animals presenting different clinical manifestations. *Veterinary Parasitology*, Vol.146, pp.235-241.
- Gomes, A.H.S.; Ferreira, I.M.R.; Lima, M.L.S.R.; Cunha, E.A.; Garcia, A.S.; Araújo, M.F.L. & Pereira-Chioccola, V.L. (2007). PCR identification of *Leishmania* in diagnosis and control of canine leishmaniasis *Veterinary Parasitology*, Vol.144, Supl. 3-4, pp.234-241.
- Gontijo, C.M.F. & Melo, M.N. (2004). Leishmaniose visceral no Brasil: quadro atual, desafios e perspectivas. *Revista Brasileira de Epidemiologia*, Vol.7, pp.338-349, 1415-790X.
- Gradoni, L. (2002). The diagnosis of canine leishmaniasis. Canine Leishmaniasis: moving towards a solution. *Proceedings of International Canine Leishmaniasis Forum, 2., 2002*, Sevilla, Spain. Salamanca: Intervet International bv, pp.7-14.
- Grosjean, N.L.; Vrable, R.A.; Murphy, A.J. & Mansfield, L.S. (2003). Seroprevalence of antibodies against *Leishmania* spp. among dogs in the United States. *Journal of American Veterinary Medical Association*, Vol.222, No.5, pp.603-606.
- Ikeda-Garcia, F.A. & Feitosa, M.M. (2006). Métodos de diagnóstico da leishmaniose visceral canina. *Clínica Veterinária*, No.62, pp.32-38.
- Ikeda-Garcia, F.A. & Marcondes, M. (2007). Métodos de diagnóstico da leishmaniose visceral canina. *Cínica Veterinária*. Ano 12, No.71, pp.34-42, ISSN 1413-571X.
- Ikonomopoulos, J.; Kokotas, S.; Gazouli, M.; Zavras, A.; Stoitsiou, M. & Gorgoulis, V.G. (2003). Molecular diagnosis of leishmaniosis in dogs. Comparative application of traditional diagnostic methods and the proposed assay on clinical samples. *Veterinary Parasitology*, Vol.113, pp.99-103.
- Lachaud, L.; Machergui-Hammami, S.; Chabbert, E.; Dereure, J.; Dedet, J.P. & Bastien P. (2002a). Comparison of Six PCR Methods Using Peripheral Blood for Detection of Canine Visceral Leishmaniasis. *Journal of Clinical Microbiology*, Vol.40, pp.210-215.
- Lachaud, L.; Chabbert, E.; Dubessay, P.; Dereure, J.; Lamothe, J. & Dedet, J.P. (2002b). Value of two PCR methods for the diagnosis of canine visceral leishmaniasis and the detection of asymptomatic carriers. *Parasitology*, Vol.125, No.3, pp.197-207.
- Lainson, R.; Shaw, J. J.; Silveira, F.T. & Braga, R. (1987). American visceral leishmaniasis: on the origin of *Leishmania (Leishmania) chagasi*. *Transactions Royal Society of Tropical Medicine Hygiene*, Vol. 81, pp. 517.
- Lindoso, J.A.L. & Goto, H. (2006). Leishmaniose visceral: situação atual e perspectivas futuras. *Boletim Epidemiológico Paulista*, Vol.3, No.26.
- Lukes, J.; Mauricio, I.L.; Schönián, G.; Dujardin, J.C.; Soteriadou, K.; Dedet, J.P.; Kuhls, K.; Tintaya, K.W.Q.; Jirku, M.; Chocholová, E.; Haralambous, C.; Pratiang, F.; Obornik, M.; Horák, A.; Ayala, F.J. & Miles, M.A. (2007). Evolutionary and geographical history of the *Leishmania donovani* complex with a revision of current taxonomy. *PNAS*, Vol.104, No.22, pp.9375-9380.
- Luvizotto, M.C.R.; Biazzone, L.; Eugenio, F.R.; Andrade, A.L.; Moreira, M.A.B. (1999). Leishmaniose visceral canina autóctone no município de Araçatuba-SP. *Anais do*

- Congresso Brasileiro de Clínicos Veterinários de Pequenos Animais, 2., Águas de Lindóia, Sao Paulo, Brazil, p.24.
- Luz, Z.M.P.; Pimenta, D.N.; Cabral, A.L.L.V.; Fiúza, V.O.P. & Rabello, A. (2001). A urbanização das leishmanioses e a baixa resolatividade diagnóstica em municípios da região metropolitana de Belo Horizonte. *Revista da Sociedade Brasileira de Medicina Tropical*, Vol.34, No.3, pp.249-254.
- Machado, J.G. (2004). *Comparação do diagnóstico sorológico da Leishmaniose visceral Canina entre laboratórios de Belo Horizonte, 2003-2004*. 48f. Dissertação (Mestrado) Universidade Federal de Minas Gerais, Belo Horizonte.
- Madeira, M.F.; Schubach, A.; Schubach, T.M.P.; Pacheco, R.S.; Oliveira, F.S.; Pereira, S.A.; Figueiredo, F.B.; Baptista, C. & Marzochi, M.C.A. (2006). Mixed infection with *Leishmania (Viannia) braziliensis* and *Leishmania (Leishmania) chagasi* in a naturally infected dog from Rio de Janeiro, Brazil. *Transactions of Royal Society of Tropical Medicine Hygiene*, Vol.100, pp.442-445.
- Moreira, M.A.B.; Luvizotto, M.C.R.; Garcia, J.F.; Corbett, C.E. & Laurenti, M.D. (2007). Comparison of parasitological, immunological and molecular methods of the diagnosis of leishmaniasis in dog with different clinical signs. *Journal of Veterinary Parasitology*, Vol.42, pp.65.
- Murray, H.W.; Berman, J.D.; Davies, C.R. & Saravia, N.G. (2005). Advances in leishmaniasis. *Lancet*, Vol.366, pp.1561-1577.
- Nicolas, L.; Prina, E.; Lang, T. & Milon, G. (2002). Real-time PCR for detection and quantitation of *Leishmania* in mouse tissues. *Journal of Clinical Microbiology*, Vol.40, No.5, pp.1666-1669.
- Nunes, V.L.B.; Galati, E.A.B.; Nunes, D.B.; Zinezzi, R.O.; Savani, E.S.M.M.; Ishikawa, E.; Camargo, M.C.G.O.; D'Áuria, S.R.N.; Cristaldo, G. & Rocha, H.C. (2001). Ocorrência de leishmaniose visceral canina em assentamento agrícola no Estado de Mato Grosso do Sul, Brasil. *Revista da Sociedade Brasileira de Medicina Tropical*, Vol.34, No.3, pp.301-302.
- Reis, A.B. (2001). *Avaliação de parâmetros laboratoriais e imunológicos de cães naturalmente infectados com Leishmania (Leishmania) chagasi, portadores de diferentes formas clínicas da infecção*. 176f. Tese (Doutorado) Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.
- Reithinger, R. & Dujardin, J.C. (2007). Molecular diagnosis of leishmaniasis: current status and future applications. *Journal of Clinical Microbiology*, Vol.45, No.1, pp.21-25.
- Rosário, E.Y. (2002). *Avaliação de testes sorológicos utilizando antígenos brutos e recombinantes para o diagnóstico da leishmaniose visceral canina*. 99f. Dissertação (Mestrado), Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.
- Rosypal, A.C.; Cortés-Vecino, J.A.; Gennari, S.M.; Dubey, J.P.; Tidwell, R.R. & Lindsay, D.S. (2007). Serological survey of *Leishmania infantum* and *Trypanosoma cruzi* in dogs from urban areas of Brazil and Colombia. *Veterinary Parasitology*, Vol.149, p.172-177.
- Santiago, M.E.B.; Vasconcelos, R.O.; Fattori, K.R.; Munari, D.P.; Michelin, A.F. & Lima, V.M.F. (2007). An investigation of *Leishmania* spp. in *Didelphis* spp. from urban and peri-urban areas in Bauru (Sao Paulo, Brazil). *Veterinary Parasitology*, Vol.150, pp.283-290.
- Savani, E.S.M.M.; Nunes, V.L.B.; Galati, E.A.B.; Castilho, T.M., Araujo, F.S.; Ilha, I.M.N.; Camargo, M.C.G.O.; D'Áuria, S.R.N. & Floeter-Winter, L.M. (2005). Occurrence of co-infection by *Leishmania (Leishmania) chagasi* and *Trypanosoma (Trypanozoon) evansi*

- in a dog in the state of Mato Grosso do Sul, Brazil. *Memórias do Instituto Oswaldo Cruz Rio de Janeiro*, Vol.100, No.7, pp.739-741.
- Silva, E.S.; Gontijo, C.M.; Pirmez, C.; Fernandez, O.; Brazil, R.P. (2001a). Short report: detection of *Leishmania* DNA by polymerase chain reaction on blood samples from dogs with visceral leishmaniasis. *American Journal of Tropical Medicine Hygiene*, Vol.65, No.6, p.896-898.
- Silva, E.S.; Gontijo, C.M.F.; Pacheco, R.S.; Fiuza, V.O.P.; Brazil, R.P. (2001b). Visceral leishmaniasis in the metropolitan region of Belo Horizonte, State of Minas Gerais, Brazil. *Memórias do Instituto Oswaldo Cruz Rio de Janeiro*, Vol.96, No.3, pp.285-291.
- Singh, S. & Sivakumar, R. (2003). Recent Advances in the diagnosis of Leishmaniasis. *Journal of Postgraduation Medicine*, Vol.49, pp.55-60.
- Slappendel, R.J. & Ferrer, L. (1998). *Leishmaniasis*. Infectious Diseases of the dog and the cat. pp. 450-457, ISBN 0721623395. Philadelphia, Pensilvania, USA.
- Soares, M.J.V.; Moraes, J.R.E. & Roselino, A.M.F. (2005). Polymerase chain reaction in detecting *Leishmania* spp. in symptomatic and asymptomatic seropositive dogs. *Journal of Venomous Animals and Toxins including Tropical Diseases*, Vol.11, No.4.
- Solano-Gallego, L.; Morell, P.; Arboix, M.; Alberola, J.; Ferrer, L. (2001). Prevalence of *Leishmania infantum* infection in dogs living in an area of canine leishmaniasis endemicity using PCR on several tissues and serology. *Journal of Clinical Microbiology*, Vol.39, pp.560-563.
- Strauss-Ayali, D.; Jaffe, C.L.; Burshtain, O.; Gonen, L.; Baneth, G. (2004). Polymerase chain reaction using noninvasively obtained samples, for the detection of *Leishmania infantum* DNA in dogs. *Journal of Infectious Diseases*, Vol.189, pp.1729-1733.
- Sundar, S. & Rai, M. (2002). Laboratory diagnosis of visceral leishmaniasis. *Clinical Diagnosis on Laboratory Immunology*, Vol.9, No.5, pp.951-958.
- Troncarelli, M.Z.; Machado, J.G.; Camargo, L.B.; Hoffmann, J.L.; Camossi, L.; Greca, H.; Faccioli, P.Y. & Langoni, H. (2008). Associação entre resultados sorológicos no diagnóstico da leishmaniose e da tripanossomíase canina, pela técnica de imunofluorescência indireta. *Veterinária e Zootecnia*, Vol.15, No.1, pp.40-47.
- Troncarelli, M.Z.; Camargo J.B.; Machado, J.G.; Lucheis, S.B. & Langoni, H. (2009). *Leishmania* spp. and/or *Trypanosoma cruzi* diagnosis in dogs from endemic and nonendemic areas for canine visceral leishmaniasis. (2009). *Veterinary Parasitology*. Vol.164, No.2-4, pp.118-123. ISSN 0304-4017.
- Zanette, M.F.; Feitosa, M.M.; Ikeda, F.A.; Rossi, C.N.; Camacho, A.A. & Souza, A.I. (2006). Ocorrência de reação cruzada entre doença de Chagas e Leishmaniose visceral canina pela técnica de ELISA. *Anais do congresso brasileiro da Anclivepa*, 27., Congresso da Fiavac, 3., 2006, Vitória - Espírito Santo, Brazil.
- Zijlstra, E. E.; Nur, Y.; Desjeux, P.; Khalil, E.A.G.; El-Hassan, A. M. & Groen, J. (2001). Diagnosing visceral leishmaniasis with the recombinant K39 strip test: experience from Sudan. *Tropical Medicine International Health*, Vol.6, pp.108-113.
- Zivicnjak, T.; Martinkovic, F.; Marinculic, A.; Mrljak, V.; Kucer, N.; Matijatko, V.; Mihajevic, Z. & Baric-Rafaj, R. (2005). A seroepidemiologic survey of canine visceral leishmaniasis among apparently healthy dogs in Croatia. *Veterinary Parasitology*, Vol.131, pp.35-43.



## **Zoonosis**

Edited by Dr. Jacob Lorenzo-Morales

ISBN 978-953-51-0479-7

Hard cover, 436 pages

**Publisher** InTech

**Published online** 04, April, 2012

**Published in print edition** April, 2012

Zoonotic diseases are mainly caused by bacterial, viral or parasitic agents although "unconventional agents" such as prions could also be involved in causing zoonotic diseases. Many of the zoonotic diseases are a public health concern but also affect the production of food of animal origin thus they could cause problems in international trade of animal-origin goods. A major factor contributing to the emergence of new zoonotic pathogens in human populations is increased contact between humans and animals. This book provides an insight on zoonosis and both authors and the editor hope that the work compiled in it would help to raise awareness and interest in this field. It should also help researchers, clinicians and other readers in their research and clinical usage.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Marcella Zampoli Troncarelli, Deolinda Maria Vieira Filha Carneiro and Helio Langoni (2012). Visceral Leishmaniosis: An Old Disease with Continuous Impact on Public Health, Zoonosis, Dr. Jacob Lorenzo-Morales (Ed.), ISBN: 978-953-51-0479-7, InTech, Available from:

<http://www.intechopen.com/books/zoonosis/visceral-leishmaniosis-an-old-zoonosis-with-continuous-impact-on-public-health>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.