Introduction
Visceral leishmaniasis is an infectious disease transmitted by sandflies, and caused by various species of *Leishmania* parasites. Distinct species occur in parts of the Old World and New World, and can infect people, domestic and wild animals. These parasites cause a wide spectrum of diseases in people, and it is estimated that the annual occurrence of human visceral leishmaniasis (HVL) cases worldwide is 500,000 [1]. Infection with *Leishmania* is also a frequent cause of clinical disease in the dog, especially in tropical, sub-tropical and temperate areas, and is less common in cats. According to the human clinical criteria, canine leishmaniasis is classified as visceral [2], although the term "generalized canine leishmaniasis" might be more appropriate because it involves visceral and cutaneous tissues.

Etiology
*Leishmania* is a genus of protozoa of the order Kinetoplastida, and family Trypanosomatidae. *Leishmania* parasites are diphasic protozoan parasites whose life cycle includes two hosts, a vertebrate (rodents, canids or humans), and an insect (sandfly)(Fig. 1). Canine visceral leishmaniasis (CVL) in the Old World is the result of infection with *Leishmania infantum*, which is considered a distinct species [3] included in the *L. donovani* complex or creating its own complex [4]. However, it has also been considered as a subspecies, *L. donovani infantum* [5]. Recently, *L. tropica* was found to be the cause of a case of CVL in Morocco [6]. In the New World, *Leishmania chagasi* is considered as the cause of CVL. *L. chagasi* is currently thought to be synonymous to *L. infantum* based on a variety of genetic methods [7]. The low genetic variability of *L. chagasi* is consistent with recent importation to the New World and it is thought that *L. chagasi* is the result of the introduction of dogs from Europe infected with *L. infantum* that arrived with settlers. *L. infantum* infections occur mainly in countries in the Mediterranean basin, the Middle East, and Portugal [8-13]. Sporadic cases have been reported in the northern parts of France, Switzerland and the Netherlands, regions in which no sandflies are found, and in other non-enzootic areas (United States and Canada) [14-16]. Infection with *L. chagasi* is found in Central and South America. *Leishmania* spp. that cause visceral leishmaniasis in dogs, cats, and humans are listed in Table 1.

Epidemiology
The natural infection cycle of *Leishmania* parasites involves an insect vector. In Old World CVL the vectors are sandflies from the genus *Phlebotomus*, whereas in the New World the vectors are from the genus *Lutzomyia*. Sandflies have a limited flight range (few kilometers), and are generally most active at dawn and dusk, and in certain seasons. When a female sandfly feeds on an infected dog, it ingests the intracellular nonflagellated form, amastigotes, in macrophages. The released amastigotes transform to the flagellated form, the promastigotes (Fig. 2). The promastigotes attach to the sandfly's gut wall where they multiply, and within a few days, the gut is packed with promastigotes. When the female gets another blood meal after laying her eggs, promastigotes may be injected to the vertebrate host's skin. The promastigotes are then phagocytosed by skin macrophages, loose their flagella and multiply as amastigotes.
Figure 2. *Leishmania infantum* promastigotes. - To view click on figure -

In many areas where HVL occurs, there is an enzootic or sylvatic transmission cycle involving wild canids (including feral and stray dogs), and domestic dogs [17] (Fig. 1). Other wild animals (rats and opossums), were reported to be infected with *Leishmania* but there is no current evidence concerning the role of these animals as reservoir. Anthroponotic transmission from human to human is found in visceral leishmaniasis caused by *L. donovani* and is prevalent in India and Sudan. Feline visceral leishmaniasis was described in sporadic cases [18-22], and therefore cats are not considered as an important reservoir for the infection of people.

**Pathogenesis**

*Leishmania* parasites multiply initially in macrophages at the site of infection. If the host fails to generate an effective protective immune response, the parasites disseminate from the skin and spread in mononuclear phagocytes to the bone marrow, spleen and liver, to cause a chronic, possibly fatal disease. Studies in murine models have determined that the generation of a protective immune response against leishmaniasis is T cells mediated, manifested by a strong proliferative response of peripheral blood lymphocytes to leishmanial antigens, and mediated by cytokines, such as interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNFα), which are required for the activation of macrophages and killing of intracellular parasites [23-27]. Hence, the chronicity of the disease and the high mortality in untreated cases has been associated with diminished T cell response. Dogs with chronic experimental or natural *L. infantum* infection can be classified as asymptomatic apparently resistant to clinical disease, and symptomatic susceptible to the disease [28, 29]. It was shown that 3 years after experimental infection with *L. infantum*, asymptomatic dogs responded to *L. infantum* antigen both by lymphocyte proliferation assays in vitro and by the delayed-type hypersensitivity reaction, whereas no serum antibodies towards *L. infantum* antigen were detected [28]. Yet, symptomatic dogs failed to respond to cell mediated assays both in vitro and in vivo, and showed a significant humoral response. Another study likewise demonstrated the lack of specific T cell response to leishmanial antigen as the main feature of experimental and natural symptomatic infections [30]. In addition, *L. infantum*-infected macrophages are lysed in a major histocompatibility complex restricted manner by CD8 cells mostly, and also CD4 cells from asymptomatic dogs [31]. Significantly higher levels of IL-2 and TNFα were found in supernatants from stimulated peripheral mononuclear cells from asymptomatic dogs when compared with cells from symptomatic dogs [28]. Moreover, peripheral blood mononuclear cells (PBMC) from asymptomatic dogs produced IFN-γ upon parasite antigen-specific stimulation, whereas lymphocytes from symptomatic dogs did not [31]. These findings are in accordance with the observation that self resolving *L. major* infection in mice is mediated by Th1 cells, characterized by production of IFN-γ, TNF, IL-2, IL-3, IL-12 and IgG2, while chronic to fatal disease is mediated by Th2, which is featured by IL-3, IL-4, IL-5, IL-6 , IL-9, IL-10 and IgG1 production [32]. A correlation between disease progression and the IgG isotype's levels in both experimental and natural *L. infantum* infections in dogs was

<table>
<thead>
<tr>
<th>Table 1: Geographical distribution and clinical manifestation of <em>Leishmania</em> spp. infecting dogs, cats and humans</th>
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</thead>
<tbody>
<tr>
<td><strong>Parasite</strong></td>
</tr>
<tr>
<td><strong>Old World</strong></td>
</tr>
<tr>
<td><em>L. tropica</em></td>
</tr>
<tr>
<td><em>L. donovani</em></td>
</tr>
<tr>
<td><em>L. infantum</em></td>
</tr>
<tr>
<td><strong>New World</strong></td>
</tr>
<tr>
<td><em>L. braziliensis</em></td>
</tr>
<tr>
<td><em>L. chagasi</em></td>
</tr>
<tr>
<td><em>L. mexicana</em></td>
</tr>
</tbody>
</table>

The parenthesis indicates a less common clinical manifestation of the disease

demonstrated both by ELISA and western blotting. Anti-Leishmania IgG1 antibodies were considerably higher in symptomatic compared to asymptomatic dogs [33, 34]. These observations can suggest that a dichotomous response to *L. infantum* infection exists in dogs, similar to that found with *L. major* in mice, but are controversial as other studies failed to demonstrate this trend as clearly [35, 36]. When clinical signs develop, lymphoid organs become depleted in the T cells regions, and the B cells zones proliferate. The overall proliferation of B cells, macrophages, plasma cells and the attraction of eosinophils, results in hepatosplenomegaly, generalized lymphoadenomegaly, and hyperglobulinemia. The increased production of immunoglobulins is non-protective and is potentially damaging. Some specific antibodies will opsonize amastigotes causing their phagocytosis by macrophages, enabling the parasite to continue multiplying in the cell. The most hazardous result of the uncontrolled B cell proliferation and the impaired T cell regulation is the production of large amounts of circulating immune complexes (CIC). Circulating immune complexes concentrations were significantly higher in dogs with naturally acquired *Leishmania* infection, than in uninfected control dogs [37]. Deposition of CIC in blood vessels walls may result in vasculitis, polyarthritis, uveitis and glomerulonephritis. CIC may also include cryoglobulins that precipitate in the blood vessels of the extremities and cause ischemic necrosis when exposed to cold temperature [21]. Autoantibodies may also be produced in leishmaniasis, and cause immune mediated thrombocytopenia and/or anemia. Thrombocytopenia, diminished collagen-induced platelet aggregation, prolonged prothrombin time and increased fibrin degradation products (FDP) were found in dogs experimentally infected with *Leishmania*, and were suggested to be a result of a compensated DIC [38]. The generalized non-pruritic skin lesions observed in CVL are the result of the dissemination of *L. infantum* parasites throughout the body [39]. It has been suggested that generalized nodular lesions indicate a less effective immune response than that associated with generalized alopecia [40].

**Signalment and History**

Dogs from all age groups, many breeds and both genders were found to be infected with leishmaniasis [14], although symptomatic infection is uncommon in young immature dogs. In a recent retrospective study, infected dogs ages ranged from nine months to 15 years, with a 5 years median [41]. The main complaints reported by owners in that study were skin lesions (50.6%) (Fig. 3, 4, 5, 6, 7) progressive loss of weight (25.3%), decreased appetite (16.5%), and exercise intolerance (10.8%). Other complaints reported in several studies include: depression, ocular signs, epistaxis, polyuria -polydipsia, diarrhea, vomiting, melena, sneezing, coughing, and lameness [41-43].

**Figure 3.** Massive skin exfoliation on the back of a *L. infantum* infected dog. - To view click on figure -

**Figure 4.** Onychogryposis in canine visceral leishmaniasis. - To view click on figure -

**Figure 5.** Ulcerative dermatitis of the hind leg of a dog infected with *L. infantum*. - To view click on figure -
Clinical Signs

Clinical signs have been reported to appear 3 months to 7 years after infection [43]. The major clinical signs of CVL are described in Table 2, the most common skin lesions are listed in Table 3, and the ocular signs mentioned in Table 4.

Table 2: Major clinical signs in CVL

<table>
<thead>
<tr>
<th>Major clinical signs seen in CVL</th>
<th>Percentage from dogs with CVL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin involvement</td>
<td>81-89% * * *</td>
</tr>
<tr>
<td>Lymphadenomegaly</td>
<td>65.2-90% * * *</td>
</tr>
<tr>
<td>Pale mucous membranes</td>
<td>58% *</td>
</tr>
<tr>
<td>Ocular signs</td>
<td>18% *</td>
</tr>
<tr>
<td>Emaciation \ Cachexia</td>
<td>10.1-47.5% * * *</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>9.5-53.3% * * *</td>
</tr>
<tr>
<td>Fever</td>
<td>4-36% * * *</td>
</tr>
<tr>
<td>Epistaxis</td>
<td>6.3-10% * * *</td>
</tr>
<tr>
<td>Arthropathies</td>
<td>3.2-4% * * *</td>
</tr>
<tr>
<td>Ascites</td>
<td>1.3-3% * * *</td>
</tr>
</tbody>
</table>

* [41]; + [43]; - [42]

Table 3: Major skin lesions in CVL

<table>
<thead>
<tr>
<th>Types of skin lesions seen in CVL</th>
<th>Percentage from dogs with CVL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exfoliative dermatitis</td>
<td>56-64.1% * * *</td>
</tr>
<tr>
<td>Ulcerations</td>
<td>34.4-40% * * *</td>
</tr>
<tr>
<td>Onychogryposis\Abnormal nails</td>
<td>20-30.5% * * *</td>
</tr>
<tr>
<td>Nasal hyperkeratosis</td>
<td>18.8% *</td>
</tr>
<tr>
<td>Digital hyperkeratosis</td>
<td>14.1% *</td>
</tr>
<tr>
<td>Nodules</td>
<td>2.3-6% * * *</td>
</tr>
</tbody>
</table>

* [41]; + [43]; - [42]
Clinicopathologic Findings
The typical clinicopathologic findings associated with CVL are listed in Table 5.

<table>
<thead>
<tr>
<th>Clinicopathologic findings</th>
<th>Percentage from dogs with CVL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperproteinemia</td>
<td>63.3-72.8% a,c</td>
</tr>
<tr>
<td>Hyperglobulinemia</td>
<td>70.6-100% b,c</td>
</tr>
<tr>
<td>Hypoalbuminemia</td>
<td>68-94% b,c</td>
</tr>
<tr>
<td>Decreased albumin/globulin ratio</td>
<td>76% c</td>
</tr>
<tr>
<td>Nonregenerative anemia</td>
<td>60-73.4% a,c</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>29.3-50% b,c</td>
</tr>
<tr>
<td>Leukocytosis</td>
<td>24% c</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>22% b</td>
</tr>
<tr>
<td>Increased serum liver enzymes activities</td>
<td>16% c</td>
</tr>
<tr>
<td>Elevated urea and creatinine</td>
<td>16-45% b,c</td>
</tr>
<tr>
<td>Mild/severe proteinuria</td>
<td>71.5-85% b,c</td>
</tr>
<tr>
<td>Hyaline/fine granular urine casts</td>
<td>Approaching 100% c</td>
</tr>
</tbody>
</table>

ª[41]; º[43]; [42]

Diagnosis

Serology
Dogs with CVL infection, either symptomatic or asymptomatic, will almost always demonstrate a specific humoral response. Therefore, various serological methods for the detection of anti-Leishmania antibodies have been developed. These include indirect immunofluorescence assays (IFA), direct agglutination assays (DAT), enzyme-linked immunosorbent assay (ELISA), competitive-ELISA, Dot-ELISA, slide-ELISA, and western blotting [40-47]. In general, good sensitivities and specificities are gained with these methods which are mostly based on the use of crude antigens. Seroreactivity with the recombinant antigen rK39 by ELISA [48], correlated well with the appearance of acute CVL [36, 49]. The use of crude Leishmania antigens is thought to underestimate the prevalence of CVL [50]. Purified Leishmania antigens for the diagnosis of CVL are of considerable importance in South America where cross-reactions were demonstrated between Leishmania spp. and trypanosomes. Diminishing antibody titers are not indicative of parasitological cure after treatment, although a decrease in the titer, and in the number and/or intensity of low molecular bands by western blot is coincidental with clinical improvement of treated dogs [51, 52].
Direct organism identification

Cytology and histology - *Leishmania* amastigotes can be demonstrated in impression smears made from fine needle aspirates of lymph nodes, spleen or bone marrow, and stained with Giemsa stain or a quick stain such as Diff-Quick ®. Amastigotes are round to oval parasites, which contain a round basophilic nucleus, and a small rod-like kinetoplast. Amastigotes are contained within macrophages, or may be found free in cytologic preparations after freeing from ruptured cells. Impression smears can also be made from dermal lesions, such as those found on the tip of the ear, after scraping of the skin (Fig. 8).

In vitro cultivation - Culture of aspirates from the spleen, lymph nodes, skin and bone marrow in Novy-MacNeal-Nicolle medium (NMN) or Schneider's Drosophila medium is used to identify patent infection and for further characterization of isolates.

In vivo cultivation - Diagnosis may also be established by the inoculation of hamsters with infected tissues and monitoring their clinical signs. This technique is used mostly in research.

Immunohistochemistry - An indirect peroxidase reaction using sera obtained from infected dogs was shown to be useful in the detection of *L. infantum* in formalin-fixed, paraffin-embedded sections of canine skin and lymph nodes [53].

Polymerase chain reaction (PCR) - Initial successful evaluation of a PCR for the identification of *Leishmania* spp. in lymph node aspirates and whole blood from dogs was performed using primers directed against a sequence of the small-subunit (SSU) rRNA gene, which has more than a hundred repeats in the *Leishmania* genome [54]. When a PCR directed against the *Leishmania* spp. kinetoplast DNA (kDNA) was compared with serology, it was more sensitive [55, 56], and demonstrated 100% sensitivity and specificity when lymph node aspirates were used, and 85% and 80%, respectively, with whole blood samples [56]. The same primers were also successful in identifying *Leishmania* spp.-specific DNA in paraffin-embedded skin biopsies from dogs [57]. Currently, PCR seems to be a sensitive and specific method for the diagnosis of CVL in dogs.

Identification of *Leishmania* spp.

The identification and characterization of *Leishmania* spp. is important in understanding the epidemiology and transmission of leishmaniasis. Isoenzyme analysis is the "gold standard" for strain characterization but is time consuming, since each isolate must be examined by multiple enzyme reactions [58]. PCR using kDNA primers specific for New World or Old World species has been used successfully [55, 59], however, species-specific PCR requires the design of individual primer sets for each species and subspecies. Random amplified polymorphic DNA (RAPD), and arbitrary primer (AP)-PCR, based on fragment length polymorphism utilize nonspecific primers which give rise to a variety of amplification products [60, 61]. AP or RAPD-PCR can't be used for direct diagnosis because false positives can occur with host contaminating DNA. A newly developed PPIP (permissively primed intergenic polymorphic) -PCR can distinguish by simple means between Old World *Leishmania* spp. with no false positives due to host DNA contamination [62].

Treatment

Human visceral leishmaniasis is commonly treated and cured in people that are not HIV+ with very high success rates. Antileishmanial drugs used in human therapy include pentavalent antimonials (selectively inhibit the protozoal enzymes required for glycolytic and fatty acid oxidation), amotosidine (an aminoglicoside antibiotic), pentamidine (interferes with nuclear metabolism), liposomal amphotericin B (a powerful anti-leishmanial agent), and allopurinol (a purine analogue). Canine visceral leishmaniasis is more resistant to therapy as compared to people and parasitological cure is rarely attained. As with humans, the most frequently used drugs for the treatment of CVL are pentavalent antimonials, such as meglumine antimoniate and sodium stibogluconate. The subcutaneous route of meglumine administration is the most commonly used, however, reported dose regimens for meglumine antimoniate vary from 200 - 300 mg/kg IV every other day [63], to 100 mg/kg IV or SC daily for 3 - 4 weeks [43]. Both pentavalent antimonials are expensive and can induce side effects. Treatment with antimonials is considered especially risky when administered daily for more than 2 months to patients with either cardiac, renal, or hepatic insufficiency. During the therapy, a significant clinical improvement is usually noted but relapses are very common. Moreover, long-term treatment can induce resistance of *Leishmania* to pentavalent antimonials [64]. The value of aminosidine in CVL therapy is controversial because of its partial efficacy and high toxicity. Treatment with L.V. administration of liposomal amphotericin B resulted in clinical cure of CVL but almost all treated dogs remained positive for parasites in lymph nodes aspirates [65]. Allopurinol inhibits the growth of *Leishmania* spp. by blocking RNA synthesis. Allopurinol as a sole treatment for CVL was attempted in dosages of 10-15 mg/kg/day P.O. for 2-24 months, and resulted in clinical cure, no adverse effects even in prolonged treatment periods, but relapses did occur [66, 67].
combination of allopurinol and antimony treatment (100mg/kg/day of meglumine and concurrent 30 mg/kg/day of allopurinol P.O.) until clinical remission, followed by maintenance treatment of allopurinol (20 mg/kg/day) for one week a month, resulted in clinical remission for a follow-up period of 10-44 months [66]. In another study, dogs were followed for 5 years after being treated with a combination of these drugs for 1 month, followed by an 8 months period of treatment with 30 mg/kg/day allopurinol. These dogs were compared to dogs treated only with meglumine or allopurinol [68]. Dogs that received the combination therapy with allopurinol maintenance showed a significantly higher rate of clinical cure compared to the other groups. Although both studies showed prolonged clinical cure in dogs receiving this combination therapy, their parasitological status was not evaluated after treatment. Nevertheless, allopurinol is advantageous as a maintenance drug (can be given P.O., inexpensive, no major side effects even after extended periods of treatment), hence either the combination therapy of meglumine and allopurinol or solely allopurinol for long periods, seems to be the main therapy regimens that can be currently recommended for the treatment of CVL.

Prevention And Control
Since the dog is considered the major reservoir for HVL, campaigns to control CVL are usually aimed at reducing the risk of infection to people, rather than to protect dogs. The results of control efforts in Brazil designed to reduce the incidence of HVL by culling seropositive dogs have been disappointing [69, 70]. Mathematical methods which compared the effectiveness of various methods for controlling HVL and CVL indicated that spraying with insecticides would possibly be the most effective control method [71]. Evaluation of a deltamethrin collar for the control of sandfly bites in southern France showed protection of dogs from the majority of bites for a complete sandfly season [72]. Further research is needed to evaluate the effect of using this method in an endemic area on the prevalence of HVL as well. An effective vaccine against CVL or HVL is currently unavailable, although much research is being carried out in this area [73, 74]. Protection of the individual dog may include keeping the animal indoors from 1 hour before sunset to 1 hour after dawn during the vector season. Repellents and insecticides to stop the entry of sandflies into the house should also be used.

Public Health Considerations
Visceral leishmaniasis in humans is caused by the L. donovani subspecies (Table 1). Estimates indicate that these parasites are responsible for 500,000 cases of HVL annually among the 200 million people that are at risk for infection [1]. Sick people have hepatosplenomegaly, irregular fever, anemia, marked hypergammaglobulinemia, and a high mortality rate if untreated. The disease was traditionally present mostly in rural areas, but is now becoming more common in suburban and urban settings. The major risk group for HVL caused by L. infantum and L. chagasi has traditionally been young children and was often associated with malnutrition. With the emergence of HIV, the infection appeared in patients with AIDS and has been posing an important and often fatal secondary infection in immunocompromised people [75]. A recent report by the World Health Organization (WHO) indicated that people with AIDS have become the largest risk group for HVL in southern Europe, and that their coinfection is expected to present an increasing problem in areas were HIV and HVL overlap, especially in Brazil, Africa, and India [76]. The spread of leishmaniasis has also been demonstrated to occur by means of sharing needles by intravenous drug users [77]. This mode of infection threatens to extend the traditional boundaries of the disease in humans out of the regions where vector sandflies are present. In India and Sudan, HVL is caused by L. donovani and is thought to be an anthroponosis. In most other endemic areas of the world, HVL is mainly caused by L. infantum and L. chagasi, and is a zoonosis, with dogs and wild canids acting as the natural reservoirs for the parasites. However, some studies in Honduras and Brazil did not attribute significant risk for acquiring HVL in the presence of dogs [69, 70, 78, 79]. These studies suggest that in the New World dogs are not the major reservoirs and that the human-vector–human transmission is of greater importance. This was also one of the hypotheses in a study that examined if the elimination of the majority of seropositive dogs diminished the incidence of HVL [70]. Another possibility mentioned is that serology is not sufficient as a criterion for eliminating infected dogs. The elimination of seropositive dogs in the western society might not be an acceptable solution, as pets are considered an integral part of the family. Treatment of dogs presents a dilemma to veterinarians and public health officials in endemic areas, since in the presence of sandflies there is a remote risk of transmission of the disease from pets to members of the owner's family, and to other people in their community. In non-endemic areas, potential zoonotic foci can be created this way.

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