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# CANINE VISCERAL LEISHMANIOSIS: CURRENT SITUATION

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## ABSTRACT

A review on the literature on canine leishmaniasis is performed. The newest contributions to the etiology, epizootology, pathogenesis, clinical manifestation, diagnostics, therapy and prevention of the disease are emphasized. A lot of data are cited, mainly focused on the epidemiology and epizootology of the disease in Bulgaria.

**Key words:** *Leishmania infantum* , epizootology, clinical manifestation, therapy, prevention, Bulgaria

Leishmaniasis is cause by protozoa, belonging to the *Leishmania* genus that could infect a number of vertebrates, including dogs and men. The clinical criteria for classification of the disease in humans determined three forms of the disease – visceral, cutaneous and mucocutaneous. In dogs, the visceral form that is also accompanied with cutaneous changes, is the commonest and that is why, some authors consider that it is more correct to talk about a generalized leishmaniasis [1,2,3] or also, European canine leishmaniasis [4].

**ETIOLOGY**. Leishmania are protozoa from the Tripanosomatidae family. The species responsible for the disease are *Leishmania donovani infantum* (*L.infantum*) for the Old World and *Leishmania donovani chagasi* (*L.chagasi*) for the New World. There are separate *L.infantum* strains, and the commonest among them is Zymodeme MON-1 [5].

Leishmania are encountered in two forms – amastigote (non-flagellate, 2-5 x 1.5-3.5 μm) that parasitizes intracellularly in host macrophages and promastigote (flagellate, 12-25 x 1.5-3.5 μm) that lives in the body of bloodsucking arthropods from the genus *Phlebotomus (in the Old World*) and the genus *Lutzomya* (in the New World); the latter comprising about 700 species. Phlebotominae are also called "sand flies".

**PREVALENCE.** Leishmaniasis is characterized with an endemic distribution pattern. In men, it is found in 88 countries – 72 developing ones, and 13 out of them are among the poorest in the world [6].

In dogs, *L.infantum* is a great concern in the Mediterranean countries and the Middle East [7-12]. More detailed results from recent surveys show the following picture of seroprevalence: Portugal – 8.5% [13], Cyprus – 1.7-10.0% [8], Albania – 12.9% and one case with *L.infantum* isolate [14,15], Israel – 3.6-15% [16], Iran – 14.28–21.6% [17], Venezuela – 25% [18], Greece – 3.7-38.8 [19-21], Italy – 22.1-30.3% [22], Spain – 3-35% [23], France – 10-40 % [4], Bosnia and Herzegovina – 45% [24], Malta – 28.9-52% [25], Turkey – 65-76% [26]. Leishmaniasis is also encountered in non-endemic regions – Austria, Belgium, Germany, Switzerland, Holland, England, Canada and the USA [27-30].

Studies on canine leishmaniasis in Bulgaria have been performed for the first time in 1941 by Drenovski. Out of 100 examined fogs from Petrich, 81% were seropositive for leishmaniasis. Later, the serological investigations carried out in the same region, showed 13.80% seroprevalence in 160 dogs. Clinical manifestation of the disease was not observed [31]. Further studies in our country have been performed by the team of Dr. Filipov from the National Centre of Infectious

and Parasitic Diseases. Blood sera from digs from regions in South Bulgaria (Radnevo, Petrich, Asenovgrad) with incidence of human visceral leishmaniasis were tested. The results of all 122 canine samples were found to be negative [32]. Kostova et al. [33] analyzed 50 canine sera from stray, domestic and police dogs living in the regions of Burgas, Kardjali and Sofia by means of ELISA and IFA. The following titres were reported: 1:40 in 2 dogs, 1:80 also in 2 dogs and 1:320 in one dog. No correlation was found between the incidence of the disease among people and dogs.

In 2004, Tsachev et al. [34] performed a seroepidemiological screening for leishmaniasis in 11 regions in Bulgaria – Plovdiv, Stara Zagora, Yambol, Burgas, Blagoevgrad, Varna, Silistra, Rousse, Veliko Tarnovo, Pleven, Montana. Using IFA, 220 blood sera were tested, but no seropositive ones were detected. In 2006 Tsachev [35] continued its studies and for the first time in our country, observed clinical leishmaniasis in two dogs in Petrich. The first one was a 4-year-old female Rottweiler with a history of cutaneous changes from one year that initially affected only the head, and then – the limbs. The dog was unsuccessfully treated for dermatophytosis and demodecosis. The clinical examination revealed alopecia on both ears, blepharitis, conjunctivitis, periorbital alopecia, exfoliative dermatitis, nasal and auricular hyperkeratosis, pale mucous coats. The lymph nodes were not enlarged and the body temperature was also normal. The appetite of the dog was decreased and it was visibly emaciated. The second patient was also a female Rottweiler at the age of 3 years. The clinical examination showed alopecia around the eyes, on ears, exfoliative dermatitis, pale mucous coats, nasal and auricular hyperkeratosis. The popliteal lymph nodes were enlarged and the body temperature – normal. The dog had a decreased appetite and was losing weight. In both cases, the diagnosis was conformed by contemporary methods: indirect immunofluorescence assay, immunochromatography, aspiration cytology of lymph fluid, PCR.

**EPIZOOTOLOGY**. The sources of the diseases are infected animals or people. The disease vectors (Phlebotomus) ingest amastigotes with sucked blood. Amastigotes begin to reproduce very quickly in the intestinal tract, become transformed in promastigotes and after 8 to 10 days, become infective. Only female Phlebotomus flies are haematophages. The definitive hosts – dog, man etc. – become infected with the bite of infected sand flies and thus, promastigotes occur in neutrophils and macrophages. They pass into amastigote form and for 12-24 hours could reach counts up to 200 per cell. Leishmania organisms are detected in the liver, the spleen, bone marrow, lymph nodes, blood monocytes etc.

Apart men and dogs, other reservoirs of the agent are foxes, wild rodents, cats, camels and horses. As shown by experiments with dogs and laboratory animals, the genetic predisposition of the host is essential for the infection [36].

The vectors of *L.infantum* are phlebotominae – small insects with a size of 1-5 mm. They are flying low and usually suck blood from the skin around the eyes, under the eyebrows and around the nose. Generally, the bloodsucking takes place when the dog is resting or sleeps. Phlebotominae are intensively reproduced between May and November and are the most active mainly in the evening – from 21 h to 5 h, and in dark areas (caves, basements) – during the entire 24-hr period. During the day the flies are hiding in the dark, including on bushes and trees (peaches, almond trees). Their life span is from 14 to 45 days and they begin to suck blood as early as the first 24-48 h of life, and afterwards – at every 3 to 5 days. Females lay down 40-70 eggs in dark places that pass consecutively through the stages of larva, nymph and imago for 28-82 days. In the Mediterranean basin, 19 Phlebotomus species are detected, and in Greece there are 12 (*Phlebotomus neglectus, P.perfiliewi, P.tobbi, P.bacanicus, P.simici, P.papatacii, P.serengeti, P.similis, P.alexandri, P.mascitii, Sergentomyia dentata* and *S.minuta* [74]. In Bulgaria, the incidence of sand flies is not quite clear. There are data about *P.papatacii* and *P.shinensis,* which are encountered in the Trakia valley and the Danube valley [37].

**PATHOGENESIS**. When leishmania penetrate through the host's skin, they enter the neutrophils and macrophages. If the organism does not manage to generate an efficient immune defense, via the mononuclear phagocytes the protozoa reach the bone marrow, the spleen and the liver.

The investigations in mice showed that the Th1 cells responsible for the protective immunity against leishmaniasis and the cytokines $IFN_{-Y}$, $TNF\text{-}_{a}$, secreted by them, are necessary for activation of macrophages and the destruction of intracellular agents [38-41]. It is shown that in untreated animals with

chronic manifestation of the disease, the T-cellular immune response was significantly reduced.

A correlation between the development of the disease and the level of isotypes in both experimental and natural *L. infantum* infection was demonstrated by ELISA and western blotting. Anti-*Leishmania* IgG1 antibodies were statistically significantly higher in animals with clinical disease compared to asymptotic animals [8,42]. The increased production of immunoglobulins is however non-protective and hence, really "damaged", Circulating immune complexes resulting in uveitis, polyarthritis, vasculitis and glomerulonephritis, do appear. Autoantibodies could be also formed and they are the cause for immune-mediated thrombocytopenia and anaemia.

***Table 1***. *Clinical changes –[a] [43] ;[b] [45];[c] [44].*

| **Principal clinical signs** | |
|---|---|
| **Signs** | **Incidence, %** |
| Cutaneous changes | 81 - 89% [a,b] |
| Lymphadenomegaly | 65.2 - 90% [a,b,c] |
| Pale mucous coats | 58% [a] |
| Ocular changes | 18% [c] |
| Cachexia | 10.1 - 47.5% [a,b,c] |
| Splenomegaly | 9.5 - 53.3% [a,b,c] |
| Fever | 4 - 36% [b,c] |
| Epistaxis | 6.3 - 10% [a,c] |
| Arthropathies | 3.2 - 4% [a,c] |
| Ascites | 1.3 - 3% [a,c] |
| **Cutaneous changes** | |
| **Alterations** | **Incidence, %** |
| Exfoliative dermatitis | 56 - 64.1% [a,c] |
| Ulcerations | 34.4 - 40% [a,c] |
| Onychogryposis | 20 - 30.5% [a,b,c] |
| Nasal hyperkeratosis | 18.8% [a] |
| Digital hyperkeratosis | 14.1% [a] |
| Nodules | 2.3 - 6% [a,c] |
| **Ocular changes** | |
| **Alterations** | **Incidence, %** |
| Conjunctivitis | 24 - 32.5% [a,b] |
| Blepharitis | 12% [a] |
| Keratitis | 7.5% [b] |
| Keratoconjunctivitis sicca | 5.1% [a] |
| Uveitis | 1.3 - 8.2% [a,b] |

**CLINICAL FINDINGS.** Leishmaniasis is a disease of dogs from various ages, breeds, both male and female [27], the asymptotic course being prevalent in young animals. Some retrospective studies have shown that the most commonly affected age groups were between 9 months and 15 years (average age 5 years) [43]. The owners of ill animals notice initially the cutaneous changes (50%), the progressive weight loss (25.3%), loss of appetite (16.5%) and the exhaustion (10%). Other clinical signs accompanying the diseases are the depression, ophthalmic changes, vomiting, melena, sneezing, cough and lameness [43-45]. The clinical picture in dogs at the age of 3 months and 7 years is given in Table 1.

***Table 2**. Blood laboratory changes – [a] [43] ; [b] [45]; [c] [44].*

| **Alterations in blood and urine parameters** | |
|---|---|
| **Changes** | **Incidence, %** |
| Hyperproteinaemia | 63.3 - 72.8% [a,c] |
| Hyperglobulinaemia | 70.6 - 100% [b,c] |
| Hypoalbuminaemia | 68 - 94% [b,c] |
| Reduced albumin/globulin ratio | 76% [c] |
| Non-regenerative anaemia | 60 - 73.4% [a,c] |
| Thrombocytopenia | 29.3 - 50% [b,c] |
| Leukocytosis | 24% [c] |
| Leukopenia | 22% [b] |
| Elevated activities of liver enzymes | 16% [c] |
| Increased urea and creatinine | 16 - 45% [b,c] |
| Medium/strong proteinuria | 71.5 - 85% [a,b] |
| Fine hyaline granules in urine | Up to 100% [a] |

**LABORATORY INVESTIGATIONS.** The typical haematological, blood and urine biochemical alterations are presented in Table 2.

**DIAGNOSTICS**. **Serology.** The infection of the host is always related to appearance of anti-leishmania antibodies. Specific serological techniques are developed – indirect immunofluorescence assay (IFA), direct agglutination test (DAT), ELISA, competitive-ELISA, Dot-ELISA, slide-ELISA and western blotting [27, 43-48]. **Cytology and histology.** The *Leishmania* amastigotes are well demonstrated on imprint preparations of lymph aspirate, spleen and bone marrow, stained by Giemsa or by Diff-Quick ®. They are detected in macrophages and outside them, with a round to oval shape containing basophilic nuclei. Specimen for analysis could be obtained from cutaneous lesions after scraping or aspiration with a needle and syringe. **In vitro cultivation.** The commonly used cultivation media are those of Novy-MacNeal-Nicolle (NMN) and Schneider's Drosophila. Suitable specimens for in vitro cultivation are lymph aspirate, spleen and bone marrow. **In vivo cultivation.** It is rarely performed, only for scientific purposes. Hamsters are used

and the clinical manifestation of leishmaniasis is monitored. **Immunohistochemistry.** It is used on skin and lymph node histological preparations by means of the indirect peroxidase technique [49].**Polymerase chain reaction (PCR**). This reaction is convenient for detection of rRNA gene sequences [50] in lymph nodes and blood. It is possible for look for *Leishmania* spp. kinetoplast DNA (kDNA) in skin bioptates (100% sensitivity and 100% specificity) [51, 52]. This technique is still under development in order to increase its sensitivity. **Identification of *Leishmania* spp.** It is extremely important to perform the identification in order to the proper characterization of the epidemiological picture and especially the transmission of the agents. The isoenzyme analysis is a "gold standard" but it requires a long time because each isolate should be tested with multiple enzymatic reactions [53]. PCR uses successfully kDNA primers specific for both the Old and the New Worlds [54, 55].

**THERAPY**. The most commonly used medications are: ▪ Antimony-containing compounds. Pentavalent preparations (Glucantime and Pentostam) are the most commonly used. They inhibit the glycolytic enzymes of leishmania. The typical course of treatment includes 100 mg/kg/day S.C. or I.V. for 3–4 weeks [27]. After the first injections, sometimes the next ones are painful. The therapy is expensive; especially for large-sized dogs and a drug resistance could be observed [56].▪ **Allopurinol.** Allopurinol is an analogue of hypoxanthine. Its mechanism of action included incorporation in the RNA and interference with the protein synthesis. Usually, allopurinol is administered at a daily dose of 20 mg/kg per os. The preparation is not expensive and it has a relatively low toxicity. It is applied for an extended period and most frequently, combined with antimony-containing preparations – 10–15 mg/kg/day P.O. for 2-24 months. Its clinical effect is marked, it has no side effect but a relapse is possible [57, 58].▪ **Pentamidine.** It is dosed at 4 mg/kg I.M. per 48 hours. The injection is painful, and the needed course of treatment is at least with 15 injections [59].▪ **Amphotericin B.** Amphotericin B is the first antifungal preparation. It impairs the cellular permeability of leishmania. Possesses a renal toxicity. Its liposome variant (AmBisome) reduces the side effects, but the therapy with this preparation is expensive [60].▪ **Paromomycin Aminosidine** is a ribosome inhibitor. It is applied at a daily dose of 10 mg/kg – twice IM or SC. The product is nephro- and ototoxic.▪ **Miltefosine.** Miltefosine is a phospholipid preparation, effective in the therapy of men and mice. Its liposome variant is now under investigations for use in dogs.▪ **Glucocorticoids** (prednisolone 1-2 mg/kg) – could be used in subacute renal failure together with allopurinol [4].

Even after the applied therapy, accompanied with clinical remission and reduction in the antibody titres, the complete elimination of leishmania from the organism is often not possible [61].

**PREVENTION AND CONTROL**.

**Immunoprevention** -numerous antigens have been used to make vaccines: killed leishmania, attenuated leishmania, recombinant and synthetic proteins, non-protein antigens, immunogens expressed in bacteria and viruses, most of them in murine models and a limited number in men [62]. For a pity, there is still no reliable means for active immunoprevention despite the great number of studies on this subject [63, 64]. The experiments with using DNA-LACK plasmid were neither successful. Another vaccine-virus rVV-LACK had a result in 3 out of 5 experimental animals [65]. **Sanitary prevention** – euthanasia of infected dogs is practiced in some countries. In Greece, it is regulated by law. This practice is however unacceptable from ethical point of view because of the availability of many effective therapeutic means. **Medical prevention** – medical prevention is rather an empirical measure. The treatment at every 5- months could prevent the

relapse, but is not recommended (toxic effect, potential onset of resistance). The prevention via a "scientific approach" requires routine serological checkups of immunity, at last once per year. **Vaccinations** – for now, vaccines for routine application with the necessary reliability are not available on the market. **Insecticide preparations** – they are recommended to preserve the dogs from being bitten by phlebotominae One of the most popular preparations for control of leishmaniasis is Scalibor (Intervet). It contains deltametrin with 90% protectivity against sand flies for 7.5 months [66, 67].

A good measure for prevention is to bring the dog indoors one hour before sunrise and 1 hour before sunset – the period when sand flies are not active [60, 68].

**PUBLIC HEALTH** . The visceral leishmaniasis in people living in our region is causes by *L. Donovani.* The data of the WHO report more than 350 million of people at risk for infection, and over 12 million already infected [69]. Every year, between 100 and 150 thousand new cases are registered and many others remain unrecognized. The statistical data evidence that the disease is the most common among children and HIV-positive, and in people with AIDS is an extremely dangerous secondary infection [70]. The distribution of leishmaniasis by intravenous applications of various substances, including narcotics, is also a serious concern [71].

Recent studies in Bulgaria showed a serious increase in the number of affected people – from 1988 to 2002, 67 clinical patients are registered, including a 4-month-old baby [72]. The geographical prevalence of the disease is well demonstrated – primarily in South Bulgaria, the morbidity rate in the regions of Stara Zagora being 50.7%. [73].

*The annual records of new infections in Bulgaria and the lack of knowledge upon the diagnostics of leishmaniasis in people illustrates plainly the importance of the problem and the need of a complex approach for its solution from both medical and veterinary specialists [72].*

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