

Premature parturition in a female dog with Visceral Leishmaniasis: Case report

ABSTRACT

Background: The visceral leishmaniasis (VL) is a parasitic disease that affects dogs usually transmitted by the presence of the vector, but other forms of transmission have already been reported, including the possibility of vertical transmission.

Case: This article reports the case of a Schnauzer female dog, two years and six months old, presenting apathy and weight loss in which the diagnosis for VL was suggested, confirming the positivity during pregnancy. Clinical and laboratorial findings were aggravated during gestation with the occurrence of preterm delivery at fifty days of coverage.

Discussion: Despite the scarcity of reports in the literature about the possibility of vertical transmission of the disease in canines, the presence of amastigote forms of *Leishmania* sp. in the uterus, vagina, vulva and mammary gland have been reported.

Conclusion: It is worth noting that female dogs parasitized with the disease should be removed from reproduction for treatment, as the disease may alter the general clinical picture of the animal and compromise the development of the fetus, causing miscarriage or premature parturition.

Keywords: canine; diagnosis; Leishmaniasis; prematurity.

1. INTRODUCTION

Visceral Leishmaniasis (LV) is a widely distributed anthroponosis and is currently among the world's top tropical diseases. In Latin America, the disease has been described in at least 12 countries, with most reported cases occurring in Brazil, especially in the northeast region [1].

Leishmania sp. is a heteroxene parasite, which requires two hosts to complete its life cycle. One of the hosts is vertebrate, mammalian, usually domestic or wild canids and man. The other host is invertebrate, dipterous, hematophagous, of the Family Psychodidae [1,2]. Dogs are the most important reservoirs of VL in urban areas, mainly due to increased proximity to human beings.

The transmission of the disease usually occurs in the presence of the vector, but there are reports in the literature about other forms of transmission such as blood transfusion and the use of needles and syringes shared among injecting drug users [3,4]. The vertical and sexual transmission of *Leishmania* sp. between humans has been described [5,6].

This disease can be characterized by a range of clinical manifestations ranging from various degrees of dermatopathies, lymphadenopathy, hepatosplenomegaly, onychogriphosis, weight loss and ocular lesions [7], in addition to other atypical manifestations such as neurological disorders, nephropathies, heart diseases [8,9] and more recently the involvement of the genital system of dogs, with reports of elimination of the parasite in the semen of positive dogs [10-13].

The infection vertical transmission in dogs, has already been suggested by Masucci *et al.* [14] and Dubey *et al.* [15], although previous evidence has been published indicating that this type of transmission does not occur in the canine species [16]. Amastigotes of *Leishmania* sp. have been previously described in the uterus, vagina, vulva and mammary gland of female dogs [17,18], as well as in other canine reproductive system organs, with evidence of possible transmission venereal disease.

In this way, this work aimed to describe a case report of a pregnant female dog with positive diagnosis for Visceral Leishmaniasis, with the occurrence of premature parturition of her pups stillborn.

2. CASE DESCRIPTION

This paper reports a Schnauzer female dog, in estrus phase, two years and six months old, weighing 5kg. The animal owners sought veterinary assistance, because it had not never been pregnant even though it had been mating by a Schnauzer male dog in the previous estrus phase.

The veterinary care was carried out on June 2016. At that time, the general and reproductive clinical evaluation of the animal was made, with collection of material for vaginal cytology by cotton-tipped swab, and collection of blood by the jugular vein in a vacuum tube with anticoagulant for hemogram and without anticoagulant for the hormonal dosage (progesterone).

The vaginal cytology was performed by smear on a microscope slide and stained by diff quick stain kit, at the Laboratory of Animal Reproduction Biotechnology of the Federal University of Piauí (LBRA/UFPI). The hemogram was performed at the Laboratory of Clinical Pathology of the Federal University of Piauí Veterinary Hospital (HVU/UFPI) and

the hormonal dosage was performed in the Bioanalysis Laboratory by means of the Vitros 5600 Immunometric Automation technique.

Between the 10th and 12th of June the bitch mated with the dog. The ultrasonography was performed at UFPI to confirm pregnancy after 35 days of mating process.

A few days after pregnancy diagnostic, the disease was confirmed serologically by TRDPP® and ELISA, using commercial Bio-Manguinhos kits, following the manufacturer's recommendations. Parasitologically by detection of amastigote forms of *Leishmania* sp. by aspiration of popliteal lymph nodes and bone marrow on slides made and stained by the GIEMSA method (Figure 1) and by detection of promastigotes forms of *Leishmania* sp. by means of sowing of aspirations of popliteal lymph nodes and bone marrow in NNN culture medium enriched with Schneider's. The culture media were placed in the Incubator Chamber B.O.D., where three optical microscopy readings were performed, interspersed, on the 5th, 7th and 10th day after sowing, confirming the positivity of the disease in the animal.

The diagnosis of VL was also performed in the dog that crossed with this bitch being negative for the disease. These tests were performed at the Animal Health Laboratory of the Federal University of Piauí (LASAN/UFPI).

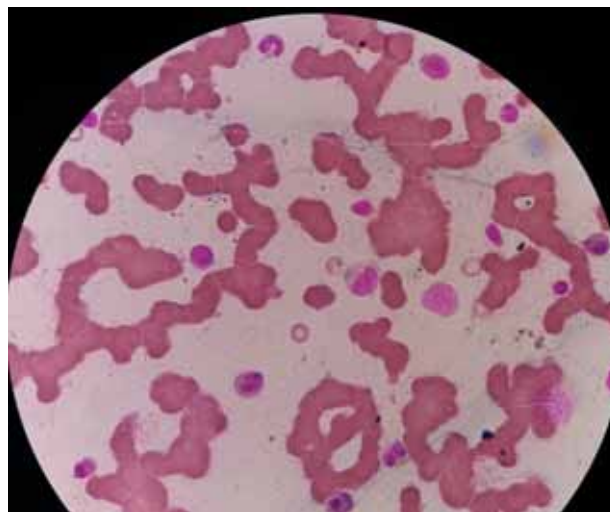


Figure 1. Amastigotes forms of *Leishmania* sp. in bone marrow of female dog of this study. 100x Objective. Source: Personal Archive.

The results of hemogram and serum biochemistry, as shown in Table 1, revealed urea, creatinine, ALT/TGP, alkaline phosphatase and GGT within normal for the canine

specie, but the results of total protein, albumin and globulin were altered, occurring including the inversion of the albumin/globulin ratio.

Table 1. Laboratory tests (hemogram and serum biochemistry) of pregnant female dog after confirming the diagnosis of VL.

Exam	Results	Reference Value
Blood Cells($\times 10^6/\mu\text{L}$)	2.5	5.5-8.5
Hemoglobin(g/dL)	5.5	12-18
Hematocrit(%)	18.3	37-55
Leukocytes(Cel/ μL)	3200	6-17.000
Segmented(%)	70	60-77
Lymphocytes(%)	20	12-30
Eosinophils(%)	04	2-10
Monocytes(%)	04	3-10
Platelets($10^3/\mu\text{L}$)	100	200-500
Urea(mg/dL)	33.0	21.4-59.9
Creatinine(mg/dL)	0.5	0.5-1.5
ALT/TGP(U/L)	53.0	21.0-73.0
Alkaline Phosphatase(U/L)	91.0	20.0-156.0
GGT(U/L)	9.0	0-10
Total Protein(g/dL)	8.2	6.0-8.0
Albumin(g/dL)	2.0	2.6-3.3
Globulin(g/dL)	6.1	2.7-4.4

The animal was monitored by clinical and laboratorial evaluation until the day of parturition, which occurred on august 2016.

3. DISCUSSION

The female dog vaginal cytology results showed the presence of anucleated and keratinized superficial cells suggestive of the estrous phase and the progesterone dosage presented 2.56 ng/mL, confirming the ovulatory phase of the animal, so it was mated 2-4 days after the tests, in the time feasible to fertilization. At 35 days after the mating was performed ultrasonography confirming the pregnancy and the presence of five viable fetuses,

a considerable increase in the size of the spleen with bulging margins was also observed, with no other alterations in other organs.

The female dog was not gaining weight during the gestational period and the hemogram revealed normocytic normochromic anemia, thrombocytopenia and leucopenia, and an examination for VL was requested. Clinical signs such as progressive weight loss, despite normal appetite, may be commonly reported in dogs with VL, and anemia and thrombocytopenia are constant hematological findings in animals with the disease [19,20].

The anemia it can occur through different mechanisms: inflammatory response due to infection, the chronic character of the disease erythropoiesis may be reduced, lysis of red blood cells, blood loss and erythrocyte decrease by the production of autoantibodies that lead to hepatic and splenic sequestration [20] and the occurrence of thrombocytopenia in positive dogs for VL is due to vascular wall alteration due to vasculitis to immunocomplexes, in addition to thrombocytopoietic disorders and the presence of antiplatelet immunoglobulins [21].

A marked biochemical alteration observed in animals with Leishmaniasis is dysproteinemia, consequent to a hyperproteinemia associated with hypergammaglobulinaemia and hypoalbuminemia [20]. Other studies report that most of the changes in the serum proteinogram of dogs infected with *Leishmania* sp. are caused by disorders of liver synthesis, renal loss in cases of glomerular disease, or even associated with a number of other chronic diseases [19].

On the 50-52th day after mating, the female dog began a birthing behavior, becoming restless, with increased urination, looking for a place in the residence to hide and a few hours later began the process of calving the babies one by one. They were developed, but at sizes below the confirmed gestational age, as observed in Figure 2.



Figure 2. (A) Pregnant female dog and (B,C) premature puppies.

The hematological and biochemical changes observed in this female dog may have been sufficient to compromise fetal development and accelerate childbirth. Cases of vertical transmission of *Leishmania* already been confirmed by molecular and immunohistochemistry techniques in two stillborn pups from a female dog infected submitted to cesarean [22].

Despite the scarcity of reports in the literature about the possibility of this type of transmission of the disease in female dogs, the presence of amastigote forms in the mammary gland has been described, where they were observed inside the macrophages of the lactating sinus, indicating the possibility of elimination of the parasite also through of breast milk [18].

In Brazil and in the world there are few reports of Visceral Leishmaniasis in gestation of women and in the canine specie, and of those available, the origin of the cases is of regions known to be endemic to the disease. In this sense, new studies must be performed for epidemiological investigation of the transplacental transmission of the disease.

4. CONCLUSION

Visceral leishmaniasis dogs should be excluded from reproduction and should not be fertilized until an appropriate therapeutic protocol is made, since the disease can alter the clinical and laboratory status of the animal and compromise the development of the fetus, causing abortion or the occurrence of premature births.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

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