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BRIEF COMMUNICATION

PRESENCE OF *Leishmania* AMASTIGOTES IN PERITONEAL FLUID OF A DOG WITH LEISHMANIASIS FROM ALAGOAS, NORTHEASTERN BRAZIL

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SUMMARY

The goal of this short communication is to report the uncommon presence of intracellular amastigotes of *Leishmania* in peritoneal fluid of a dog with leishmaniasis from Alagoas State, Brazil. Physical examination of an adult male rottweiler suspected to be suffering of leishmaniasis revealed severe loss of weight, ascitis, splenomegaly, moderately enlarged lymph nodes, onychogryphosis, generalized alopecia, skin ulcers on the posterior limbs, and conjunctivitis. Samples of bone marrow, popliteal lymph node, skin ulcer, and peritoneal fluid were collected and smears of each sample were prepared and stained with hematoxylin and eosin. Numerous amastigotes were detected in bone marrow, popliteal lymph node, and skin ulcer smears. Smears of peritoneal fluid revealed the unusual presence of several free and intracellular amastigotes of *Leishmania*. Future studies are needed to determine whether the cytology of ascitic fluid represents a useful tool for diagnosis *Leishmania* infection in ascitic dogs, particularly in those living in areas where canine leishmaniasis is enzootic.

KEYWORDS: Canine leishmaniasis; *Leishmania* sp.; Peritoneal fluid; Diagnosis.

Zoonotic visceral leishmaniasis (ZVL) is historically endemic in Alagoas State. In such state, as observed in other Brazilian northeastern states[5], most of cases are from the east region and children up to 15 years of age are usually affected by the disease[12]. However, several aspects of ZVL epidemiology in Alagoas, such as species of *Leishmania* involved in canine and human infection, canine seroprevalence, phlebotomine sandfly fauna, and pattern of transmission in urban and rural areas, remain to be further investigated.

Domestic dogs are considered to be the principal host reservoir of *Leishmania infantum* (= *L. chagasi*), the causative agent of ZVL[2,5]. Hence, canine leishmaniasis (CanL) has a particular importance not only for the clinical veterinarians, but also to public health. In this way, the goal of this short communication is to report the unusual finding of *Leishmania* amastigotes in peritoneal fluid of a dog with leishmaniasis from the municipality of Maragogi, north coast of Alagoas, Brazil.

An adult male rottweiler suspected to be suffering of leishmaniasis was referred to the Veterinary Hospital of the Federal Rural University of Pernambuco, during June 2004 with a one-month history of a seriously deteriorated health condition. Physical examination revealed severe loss of weight, ascitis, splenomegaly, moderately enlarged lymph nodes, onychogryphosis, generalized alopecia, skin ulcers on the posterior limbs, and conjunctivitis. After appropriate immobilization, aspirates from bone marrow, popliteal lymph node, and peritoneal fluid were obtained, as well as imprints of the skin ulcers were made. Duplicate smears were prepared from each sample and stained with hematoxylin and eosin (H&E) (Panótico Rápido LB - Laborclin).

The microscopic examination revealed numerous amastigotes in bone marrow (Fig. 1), skin ulcer, and popliteal lymph node. The cytology of peritoneal fluid smears revealed the presence of macrophages, lymphocytes, polymorphonuclear cells, and unexpectedly of *Leishmania* amastigotes (Fig. 2). The finding of *Leishmania* amastigotes in bone marrow and, mainly, the observed clinical expression suggest that the causative agent involved in this case of CanL from Alagoas was *L. infantum*, though the species was not determined.

To date, there is no concise description on the presence of amastigotes in peritoneal fluid of dogs with leishmaniasis. In a recent study carried out in Barra de Guaratiba, Rio de Janeiro State, the authors affirmed that all imprints made from skin, spleen, liver, heart, lung, kidney, ascitic fluid, and lymph node of five dogs submitted to euthanasia and necropsy were found to be positive for the presence of *Leishmania* amastigotes[17]. Unfortunately, the authors did not provide further information, i.e., details regarding these findings. *Leishmania*

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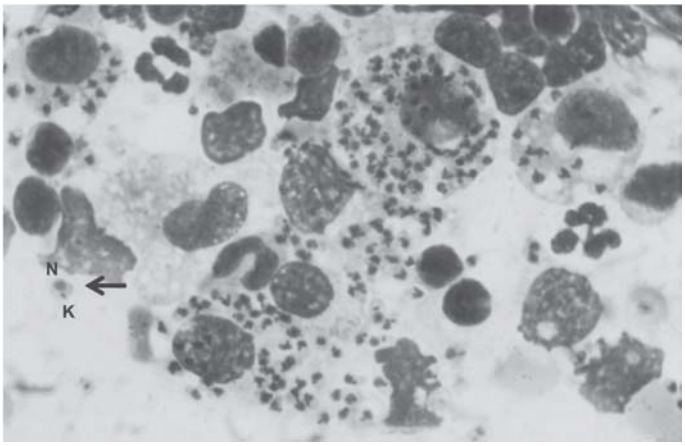

Fig. 1 - Over a thousand of *Leishmania* amastigotes in a bone marrow smear. Note the nucleus (N) and kinetoplast (K) of a typical free amastigote (arrow) (H&E; x1000).

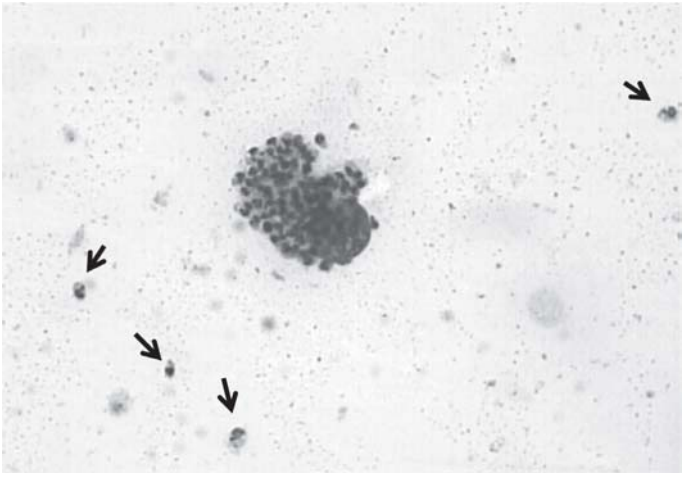

Fig. 2 - Some free (arrows) and several intracellular amastigotes of *Leishmania* in peritoneal fluid smear (H&E; x1000).

amastigotes have been detected in lymph nodes, liver, bone marrow, pancreas, joints, bone, urinary bladder, intestinal lamina propria, lung, thyroid[1], choroid plexus[11], urine, semen[14], endothelial cells, and fibroblasts[6] of infected dogs. Rarely, *L. infantum* has been described in peripheral blood of dogs[4,15].

During the course of *L. infantum* infection, there is a generalized spreading of the amastigote forms and many organs may be colonized. However, the dissemination of the parasites is dependent of many factors, such as infectivity of *Leishmania* species, tropism of the strain, vertebrate host immune response, and also components of phlebotomine sandfly saliva. After the inoculation by the bite of an infected female phlebotomine sandfly, the promastigotes are phagocyted rapidly by the cells of the host immune system, especially macrophages that are the principal effector cell in both innate and acquired immune responses to leishmaniasis. Inside the lisosomal vacuoles present in phagocytic cells of the vertebrate host, the promastigotes differentiate into amastigotes, which proliferate and colonize other cells, establishing the infection[3]. It is well documented, for example, that *L. infantum* amastigotes may be encountered in the intact skin of naturally infected dogs, though the mechanism of dissemination of the parasite in the skin of dogs is not well understood[8].

Previous studies concerning the infectivity of dogs naturally parasitized by *L. infantum* to sandflies showed divergent results[9,16,18]. In general, asymptomatic dogs are either non-infective[18] or modest sources of infection[15], while symptomatic dogs are often infective[18]. Indeed, every infected dog must be seen as a potential risk to phlebotomine sandfly infection. In the same way, since the dogs are often next or in human dwellings, they play a key role in the zoonotic transmission cycle. As a consequence, 850 000 dogs are screened annually and 20 000 of them are culled as a control measure against ZVL in Brazil[13]. A recent study showed that dog-culling programs do not reduce the incidence of *Leishmania* infection in dogs[10]. Perhaps, these programs have failed due to the fact that either susceptible puppies or already infected dogs usually replace the destroyed dogs, but also because other vertebrate host might be acting as reservoirs.

Nowadays, new strategies for control of CanL are available (e.g. insecticide-impregnated dog collars) or are being developed (e.g. sandfly saliva-based vaccine)[13]. However, an effective treatment of CanL is still to be discovered. There are also a number of novel tools to diagnose *Leishmania* infection in dogs, though some of these are more expensive and less reproducible (e.g. PCR-based methods) than traditional methods[13]. Hence, the serological methods remain as the most widely used tools for the detection of *Leishmania* infection in dogs. Indeed, despite of its known low sensitivity, the parasitological methods, even associated to clinical and epidemiological features, may be seen as practical and less expensive alternatives to be used, mainly in symptomatic dogs.

Experimentally, canine peritoneal cells might be used as a model for the screening of drugs against *Leishmania* parasites and also to study the pathogenesis of some *Leishmania* species, such as *L. infantum*[7]. On the other hand, the possible implications, if there are any, of the presence of intracellular *Leishmania* amastigotes in peritoneal macrophages of naturally infected dogs are not well understood. In HIV/*Leishmania* co-infected patients, for example, the cytology of ascitic fluid can be helpful in diagnosing *Leishmania* infection[19]. In the case of CanL, further investigations will be needed to determine whether this technique represents a useful tool for diagnosis *Leishmania* infection in ascitic dogs, particularly in those living in areas where CanL is enzootic. In the same way, this finding indicates that at least some aspects of *Leishmania*-dog interaction remain to be discovered.

RESUMO

Presença de formas amastigotas de *Leishmania* em fluido peritoneal de cão com leishmaniose proveniente de Alagoas, nordeste do Brasil

O objetivo desta comunicação é descrever a presença incomum de formas amastigotas de *Leishmania* em fluido peritoneal de um cão com leishmaniose proveniente do Estado de Alagoas, nordeste do Brasil.

O exame físico de um cão macho adulto da raça rottweiler, apresentando suspeita de leishmaniose, revelou perda de peso severa, esplenomegalia, linfonodos moderadamente aumentados, ascite, onicogrifose, alopecia generalizada, conjuntivite e presença de lesões cutâneas ulceradas localizadas nos membros posteriores. Foram coletadas amostras de medula óssea, linfonodo poplíteo, fluido peritoneal e úlcera cutânea. A partir das amostras, foram elaborados esfregaços, os quais foram corados pela hematoxilina e eosina. Inúmeras formas amastigotas foram detectadas na medula óssea, linfonodo poplíteo e úlcera cutânea. Esfregaços de fluido peritoneal revelaram a presença, não usual, de várias formas amastigotas livres e intracelulares. Futuros estudos serão necessários a fim de determinar se a citologia de líquido ascítico representa uma ferramenta útil para o diagnóstico da infecção por *Leishmania* em cães com ascite, particularmente naqueles que vivem em áreas onde a leishmaniose canina é enzoótica.

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