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CASE REPORT
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First Autochthonous Case of Canine Visceral Leishmaniasis in the Center of Rio Grande do Sul State, Brazil

Aleksandro Schafer Da Silva[1], Raqueli Teresinha França[2], Virginia Bodelão Richini-Pereira[3], Helio Langoni[3], Silvia Gonzalez Monteiro[1] & Sonia Terezinha dos Anjos Lopes[2]

ABSTRACT

***Background*:** Leishmaniasis is one of the most important vector-borne diseases of humans. This parasitic disease can be caused by many species of *Leishmania.* In humans, different species of the parasite are associated with different forms of the disease, cutaneous and visceral. Among domesticated animals, dogs are the most important species in the epidemiology of this disease. *Leishmania chagasi*, an important zoonosis, is well established as the agent of visceral leishmaniasis in Brazil. The disease is endemic in north, northeast, midwest and southeast, and is transmitted to mammals by hematophagous insects such as the *Lutzomyia longipalpis.* In 2008, our research group has diagnosed a case of canine leishmaniasis in the municipality of Uruguaiana and subsequently there were several cases in the city and the neighbor municipality of São Borja. Most Brazilian states are endemic for leishmaniasis, with the exception of Rio Grande do Sul. In southern Brazil, the reports of humans and dogs infected by *Leishmania* spp. are the source of endemic area in the country. Therefore, the aim of this study is register the first clinical case of canine visceral leishmaniasis in the municipality of Santa Maria, RS.

***Case*:** In october 2010, a veterinary clinic of Santa Maria received a canine, female, Doberman, with two years of age. The animal had severe skin lesions on the head and limbs, pale mucous membranes, and enlarged lymph nodes. According to the owner, the animal showed progressive weight loss and anorexia for more than five days. During the clinical examination the blood was collected for hemogram and cytology of lymph nodes was performed by puncture aspiration with a fine needle. In the erythrogram, it was observed a decrease in the total number of erythrocytes (2.8 x 10^6/μL), hematocrit (21%), hemoglobin (6.8 g/dL) and platelets (98 x 10^3/μL). In the leucogram, any alteration was observed. The cytology of lymph nodes showed amastigotes forms, suggestive of the *Leishmania* spp. Based on this finding; we performed the blood collection for PCR, to confirm parasitism and to determine the species of *Leishmania*. At the molecular test was used PCR-specific for *L. chagasi*, and the result was positive.

***Discussion*:** This is the first autochthonous clinical case in the central region of the RS, non-endemic area for leishmaniasis. In serological studies of visceral leishmaniasis it was diagnosed in five asymptomatic dogs in the municipalities of Santa Maria, Julio de Castilhos and Itaara, however not confirmed by molecular analysis. In the municipalities of Cruz Alta and Uruguaiana cases of *L. chagasi* have been reported in dogs which previously resided in *Leishmania* sp. endemic areas. The municipality of São Borja had the first record of *L. longipalpis* in the RS during the leishmaniasis outbreak in 2008-2009. In the central region of the RS vector has not been found, but because in this first autochthonous case dog in Santa Maria believe that the parasite is present and/or doing other insect transmission of leishmaniasis. Clinical signs associated with hematologic and coagulation disorders observed in the canine are commonly described in symptomatic dogs in endemic regions. This case of autochthonous leishmaniasis reinforces the idea of the vector presence in Santa Maria, center of the RS. We believe that canine leishmaniasis is an emerging disease in the southern region of Brazil.

Keywords: *Leishmania chagasi,* dog, non-endemic area.

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[1]Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Maria (UFSM). [2]Departamento de Pequenos Animais da UFSM. [3]Universidade Estadual Paulista (UNESP), Campus Botucatu, Faculdade de Medicina Veterinária e Zootecnia, Departamento de Higiene Veterinária e Saúde Pública, Brasil. CORRESPONDÊNCIA: A.S. Da Silva [aleksandro_ss@yahoo.com.br - FAX +55 (55) 3220-8958]. Campus Universitário, Prédio 20, Sala 4232, Camobi, CEP 97105-900 Santa Maria, RS, Brasil.

INTRODUCTION

Leishmania chagasi (syn. *Leishmania infantum*), an important zoonosis, is well established as the agent of visceral leishmaniasis in Brazil. The disease is endemic in north, northeast, midwest and southeast, and is transmitted to mammals by hematophagous insects such as the *Lutzomyia longipalpis* [1,5]. This disease is responsible for the euthanasia of thousands of seropositive dogs every year, as well as the treatment of many humans living in endemic areas. The brazilian program of eradication and control of leishmaniasis is failing to reduce the number of cases in endemic regions and the parasite is spreading to not infected areas like the south regions of the country [7].

In 2008, our research group has diagnosed a case of canine leishmaniasis in the municipality of Uruguaiana and subsequently there were several cases in the city and the neighbor municipality of São Borja, the main affected locality as described by researchers [7]. Since then, several cases have been diagnosed in dogs in the RS, but these animals which were infected or lived or walked by municipalities where occurred the leishmaniasis outbreak in 2008 and 2009. Any autochthonous cases were recorded in the central region of the RS, so it seemed appropriate to register the first clinical case of canine visceral leishmaniasis in the municipality of Santa Maria.

CASE REPORT

In october 2010, a veterinary clinic of Santa Maria received a canine, female, Doberman, with two years of age. The animal had severe skin lesions on the head and limbs, pale mucous membranes, and enlarged lymph nodes. According to the owner, the animal showed progressive weight loss and anorexia for more than five days. During the clinical examination the blood was collected for hemogram and cytology of lymph nodes was performed by puncture aspiration with a fine needle.

In the erythrogram, it was observed a decrease in the total number of erythrocytes (2.8 x 10^6/µL), hematocrit (21%), hemoglobin (6.8 g/dL) and platelets (98 x 10^3/µL) compared to reference values [2]. In the leucogram, any alteration was observed. The cytology of lymph nodes showed amastigotes forms, suggestive of the *Leishmania* spp. (Figure 1). Based on this finding, we performed the blood collection for PCR, to confirm parasitism and to determine the species of *Leishmania*. DNA from blood sample was extracted using illustrate blood genomic Prep Min Spin Kit[1]. PCR reaction was performed using the specific primers of kinetoplast minicircle DNA, Lc14 (5'-GCACGTTATATCTACAGGTTGAG-3') and Lc15 (5'-TGTTTGGGATTGAGGTAATAGTGA-3'), designed in NUPEZO (FMVZ – UNESP - Botucatu). PCR mix containing reaction buffer (10 mM TrisHCl, 50 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.2 U *Platinum* Taq[2], 10 ng of extracted DNA, and 10 mM of each primers that produce a 167 bp. The amplified products were analyzed by electrophoresis on 1.5% agarose gel stained with SYBR®[3] safe. At the molecular test was used PCR-specific for *L. chagasi*, and the result was positive. Euthanasia was performed after diagnosis as recommended by the Ministry of Health/Brazil.

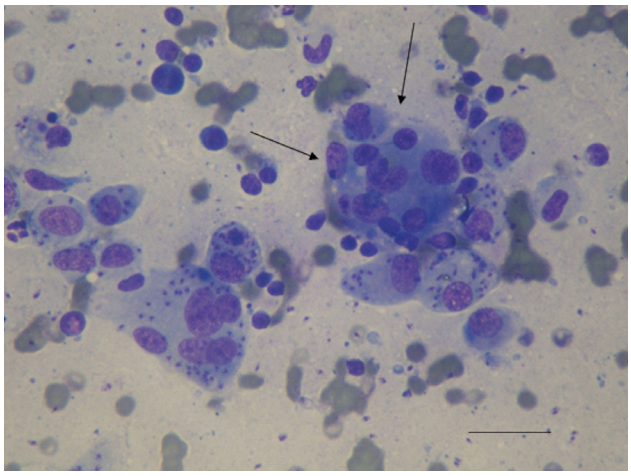

Figure 1. *Leishmania chagasi* amastigotes inside macrophages obtained from the cytology of lymph nodes from one dog. Smear was stained by the panoptic method, and visualized at a magnification of ×1,000 (BAR: 5µm).

DISCUSSION

This is the first autochthonous clinical case in the central region of the RS, non-endemic area for leishmaniasis. In 1998, researchers described the pathological findings of five dogs infected with *Leishmania* spp. in Santa Maria, RS [8]. However, later the laboratory confirmed through immunohistochemical techniques, that the animals had rangeliosis [3], and not leishmaniasis as described above. In serological studies of visceral leishmaniasis it was diagnosed in five asymptomatic dogs in the municipalities of Santa Maria, Julio de Castilhos and Itaara [6], however not confirmed by molecular analysis. In the municipalities of Cruz Alta [4] and Uruguaiana [7] cases of *L. chagasi* have been reported in dogs which previously resided in *Leishmania* sp. endemic areas.

Our research group has installed traps in many regions of Santa Maria in the years 2009 and 2010 in search of the insect *L. longipalpis*, vector of leishmaniasis. So far were collected and identified 217 mosquitos, all Culicidae (study in progress). These data are similar to those described by researchers in the central region of the state, ie, found no phlebotomine insect [6]. The municipality of São Borja had the first record of *L. longipalpis* in the RS during the leishmaniasis outbreak in 2008-2009 [9]. Therefore, the vector *L. longipalpis* can be distributed in other cities, with risks of outbreaks and autochthonous cases in the state. We believe that this vector occurs in Santa Maria and it has been the transmitter of canine visceral leishmaniasis, reported here.

Clinical signs associated with hematologic and coagulation disorders observed in the canine are commonly described in symptomatic dogs in endemic regions [1]. This case of autochthonous leishmaniasis reinforces the idea of the vector presence in Santa Maria, center of the RS. We believe that canine leishmaniasis is an emerging disease in the southern region of Brazil.

SOURCES AND MANUFACTURERS

[1]GE Healthcare, São Paulo, Brazil.
[2]Invitrogen, Carlsbad, California, USA.
[3]Invitrogen, Carlsbad, California, USA.

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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