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Short Communication

Prevalence of zoonotic visceral leishmaniasis in dogs in an endemic area of Brazil

***Danillo de Souza Pimentel*[1], *Rafael Antonio Nascimento Ramos*[2],[3], *Marília de Andrade Santana*[2],[4], *Carina Scanoni Maia*[5], *Gílcia Aparecida de Carvalho*[6], *Hernande Pereira da Silva*[7] *and Leucio Câmara Alves*[2]**

[1]. Centro de Estudos Superiores de Maceió, Maceió, Alagoas, Brasil. [2]. Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brasil. [3]. Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, Bari, Italy. [4] Departamento de Medicina Veterinária, Universidade Federal da Bahia, Salvador, Bahia, Brasil. [5]. Departamento de Biologia, Universidade Federal de Campina Grande, Cuité, Paraíba, Brasil. [6]. Unidade Acadêmica de Garanhuns, Universidade Federal Rural de Pernambuco, Garanhuns, Pernambuco, Brasil. [7]. Departamento de Tecnologia Rural, Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brasil.

ABSTRACT

Introduction: The northeast region of Brazil is endemic for zoonotic visceral leishmaniasis (ZVL). The aim of this study was to determine the prevalence of infection in dogs in Petrolina. **Methods:** Blood samples were collected from dogs (n = 600), and bone-marrow biopsy was performed in animals with positive serological test results that presented clinical signs of ZVL. The serological analyses were performed using an enzyme-linked immunosorbent assay (ELISA) (S7®Biogene). **Results:** Of the 600 dogs tested, 19% (115/600) presented anti-*L. infantum chagasi* antibodies. **Conclusions:** Our data are important because canine infection is an important risk factor for the human disease.

Keywords: Leishmaniasis. Dog. Serology. Geographic information system.

Zoonotic visceral leishmaniasis (ZVL) is an important disease caused by protozoans belonging to the genus *Leishmania* (Tripanosomatida: Trypanosomatidae), which is present worldwide, particularly in India, Nepal, Bangladesh, Sudan, and Brazil[1]. *Leishmania* (*Leishmania*) *infantum chagasi* is the main species causing ZVL in Brazil. It is transmitted by sand flies, the species *Lutzomyia longipalpis* being the most important vector in the New World. Although wild species may be infected by this protozoan, the domestic dog is considered the main reservoir in Brazil. In dogs, ZVL displays a multifaceted clinical picture, varying from asymptomatic to severe clinical signs and death[2].

In Brazil, ZVL has been reported in different regions with different rates of prevalence. Indeed, the northeast region of Brazil is traditionally endemic for ZVL, accounting for about 40% of the annual cases that occur throughout the country[3]. In this area, the humans, animal reservoir, and vector share the same ecological niche, which contributes to the persistence of the disease.

Interestingly, *Leishmania infantum chagasi* has become an *urban parasite* over recent years, and the phenomenon *urbanization* is present in several regions of the country[4] [5]. Because the City of Petrolina (State of Pernambuco, northeast region, Brazil) is a *hot spot* for ZVL, with more than 100 human cases diagnosed between 2001 and 2010[6], this study aimed to determine the prevalence of infection in dogs residing in that area.

The study was performed in the municipality of Petrolina, located in the State of Pernambuco, Brazil. With a total area of 4,756.8km², this municipality presents a human and dog population of 281,851 and 30,000 inhabitants, respectively. The selection of the animals to be evaluated was based on a retrospective cross-sectional study of the registered human cases of ZVL that occurred from 2001 to 2010. Therefore, blood samples of dogs living in an area up to 100 meters away from the residence of the human patient (previously diagnosed with ZVL) were collected.

Animals from both urban (i.e., Areia Branca, Fernando Idalino, Gercino Coelho, Henrique Leite, João de Deus, José e Maria, Ouro Preto, and Pedro Raimundo neighborhoods) (n = 485) and rural (i.e., Izacolândia neighborhood) (n = 115) areas were sampled. After sampling, the obtained sera were stored at -20°C until serological analysis. The serological analyses were performed using an enzyme-linked immunosorbent assay (ELISA) (S7®Biogene) based on a recombinant peptide. Samples were tested in duplicate, including blank, negative, and positive controls, being the control sera provided in the ELISA kit. The reaction was performed in a spectrophotometer and analyzed by a wavelength of 450nm. All procedures and determination of cutoff were performed following the instructions provided in the ELISA kit test. According to the presence or absence of clinical signs compatible with ZVL, the dogs were classified as

Corresponding author: Dr. Leucio Câmara Alves. Laboratório de Doenças Parasitárias dos Animais Domésticos/UFPE. Av. Dom Manoel de Medeiros s/n, Dois Irmãos, 52171-900 Recife, Pernambuco, Brasil.
Phone: 55 81 3320-6422
e-mail: leucioalves@gmail.com; rafaelaanramos10@yahoo.com.br
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symptomatic or asymptomatic[2], and bone-marrow biopsy was performed in symptomatic animals with positive serological test results (n = 43). Briefly, the skin surface was clipped of hair and aseptically prepared. Then, bone marrow was aspirated directly from the manubrium of the sternum using sterile syringes and needles (1.6 × 40mm). Smears were prepared on glass slides, stained with Diff-Quick® (Dade, Miami, FL, USA), and examined under an optical microscope (at 100× magnification).

Data of positivity were statistically analyzed through the chi-squared test, using the software BioEstat 5.0.

Of the 600 dogs tested, 19% (115/600) presented anti-*L. infantum chagasi* antibodies. In particular, 17% (84/485) and 27% (31/115) of the animals studied in the urban and rural areas were serologically positive ($p < 0.05$), respectively (**Table 1**). Among the serologically positive animals, 37.4% (43/115) displayed clinical signs, with dermatopathy, enlargement of lymph nodes, and weight loss being the most frequent clinical signs. Amastigote forms of *L. infantum* were detected in 39.5% (17/43) of the symptomatic serologically positive dogs (**Table 1**).

The age of animals herein studied varied from 6 months to >10 years. In addition, 322 animals were female and 278 were male. No difference was observed between the positivity of animals of different sexes and ages ($p > 0.05$).

In any serological test, cross-reactions with other pathogens may occur, particularly in the case of trypanosomatids[5]. However, considering the endemicity of the area studied, the results obtained herein are pivotal to better understand the current situation of canine leishmaniasis in Petrolina. In this study, the prevalence of infection by *L. infantum chagasi* in dogs in an endemic area was studied. The positivity rate of 19% in dogs reported here emphasizes the high-risk situation in this high-endemic area for ZVL. Over the preceding years, several human cases have been diagnosed in this area, specifically more than 100 cases between 2001 and 2010[6]. Indeed, approximately 40% of the annual cases of ZVL occur in the northeast region of Brazil[3]. In this area, humans, dogs, and vector share the same ecological niche, which contributes to the persistence of the infection in a given population.

A higher positivity was observed in dogs from the rural study area (27%) that in those from the urban one (17%). This rural region (i.e., Izacolândia) is characterized by basic housing conditions and sanitation and extremely poor garbage-disposal practices (**Figure 1**). In addition, people living in this area have low socioeconomic status, live together with animals, and accumulate organic matter. Importantly, the City of Petrolina, located in the northeast region of Brazil, is another example of *urbanization* of ZVL. Although, leishmaniasis has been historically considered a rural disease, over recent years, a gradual *urbanization* has been reported *urbanization*[7], especially in some cities in the northeast region of Brazil, such as Teresina, Natal, and São Luis. Recently, a similar situation has been reported in the City of Governador Valadares[8]. In the previous study, a reemerging focus of ZVL has been attributed to this phenomenon (i.e., urbanization). Interestingly, the same conditions described in our study, such as basic sanitation, poor garbage-disposal practices, and presence of accumulated organic matter, have been evidenced there.

FIGURE 1 - The rural region of Petrolina (i.e., Izacolândia) characterized by basic housing conditions and sanitation, and extremely poor garbage-disposal practices.

The areas that presented a high positivity were those in which the sanitation and garbage disposal were inadequate (data not shown), being areas close to a forest environment modified by humans. In this context, it has been speculated that in places with regular garbage collection, the rate of ZVL is lower. The accumulation of solid waste may contribute to the colonization of the sand fly, because the environment becomes rich in organic matter. In addition, it may attract synanthropic animals (e.g., rodents and marsupials), which may be involved in the epidemiological life cycle of the disease[9].

Clinical signs such as dermatopathy, enlargement of lymph nodes, and weight loss observed in the animals studied herein resembled those observed in dogs living in ZVL-endemic areas[10]. Only about one third of the serologically positive animals displayed clinical signs.

TABLE 1 - Total and positive numbers of animals examined with the ELISA test and bone-marrow smears.

	Serology	Serology positive		Bone marrow smears	Bone marrow positive	
Area	**(n)**	**n**	**%**	**(n)**	**n**	**%**
Urban	485	84	17.0	32	11	34.0
Rural	115	31	27.0	11	6	54.0

ELISA: enzyme-linked immunosorbent assay.

In general, the prevalence of ZVL in dogs varies widely in different areas of Brazil[8] [11]. In particular, the municipality of Petrolina is considered a *hot spot* for this disease. Most likely, the modification of the environment by humans and the sanitary problems, still present in many areas of the country, have contributed to this current picture. Recently, a serological survey was performed in cats from the same area, with prevalence reaching 3.9%[12]. The putative role of these animals in the epidemiology of the disease has been speculated, but more data are needed to better clarify this hypothesis.

This research was the first large serological survey on ZVL performed in Petrolina. Data herein reported are of major importance, because the prevalence of canine infection is considered the most important risk factor for the occurrence of the disease in humans in Brazil[13]. Future studies focusing on assessment of the local sand-fly fauna and the role of wild species must be conducted in this area. These future data will contribute to obtain a complete and clear picture of leishmaniasis is this area of high endemicity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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