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Short communication

Serological evidence of *Histoplasma capsulatum* infection among dogs with leishmaniasis in Brazil

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ABSTRACT

Histoplasmosis is a systemic infection caused by the fungus *Histoplasma capsulatum*. Environmental sources of infection for humans and animals in certain regions and the prevalence of infection in animals are frequently unknown. Because of the clinical and epidemiological similarities between histoplasmosis and leishmaniasis in northeastern Brazil, we decided to investigate the serologic evidence of *H. capsulatum* in dogs, considering that these animals can act as sentinels for histoplasmosis. A total of 224 serum samples from dogs were tested for antibodies against *H. capsulatum* through immunodiffusion. A total of 128 (57.14%) samples were positive for leishmaniasis by indirect immunofluorescence assay and four (1.78%) samples were positive for antibodies against *H. capsulatum*. Immunological evidence of the co-existence of histoplasmosis and leishmaniasis in dogs living in urban areas was observed. Diagnosis and clinical management of these diseases in endemic areas should be improved by veterinarians.

Histoplasmosis is a systemic disease caused by the dimorphic fungus *Histoplasma capsulatum*. In the Americas, this mycosis is caused by *H. capsulatum* var. *capsulatum*. It can be acquired after inhalation of infectious conidia from soil contaminated with bird or bat droppings, as well as from rotting wood. The fungus has been isolated from confined spaces, such as chicken breeding facilities and caves where bat guano is abundant, as well as from open spaces such as parks where bird droppings are frequently found (Taylor et al., 2005).

Although histoplasmosis can occur in immunocompetent individuals as an occupational disease, it is the most common systemic mycosis reported in the immunosuppressed population (Kauffman, 2008). In Brazil, a survey conducted among 3583 AIDS patients between 1996 and 2006 revealed that histoplasmosis was the primary cause of death in 10.1% of these individuals (Prado et al., 2009). In northeastern Brazil, epidemiological data on human histoplasmosis are very scarce. In a retrospective study conducted among 378 HIV patients in the northeastern state of Ceará, disseminated histoplasmosis was detected in almost 44% of the patients, with a high mortality rate (Daher et al., 2007). The environmental micro niches of infection for these patients are often unknown.

Histoplasmosis is also a common disease in animals (Bromel and Sykes, 2005), mainly domestic cats and dogs (Ueda et al., 2003; Kobayashi et al., 2009). In these small animals, the infection may be clinically unapparent or develop into an acute or chronic disease, with local granulomatous response in the respiratory tract or fungal dissemination through lymphatic and hematogenous routes (Bromel and Sykes, 2005).

Epidemiological data on animal histoplasmosis in Brazil are even rarer and the prevalence of this mycosis is unknown. In Brazil, one of the most important diseases in dogs is leishmaniasis, which is a widely found zoonosis in the northeastern region (Silva et al., 2007). Considering the clinical and laboratory similarities between histoplasmosis and leishmaniasis (São Thiago et al., 1998; Guimarães et al., 2006) and the overlapping endemic areas for these infections in northeastern Brazil (Soares et al., 2008; Deus Filho et al., 2009), we decided to investigate the serological evidence of histoplasmosis among dogs suspected of having leishmaniasis.

The study was approved by the ethics committee of Ceará State University (Process 07381395-8). Serologic detection of histoplasmosis was performed during 2008, 2009 and 2010 in 224 serum samples from dogs (*Canis familiaris*) of different breeds and ages. From January to May 2008, 66 samples from dogs were collected from animals held in the Zoonosis Control Center in Fortaleza

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Table 1
Data on dogs included in the study, collection period and histoplasmosis/leishmaniasis positivity.

Sex		Age (yr)		Breed		Origin		Collection period			Positive for histoplasmosis (n)	Positive for leishmaniasis (n)	Positive for both leishmaniasis and histoplasmosis (n)
Male	Female	1–10	11–20	Mongrel	Others	Fortaleza	Sobral	January–May 2008	January–July 2009	January–July 2010			
134	90	212	12	168	56	163	61	66	97	61	4	128	3

(3°43′S-38°32′W). From January to July 2009, a total of 97 dog serum samples were collected in veterinary clinics in Fortaleza. From April to June 2010, 61 samples from dogs were collected at the Zoonosis Control Center in the city of Sobral (3°41′S-40°20′W).

A total of 3 mL of blood was aseptically drawn from each animal by a veterinarian of our research group. The serum was then obtained by centrifuging at 1500 g for 15 min, at 28 °C. The serum samples were stored at -20 °C until tested for the presence of *Histoplasma* antibodies by immunodiffusion (ID), using the *Histoplasma* ID Antigen (H & M), according to the supplier's instructions (Immy Immunodiagnostics, Inc., USA). Positive serum samples were tested for cross-reactions with coccidioidal antibodies using *C. immitis* IDCF antigen (Immy Immunodiagnostics, Inc., USA). Each serum sample was also tested for visceral leishmaniasis by indirect immunofluorescence assay (IFI), according to the supplier's instructions (IFI-leishmaniose-visceral-canina-Bio-Manguinhos, Brazil).

Of the 224 canine samples studied, 134 were from males and 90 were from females, at ages varying from 1 to 20 years old. With regard to breed, most of them were mongrels ($n = 168$) and the others were distributed as follows: Poodle ($n = 15$), Cocker Spaniel ($n = 6$), German Shepherd ($n = 4$), Boxer ($n = 6$), Pointer ($n = 3$), Pekinese ($n = 3$), Rottweiler ($n = 6$), Pit Bull ($n = 4$), Yorkshire ($n = 3$), Beagle ($n = 3$), Siberian Husky ($n = 2$) and Doberman ($n = 1$). The positivity to *Leishmania* antibodies showed different results according to the origin of the animals. Of the 66 animals in the Fortaleza Zoonosis Control Center, 50 (75.75%) were positive for visceral leishmaniasis. All 61 dogs from the Sobral Zoonosis Control Center were positive for visceral leishmaniasis and they were mostly symptomatic for the disease. Of the animals from veterinary clinics ($n = 97$), 32 (32.98%) were positive for visceral leishmaniasis by IFI (Table 1).

At the moment of serum collection, the animals showed at least one of the following clinical signs: onychogryphosis, emaciation, orbital alopecia, apathy and skin lesions with ulcerated margins, especially in the extremities (limbs, nose, ears) (Fig. 1A). Clinical signs were detected in all animals, even though some of them were not positive for *Leishmania* or *Histoplasma* antibodies.

Histoplasma antibodies were detected in 1.78% of the dog serum samples (four dogs) (Fig. 1B), of which three were also positive for leishmaniasis. These dogs were from the Fortaleza Zoonosis Control Center. Another dog was positive only for histoplasmosis and it was from a private veterinary clinic in Fortaleza. No cross-reactions with coccidioidal antibodies were detected. Even though all 61 dogs from the Sobral Zoonosis Control Center were positive for visceral leishmaniasis, none of them were positive for histoplasmosis.

In Brazil, visceral leishmaniasis is caused by the protozoon *Leishmania chagasi* and dogs represent the most important reservoir of the parasite. This parasitic disease is of extreme relevance because of its high prevalence in the country, particularly in the Northeast (Lima et al., 2003; Silva et al., 2007). São Thiago et al. (1998) reported one case of human histoplasmosis, where the patient suffered from typical lesions on the hard palate, similar to cutaneous leishmaniasis. Considering the possibility of initial misdiagnosis because of the clinical similarity between leishmaniasis and histoplasmosis in dogs, we decided to investigate, for the first time, the occurrence of histoplasmosis in dogs that were positive or suspect for this parasitic disease.

The ID technique was chosen because of its low cost, ease of use and high specificity (Guimarães et al., 2006). By way of ID tests, serological response towards two important antigens of *H. capsulatum* can be detected: the M and H antigens. M precipitins are detected earlier in patients suffering from acute pulmonary histoplasmosis and are considered an immunological signature of this disease. H precipitins can be detected in patients with acute and/or progressive disease, although they are present in only 7% of patients with the former clinical presentation. Detection of both the "M" and

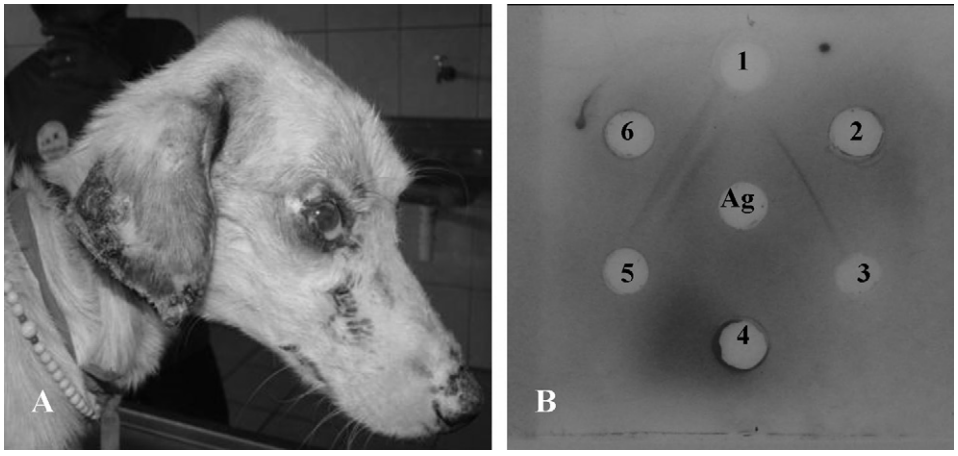

Fig. 1. Male dog, mongrel, positive for leishmaniasis, presenting characteristic skin lesions, such as ulcerated cutaneous lesions on ears and nose. This animal was from the Fortaleza Zoonosis Control Center (A). Demonstrative figure of an immunodiffusion test for detection of antibodies against *H. capsulatum*. Standard fungal antigen preparation (Ag) was placed in the central well and suspect serum samples were deposited in wells 1, 2, 3 and 4. Negative and positive controls were put in wells 5 and 6, respectively. The M precipitin line was detected in the serum sample placed in well 2, which was also positive for leishmania by way of indirect immunofluorescence assay. The positive control showed both M and H bands (B).

"H" bands is highly suggestive of active histoplasmosis, regardless of other immunological tests (Guimarães et al., 2006; Kauffman, 2007). Previous authors have also used ID reactions in epidemiological surveys (Cermeño et al., 2009; Canteros et al., 2010). In comparison to other techniques, such as complement fixation, latex agglutination and ELISA, ID lacks sensitivity, although it can reach 100% specificity (Guimarães et al., 2006). Therefore, the ease and high specificity of ID makes it suitable for epidemiological inquiries. In this study we were able to detect specific antibodies to *H. capsulatum* among three dogs with leishmaniasis.

Even though our results could be underestimated, they represent evidence of histoplasmosis among the investigated animals. Further studies should be conducted in order to determine the actual prevalence of histoplasmosis and leishmaniasis coinfection among dogs in Brazil.

Among the clinical findings of the canine population studied, the great frequency of cutaneous lesions suggests the need to perform differential diagnosis between these infections, because in dogs both histoplasmosis and leishmaniasis can be manifested with nodular or ulcerated cutaneous lesions on the ears, nose and limbs (Ueda et al., 2003; Bromel and Sykes, 2005; Massunari et al., 2009). Additionally, laboratory tests can present results that are similar for both diseases, such as non-regenerative normocytic and normochromic anemia, thrombocytopenia, hypoalbuminemia, hyperglobulinemia, azotemia and increased enzymatic activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Concomitant unspecific clinical signs, such as lethargy, adenomegaly, hepatosplenomegaly and diarrhea, were observed in many of the animals assessed in this study. Additionally, fever and anorexia were observed in a few of them. We observed that respiratory signs are not always present (Lima et al., 2003; Bromel and Sykes, 2005). In our study, many dogs presented skin lesions that were highly suggestive of leishmaniasis, but these lesions could easily be mistaken for nodular or ulcerated cutaneous histoplasmosis (Ueda et al., 2003; Bromel and Sykes, 2005).

In the present study immunological proof of the coexistence of histoplasmosis and leishmaniasis in dogs living in urban areas was shown for the first time. Veterinarians should be aware of this possibility and consider it when establishing diagnosis, especially in animals from regions considered endemic for both diseases. The results presented here indicate the need for further research into the real prevalence of histoplasmosis and leishmaniasis coinfection among dogs.

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