Paracoccidioides brasiliensis infection in dogs from Western Brazilian Amazon[1]

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ABSTRACT.- Corte A.C., Gennari S.M., Labruna M.B., Camargo L.M.A., Itano E.N., Freire R.L., Camargo Z.P. & Ono M.A. 2012. ***Paracoccidioides brasiliensis* infection in dogs from Western Brazilian Amazon.** *Pesquisa Veterinária Brasileira 32(7):649-652.* Departamento de Ciências Patológicas, Universidade Estadual de Londrina, Londrina, PR 86051-980, Brazil. E-mail: marioono@uel.br

The objective of the study was to evaluate *Paracoccidioides brasiliensis* infection in urban dogs from the municipality of Monte Negro, Rondonia, Western Brazilian Amazon. The serum samples (n=126) were analyzed by indirect ELISA and the immunodiffusion test using *P. brasiliensis* gp43 and exoantigen as antigens, respectively. A positivity of 54.8% was observed only in the ELISA test and no statistical difference was observed in the seroprevalence in relation to age or sex. This is the first paracoccidioidomycosis survey carried out with dogs from the Western Brazilian Amazon. The higher positivity rates of *P. brasiliensis* infection observed in this study suggest that veterinarians must be alert to detect new cases of natural disease in dogs living in paracoccidioidomycosis endemic areas.

INDEX TERMS: *Paracoccidioides brasiliensis*, paracoccidioidomycosis, epidemiology, Amazon, dogs.

RESUMO.- [**Infecção por *Paracoccidioides brasiliensis* em cães da Amazônia Ocidental Brasileira.**] O objetivo deste estudo foi avaliar a infecção por *Paracoccidioides brasiliensis* em cães urbanos do município de Monte Negro, Rondônia, Amazônia Ocidental Brasileira. As amostras de soro (n=126) foram analisadas por meio dos testes de ELISA indireto e imunodifusão utilizando gp43 de *P. brasiliensis* e exoantígeno como antígenos, respectivamente. Uma positividade de 54,8% foi observada apé isso mesmoenas no teste de ELISA e nenhuma diferença estatística foi observada na soroprevalência em relação ao sexo ou à idade. Este é o primeiro estudo epidemiológico de paracoccidioidomicose realizado com cães da Amazônia Ocidental Brasileira. A alta positividade de infecção por *P. brasiliensis* observada neste estudo sugere que os veterinários devem estar atentos para detectar a doença natural em cães de áreas endêmicas para paracoccidioidomicose.

TERMOS DE INDEXAÇÃO: *Paracoccidioides brasiliensis*, paracoccidioidomicose, epidemiologia, Amazônia, canino.

INTRODUCTION

Paracoccidioides brasiliensis is the ethiological agent of paracoccidioidomycosis, a systemic granulomatous mycosis that mainly affects rural workers living in Latin American countries (Brummer et al. 1993). The infection occurs by inhalation of fungus propagules while working with the soil, the probable habitat of *P. brasiliensis* (Negroni 1966, Albornoz 1971, Restrepo, 1985, Silva-Vergara et al. 1998, Terçarioli et al. 2007).

Epidemiological studies have shown that paracoccidioidomycosis occurs in several species of domestic and wild animals such as dogs (Mós & Fava-Netto 1974, Ono et al. 2001, Silveira et al. 2006, Canteros et al. 2010, Fontana et al. 2010), cows (Silveira et al. 2008), horses (Conti Diaz et al. 1972, Costa & Fava-Netto 1978, Corte et al. 2009), armadillos (Fernandes et al. 2004), sheep (Costa & Fava-Netto 1978, Oliveira et al. 2011), monkeys (Corte et al. 2007),

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guinea pigs, raccoons, grisons, porcupine (Richini-Pereira et al. 2008) and chickens (Gonçalves et al. 2011). The fungus was also isolated from armadillos (Naiff et al. 1986, Bagagli et al. 1998, Corredor et al. 1999, Silva-Vergara et al. 2000) penguin faeces (Gezuele 1989), and recently, natural paracoccidioidomycosis was reported in two dogs (Ricci et al. 2004, Farias et al. 2011), a cat (Gonzalez et al. 2010) and a two-toed sloth (Trejo-Chávez et al. 2010).

The first experimental study with paracoccidioidomycosis in dogs was reported in 1911 (Pereira & Vianna 1911). Several decades later the natural infection by *P. brasiliensis* was reported in dogs from São Paulo (74.3-78.2%), Paraná (14.8-89.5%), Mato Grosso do Sul (67.8%), Minas Gerais (53.7%), in Brazil (Mós & Fava-Netto 1974, Ono et al. 2001, Silveira et al. 2006, Fontana et al. 2010) and Argentina (2.2%) (Canteros et al. 2010).

Taking into account that *P. brasiliensis* probably lives in soil as a saprobe, the dog's habit of sniffing and digging the soil could expose the animal to *P. brasiliensis* infection (Ono et al. 2001).

The objective of this study was to evaluate the infection by *P. brasiliensis* in dogs from Monte Negro, a municipality located in the Western Amazonian Region, Brazil.

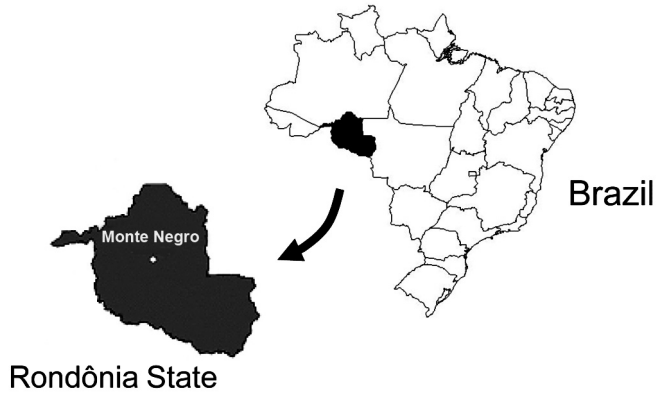

Fig.1. Map showing the municipality of Monte Negro in the state of Rondônia, Brazil.

MATERIALS AND METHODS

Study area

The municipality of Monte Negro is located in Rondônia State, in the Western Region of the Brazilian Amazon (10°18' S, 63°14' W). The climate is hot and humid (25-29°C) with two hallmark seasons, the rainy season (October to April 1440 mm mean rainfall) and the dry season (June to August, 559 mm mean rainfall).

Animals

Blood samples were collected by venipuncture from 126 dogs (72 males and 54 females) living in the urban area of Monte Negro Municipality and at least one animal was sampled in the 85 blocks with dogs. The animals were classified as puppy (<1 year), young (1-2 years) and adult (>2 years).

Paracoccidioides brasiliensis antigens

Exoantigen.The exoantigen was obtained as previously described (Camargo et al. 1988), using the *P. brasiliensis* isolate B-339.

Gp43 antigen. The gp43 antigen was purified from *P. brasiliensis* exoantigen by immunoaffinity chromatography as previously described (Puccia and Travassos 1991) and the protein concentration was determined by the Bradford method using BSA as standard (Bradford 1976).

Immunodiffusion test

The serum samples from *P. brasiliensis* immunized animals were analyzed by the immunodiffusion test as previously described (Camargo et al. 1988) using *P. brasiliensis* exoantigen as reagent.

ELISA with gp43

The 126 serum samples from urban dogs were analyzed by ELISA using gp43 as antigen. Flat bottom Microtitre polystyrene plates (Costar Corporation, Corning, NY,USA) were coated at 4°C overnight with 250 ng well of gp43 in carbonate buffer, pH 9.6. After washing with PBS-T (PBS with Tween 20) the wells were blocked with 2% skim milk in PBS for 1 hour at 37°C. After washing with PBS-T the serum samples, diluted 1:100 in PBS-1% skim milk were incubated at 25°C for 1 hour. The plates were washed with PBS-T and the anti-dog IgG-peroxidase conjugate (Sigma, St Louis, MO, USA) was added followed by incubation for 1 hour at 37°C. After washing with PBS-T the substrate-chromogen solution (H_2O_2/TMB) was added. The reaction was stopped by adding H_2SO_4 4N (50µl/well). The absorbance (450 nm) was analyzed in a Microplate Reader (Biotek Instruments Inc., Winooski, VT, USA). All serum samples were analyzed twice. The positive and negative controls were a serum sample from a dog immunized with *P. brasiliensis* and a pool of sera from young urban dogs, respectively. Sera with two-fold or more the absorbance of the negative control were considered positive.

Statistical analysis

The statistical analysis was performed with the program EpiInfo® 6.0 (Dean et al. 1994) using the chi-square test and Yates´ qui-square test. The difference was considered significant when P was less than 0.05.

RESULTS

A 54.8% positivity was observed in the ELISA test although no reactivity was detected by the immunodiffusion test and the positivity rates observed in males (52.7%) and females (57.6%) were not significantly different (Table 1).

No statistical difference was observed in relation to age in puppies (37.5%) compared to adults (57%) and young animals (58.8%) (Table 1).

Table 1. Reactivity to *Paracoccidioides brasiliensis* in 126 serum samples from urban dogs evaluated by ELISA and Immunodiffusion (ID), according to sex and age

	Positivity n (%)	
	ELISA	ID
Male	39 (52.7)	0 (0)
Female	30 (57.6)	0 (0)
Adult	53 (57.0)	0 (0)
Young	10 (58.8)	0 (0)
Puppy	6 (37.5)	0 (0)
TOTAL	69 (54.8)	0 (0)

DISCUSSION

Infection by *Paracoccidioides brasiliensis* of domestic and wild animals has been observed in epidemiological studies using immunological and molecular methods. In this study the infection by *P. brasiliensis* was evaluated in dogs from the Brazilian Western Amazonian region.

The positivity observed by ELISA in this study is similar to other seroepidemiological studies of paracoccidioidomycosis in urban dogs from Paraná (Ono et al. 2001) and Minas Gerais (Fontana et al. 2010) Brazilian states, which reported positivity rates of 51.5% and 53.7%, respectively.

The immunodiffusion with exoantigen is the test of choice for diagnosis of paracoccidioidomycosis in humans (Camargo 2008). The lack of reactivity observed in the immunodiffusion test probably is due its lower sensitivity when compared with ELISA and suggests that dogs were infected by *P. brasiliensis* but without disease as observed in other seroepidemiological studies (Ono et al. 2001, Fontana et al. 2010). Individuals living in paracoccidioidomycosis endemic areas are frequently infected by *P. brasiliensis* although most of them will not develop the disease (Franco 1986).

No significant difference was observed in relation to sex as observed in other seroepidemiological studies carried out by our group to evaluate paracoccidioiomycosis infection in dogs, monkeys and chickens (Ono et al. 2001, Silveira et al. 2006, Corte et al. 2007, Oliveira et al. 2011).

Although no statistical difference was observed in relation to age a trend of lower seroprevalence was observed in puppies. In another study carried out by our group to evaluate infection by *P. brasiliensis* in urban dogs from Mato Grosso do Sul, a significantly lower positivity was observed in dogs under one year old (Silveira et al. 2006). In studies on experimental paracoccidioidomycosis in dogs, puppies were more susceptible to developing paracocccidioidomycosis than adults (Ono et al. 2003, Eisele et al. 2004).

This is the first study of *P. brasiliensis* infection in dogs from the Western Brazilian Amazon. The higher positivity rates of *P. brasiliensis* infection observed in this study reinforces that dog is a useful and sensitive epidemiological marker of paracoccidioidomycosis and suggest that veterinarians must be alert to detect new cases of natural disease in dogs living in paracoccidioidomycosis endemic areas.

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