

SEROLOGICAL EVIDENCE OF EXPOSURE TO *Ehrlichia canis* IN CATS

EVIDÊNCIAS SOROLÓGICAS DA EXPOSIÇÃO DE GATOS A Ehrlichia canis

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Abstract

The aim of the present study was to estimate the occurrence of *Ehrlichia canis* in cats from the semiarid region of Northeast of Brazil. Sera of 101 healthy cats were submitted by Indirect Immunofluorescence Assay (IFA), and considered positive when antibody titers ≥ 40 were obtained. Seroprevalence of 35.6% (36/101) was found, with the following titers: 40 (15 animals); 160 (6); 320 (1); 640 (3), and 2,560 (11). No statistical differences were observed when comparing county of origin, gender, age, breed, and modus vivendi (pet and stray cats), and no ticks were observed in any of the cats. This study revealed exposure to *E. canis* in cats of the Semiarid Northeast of Brazil.

Keywords: Caatinga; ehrlichiosis; feline; hemoparasite; semiarid.

Resumo

O objetivo do presente estudo foi estimar a ocorrência de *Ehrlichia canis* em gatos na região semiárida do Nordeste. Soros de 101 gatos saudáveis foram submetidos à Reação de Imunofluorescência Indireta (RIFI), sendo considerados positivos títulos ≥ 40 . Soroprevalência de 35,6% (36/101) foi encontrada, com os seguintes títulos: 40 (15 animais); 160 (6); 320 (1); 640 (3) e 2560 (11). Não foram observadas diferenças estatísticas quanto ao município de origem, gênero, faixa etária, raça e *modus vivendi* (gatos domiciliados e errantes) e não foram observados carrapatos em qualquer um dos animais testados. Este estudo revelou exposição de gatos a *E. canis* na região semiárida do nordeste brasileiro.

Palavras-chave: Caatinga; erliquiose; felinos; hemoparasita; semiárido.

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Introduction

Ehrlichia sp. is a gram-negative, pleomorphic, obligate intracellular bacteria, belonging to Anaplasmataceae family, Rickettsiales order, that affects leukocytes and thrombocytes, and potentially

infects a large variety of mammal species⁽¹⁾. Transmission to the host occurs predominantly via vector through the bite of an infected tick, an event that can be attributed to the high prevalence of ehrlichiosis in tropical and subtropical regions, due to the geographical distribution of vectors associated to transmissibility⁽²⁾.

Indirect Immunofluorescence Assay (IFA) is traditionally used for the diagnosis of human and canine ehrlichiosis⁽³⁾, and can also be used for diagnosis of feline ehrlichiosis⁽⁴⁾.

The first evidence for naturally occurring ehrlichiosis in cats was provided in 1986 by Charpentier and Groulade in France⁽⁵⁾. In Brazil, the first report was in 1998, by hematoscopy, through morulae observation of *Ehrlichia* sp. in leukocytes of a cat with clinical signs similar to those described in dogs with this disease⁽⁶⁾.

Ehrlichia sp. has been described in wild and domestic felids in various regions of the world, through hematoscopy, serology, and molecular techniques, feline ehrlichiosis being caused especially by *E. canis*⁽⁷⁻¹⁶⁾. However, most surveys regarding *E. canis* occur in dogs and the reports about its occurrence in cats still remain scarce⁽⁴⁾.

Rhipicephalus sanguineus is the most important tick that parasitizes dogs in Brazil⁽¹⁷⁾. Although rarely found in cats, this tick is seen as the main vector of feline ehrlichiosis⁽¹⁸⁾. Predominant signs reported in cats include fever, anorexia, and lethargy. Myalgia, dyspnea, anemia, thrombocytopenia, and pancytopenia have been described as well. However, in most cases, the cats are asymptomatic^(8, 19).

Little is known about the exposure of the Brazilian feline population to *E. canis* because most studies focus on canine infections⁽²⁰⁾. The present study aimed to verify the occurrence of anti-*E. canis* antibodies in cats and the possible associated risk factors in the counties of Juazeiro, Bahia State (BA), and Petrolina, Pernambuco State (PE), located in the middle region of the São Francisco Valley, Semiarid of Northeast of Brazil, belonging to Petrolina-Juazeiro Pole of the Integrated Network for Economic Development.

Materials and methods

This cross-sectional study was conducted in the counties of Juazeiro, BA (9° 24' 42" S; 40° 29' 55" W) and Petrolina, PE (9° 23' 55" S, 40° 30' 3" W), semiarid tropical weather and Caatinga biome region.

From September 2012 to July 2013, convenience sampling was conducted, corresponding to 101 clinically healthy cats without restriction of age, breed or sex, originated from routine vaccines or other periodic control practices of a veterinary clinic, and animals from the Center of Zoonosis Control of both counties.

Peripheral blood samples were collected from the jugular, left or right cephalic vein, in dried tubes, properly identified, then centrifuged at 3,500 rpm for 10 min to obtain the serum, within 24 hours after collected. Sera were stored in 1.5 mL microtubes at -20 °C until examination. Information about county of origin, sex, age, breed, and modus vivendi (pet and stray cat) were obtained. The samples were collected following the ethical standards of animal experimentation established by the Committee of Ethics and Deontology Studies and Research of the Federal University of São Francisco Valley (protocol number 11/161012).

The occurrence of anti-*E. canis* IgG antibodies was assessed by IFA, using *E. canis* strain Cuiabá 16⁽²¹⁾ as antigen with the cut-off point at an initial dilution of 1:40^(9,22,23). Commercial fluorescein

isothiocyanate-conjugated anti-cat IgG (Sigma-Aldrich, USA) was used as conjugate at a dilution of 1:1000, and the antigen preparation and IFA technique were performed as previously described⁽²⁴⁾. Both positive and negative control sera were included in each assay.

Statistical evaluation was carried out by the Chi-Square test (X^2) or Fischer's exact test with a 95% confidence interval.

Results

Among the 101 sampled animals, 56 (55.4%) cats were from Juazeiro (BA), while 45 (44.6%) were from Petrolina (PE); 59 (58.4%) were female and 42 (41.6%) were male. A total of 63 (62.4%) cats were kittens (12 months of age or less) and 38 (37.6%) were adults, among whom there were 4 seniors (over 7 years old); 95 (94.1%) were of undefined breed, while six (5.9%) were Siamese breed; 47 (46.5%) were pets, versus 54 (53.5%) stray cats. Seroprevalence of cats for *E. canis* in this study was 35.6%, and the positive samples showed the following titers: 40 (15 animals); 160 (6); 320 (1); 640 (3), and 2,560 (11) (Table 1).

Table 1. Titers of anti-*Ehrlichia canis* antibodies in cats from semiarid region of northeastern Brazil obtained by Indirect Immunofluorescence Assay from September 2012 to July 2013

IFA titers	<i>n</i>	%
<i>Negative</i>	65	64.3
40	15	14.9
160	6	5.9
320	1	1.0
640	3	3.0
2560	11	10.9
Total	101	100%

No significant differences ($p > 0.05$) were found for any of the analyzed variables (Table 2).

Table 2. *Ehrlichia canis* serology in cats from the semiarid northeast Brazil from September 2012 to July 2013: county of origin, gender, age, breed and modus vivendi

Epidemiological aspects	Tested animals		Seropositive animals		P
	<i>N</i>	%	<i>n</i>	%	
County of origin					
Juazeiro, BA	56	55.4	18	32.1	0.541
Petrolina, PE	45	44.6	18	40.0	
Gender					
Male	42	41.6	14	33.3	0.842
Female	59	58.4	22	37.3	
Age					
Younger (≤ 12 months)	63	62.4	18	28.6	0.089
Adults (> 12 months)	38	37.6	18	47.4	
Breed					
Undefined	95	94.1	34	35.8	0.750
Defined	6	5.9	2	33.3	
Modus vivendi					
Pet	47	46.5	13	27.7	0.175
Stray	54	53.5	23	42.6	

Discussion

IFA results obtained in the present study are in accordance with Braga et al.⁽²³⁾, who found seropositivity of 41.5% to *E. canis* (cut-off 1:40) among 93 pet cats from Cuiabá, Mato Grosso State (tropical wet and dry climate and Cerrado biome region). This similarity suggests the dissemination of the agent within the host population may be associated with abiotic factors (climate), whereas the higher temperature favors the dispersion of ticks and, hence, the vector-borne diseases^(2,25).

Braga et al.⁽¹³⁾ found 5.5% of individuals reagent (cut-off 1:64) to the same agent among 200 pet cats in São Luís, Maranhão State (tropical monsoon climate region, palm tree forest biome). This prevalence is in agreement with the canine ehrlichiosis profile at the locality⁽²⁰⁾, indicating that the epidemiological behavior of feline ehrlichiosis is similar to the canine ehrlichiosis in that region, as described in Cuiabá by Braga et al.⁽¹⁴⁾. We found the same association comparing seropositivity in cats (present study) and dogs⁽²⁰⁾ at matching geographic location. Related epidemiological profile between cats and dogs was also found in Central Italy⁽²²⁾ and Portugal^(16,26,27). This association suggests once more that the major factor implicated in ehrlichiosis prevalence is that associated to the vector biology in a tick-free environment rather than host factors, including host species⁽²⁸⁾.

However, other reports found lower rates in cats than those detected in dogs, conjecturing that cats might be more resistant to ehrlichiosis⁽²⁹⁾, or cats are infected less frequently as they remove ticks during self-cleaning⁽²²⁾.

André et al.⁽³⁰⁾ detected positivity of 21% in *Ehrlichia* spp. in wild captive felids in Brazil, among which 52% contained ehrlichial DNA closely related to *E. chaffeensis*, a species associated to human monocytic ehrlichiosis⁽³¹⁾.

None of the cats were parasitized by ticks at the time of blood collection; although, some owners have reported the presence of ticks on their animals elsewhere. Exposure to ticks has been reported in about 30% of feline ehrlichiosis cases⁽³²⁾. In the present survey, information about vector exposure is not complete. This lack of data precludes definitive assertions regarding this association.

None of cats in the present study presented clinical signs, such as those found by Correa et al.⁽³³⁾. It is known that clinical cases of ehrlichiosis in these animals are not common⁽⁸⁾. This aspect suggests that the felines might act as potential asymptomatic reservoirs and sentinels of *E. canis* and other vector-borne agents^(16,33).

No statistical differences were observed when comparing the presence of antibodies in cats according to the county of origin, gender, age, breed, and modus vivendi, similarly to previous reports^(14,22). However, the high frequency of positive adult and stray cats demonstrated in this work should be explained by the higher time of exposure and higher possibility of contact with the vectors, respectively.

Conclusion

These results revealed the cats of Semiarid Northeast of Brazil were exposed to *E. canis*. Considering this fact, adopting effective prophylaxis and control measures against the vector becomes necessary to prevent infection of cats by *E. canis* and other vector-borne diseases, as well as their potential transmission to other animal species and to human beings.

Further investigations about *E. canis* and other vector-borne agents are needed to better understand

and define the role of cats in the epidemiology of ehrlichiosis.

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