

Research Paper

Detection of *Ehrlichia canis* in domestic cats in the central-western region of Brazil

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Abstract

Ehrlichiosis is a worldwide distributed disease caused by different bacteria of the *Ehrlichia* genus that are transmitted by arthropod vectors. Its occurrence in dogs is considered endemic in several regions of Brazil. Regarding cats, however, few studies have been done and, consequently, there is not enough data available. In order to detect *Ehrlichia* spp. in cats from the central-western region of Brazil, blood and serum samples were collected from a regional population of 212 individuals originated from the cities of Cuiabá and Várzea Grande. These animals were tested by the Immunofluorescence Assay (IFA) and the Polymerase Chain Reaction (PCR) designed to amplify a 409 bp fragment of the *dsb* gene. The results obtained show that 88 (41.5%) cats were seropositive by IFA and 20 (9.4%) cats were positive by PCR. The partial DNA sequence obtained from PCR products yielded twenty samples that were found to match perfectly the *Ehrlichia canis* sequences deposited on GenBank. The natural transmission of *Ehrlichia* in cats has not been fully established. Furthermore, tick infestation was not observed in the evaluated cats and was not observed any association between age, gender and positivity of cats in both tests. The present study reports the first serological and molecular detection of *E. canis* in domestic cats located in the endemic area previously mentioned.

Key words: Ehrlichiosis, feline, PCR, IFA, ticks.

Introduction

Ehrlichiosis is a worldwide distributed disease caused by microorganisms of *Ehrlichia* genus that is transmitted by arthropod vectors (Groves *et al.*, 1975; Couto, 1998). *Ehrlichia* spp. belongs to the Rickettsiales order and the Anaplasmataceae family (Dumler *et al.*, 2001; Paddock and Childs, 2003). It is an exclusive Gram-negative intracellular parasite, located in cytoplasmic vacuoles of mature or immature hematopoietic and endothelial cells, and found in peripheral blood or tissue (Unver *et al.*, 2001).

Some domestic and wild mammals, such as canines and ruminants, are reservoirs of *Ehrlichia* spp., but domes-

tic cats also can be considered as reservoirs for the agent and infection source for other animals, including humans (Stubbs *et al.*, 2000). The first case of feline ehrlichiosis was described in the 80's (Charpentier and Groulade, 1986) and, since then, several cases of this ehrlichiosis have been reported (Stubbs *et al.*, 2000). *Ehrlichia* species that naturally infect cats were not totally characterized. Few studies in order to clarify the *Ehrlichia* species that infect cats were conducted, but monocytic, lymphocytic and, granulocytic inclusions were detected in cats with febrile illness and thrombocytopenia suggesting that other rickettsial species could act on feline ehrlichiosis infection.

In Brazil, *Ehrlichia* infection was first reported by Almosny and Massard (1999) throughout the observation of ehrlichial morula in blood smears, and in 2009 it was first detected by PCR by Oliveira *et al.* (2009), both studies accomplished in southeast region of Brazil. Recently, Braga *et al.* (2010) reported the first surveillance of feline ehrlichiosis in a free-living, healthy cat population in the northeast region of Brazil.

In addition, natural transmission of *Ehrlichia* in cats has not been fully established (Amyx and Huxsoll, 1997). However, *Rhipicephalus sanguineus* tick demonstrates wide geographical distribution in Brazilian urban areas (Labruna and Pereira, 2001) and it was previously reported in cats from the northeast region of Brazil (Ferreira *et al.*, 2009; Ferreira *et al.*, 2010), what suggests the transmission of *Ehrlichia* by *R. sanguineus*.

This study investigates the presence of *Ehrlichia* spp. in domestic cats from the metropolitan region of Cuiabá, which is an endemic area to these bacteria in Mato Grosso state.

Materials and Methods

Between May and December 2011, 212 whole blood EDTA and serum samples were collected from domestic cats. The samples were collected in animals originally from the Zoonosis Control Center (ZCC) in Cuiabá (15°35'56" S 56°06'01" W) and in Várzea Grande (15°38'49" S; 56°07'58" W) cities (IBGE, 2010) (Figure 1), from animals treated in the Veterinary Hospital of the Federal University of Mato Grosso State (HOVET-UFMT) and also from animals that lived in a shelter for dogs and cats. All samples were stored at -20 °C until analysis. Samples collection was in agreement with the Ethical Principles for Animal Research established by the Brazilian Society of Science in Animals of Laboratory (SBCAL) under the institutional Committee for Ethics in Animal Research (protocol number UFMT 23108.017751/11-7).

The collected blood samples were subjected to a DNA extraction using the Axyprep Blood Genomic DNA Miniprep Kit (Axygen Biosciences, Zhejiang Province, Hangzhou, China). The DNA was then used as a template for a PCR assay with primers *dsb*-330 (5'-GAT GAT GTC TGA AGA TAT GAA ACA AAT -3') and *dsb*-728 (5'-CTG CTC GTC TAT TTT ACT TCT TAA AGT -3'), designed to amplify a 409-bp fragment of the *dsb* gene of *Ehrlichia* spp., following Doyle *et al.* (2005). PCR negative control (ultrapure - MilliQ®) and positive control (DNA from São Paulo strain of *E. canis* obtained from DH82 culture) were used in each reaction.

After that, amplicons were purified using illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare Bio-Sciences, England, Buckinghamshire, UK), and subjected to DNA sequencing using the Big Dye Kit (Applied Biosystems/Perkin Elmer, Foster City, California, USA) on an ABI-PRISM 3100 Genetic Analyzer.

Both products were used in accordance with the manufacturer's instructions. The sequences were evaluated using the SeqMan software (Lasergene, DNASTar, Madison, WI USA.) and similarity was analyzed using the program Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1990) to check for homology with corresponding sequences available on GenBank.

The presence of anti-*Ehrlichia* spp. antibodies was evaluated by the Immunofluorescence Assay (IFA) using São Paulo strain of *E. canis* as antigen with a cut-off point at an initial dilution of 1:40 (Aguirre *et al.*, 2004). Commercial fluorescein isothiocyanate-conjugated anti-cat IgG (Sigma-Aldrich, Saint Louis, Missouri) was used as conjugate at dilution of 1:1000. The antigen preparation and the IFA were executed as previously described (Aguar *et al.*, 2007). For IFA standardization, serums of PCR positive cats were tested to be used as positive control and a previously non-reactive serum (negative control) was included in each assay.

Data concerning age, sex and tick parasitism in cats were used for risk factor analysis. To define age, cats with corporal mass less than 1.5kg were considered young and cats weighing 1.5 kg or more were considered adults based on their dental arcade, according to study of Sharif *et al.* (2007).

The association between *Ehrlichia* spp. infection in cats and independent variables was performed by Qui-Square test (χ^2) or Fisher exact test when applicable. Analysis of the association among different IFA endpoint titers and positive or negative PCR cats were performed by Wilcoxon test. The statistical software EPIINFO 3.5.3 was used for this analysis and $p \leq 0.05$ was considered significant.

Results

Among the 212 evaluated cats, 93 (43.8%) of the samples were from HOVET, 31 (14.7%) were from the mentioned shelter, 23 (10.8%) and 65 (30.7%) were from the ZCC of Cuiabá and Várzea Grande, respectively. One hundred two animals (48.1%) were female and 110 (51.9%) male. Fifty nine (28.6%) were young and 147 (71.4%) were adult. Age information was not available for six cats.

Twenty (9.4%) cats were PCR positive for *Ehrlichia* spp. and nucleotide sequences generated in PCR were identical to each other and between sequences of *E. canis* Uberlandia (GU586135) and *E. canis* str. Jake (CP000107), deposited on Genbank. According to their origin, 8 (2.6%) positive cats were from HOVET, 1 (4.3%) from the ZCC of Cuiabá and 11 (16.9%) from the ZCC of Várzea Grande. No samples from shelter cats were positive on PCR ($p \leq 0.05$). Eight (40.0%) of the positive animals were young and 12 (60.0%) were adults ($p > 0.05$). Both gender

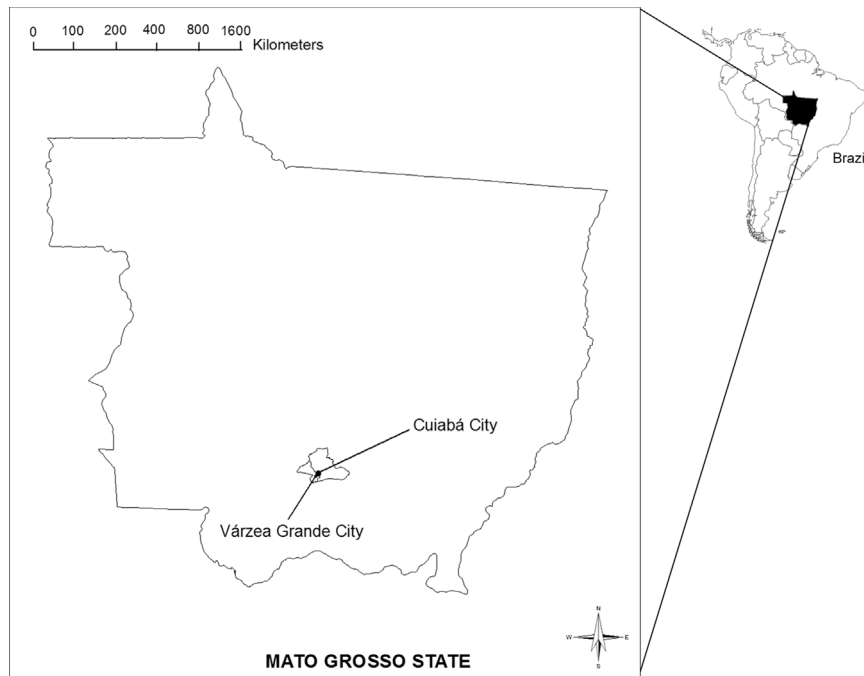


Figure 1 - Location of Cuiabá and Várzea Grande cities, Mato Grosso State. Cuiabá, 2013.

had the same proportion (50.0%; $p > 0.05$) of positive samples.

Serologic tests resulted in 88 (41.5%) seropositive cats for *E. canis*. Forty-two (45.1%) cats were from HOVET, 3 (13.0%) from the ZCC of Cuiabá, 27 (41.5%) from the ZCC of Várzea Grande and 16 (51.6%) from the shelter ($p \leq 0.05$). Twenty-six (31.7%) cats were young and 59 (71.9%) were adults. Forty-five (51.1%) seropositive samples were from female and 43 (48.9%) from male animals ($p > 0.05$). Antibody titers ranged from 40 to 40960. Two cats (2.2%) had titers of 40, 14 (16.0%) cats of 80, 31 (35.2%) of 160, 15 (17.0%) of 320, 14 (16.0%) of 640, 10 (11.4%) of 1280, one (1.1%) of 10240, and one (1.1%) of 40960. No significant association was observed between IFA antibodies titles and PCR result.

Twelve (5.6%) cats were positive for both molecular and serological tests and eight PCR-positive cats were negative by serology ($p > 0.05$). No ticks were found in the evaluated cats.

Discussion

Research about the presence of *Ehrlichia* spp. in domestic cats has been conducted in some countries, such as Spain (Aguirre *et al.*, 2004), Sweden (Bjoersdorff *et al.*, 1999), France (Beaufils *et al.*, 1995), United States (Bouloy *et al.*, 1994), and Kenya (Buoro *et al.*, 1989). In particular, the present study detected the highest frequency of *Ehrlichia* spp. infection in domestic cats in Brazil.

Seroprevalence detected evidence of exposure to *Ehrlichia* spp. in a feline population in the central-western region of Brazil. However, the occurrence of cross-reac-

tivity with other Anaplasmataceae family agents cannot be ruled out (Wen *et al.*, 1997). Amplified DNA samples analyzed by sequencing reactions were identical to the sequences of *E. canis* available on GenBank, what suggests that a single strain of *E. canis* was infecting dogs and cats, collaborating with the findings of Oliveira *et al.* (2009). The central-western region of Brazil was considered to be endemic for *E. canis* in dogs (Silva *et al.*, 2010; Sousa *et al.*, 2010; Melo *et al.*, 2011). Furthermore, *E. canis* DNA was detected in *R. sanguineus* ticks, assuming potential risk factor for the disease (Almeida *et al.*, 2012).

Ehrlichia is transmitted through the bite of an infected tick, mainly in tropical and subtropical regions (Rodriguez-Vivas *et al.*, 2005). Although tick exposure has been reported in about 30% of feline ehrlichiosis cases (Lappin, 2001), reports of *R. sanguineus* parasitism in cats are rare, probably due to their licking habit and to common prophylactic measures (Breitschwerdt *et al.*, 2002). Moreover, the absence of ticks in the evaluated cats can suggest the existence of an unknown vector acting as the transmission agent in cats.

Negative PCR results in seropositive cats show the lack of *E. canis* in peripheral blood, during subclinical or chronic phases of the disease, despite their presence in spleen or other lymphoid tissue (Eberhardt *et al.*, 2006). However no association was found among different IFA antibody endpoint titers and PCR positive or negative cats. Little is known about the dynamics of *E. canis* infection in cats, so any presumption about rickettsemia in cats is speculative. The positive result in both tests leads to the assumption that these animals were possibly in the acute or

asymptomatic phase of the infection, corresponding to the period of seroconversion. Other potential hypothesis is that these animals would be through a period of resurgence as a result of immunosuppression.

No association between age and sex was found, minimizing the effect of social behavior in the epidemiology of infection in the considered region. These results are similar to the findings of Stubbs *et al.* (2000) related to age, except that they found gender predominance with higher female positivity.

Regarding the origin of cats, animals from the ZCC Várzea Grande had a higher frequency of positive PCR and the second highest frequency of anti-*E. canis* antibodies. This fact is probably due to the peculiar characteristics of the area, which contribute to expose these animals to infection. Shelter cats had a higher frequency of anti-*E. canis* antibodies, what implies that these animals were more exposed to the agent, possibly by being in direct contact with *R. sanguineus* of infected dogs. Nevertheless the study contributes to other studies that suggest the role of domestic cats as reservoirs of *E. canis*.

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