Molecular and serological detection of *Ehrlichia* spp. in cats on São Luís Island, Maranhão, Brazil

Detecção molecular e sorológica de *Ehrlichia* spp. em gatos da ilha de São Luís, Maranhão, Brasil

Maria do Socorro Costa de Oliveira Braga; Marcos Rogério André; Carla Roberta Freschi; Márcia Cristina Alves Teixeira; Rosangela Zacarias Machado

1Universidade Estadual do Maranhão – UEMA
2Universidade Estadual Paulista – UNESP

Received April 26, 2011
Accepted July 12, 2011

**Abstract**

Ehrlichiosis is a tick-borne disease that affects both humans and animals. The few existing reports on ehrlichiosis in Brazilian cats have been based on observation of morulae in leukocytes and, more recently, on molecular detection of *Ehrlichia* sp. In this study, we assessed occurrences of *Ehrlichia* sp. in the blood of 200 domestic cats in São Luis, Maranhão. Of the 200 animals tested, 11 (5.5%) were seropositive for *Ehrlichia* sp. and two (1%) were positive for *Ehrlichia* sp. in PCR. We also performed DNA sequence alignment to establish the identity of the parasite species infecting these animals, using the gene 16S rRNA. One cat presented infection with *Ehrlichia* sp. with 98% identity with *E. canis*, and another cat infected with *Ehrlichia* sp. showed 97% identity with *E. chaffeensis*. This is the first study on molecular detection of *Ehrlichia* sp. among domestic cats in São Luis, Maranhão.

**Keywords:** Cats, *Ehrlichia* sp., serology, PCR, Brazil.

**Resumo**

Erliquiose é uma enfermidade transmitida por carrapatos que afeta seres humanos e animais. Os poucos relatos de erliquiose em gatos, no Brasil, são baseados na observação de mórulas em leucócitos e, mais recentemente, na detecção molecular de *Ehrlichia* sp. Neste estudo, foi avaliada a ocorrência de *Ehrlichia* sp. no sangue de 200 gatos de São Luís, Maranhão. Dos 200 animais testados, 11 (5,5%) foram soropositivos para *Ehrlichia* sp. e dois (1%) foram positivos na PCR para *Ehrlichia* spp. O alinhamento de sequências de DNA baseado no gene 16S rRNA foi conduzido para estabelecer a identidade da espécie de parasito que infectou estes animais. Um gato apresentou infecção por uma espécie de *Ehrlichia* sp. com 98% de identidade com *E. canis*; e outro mostrou-se infectado por *Ehrlichia* sp. com 97% de identidade com *E. chaffeensis*. Este estudo traz a primeira detecção molecular de *Ehrlichia* sp. em gatos de São Luís, Maranhão.

**Palavras-chave:** Gatos, *Ehrlichia* sp., sorologia, PCR, Brasil.

**Introduction**

Ehrlichiosis is an emerging tick-borne disease that affects both humans and animals (WALKER; DUMLER, 1996). The clinical signs and abnormal laboratory findings relating to ehrlichiosis are similar in felids and canids (ALMOSNY et al., 1998). Suggestive morulae from agents in the family Anaplasmataceae have been detected among cats in France (BEAUFILS et al., 1999), Sweden (BJÖERSDORFF et al., 1999) and Italy (TARELLO, 2005). Some serological studies have demonstrated that antibodies against agents in the family Anaplasmataceae are present in cat serum (MATTHEWMAN et al., 1996; AGUIRRE et al., 2004; ORTUÑO et al., 2005; SOLANO-GALLEGO et al., 2006). The presence of DNA from *Ehrlichia* sp. has been detected among domestic cats in the United States (BREITSCHWERDT et al., 2002), Taiwan (YIN-CHIACHUN et al., 2003), Spain (TABAR et al., 2007) and Brazil (OLIVEIRA et al., 2009). DNA from *Anaplasma phagocytophilum* has been detected in the blood of domestic cats in Sweden (BJÖERSDORFF et al., 1999) and the United States (LAPPIN et al., 2004).

Otherwise, very little is known about the presence of ehrlichiosis-based pathogens in Brazilian cats. In Brazil, the first occurrence of feline ehrlichiosis was reported in 1998, in which morulae of mono and polymorphonuclear leukocytes were observed in
Material and Methods

Between October 2008 and January 2009, EDTA whole blood and serum samples were collected from 200 domestic cats in São Luís, Maranhão, Brazil. The blood and serum samples were stored at –20 °C.

The presence of anti-
Ehrlichia canis
antibodies in the serum samples from each animal was detected by means of the indirect immunofluorescent assay (IFA). Antigen slides were removed from storage and allowed to thaw at room temperature for 30 minutes. Ten microliters of twofold dilutions of serum at 1:64 (cut-off) were placed in wells on antigen slides. Antigens of
Ehrlichia canis
were obtained by culturing DH182 cells infected with
E. canis
(Jaboticabal strain) at the Immunoparasitology Laboratory, UNESP (AGUIAR et al., 2007b). Known positive canine serum (titer 1:2,560) was obtained from a symptomatic dog with ehrlichiosis at Governador Laudo Natel Veterinary Hospital, UNESP, Jaboticabal, São Paulo. When highly positive serum was obtained from a feline (titer of 2,560), it was used as a positive control. Test serum samples that had been analyzed using a canine positive control were reprocessed again using a feline positive control. The negative control serum sample was obtained from a cat that had not been exposed to this agent, according to IFA results.

The slides were incubated at 37 °C in a moist chamber for 30 minutes, washed three times in PBS (pH 7.2) for 5 minutes, and air dried at room temperature. Anti-cat conjugate (dilution of 1:100) for feline samples and anti-dog conjugate for controls (dilution of 1:80) were diluted in accordance with the manufacturer’s instructions and then added to each well. These slides were incubated again, washed, dried, and overlain with buffered glycerin (pH 8.7), covered with glass coverslips and examined using a fluorescence microscope (NAKAGHI et al., 2008).

DNA was extracted from 200 µL of EDTA whole blood from felines, using the QIAamp DNA blood mini-kit (QIAGEN, Valencia, California, USA), in accordance with the manufacturer’s instructions. Each sample of extracted DNA was used as a template in a nested PCR with genus-specific primers (478 bp) and species-specific primers for
Ehrlichia canis
(358 bp) (MURPHY et al., 1998) and
E. chaffeensis
(410 bp) (KOCAN et al., 2000). A positive DNA control for
Ehrlichia canis
was obtained from a dog that had been experimentally infected with the Jaboticabal
E. canis
strain. The positive control for
Ehrlichia chaffeensis
was kindly supplied by J. Stephen Dumler, Department of Pathology, Johns Hopkins School of Medicine, Baltimore, Maryland, USA.

Results and Discussion

Eleven (5.5%) of the 200 cats tested were seropositive for
E. canis
antigens, according to IFA. The antibody titers ranged from 64 (cut-off) to 512 for
E. canis
. Three animals showed antibody titers of 64 and seven animals were seroreactive at a dilution of 1:160. Only one animal showed antibody titer of 512.

Two (1%) of the 200 samples were positive for
Ehrlichia sp.
PCR based on 16S rRNA. One sample (cat#45) was positive for
E. canis
nPCR and another one (cat#211) for
E. chaffeensis
nPCR. Both of the PCR-positive animals were negative in the serological test. DNA sequencing using the 16S rRNA gene showed that the
Ehrlichia sp.
DNA obtained from cat#45 (GenBank access number JN123433) was closely related (98.0% identity) to
E. canis
from dogs in Tunisia (EU781695), Taiwan (EU143637) and Italy (EU439944). The
Ehrlichia sp.
DNA obtained from cat#211 (GenBank access number JN123434) was closely related (97.0% identity) to
E. chaffeensis
isolated in Arkansas (AF416764),
Ehrlichia sp.
from Boophilus microplus in Tibet (AF414399) and
Ehrlichia sp.
from deer in Japan (AB454074). While the
Ehrlichia sp.
isolate from cat#45 clustered together with
E. canis
isolates, the
Ehrlichia sp.
from cat#211 clustered together with
E. chaffeensis
isolates (Figure 1).

To our knowledge, the present study is the first to show the presence of DNA from
Ehrlichia spp.
asymptomatic cats in the state of São Paulo, Brazil. The
Ehrlichia DNA
has been detected among cats in the United States (BREITSCHWERDT et al., 2002), Spain (TABAR et al., 2007) and Brazil (OLIVEIRA et al., 2009).

Ehrlichia spp.
DNA and antibodies to this agent have been detected in blood samples from wild felids that were kept in captivity in the state of São Paulo and the Brazilian Federal District (ANDRÉ et al.,
Furthermore, DNA from *Anaplasma phagocytophilum* has been detected among cats in Sweden (BJÖERSDORFF et al., 1999) and the United States (LAPPIN et al., 2004). The low percentage of positive animals in the present study and other studies can possibly be explained by considering the fact that cats are more resistant to *Ehrlichia* infection than dogs are, and they interact differently with the tick vector. Most cats rapidly remove ticks when they become infested, and thus the minimum tick attachment time of 24-48 hours that is thought likely to be required for transmission of most tick-transmitted infections may not be achieved (KIDD; BREITSCHWERDT, 2003). It is also possible that cats have lower copy numbers of *Ehrlichia* DNA than shown by dogs, thereby resulting in false negative results (EBERHARDT et al., 2006). Moreover, these results show that *Ehrlichia* infections in cats are uncommon.

![Phylogenetic position of Ehrlichia sp. isolates from Brazilian domestic cats on São Luís island, based on 16S rRNA sequences (300 bp). The tree was constructed using the neighbor-joining method and the numbers on the tree indicate bootstrap values for the branch points. Accession numbers and place of origin of the isolates are shown beside the sequence names.](image)

The observed low antibody titers may result from a low humoral immune response to *Ehrlichia* sp. among cats, or may result from cross-reactivity with other Anaplasmataceae species (ORTUÑO et al., 2005). The occurrence of positive PCR and negative serological results suggests that genes from other organisms that are closely related to *E. canis* may also have been amplified, but were distinct enough not to induce cross-reacting antibodies (EBERHARDT et al., 2006).
Although it has been suggested that feline ehrlichiosis is transmitted by ticks, the transmission mechanism still remains imperfectly understood (SHAWN, 2001). In Brazil, *E. canis* is transmitted to dogs by the tick *Rhipicephalus sanguineus* (AGUIAR et al., 2007a). Although *E. chaffeensis* DNA has been detected in Brazilian marsh deer, there is no information about its transmission route (MACHADO et al., 2006). Although the samples for the present study were collected during the summer, ticks were found attached in only one of the sampled animals (*Rhipicephalus sanguineus* ticks in one cat that was negative to both PCR and serological tests; data not shown). It should be noted that exposure to vectors among felids is less frequent than among dogs. Alternatively, felids may remove the vectors before hemoparasite transmission occurs (LAPPIN et al., 2006). At present, the exposure route to *E. canis* among the cats studied here is unknown.

The role of cats in the epidemiology of ehrlichiosis is unknown. It has been suggested that felids are more resistant to infection than are dogs (LAPPIN et al., 2006). The presence of *Ehrlichia* sp. closely related to *E. chaffeensis* in one cat in the present study, along with reports of antibodies to *E. chaffeensis* both in humans (CALIC et al., 2004; DA COSTA et al., 2005, 2006) and in dogs (GALVÃO et al., 2002) in the state of Minas Gerais, Brazil, and the molecular detection of this agent in Brazilian marsh deer (*Blastocerus dichotomus*) (MACHADO et al., 2006), shows that there is a need for more studies on the zoonotic potential represented by domestic and wild animals in Brazil, taking into consideration the existence of human monocytic ehrlichiosis.

The present work shows that Brazilian cats have been exposed to *Ehrlichia* sp. infection. To determine whether this finding represents a real threat to the health of these animals, further studies are needed. To our knowledge, this is the first study on molecular detection of *Ehrlichia* sp. among cats in the state of Maranhão.

**Acknowledgements**

The authors would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp) for the financial support, and Dr. John Stephen Dumler from Johns Hopkins University School of Medicine, Baltimore, MD, USA, for providing the positive DNA control for *Ehrlichia chaffeensis* and a polymerase chain reaction technique. *Ann NY Acad Sci* 2004; 1026: 103-105. PMid:15604476. http://dx.doi.org/10.1196/annals.1307.013


**References**


