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Detection of hemoplasma and *Bartonella* species and co-infection with retroviruses in cats subjected to a spaying/neutering program in Jaboticabal, SP, Brazil

Detecção de hemoplasmas e *Bartonella* sp. e co-infecção com retrovírus em gatos submetidos a um programa de castração/esterilização em Jaboticabal, SP, Brasil

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Abstract

Hemotrophic mycoplasmas and *Bartonella* species are important pathogens that circulate between cats and invertebrate hosts, occasionally causing diseases in humans. Nevertheless, there are few reports on occurrences of these agents in cats in Brazil. The present study aimed to detect the presence of hemoplasma and *Bartonella* DNA by means of PCR and sequencing. FIV antigens and anti-FeLV antibodies, were studied by using a commercial kit on blood and serum samples, respectively, among 46 cats that were sampled during a spaying/neutering campaign conducted in Jaboticabal, SP. Three (6.5%) cats were positive for hemoplasmas: two (4.3%) for '*Candidatus* M. haemominutum' and one (2.2%) for both *M. haemofelis* and '*Candidatus* M. turicensis'. One of the two '*Candidatus* M. haemominutum'-infected cats was also positive for FeLV antigens and showed antibodies for FIV. Two cats (4.3%) were positive for *B. henselae*. One of them was also positive for FeLV antigens. Eight cats (17.4%) were positive for FeLV, and just one (2.2%) showed anti-FIV antibodies. *Bartonella* species and hemoplasmas associated with infection due to retroviruses can circulate among apparently healthy cats.

Keywords: Hemotrophic mycoplasmas, *Bartonella henselae*, FIV, FeLV, cats.

Resumo

Micoplasmas hemotróficos e espécies de *Bartonella* são importantes patógenos que circulam entre gatos e hospedeiros invertebrados, causando ocasionalmente doenças no homem. Apesar disto, poucos são os estudos acerca da ocorrência destes agentes entre gatos no Brasil. O presente estudo objetivou detectar o DNA de hemoplasmas e *Bartonella* sp. pela PCR e sequenciamento. Antígeno de FIV e anticorpos anti-FeLV foram estudados utilizando um "kit" comercial, em amostras de sangue e soro, respectivamente, de 46 gatos amostrados em uma campanha de castração em Jaboticabal, SP. Três gatos (6,5%) foram positivos para hemoplasmas: dois (4,3%) para '*Candidatus* M. haemominutum' e um (2,2%) para *M. haemofelis* and '*Candidatus* M. turicensis'. Um dos gatos positivos para '*Candidatus* M. haemominutum' mostrou-se também positivo na detecção de antígeno de FeLV e de anticorpos para FIV. Dois (4,3%) gatos mostraram-se positivos para *B. henselae*, sendo que um deles também se mostrou positivo para antígeno de FeLV. Oito gatos (17,4%) foram positivos para FeLV, e apenas um gato mostrou anticorpos anti-FIV. *Bartonella* sp. e hemoplasmas associados à infecção por retrovírus podem circular entre gatos aparentemente saudáveis.

Palavras-chave: Micoplasmas hemotróficos, *Bartonella henselae*, FIV, FeLV, gatos.

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Introduction

Arthropod-borne bacterial agents have arisen as emerging pathogens over recent decades, due to ecological and climatic changes (SHAW et al., 2001). In this context, hemotrophic mycoplasmas and *Bartonella* species appear to be important pathogens that circulate between cats and invertebrate hosts, occasionally causing diseases in human beings. *Mycoplasma haemofelis* and *Bartonella henselae* have been detected in a human immunodeficiency virus-infected patient in Brazil (DOS SANTOS et al., 2008).

Feline hemotrophic mycoplasmas (hemoplasmas) comprise a group of bacteria that can induce hemolytic anemia in cats. *Mycoplasma hemofelis* is the most pathogenic species; '*Candidatus* Mycoplasma haemominutum' and '*Candidatus* Mycoplasma turicensis' are less pathogenic species (TASKER et al., 2010). Hemoplasma-infected cats show unspecific clinical signs like pallor, anorexia, lethargy, weight loss, depression, dehydration and pyrexia (MESSICK et al., 2003; TASKER et al., 2010).

The genus *Bartonella* comprises a group of fastidious Gram-negative bacteria that are very well adapted to mammalian hosts and develop long-lasting intraerythrocytic bacteremia (BREITSCHWERDT et al., 2010). Cats are the major reservoirs for *B. henselae*, *B. clarridgeiae* and *B. koehlerae* (BREITSCHWERDT; KORDICK, 2000; BREITSCHWERDT et al., 2010). Although cats rarely show clinical manifestations caused by *Bartonella* species, there have been reports of occurrences of fever, lethargy, anorexia, reproductive failure, lymphadenopathy, stomatitis, uveitis and neurological dysfunction (BREITSCHWERDT; KORDICK, 2000; BREITSCHWERDT et al., 2010; GUPTILL et al., 1998).

There are few reports on occurrences of hemoplasmas (BATISTA, 2004; BAUMANN et al., 2006; HORA, 2008; MACIEIRA et al., 2008; SANTOS et al., 2009) and *Bartonella* (STAGGEMEIER et al., 2010; SOUZA et al., 2010; CRISSIUMA et al., 2011), in infections among cats in Brazil. The present study aimed to detect the presence of hemoplasma and *Bartonella* species, FeLV antigens and FIV antibodies in blood samples from cats subjected to a spaying/neutering program in Jaboticabal, SP, Brazil.

Materials and Methods

Between January and July 2009, EDTA blood and serum samples were collected from all 46 clinically healthy cats that were presented to a free spaying/neutering program that was conducted in the city of Jaboticabal, SP, Brazil. DNA was extracted from 200 µL of whole blood sample using the QIAamp DNA Blood Mini kit (QIAGEN, Valencia, California, USA), in accordance with the manufacturer's instructions.

Partial sequences of the 16S rRNA gene of *M. haemofelis*, '*Candidatus* Mycoplasma haemominutum' and '*Candidatus* Mycoplasma turicensis' were amplified by means of PCR, in reaction mixtures of final volume 25 µL, containing 5 µL of template DNA, 10X PCR buffer, 1.0 mM of $MgCl_2$, 0.2 mM of deoxynucleotide triphosphate (dNTPs) mixture, 1.5 U of Taq DNA polymerase (Invitrogen, Carlsbad, California, USA) and 0.2 mM of primers that had previously been described (BERENT et al., 1998; FOLEY et al., 1998; SANTOS et al., 2009). Regarding the high similarity found in 16S rRNA sequences between *M. haemofelis* and *M. haemocanis*, samples that were positive in the above-described PCRs were subjected to another PCR test based on the RNAase P gene (*rnpb*), using primers, reagent concentrations and reaction conditions that had previously been described (BIRKENHEUER et al., 2002).

Screening for the genus *Bartonella* was performed by means of PCR targeting the intergenic transcribed spacer (ITS), as described previously (MAGGI; BREITSCHWERDT, 2005a; DINIZ et al., 2007). For further molecular characterization and species differentiation, samples that were positive in ITS amplification were tested for other genes: the bacteriophage-associated heme-binding protein gene (*pap31*) (MAGGI; BREITSCHWERDT, 2005b); the RNA polymerase beta subunit gene (*rpoB*) (DINIZ et al., 2007); the riboflavin synthase gene (*ribC*) (JOHNSON et al., 2003); and the citrate synthase gene (*gltA*) (NORMAN et al., 1995; WINOTO et al., 2005).

Fragments of the expected size from *M. haemofelis*, '*Candidatus* M. haemominutum' and '*Candidatus* M. turicensis', cloned into pGEM T-Easy Vector System II (Promega, Madison, Wisconsin, USA), were kindly supplied by Dr. Joanne B. Messick, Purdue University, West Lafayette, IN, USA, and were used as positive controls. *Bartonella henselae* DNA, obtained from a cat in São Luís, Maranhão, was used as the positive control in *Bartonella* PCR assays (GenBank Access Number HQ012581). Ultra-pure sterile water was used as the negative control. In order to prevent PCR contamination, the DNA extraction, reaction setup, PCR amplification and electrophoresis were performed in separate rooms.

The reaction products were purified using the Silica Bead DNA Gel Extraction Kit (Fermentas, São Paulo, SP, Brazil). Purified amplified DNA fragments from positive samples were subjected to sequence confirmation in an automatic sequencer (ABI Prism 310 Genetic Analyzer; Applied Biosystems/Perkin Elmer) and were used for subsequent phylogenetic analysis. Consensus sequences were obtained through analyzing sense and antisense sequences using the CAP3 program (http://mobyle.pasteur.fr/cgi-bin/MobylePortal/portal.py). Comparisons with sequences deposited in GenBank were made using the basic local alignment search tool (BLAST).

A commercially available immunoassay was used to detecting FeLV antigens and antibodies for FIV in cat serum samples (n = 46). This system, the Snap™ Combo FeLV antigen/FIV (feline immunodeficiency virus) antibody test kit (IDEXX Laboratories, Inc., Westbrook, Maine, USA), was used in accordance with the manufacturer's recommendations.

Results

Among the 46 sampled cats, three (6.5%) were positive for hemoplasmas: two for '*Candidatus* M. haemominutum' and one for both *M. haemofelis* and '*Candidatus* M. turicensis', based on sequencing confirmation. One of the '*Candidatus* M. haemominutum'-infected cats was positive for FeLV antigens and showed antibodies for FIV. The sequenced products showed 83% identicalness with 16S rRNA *M. haemofelis* (EU930823), 66% identicalness with rnpb *M. haemofelis* (EU078617), 100%

identicalness with '*Candidatus* M. turicensis' (EU839977), 99-100% identicalness with 16S rRNA '*Candidatus* M. haemominutum' (EU839983; AM745338) and 90-91% identicalness with '*Candidatus* M. haemominutum' (AY150990) (Table 1).

Two cats (4.3%) were positive for *B. henselae*. One of them was also positive for FeLV antigens. The analysis on the sequenced products showed that they presented 98-99% identicalness with *B. henselae* (access numbers FJ832096, DQ529247 and DQ529248) (Table 2).

Fleas were found parasitizing 10 out of the 46 animals at the time of blood collection. Eight cats were positive for FeLV antigens, and just one showed antibodies for FIV. No fleas were found in any of the hemoplasma-infected cats. One *B. henselae*-positive cat was parasitized by fleas and tested positive for FeLV antigens at the time of sample collection.

Discussion

The present study showed that hemoplasma and *Bartonella* species circulate among household cats in Jaboticabal, SP, as well as the feline retroviruses FeLV and FIV. The prevalences of *M. haemofelis*, '*Candidatus* M. haemominutum' and '*Candidatus* M. turicensis' among the sample were 2.1%, 4.3% and 2.1%, respectively. The prevalences among domestic cats around the world are 0.4% to 35% for '*Candidatus* M. haemominutum', 0.3% to 6.5% for '*Candidatus* M. turicensis', 0.125% to 10% to *Mycoplasma haemofelis* and 0 to 0.7% for '*Candidatus* M. haematoparvum' (TASKER et al., 2010). In Brazil, the prevalences of hemoplasmas among healthy and anemic cats are, respectively, 2.2% and 38% for *M. haemofelis*, 4% and 13.5% for '*Candidatus* M. haemominutum' and 0.37% and 2.7% for '*Candidatus* M. turicensis' (BATISTA, 2004; BAUMANN et al., 2006; MACIEIRA et al., 2008; HORA, 2008; SANTOS et al., 2009).

In the present study, co-infection of *M. haemofelis* and '*Candidatus* M. turicensis' was found in one cat. Previously, co-infection of *M. haemofelis* and '*Candidatus* M. haemominutum' has been reported in cats in the states of São Paulo (BATISTA, 2004), Paraná (BAUMANN et al., 2006), and Rio de Janeiro (MACIEIRA et al., 2008).

Mycoplasma haemofelis is the most pathogenic hemoplasma and it has been incriminated in hemolytic crises, even in immunocompetent cats (WILLI et al., 2007). '*Candidatus* M. haemominutum' infections may cause anemia when animals are co-infected with retroviruses, and '*Candidatus* M. turicensis' may cause this when cats are co-infected with other hemoplasmas or are immunosuppressed (WILLI et al., 2007). Although the hemoplasma-positive cats in the present study fulfilled at least one of the three situations described above, all of them were apparently healthy at the time of sample collection.

One of the '*Candidatus* M. haemominutum'-infected cats was positive for FeLV antigens and showed antibodies for FIV. Cats infected with FIV or co-infected with FIV and FeLV are likely to be at higher risk of harboring '*Candidatus* M. haemominutum' than retrovirus-negative cats (LURIA et al., 2004; HORA, 2008; MACIEIRA et al., 2008).

In our study, *Bartonella henselae* DNA was detected in two out of the 46 sampled cats (4.3%). In Brazil, few reports have been produced concerning the epidemiology of bartonellosis. *Bartonella henselae* and *B. clarridgeiae* DNA was detected in 10.6% and 6.3% of the blood samples, respectively, from 40 cats at an animal shelter in Nova Hamburgo, state of Rio Grande do Sul (STAGGEMEIER et al., 2010). Using molecular techniques, high prevalence (97.3%) of *Bartonella* spp. was found among cats in Vassouras, state of Rio de Janeiro (SOUZA et al., 2010). Also, *Bartonella* spp. DNA was detected in 17 out of 40 clinically healthy cats that were treated in a spaying/neutering program in the city of Rio de Janeiro (CRISSIUMA et al., 2011).

Cats are the main reservoirs for *B. henselae*, *B. clarridgeiae* and *B. koehlerae* (BREITSCHWERDT et al., 2010). *Bartonella henselae* is incriminated as the causative agent of cat scratch disease, ocular manifestations, encephalopathy, osteomyelitis and hepatic peliosis in humans (LAMAS et al., 2008). In Rio

Table 1. Degree of similarity according to Blast, of hemoplasma isolates among cats in Jaboticabal, SP, Brazil.

Animal	% similarity according to Blast			
	16S rRNA gene (600 bp fragment)	16S rRNA gene (1475 bp fragment)	CMt 16S rRNA gene	*Mycoplasma* sp. rnpB gene
G321	*Mf* 83% (EU930823)	*Mf* 83% (EU839978)	*CMt* 100% (EU839977)	*Mf* 66% (EU078617)
G7	*CMh* 99% (EU839983)	*CMh* 100% (AM745338)	Negative	*CMh* 90% (AY150990)
G355	*CMh* 99% (EU839984)	*CMh* 100% (AM745338)	Negative	*CMh* 91% (AY150990)

Mycoplasma haemofelis = Mf; '*Candidatus* Mycoplasma haemominutum' = CMh; '*Candidatus* Mycoplasma turicensis' = CMt.

Table 2. Degree of similarity according to Blast, *Bartonella henselae* isolates among cats in Jaboticabal, SP, Brazil.

Animal	City	% similarity according to Blast				
		ITS	PAP 31 gene	RPOB gene	gltA gene	ribC ene
G302	Jaboticabal	99% *Bh* (FJ832096)	negative	negative	99% *Bh* (HQ012580)	negative
G312	Jaboticabal	98% *Bh* (DQ529247)	99% *Bh* (DQ529248)	negative	99% *Bh* (HQ012580)	negative

Bartonella henselae = *Bh*.

de Janeiro, HIV-infected individuals and breeding cats were found to be at higher risk of *Bartonella* infection (LAMAS et al., 2010). Although the prevalence of *Bartonella* sp. infection found among the cats in Jaboticabal was low, these animals may act as a source of infection of *B. henselae* for humans through scratches contaminated by flea feces or bites contaminated with infected blood of cats (CHOMEL et al., 1996).

In Japan, where cats have been tested for antibodies to both FIV and *B. henselae*, significantly higher incidence of lymphadenopathy and gingivitis was observed among cats with serological evidence of infection with both of these organisms (UENO et al., 1996). Since FIV is associated with decreased levels of CD41 lymphocytes, these results suggest that co-infection with *B. henselae* in an immunodeficient cat can induce specific disease manifestations (BREITSCHWERDT et al., 2010). In our study, although one cat was positive for both *B. henselae* and FeLV, it was apparently asymptomatic. Cats may be more likely to show clinical signs when infected with a non-reservoir-adapted *Bartonella* species (BREITSCHWERDT et al., 2010). *Bartonella* sp. induces asymptomatic infections when parasitizing preferred hosts, through behaving as a stealth pathogen (KORDICK; BREITSCHWERDT, 1998). While some seroepidemiological studies have showed a lack of association between feline retroviral infection and seroreactivity to *B. henselae* (GLAUS et al., 1997; MARUYAMA et al., 1998; LURIA et al., 2004), a recent study found that the course of natural *B. henselae* infection in cats does not seem to be influenced by immunosuppressive viral infections, but that latent FeLV infection may predispose cats to *B. henselae* infection or persistence (BUCHMANN et al., 2010).

As in the present study, low seroprevalences of 2% and 1.5% for *B. henselae* and *B. vinsonii berkhoffii*, respectively, and low molecular prevalences of 1% and 0.5% for *B. henselae* and *B. vinsonii berkhoffii* were found among 198 dogs in the state of São Paulo (DINIZ et al., 2007).

Among cats, the flea *Ctenocephalides felis* is the main vector of *B. henselae* (CHOMEL et al., 1996). At the time of sample collection in the present study, fleas were found in one of the two *Bartonella*-infected cats. On the other hand, fleas were not found parasitizing hemoplasma-positive cats. Although a previous attempt to show experimental transmission between cats via fleas was inconclusive (WOODS et al., 2005), *C. felis* fleas are also considered to be the main vectors for hemoplasmas among cats (SHAW et al., 2001).

Conclusion

In conclusion, we showed that cats in the Jaboticabal region are exposed to retroviruses, hemoplasmas and *Bartonella* species. Retroviruses associated with hemoplasma and *Bartonella* species can circulate even in apparently asymptomatic cats. Molecular and serological surveys are needed, in order to attempt to investigate the frequency of these infections among cats and other animal species in other regions of Brazil. In addition, further studies should be conducted with the aim of elucidating the role of cats in the epidemiology of vector-borne bacterial zoonotic diseases in Brazil.

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