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MOLECULAR DETECTION OF *ANAPLASMA PLATYS* IN A NATURALLY-INFECTED CAT IN BRAZIL

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ABSTRACT

Following the accidental finding of inclusion bodies similar to *Anaplasma platys* in a stained blood smear from a cat, DNA analysis of the 16S rRNA gene was performed and 100% identity was found with different strains of *A. platys*. These data confirm that cats are susceptible to parasitism by *A. platys*.

Key words: feline, *Anaplasma platys*, molecular detection

Anaplasma platys (formerly *Ehrlichia platys*) is an obligate intracellular gram-negative bacteria from the family Anaplasmataceae (10) that causes Infectious Canine Cyclic Thrombocytopenia (ICCT), possibly transmitted through the bite of infected brown ticks *Rhipicephalus sanguineus* (24). This rickettsia has been described mainly in dogs in various regions of the world, such as Brazil (22), Spain (20), Japan (17) and others (14, 3), but *Anaplasma*-like bodies in platelets of cats and humans have been described in Brazil (23) and Venezuela (29), respectively.

Rickettsiae that infect felines are not very well characterized. However, *E. canis* DNA has been detected in blood of domestic cats in North America (2) and recently in Trinidad by reverse line blot hybridization (13). Moreover, experimental infection of cats by *Neorickettsia risticii* (formerly *Ehrlichia risticii*) has been described (8).

In the present study, a natural infection by *A. platys* in a cat is reported for the first time in the state of Pernambuco, in northeastern Brazil, using molecular tools to confirm the identity of the pathogen.

In August 2007, a 10-year-old male cat from the city of Paulista (7°55'57"S, 34°49'23"W) was admitted to a private small animal clinic in the city of Recife (8°3'0"S, 34°54'0"W), both located in the state of Pernambuco, Brazil. During the clinical examination, the animal exhibited anorexia, apathy, anury and constipation. A complete blood count (CBC), urinalysis and abdominal ultrasound exam were carried out and urinary infection was diagnosed.

During the blood smear examination with panoptic stain, inclusion-like bodies compatible with *A. platys* were observed in the platelets. No major changes were detected in the CBC, except for mild thrombocytopenia. Thirty-five days following the first blood count, leukocytosis was observed and attributed to persistence of the urinary infection, since the platelet count was normal.

Two milliliters of whole blood were collected with EDTA. DNA was isolated using the *Easy DNA* kit (Invitrogen, USA), following the manufacturer's instructions. A nested polymerase chain reaction (PCR) was conducted based on the procedure described by Martin *et al.* (16), using primers 8F (5'

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AGTTTGATCATGGCTCAG 3') and 1448R (5' TGGCGTGACGGGCAGTGT 3'), which amplify the majority of the 16S rRNA gene (31), in the first PCR round. This was followed by a second round using a specific primer for *A. platys,* PLATYSF (5' GATTTTTGTCGTAGCTTGCTATG 3'), and a specific primer for the genus *Ehrlichia,* EHR16SR (5' TAGCACTCATCGTTTACAGC 3'), in order to produce a 678-bp amplicon of the 16S rRNA gene. Amplicons were cloned in the *pGEM-T Easy* vector (Promega, USA), following the manufacturer's instructions, and sequenced three times using an automated DNA sequencer (model ABI 3130, Applied Biosystems). The Sequencher 4.1.4 program (Gene Codes, USA) was used for the editing and generation of the consensus sequence.

A BLAST search was performed (http://www.ncbi.nlm.nih.gov/BLAST) with the consensus sequence and phylogenetic analysis was performed using the MEGA 4.0 program (30). A phylogenetic tree was constructed using the neighbor-joining (NJ) method (21) and bootstrap resampling with 1000 replications was performed to statistically support the reliabilities of the nodes on the trees (12). The 16S rRNA gene from *Neorickettsia sennetsu* (accession number M73225) was used as the outgroup. The other 16S rRNA sequences used in the study were found on the Genbank database under the following accession numbers: *A. platys* from Italy EU439943, *A. platys* from Thailand EF139459, *A. platys* from Japan AF536828, *A. platys* from Brazil DQ401045, *A. platys* from China AF156784, *Anaplasma phagocytophilum* AY741099, *Anaplasma bovis* AY144729, *Anaplasma marginale* FJ226454, EF520690, *Anaplasma centrale* EF520690, *Ehrlichia canis* EU439944, *Ehrlichia ewingii* U96436 and *Ehrlichia ruminantium* U03776.

The nested PCR resulted in an amplicon of 670 bp, similar to the positive control amplified from *A. platys* DNA from a naturally infected dog (Figure 1). Following sequence editing and consensus assembly, a fragment of 16S rRNA of approximately 416 bp was obtained, which had 100% identity with sequences of 16S rRNA from *A. platys* from Italy (EU439943) and Thailand (EF139459) and 99% identity with Japanese (AF536828) and Brazilian (DQ401045) isolates. The nucleotide sequence of 16S rRNA from the cat was deposited in the Genbank under accession number FJ686112.

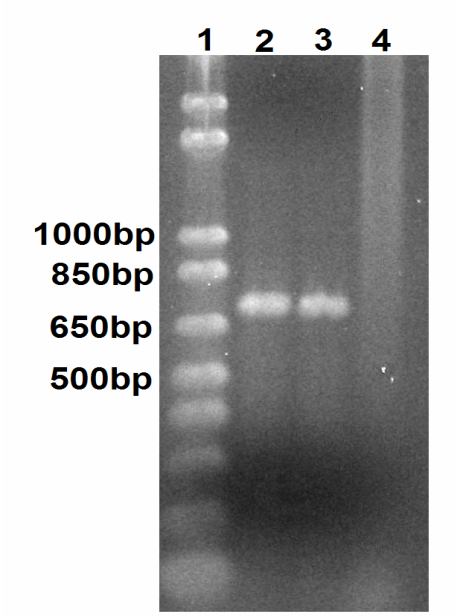

Figure 1. Agarose gel electrophoresis revealing amplification of 16S rRNA from *A. platys* in a cat in Recife, Brazil; Line 1 – DNA ladder 1Kb Plus (Invitrogen, USA); 2- Positive control (amplification of DNA extract from blood of a dog naturally infected by *A. platys*); 3 – Amplification of DNA extracted from blood of a cat with *A. platys*-like inclusion bodies; 4 – Negative control

Blood-borne pathogens in domestic cats have been attracting the attention of researchers (11, 27, 13, 28). With the use of molecular tools, a large number of pathogens previously recognized only as parasites of dogs have also been detected in domestic cats (2, 25). Recently, molecular studies (11, 28) supported by serological data (1, 27) have been conducted for the detection of a variety of tick-borne pathogens in cats. However, no success has been achieved in the amplification of DNA from *Anaplasma* and *Ehrlichia* organisms. In three recent studies with this aim (11, 27, 28), only one animal (1/100) was positive for *Ehrlichia/Anaplasma* in Spain (Barcelona region) (28). In another study, despite a seroprevalence of 10.6% and

4.9% for *E. canis* and *Anaplasma phagocytophilum,* respectively, DNA from these pathogens was not detected in any of the animals analyzed (1). It has been proposed that the discrepancy in prevalence between serological and molecular studies may result from a failure in PCR sensitivity due to the elimination of or reduction in bacteremia following drug administration and/or the action of the host immune system (28). However, the poor specificity of many serological tests should be considered (19).

The detection of *A. platys* inclusion bodies in platelets of species other than dogs is seldom described. However, in a study carried in Venezuela, *Ehrlichia* sp was found in the platelets of 12 humans in a group of 87 HIV-positive patients through a microscopic examination of stained buffy coats (29). Recently, DNA from *A. platys* was detected through PCR followed by sequencing analysis in a goat in Cyprus (4). In domestic cats, there is a report made by Santarém *et al*. (23) in a cat from Presidente Prudente in the state São Paulo, Brazil. This animal had thrombocytopenia and *A. platys*-like inclusion bodies in approximately 7% of the platelets; however, molecular confirmation was not carried out. Thus, the present report is the first molecular description of a natural infection by *A. platys* in a cat in Brazil.

The resultant phylogenetic tree revealed that *A. platys* cat from Pernambuco, Brazil, was tightly grouped with other *A. platys* isolates from dogs in various regions of the world (Figure 2). This supports the hypothesis that *A. platys* strains are neither geographically nor host segregated. *Ehrlichia* and *Anaplasma* were divided into clearly defined clades. *Ehrlichia ewingii* had the closest relationship to *E. canis,* whereas *E. ruminantum* was the most distant. *Anaplasma bovis* had the closet relationship to *A. platys*, although grouped in an independent clade and nearer to *A. phagocytophilum*. *Anaplasma marginale* clustered in a branch linked to *A. centrale*. These conclusions are consistent with other reports (9, 18).

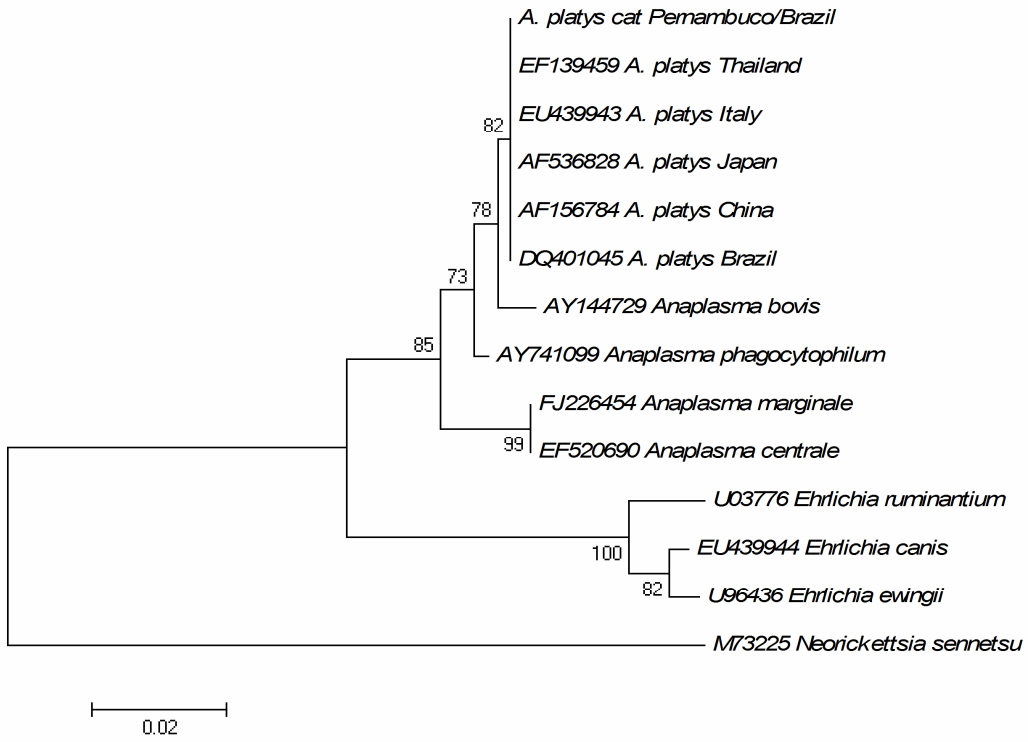

Figure 2. Phylogenetic tree based on *Anaplasma* and *Ehrlichia* 16S rRNA; Sequences were compared with the neighbor-joining method using the MEGA 4.0 program, with *N. sennetsu* as outgroup. Scale bar represents the number of mutations per sequence position. The numbers at the nodes indicate the percentage of 1000 bootstrap resamplings.

The transmission of *A. platys* to cats has not been determined. However, the brown dog tick, *R. sanguineus*, which is a potential vector of *A. platys* for dogs, has been described parasitizing cats (26), humans (7), cattle and goats (15), thereby demonstrating low host specificity. In metropolitan Recife (Pernambuco, Brazil), the only species of tick that has been identified in dogs is *R. sanguineus* (6). This observation, associated to the high frequency of infection by *A. platys* in dogs in this region and the fact that the genotype of *A. platys* cat was identical to those found in dogs in different regions of the world, indicates that the source of infection for the cat in this report was probably a dog. However, further studies are needed to clarify this matter.

The finding of *A. platys* in the cat described in this paper was accidental and no significant clinical and hematological changes associated to *A. platys* infection were observed. The epidemiological importance of this rickettsia to the feline population remains unknown. However, with the advent of PCR and sequence analysis, the constant finding of blood-borne pathogens in unusual hosts, such as *E. canis* in cats (2), *A. platys* in goats (4), *B. canis* in horses and *B. equi* in dogs (5) and others, raises the following questions: What is the real specificity of these pathogens? What are their arthropod vectors? By answering these questions, it will be possible define the real epidemiological importance of these findings.

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