Brazilian Journal of Microbiology (2007) 38:478-479
ISSN 1517-8382

ANALYSIS OF THE 16S rRNA GENE OF *ANAPLASMA PLATYS* DETECTED IN DOGS FROM BRAZIL

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Submitted: October 30, 2006; Returned to authors for corrections: March 12, 2007;Approved: June 21, 2007.

SHORT COMMUNICATION

ABSTRACT

Comparison of the partial DNA sequence of the 16S rRNA gene of *Anaplasma platys* detected in dogs from Ribeirão Preto, Brazil, to sequences of other strains previously deposited in GenBank showed that there are at least three *A. platys* strains circulating in South America.

Key words: *Anaplasma platys*, 16S rRNA gene, PCR

Anaplasma platys is a Gram-negative bacterium which develops inside dog platelets and causes canine infectious cyclic thrombocytopenia (2,3). It is mainly transmitted to dogs by the brown tick *Rhipicephalus sanguineus* and infected dogs usually do not show obvious clinical signs of the disease. Since bacteremia is usually low, the infection is difficult to be detected in blood smears and serological exams may yield false-positive results due to cross-reaction with other species. On the other hand, PCR-based methods can be designed to specifically detect *A. platys* in blood samples from dogs with high accuracy (3,4,6). The incidence of *A. platys* in Brazil is low (5) and molecular characterization involving a comparison of DNA sequences of Brazilian strains with strains around the world has not been reported. The aim of the present study was to analyze partial DNA sequences of the *A. platys* 16S ribosomal RNA gene detected by nested PCR in dogs from Ribeirão Preto, Brazil. This analysis is important for the development of specific and sensitive molecular exams, which are becoming more common among Brazilian veterinarians and can be used in epidemiological studies and vaccine development.

The *A. platys* 16S rRNA gene sequences were generated by sequencing PCR products obtained in a previous study regarding the prevalence of blood parasites in dogs from Ribeirão Preto, Brazil (Marins and cols., unpublished data). In that study, *A. platys* was detected by nested PCR using in the first reaction the Aplsense 5'-CTCAGAACGAACGC TGGCGGCAAGC-3' and Aplantisense 5'-CGTATTACCGC GGCTGCTGGC-3' primers, which amplify a fragment of the 16S rRNA gene from a broad range of species of the Anaplasmataceae family. In a second reaction, the Platys1sense 5'-GATTTTTGTCGTAGCTTGCTA-3' and Aplastintrev3 5'-GGTACCGTCATTATCTTCCC-3' primers were used to specifically amplify a 382-bp fragment of the *A. platys* 16S rRNA gene. These primers were designed based on primers previously described in the literature and sequences deposited in GenBank. The amplified products were purified and sequenced in an automated DNA sequencer (model 377, Applied Biosystems). Sequence analysis was performed using the ClustalX (7) and BLAST (1) programs.

The PCR nucleotide sequences of the seven dogs analyzed were identical and showed high similarity to sequences detected in dogs from around the world. The polymorphisms detected in these sequences are summarized in Table 1 for a fragment of 265 bp encompassing position 71 to 335 of the *A. platys* Okinawa 1 reference sequence (GenBank accession number AF536828).

The analysis of the partial DNA sequence performed in this study indicates that the strains identified in Ribeirão Preto, Brazil, are closely related to other strains described in dogs from other

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Table 1. Polymorphisms in 16S rRNA gene from different *A. platys* strains.

Strains	Nucleotide Positions[a]							
	92	94	95	96	97	187	216	217-218
A . platys Okinawa 1	G	A	G	T	A	T	A	G
A. platys Lara	·	·	·	·	·	·	·	·
A. platys Venezuela	·	·	·	·	·	·	G	·
A. platys Thailand	·	·	·	·	·	C	·	·
A. platys Gzh981	·	·	·	·	·	·	·	·
A. platys Sommieres	·	·	·	·	·	·	·	·
A. platys Okinawa	·	·	·	·	·	·	·	·
A. platys Louisana	·	·	·	·	·	·	G	-
A. platys Spain	·	·	·	·	·	·	·	·
A. platys Jaboticabal	T	T	C	C	ND	·	·	·
A. platys Ribeirão Preto 1	·	·	·	·	·	·	·	·

(a) *A. platys* Okinawa, GenBank accession number AF536828, was used as a reference sequence for nucleotide positions; where two numbers are shown there was an insertion; ·, identical base to reference strain; - , deletion; ND, not determined. The Genbank accession numbers for the other strains starting with *A. platys* Lara are AF399917, AF287153, AF286699, AF156784, AF303467, AY077619, M82801, AY530806, DQ401045, EF052620.

parts of the world, including two strains from Venezuela (3). Interestingly, not considering inaccuracy in DNA sequencing, the sequence of a Brazilian strain identified in another study (GenBank accession number DQ401045) shows at least four polymorphic differences to the strains detected in Ribeirão Preto and to the other strains, suggesting the presence of at least two *A. platys* strains in Brazil. Since molecular characterization of *A. platys* is limited, further studies including strains isolated from different geographical regions in Brazil, the complete sequence of the 16S rRNA gene and involving other genomic regions of the parasite are necessary.

ACKNOWLEDGMENTS

This study was supported by funds from Fundação de Amparo à Pesquisa do Estado de São Paulo (grauts 04/14196-5, 06/06890-4, 06/04413-4). We thank Dr. S.C. França and the staff of the Biotechnology Unit-UNAERP for general support, and K. Markendorf for revision of the English text.

RESUMO

Análise do gene 16S RNAr de *Anaplasma platys* detectado em cães do Brasil

A comparação de sequências parciais do gene 16S RNAr de *Anaplasma platys* detectadas em cães de Ribeirão Preto, Brasil, com sequências de outras linhagens previamente depositadas no GenBank indicam que existem pelo menos três linhagens de *A. platys* circulando na América do Sul.

Palavras-chave: *Anaplasma platys*, gene 16S RNAr, PCR

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