10.1111/j.1469-0691.2008.02175.x

Molecular detection of *Ehrlichia canis* in cats in Brazil

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INTRODUCTION

Ehrlichia canis is the causal agent of canine monocytic ehrlichiosis (CME) and is transmitted by *Rhipicephalus sanguineus* ticks. This bacterium has wide geographic distribution and CME is endemic in a number of tropical and subtropical regions. CME has been reported almost everywhere in Brazil.

Ehrlichia spp. naturally infecting felines have not been characterised yet. However, inclusions similar to ehrlichia have already been detected in monocytes, lymphocytes and granulocytes of cats with fever and thrombocytopenia. Molecular techniques confirmed the presence of *E. canis* in cats with clinical signs compatible with monocytic ehrlichiosis in North America [1].

Clinical signs reported in cats with ehrlichiosis are extremely varied. For the monocytic ehrlichiosis, the signs are: intermittent fever, anorexia, weight loss, vomiting and diarrhoea. Clinical-pathological discoveries include anaemia, thrombocytopenia, leukopenia and hyperglobulinaemia. It is still unknown whether cats become persistently infected or develop immunity as a result of chronic infection, similar to what happens with dogs [2].

In Brazil, Filoni *et al.* [3] reported the first serological evidence of *E. canis* in wild felines. Based on this report, the objective of this work was to confirm the presence of *E. canis* in a cat population in Brazil.

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No conflicts of interest declared.

MATERIALS AND METHODS

Blood samples were collected from 15 cats arriving for veterinary treatment at the Veterinary Hospital of the Federal University of Vicosa/MG, Brazil, from April to May 2007.

DNA was extracted from white blood cells by using the Dneasy Tissue Kit (QIAGEN) according to the manufacturer's recommendations. The DNA samples were tested by nested-PCR with primers encoding a region of the gene 16S rRNA. The primers ECC (5′-AGAACGAACGCTGGCGGCAAGC-3′) and ECB (5′-CGTATTACCGCGGCTGCTGGCA-3′) were used for the primary amplification [4], and ECAN5 (5′-CAAT TATTTATAGCCTCTGGCTATAGGA-3′) and HE3 (5′-TATA GGTACCGTCATTATCTTCCCTAT-3′) for the secondary amplification [5]. Four microlitres of DNA of each sample were used as template for the primary amplification and 1.5 μL of the product from the first reaction as template for the secondary amplification. The reaction conditions were: one cycle of 94°C for 4 min, 30 cycles of 94°C for 30 s, 60°C (for the primary amplification) or 58°C (for the secondary amplification) for 30 s, 72°C for 1 min, followed by a cycle of 72°C for 5 min. PCR products were visualised in 1.2% agarose gel stained with ethidium bromide.

A product of nested-PCR was cloned using the pGEM®-T Easy Vector System (Promega, Madison, Wisconsin, USA) according to manufacturer's recommendations. Recombinant plasmids were purified using the PureLink™ HiPure Plasmid Miniprep Kit (Invitrogen™, Carlsbad, CA, USA) and inserts were sequenced using the universal primers M13R and M13F. The sequences were compared with the corresponding homologous sequence available from BLAST of the NCBI database (http://www.ncbi.nlm.nih.gov) and with *E. canis* sequences obtained from dogs of the same study area (data yet not published, GenBank accession numbers: EU567020, EU567021, EU567022, EU567023 and EU567024).

The nucleotide sequence reported here has been deposited in GenBank with accession number EU567025.

RESULTS AND DISCUSSION

Three cats were found to be infected with *E. canis*. This is the first report of molecular detection of *E. canis* in cats in South America. The only abnormal haematological finding observed in the present study was thrombocytopenia in a cat.

The *E. canis* sequence obtained in this study showed 99% of similarity with the homologous sequences compared and 100% of similarity with the *E. canis* sequences obtained from dogs of the same study area, except for the sequence EU567024, which was different in two nucleotide bases, suggesting that possibly the same *E. canis* strain is infecting dogs and cats in Brazil.

The cats seem to be less predisposed to tick-transmitted diseases than dogs. They are likely to have innate resistance or adaptation to infection, limiting the disease development or impairing the tick-to-cat transmission of the infective agents [2].

Little is known about tick species able to infest and transmit infectious diseases to cats. However, cats infested with *Dermacentor* spp., *R. sanguineus* and *Haemaphysalis* spp. [2] are found in warm areas. It is, therefore, possible that *R. sanguineus* is the vector of *E. canis* to cats in Brazil, because it is universally distributed in the country.

CONCLUSION

The results of this work suggest that cats can be hosts of *E. canis*, showing the need for further studies to confirm the potential of cats as reservoirs and provide better knowledge of ehrlichiosis epidemiology in South America.

ACKNOWLEDGEMENTS

The authors would like to thank CNPq for a doctoral grant to L.S. Oliveira and scientific initiation grant to L.C. Mourão, and CAPES for the doctoral grant to K.A. Oliveira. We are grateful to Dr David H. Walker of The University of Texas Medical Branch for partial financial support given to this project.

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