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# First report of *Ehrlichia ewingii* detected by molecular investigation in dogs from Brazil

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## INTRODUCTION

The genus *Ehrlichia* encompasses a group of obligate intracellular bacteria that infect phagocytes, are transmitted by ticks and are related to ehrlichiosis in a number of animal species such as dogs, equines, ruminants, felines and humans. *Ehrlichia ewingii* is the causal agent of canine (CGE) and human (HGE) granulocytic ehrlichiosis and is mainly transmitted by ticks of the species *Amblyomma americanum*. CGE causes moderately severe disease and polyarthritis in chronically infected dogs. It has been described in dogs from the United States and Cameroon. *E. ewingii* has not been reported to have been propagated in cell culture and is usually identified by molecular techniques. This work aimed to verify by molecular assay the incidence of *E. ewingii* in dogs from Vicosa, Minas Gerais state, Brazil.

## MATERIAL AND METHODS

In order to detect *E. ewingii* in a population of dogs from Brazil, 100 blood samples were collected from dogs undergoing consultations or examinations at the Veterinary Hospital of the Federal University of Vicosa, Brazil, from April to May 2007. DNA was extracted from white blood cells by using the Dneasy Tissue Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's recommendations. These samples were tested for nested-PCR with genus-specific primers ECC (5′-AGAACGAACGCTGGCGGCAAGC-3′) and ECB (5′-CGTATTACCGCGGCTGCTGGCA-3′) [1] and species-specific primers for *E. ewingii* EE5 (5′-CAATTCCTTAAATAGTCTCTGACTATTAG-3′) [2] and HE3 (5′-TATAGGTACCGTCATTATCTTCCCTAT-3′) [3]. The primary amplification used as template 4 μL of DNA from each sample, and the secondary amplification used as template 1.5 μL of the product from the first reaction. The following conditions were used: 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. *E. ewingii* presence was confirmed by another reaction with PCR primers Dsb-321 (5′–TTGCAAAATGATGTCTGAAGATATGAAACA–3′) and Dsb-671-ew (5′–CAGCTCCACCAATGAATGTATTTCCAA–3′) [4] with DNA extracted from the canine blood samples that were positive by the nested-PCR. The conditions for this reaction were: one cycle of 94°C for 4 min, 40 cycles of 94°C for 30 s, 48°C for 30 s, 72°C for 1 min, followed by one cycle of 72°C for 5 min. PCR products were visualised in 1.2% agarose gel stained with ethidium bromide.

## RESULTS AND DISCUSSION

Five dogs infected by *E. ewingii* were found by nested-PCR and the reaction with primers that amplify a fragment of the dsb gene confirmed the presence of *E. ewingii* in these five samples. Four animals showed anaemia and one of them also showed thrombocytopenia. One animal infected with *E. ewingii* showed no haematological abnormality.

Formerly, the only recognised *Ehrlichia* species in Brazil were *Ehrlichia canis* and *Ehrlichia chaffeensis*, which makes this the first study to provide molecular evidence for canine infection caused by *E. ewingii* in the country. Trapp *et al.* [5] evaluated the serum antibody prevalence for *E. canis* in a population of dogs at the Veterinary Hospital of the State University of Londrina (Brazil) and did

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not exclude the possibility of infection by *E. ewingii* because the clinical signs observed in some dogs were more compatible with ehrlichiosis caused by *E. ewingii.*

In North America, *E. ewingii* is mainly transmitted by *A. americanum* ticks. As this species is not common in South America, other species of these ectoparasites should be involved in the transmission of *E. ewingii* to dogs in Brazil. Murphy *et al.* [3] found *R. sanguineus*, *A. americanum* and *Dermacentor variabilis* infected with this bacterium, suggesting that these tick species could be potential vectors for *E. ewingii*. Because *R. sanguineus* is a very common tick in Brazil, it is possible that this species is involved in the transmission of *E. ewingii* to dogs in this country.

## CONCLUSION

These results suggest that *E. ewingii* may be circulating among dogs in the studied region and show the need for further research in order to provide a better understanding of *E. ewingii* ecology in Brazil. Such studies would allow a better management of epidemiological surveillance aimed at the prevention of new *E. ewingii* infections.

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