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# *Ehrlichia canis* and *Leishmania infantum* co-infection: a 3-year longitudinal study in naturally exposed dogs

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## INTRODUCTION

Ehrlichiosis and leishmaniasis are major vector-borne diseases of dogs with a worldwide distribution. The causative agents of these diseases, *Ehrlichia canis* and *Leishmania infantum*, are intracellular pathogens that use monocyte–macrophages as host cells. The epidemiology of canine ehrlichiosis and leishmaniasis overlaps in many areas of the world, because the vector activity and transmission periods of these pathogens are similar. Whereas *E. canis* is transmitted by ticks, mainly *Rhipicephalus sanguineus*, *L. infantum* is transmitted by phlebotomine sand flies. Both infectious agents disseminate from the skin to the spleen, liver and bone marrow (BM), and activate host immune mechanisms that induce a range of immunopathological responses. The aim of the study was to longitudinally evaluate the time course and possible relationship between naturally occurring *E. canis* and *L. infantum* infections.

## MATERIALS AND METHODS

Forty-three 6-month-old naive beagle dogs (23 males) were introduced in July 2002 into an area endemic for both infections near Naples in southern Italy [1]. The dogs were kept at an open kennel without the use of insecticides. They were followed for two transmission periods until April 2004 by monitoring clinical signs (lymphadenomegaly, splenomegaly, epistaxis, onychogryphosis, dermatitis, weight loss), and indirect fluorescent antibody test (IFAT) and BM PCR for *E. canis* and *L. infantum* at eight intervals. Dogs were considered to be positive for infection if found to be reactive by serology or PCR.

IFAT for *Leishmania* was performed using a standard assay with *L. infantum* MON-1 promastigotes, using a cut-off titre of 1 : 160 [1]. IFAT for *E. canis* was performed using *E. canis* antigen in DH82 cells obtained from the American Tissue Culture Collection (ATCC) with a cut-off titre of 1 : 80. All dogs were negative for *L. infantum* and *E. canis* prior to the study.

PCR was performed on DNA extracted from BM. Nested PCR for *Leishmania* was performed with kinetoplastid-specific primers R221 and R332, targeting the small-subunit rRNA gene on first amplification, and *Leishmania*-specific primers R223 and R333 on second amplification [1]. Primers ECA and HE3 were used for amplifying a fragment of the *Ehrlichia* 16S rRNA gene, and sequence analysis of the amplicon and a BLAST search were performed to confirm *E. canis* [2].

The chi-square and Fisher's exact tests were used for statistical analyses.

## RESULTS

Twenty-nine dogs were positive for *E. canis* by PCR and 42 by IFAT. In total, 42 dogs had evidence of *E. canis* infection. Thirty-five dogs were positive for *L. infantum* by PCR and seven by IFAT, with a total of 35 presenting evidence of *L. infantum* infection. All dogs with positive PCR results for *E. canis* were also seropositive for this pathogen, and all dogs that were seropositive for *L. infantum* were also *Leishmania* PCR-positive. Thirty-four of 35 dogs that developed *L. infantum* infection also had *E. canis* infection (Fig. 1). Among these, *E. canis* infection significantly

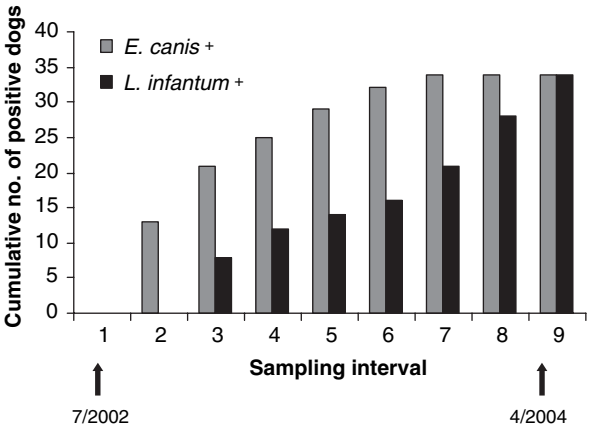


**Fig. 1.** A longitudinal presentation of cumulative numbers of dogs infected with *Ehrlichia canis* and *Leishmania infantum* as determined by combined serology and PCR. Of 43 dogs exposed to natural infection, only dogs with dual infection ($n$ = 34) are presented.

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($p < 0.05$) preceded *L. infantum* infection in 28 (82%), was found concomitantly in two (6%), and followed *L. infantum* infection in four (12%). Twenty-two of 28 (79%) cases of preceding *E. canis* infection were detected before the second transmission season. The dogs were treated with doxycyline at 10 mg/kg for 10 days on October 2002, October 2003 and December 2003 to prevent mortality due to *E. canis*, in compliance with the experimental protocol. Clinical signs were more frequent ($p < 0.01$) in dogs with dual infection (30/34; 88%) than in those with single infection (4/9; 44%).

## DISCUSSION

Canine ehrlichiosis and leishmaniasis are potentially chronic infections that are frequently detected concurrently in dogs. The impact of one pathogen on co-infection with the other is unknown. The synergistic effects of co-infection with other vector-borne pathogens such as *Anaplasma phagocytophilum* and *Borrelia burgdorferi* have been described and their mechanisms have been elucidated [3].

*E. canis* infection was evident before *L. infantum* infection in the majority of dogs with dual infections in this study. The transmission period of *L. infantum* in the study area is May to October [1], whereas the tick activity of *R. sanguineus* studied in nearby Sicily begins earlier [4]. However, it is not likely that most preceding ehrlichiosis is due to earlier transmission, as 79% of the preceding *E. canis* infections were detected before the second transmission season. These were not due to earlier *E. canis* exposure, because the study started in July, after both transmission seasons began.

The higher frequency of dogs with positive serology for *E. canis* vs. positive PCR may be explained by the lower sensitivity of BM PCR in comparison to serology or to elimination of *E. canis* by doxycyline treatment. The higher frequency of positive *Leishmania* PCR in comparison to serology is due to the known long lag period between infection with *L. infantum* and seroconversion and the possibility of infected dogs remaining seronegative [5]. The increased presence of clinical signs in dogs with dual infections supports the postulation of a synergistic pathological effect between pathogens. The study suggests that ehrlichiosis is likely to be a contributing factor to the establishment of canine leishmaniasis.

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