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Feline Leishmaniasis and Ehrlichiosis: Serological Investigation in Abruzzo Region

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Abbreviations: ELISA, enzyme-linked immunosorbent assay; FIV, feline immunodeficiency virus; FeLV, feline leukaemia virus; IFI, indirect immunofluorescent antibody; PCR, polymerase chain reaction

INTRODUCTION

Leishmaniasis and ehrlichiosis are arthropod-borne diseases, which have mainly been described in canine medicine. Natural infection and clinical disease in domestic cats caused by *Leishmania* and *Ehrlichia* appear to be rare. Whether the low prevalence of infection/disease in endemic areas is due to under-reporting or to the fact that cats have a high degree of natural resistance is unknown. Leishmaniasis shows a variety of clinical symptomatology in cats, although the parasite seems to cause both cutaneous and visceral forms. It is important to underline that feline leishmaniasis seems to be mostly characterised by strictly cutaneous forms (Pennisi, 2002; Poli *et al.*, 2002; Pennisi *et al.*, 2003; Savani *et al.*, 2004). In Italy, sero-epidemiological studies have shown a prevalence of feline leishmaniasis infection ranging from 0.9 to 68% (Pennisi, 2002; Poli *et al.*, 2002). According to a few clinical cases reported in the literature, *Ehrlichia canis* infection in cats shows a polymorphic and non-specific symptomatology (Bouly *et al.*, 1994; Beaufils *et al.*, 2002; Breitschwerdt *et al.*, 2002). A serological investigation in Tuscany showed that 50 out of 400 cats (12.5%) tested positive for ehrlichiosis (Ebani and Andreani, 2002). The purpose of this report is to perform a sero-epidemiological study on a representative sample of cats coming from several areas of the Abruzzo region where canine leishmaniasis and ehrlichiosis infections are present.

MATERIALS AND METHODS

Two hundred and three (203) cats from the Abruzzo region were submitted to serological testing for the two arthropod-borne diseases between September 2002 and March 2004.

All of the animals were submitted to clinical examination and serum blood samples were used for serological investigations. *Leishmania infantum* and *Ehrlichia canis* antibodies were detected using IFI assays and all samples were initially tested at a dilution of 1:40. For samples with positive results at a dilution of 1:40, serial 2-fold dilutions were tested until an endpoint titer was reached. All serum samples were also tested with an ELISA assay (SNAP® combo plus, IDEXX) for FIV antibodies and FeLV antigens. In the cats that showed a seropositive response to the two pathogens, more blood was collected from two to twelve months after the preliminary study, to evaluate a possible seroconversion. Finally, PCR testing was performed on blood samples to detect *Leishmania infantum* and *Ehrlichia canis* infections. In the cats that were seropositive to Leishmaniasis, the PCR was also performed on lymph node aspirates.

RESULTS

Anti-*Leishmania* antibodies were found in 33 of 203 cats tested (16.3%). The seropositive cats ranged in age from three months to 16 years; four cats always lived inside the house, six had access to indoor and outdoor environments, and the others were stray cats. 20 of these 33 subjects had an anti-*Leishmania* antibody titer of 1:40, ten cats a titer of 1:80, two cats a titer of 1:160, and one a titer of 1:320. From the analysis of the serological results concerning FIV and FeLV it emerged that 5 out of 33 *Leishmania*-positive cats were FIV positive. Abnormalities observed in the 33 cats upon physical examination included: dehydration (18.9%); localized lymphomegaly and ocular discharge (15.8%); fever, anorexia, vomiting (12.1%); weight loss, nasal discharge, cutaneous and ocular lesions (9.7%); depression, otitis, generalized lymph node enlargement, congested mucous membranes, neonatal mortality and abdominal distention (6.5%); polyuria and polydipsia, hematuria, pale mucous membranes and chronic diarrhoea (3.4%). The remaining subjects (33.6%) seemed to be asymptomatic. Since most of the *Leishmania* positive cats were strays, the study was only continued for eleven cats. On follow-up, six cats were seronegative (five of them had an initial titer of 1:40 and one of 1:80), and the antibody titer remained steady (1:40 and 1:80) for two cats, while there was a reduction in the titer for three subjects (two from 1:80 to 1:40 and one from 1:160 to 1:40). On follow-up all cats gave positive results to PCR for lymph node aspirates and only five of them for blood sample. Anti-*Ehrlichia canis* antibodies were only found for two cats (1%), a mother and son, who had been mainly living indoors, and who had antibody titers of 1:160 and 1:2560, respectively. The subject with the highest titer was a 14-year-old European long-haired, neutered cat with a history of chronic vomiting, dysorexia, polyuria and polydipsia for which a definitive diagnosis of chronic renal failure was made. Serological tests for FIV, FeLV and *Leishmania* gave negative results. Based on the high antibody titer, the cat was treated with doxycycline at the dosage of 10 mg/kg of body weight, PO, every 24 h for 28 days. The mother, a 16-year-old domestic cat, had no clinical signs. This cat gave a positive result on testing for antibodies against *Leishmania* (1:80). After a follow-up of four months, we observed a reduction in the antibody titer for the cat which had an initially higher titer (from 1:2560 to 1:160), while the other subject was negative. Results of *Ehrlichia* PCR assays performed on blood samples from both cats were negative.

DISCUSSION

Results suggest that the seroprevalence of antibodies against *Leishmania infantum* in cats living in some areas of the Abruzzo region is low (16.3%). Moreover, serological investigation has revealed that most of these cats have low antibody titers and subsequent seronegativity or reduction in the antibody titer. This finding may confirm the low susceptibility of the cat to *Leishmania* infection. The natural resistance of the cat to this infection has also been demonstrated by either absent or few clinical signs presented by naturally infected cats.

Positive serological results were only observed for stray cats and could be due to their higher probability of coming into contact with the carrier.

Our results suggest that the role of immunosuppressive viruses in triggering the infection/disease of feline leishmaniasis is not important. In fact, in our study, the presence of the *Leishmania* infection was not correlated with FIV seropositivity or FeLV antigenemia.

The lack of correlation between positive PCR for *Leishmania* for lymph node aspirates and positive PCR for blood samples may be due to an extremely low parasitemia, while the lymph nodes can be considered the first target of the parasite. The seroprevalence of the natural infection of cats to *Ehrlichia canis* was extremely low (1%), even though it seems to produce a high humoral response. The low susceptibility of cats to this infection is demonstrated both by its low seroprevalence and low antibody titers. Additionally, anti-*Ehrlichia* antibody titers show a reduction in an asymptomatic cat which did not receive any therapy during the follow-up. The negative PCR for blood samples may be due, as already reported by other authors, to the possibility of cross-reactions among similar aetiological agents, which is likely to happen while using the IFI assay (Bouly *et al.*, 1994).

In conclusion, our research shows that contact with *Leishmania infantum* and *Ehrlichia canis* is a possible event for the cats; however the epidemiological role of the cat in these two infections needs further study.

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