A molecular and serological study of exposure to tick-borne pathogens in sick dogs from Italy
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INTRODUCTION
There is limited information regarding combined serological and molecular prevalence of canine ehrlichiosis and anaplasmosis in Italy [1] and much less information is available about the epidemiological and clinical importance of Rickettsia infection in dogs in endemic areas.

Several studies have reported Rickettsia conorii seroprevalence rates ranging from 15.5% to 74% in dogs in R. conorii endemic regions [2]. The very high seroprevalences detected in dogs would suggest frequent exposure to Rickettsia. Recent studies reported the detection of Rickettsia DNA in the blood of Spanish and Italian dogs [3–5]. Although febrile illness has recently been associated with R. conorii infection in dogs from Sicily by means of seroconversion and PCR [5], evidence that R. conorii infection causes illness in dogs remains unclear.

The aims of this prospective study were to evaluate the prevalence of Ehrlichia canis, Anaplasma platys, Anaplasma phagocytophilum and Rickettsia by means of serological and molecular techniques in Italian dogs with suspected tick-transmitted diseases and to assess the usefulness of those techniques to diagnose tick-transmitted diseases.

MATERIALS AND METHODS
One hundred and thirty-five dogs were enrolled in the study from April to November 2007 from many veterinarian clinics throughout Italy. Inclusion criteria were the presence of clinical signs and clinicopathological abnormalities compatible with tick-transmitted diseases with or without recent history of tick exposure and no recent treatment with doxycycline. Sera were tested for IgG antibodies to R. conorii, E. canis and A. phagocytophilum as reported [5] and E. canis, A. platys/A. phagocytophilum and Rickettsia spp. real time PCR were performed from whole blood samples taken at the time of diagnosis.

DNA extraction was performed by the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany). Real-time PCRs for detection and quantification of a fragment of 16S rRNA gene of Ehrlichia, Anaplasma and Rickettsia DNA in blood samples were developed using LightCycler version 3.5.17 instrument (Roche). Reaction mixtures for PCRs contained 1x QuantiTect SYBR Green PCR Master Mix (Qiagen, Hilden, Germany), 5 mM MgCl2, 1 µM of each forward and reverse primers (EHR163F 5’-GGCTACGTTAGATTAGCGTAGTTG-3’ and EHR163R 5’-CTGGATACGCTTCCCTCC3′) of Ehrlichia spp., 1 µM of each forward and reverse primers (HGE162F 5’-TAGTAGTATGGGATAGCCACTAGAA-3’ and HGE162R 5’-GTGTGGCTGATCATCCT-3’) of Anaplasma spp., 1 µM of each forward and reverse primers of Rickettsia [5] and 2.5 µL of DNA template. The expected sizes of the amplification products were 146 bp (Ehrlichia), 149 bp (Anaplasma) and 171 bp (Rickettsia).

The positive samples for Anaplasma and Ehrlichia spp. were also confirmed by a real time PCR with hybridisation probes as previously reported [1]. All Anaplasma spp. positive samples by real time PCR were subjected to conventional PCR using specific primers (ApGroELF: 5’-TAGCTAAGGAAACGTAGTCCGA-3’ and ApGroELR: 5’-AAAACTCCGACCGGAGCTTCC3′) for the A. platys GroEL gene that amplify a 275 bp fragment. Amplicons were subjected to sequencing.

RESULTS

The serological and molecular results are summarised in Table 1. All Anaplasma positive samples were A. platys. The five sequences with GenBank accession numbers EU707182–EU707186 were 100% homologous to a portion of the complete genome of the A. platys GroEL gene (GenBank accession number AF478129). A. phagocytophilum seroprevalence was significantly higher in central and southern Italy (chi-square = 13; p <0.0001). Four out of five dogs positive to A. platys PCR were seronegative to A. phagocytophilum antigen, suggesting acute infection. The majority of dogs positive to A. platys PCR...
presented with fever, lethargy, anorexia and thrombocytopenia.

_Ehrlichia canis_ seroprevalence was significantly higher in southern Italy (chi-square = 9; p = 0.003). All dogs positive to _E. canis_ PCR presented high _E. canis_ antibody titres indicating chronic infection. Clinicopathological abnormalities were those commonly described in the literature such as fever and bleeding disorders.

There was no statistical association between gender, breed, lifestyle and serological or molecular outcomes.

**DISCUSSION**

The high seroprevalence rates observed indicate that Italian sick dogs are frequently exposed to tick-transmitted pathogens while molecular prevalence is much lower. The _E. canis_ serological and molecular prevalences found in the present study are in agreement with previous studies performed in Italy [1,2,4]. In addition, it seems that _E. canis_ infection is more prevalent in central and southern regions than in northern Italy [1]. Despite high _Anaplasma phagocytophilum_ and _Rickettsia_ seroprevalences found in this study in Italian sick dogs, we were unable to detect _A. phagocytophilum_ and _Rickettsia_ DNA in any of the samples. It is likely that dogs are exposed to _A. phagocytophilum_ and _Rickettsia_ and infection is rapidly cleared [5].

Based on our results and clinical observations, it is frequently difficult to find a proven aetiology in dogs with suspected tick-borne disease. For this reason, sensitive PCR and acute and convalescent titres must be applied to confirm acute or chronic infections with rickettsial agents in humans and animals.

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**REFERENCES**