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Seroepidemiology of leptospirosis, toxoplasmosis, and leishmaniosis among dogs in Ankara, Turkey

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Abstract

Seroprevalence of five different *Leptospira interrogans* serovars, *Toxoplasma gondii* and *Leishmania infantum* in stray dogs in Ankara was investigated. A total of 116 dog sera collected from apparently healthy stray dogs were tested for *L. interrogans* serovars by microscopic agglutination test (MAT), for *T. gondii* antibodies by Sabin–Feldman dye test (SFDT), and for *L. infantum* antibodies by indirect fluoresence antibody test (IFAT). Of the 116 dogs, 51 (43.96%) were seropositive for leptospirosis, 72 (62.06%) for *T. gondii* and 3 (2.58%) for *L. infantum*. No statistically significant difference was observed between male and female dogs in the seroprevalences of toxoplasmosis and leptospirosis ($P > 0.05$), but statistically significant difference was observed among different age groups in the seroprevalences of toxoplasmosis and leptospirosis ($P < 0.05$). Although the seroprevalence of *L. infantum* was low, asymptomatic animals should be considered as a reservoir for the spread of the disease.

Keywords: *Leptospira interrogans*; *Toxoplasma gondii*; *Leishmania infantum*; Stray dogs

1. Introduction

Leptospirosis, toxoplasmosis and leishmaniosis are important worldwide zoonotic infections affecting both dogs and human beings. *Leptospira interrogans* serovars *canicola* and *icterohaemorrhagie* are considered to be the most significant serovars in dogs (Rentko et al., 1992; Faine, 1994). Widespread use of a bivalent vaccine against serovars *canicola* and *icteroheamorrhagie* decreased the incidence of homologous infections with both serovars, but infections with other serovars have been reported to cause acute clinical infections in dogs in Europe and the USA (Hanson, 1982; Adin and Cowgill, 2000; Scanziani et al., 2002). *Toxoplasma gondii*, an obligate intracellular parasite, is causative agent of the toxoplasmosis, and felids are the only known definitive host for this protozoan (Dubey and Beattie,

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1988). Visceral leishmaniosis (VL) caused by *Leishmania infantum* is endemic zoonotic disease in the Mediterranean basin. Dogs are the reservoir of this protozoan parasite, which is transmitted among canines and to humans by phleobotomine sand flies (Bettini and Gradoni, 1986; WHO, 1993).

Studies carried out in different provinces of Turkey showed that the seroprevalence of leptospirosis, toxoplasmosis and leishmaniosis in dogs are 10.97–26.90% (Ülgen et al., 1997; Özdemir and Diker, 1999), 11.7–85.57% (Çakmak et al., 1996; Babür et al., 1997; Aktaş et al., 1998; İnci et al., 2002; Eren et al., 2002) and 0–7% (WHO, 1993; Coşkun et al., 1997; Özbel et al., 2000; Ertabaklar et al., 2001; Gültekin et al., 2003), respectively. However, the situation in Ankara, the capital, is not well documented (Çakmak et al., 1996; Özdemir and Diker, 1999). Moreover, there is no data available on leishmaniosis in dogs.

Serology is the most widely used tool for diagnosis of these diseases (Abranches et al., 1991; Dubey and Beattie 1988; OIE, 2000). Since the majority of clinical patterns of these diseases in dogs may vary considerably from apparently healthy or inapparent infections to severe systemic disease (Beyer and Shevkunova, 1986; Rentko et al., 1992; Noli, 1999).

It is well known that stray dogs is a potential risk for public health in big cities, so is in Ankara. There is a need to clarify epidemiologic status of these diseases. In this study, therefore, we aimed to determine seroprevalence of five different *L. interrogans* serovars, *T. gondii* and *L. infantum* in stray dogs in Ankara, using serological tests of microscopic agglutination test (MAT), Sabin–Feldman dye test (SFDT) and indirect fluorescence antibody test (IFAT), respectively.

2. Material and methods

2.1. Study area

Ankara is located in the central Anatolia with an altitude of 861 m (39°37′N–32°53′E). The region has subtropical climate having a dominate steppe climate. Therefore, the summers are warm and dry and the winters are cold and snowy. Annual precipitation in Ankara is about 350 mm and average temperature is 11.7 °C.

2.2. Animals and blood collection

A total of 116 sera were collected from apparently healthy dogs, consisting of 67 females (57.76%) and 49 males (42.24%) in Ankara. Age and gender of animals were recorded. Serum samples were stored at −20 °C until serological analyses were performed.

2.3. Serological testing

2.3.1. Microscopic agglutination test (MAT)

Serum samples were tested for leptospirosis by using the following serovars as antigen: serovar *grippotyphosa* (serogroup Grippotyphosa), serovar *icterohemorrhagie* (serogroup Icteroheamorrhagie), serovar *bratislava* (serogroup Australis), serovar *pomona* (serogroup Pomona), and serovar *canicola* (serogroup Canicola). MAT titres equal to or higher than 1/100 were considered to be positive (Cole et al., 1973).

2.3.2. Sabin–Feldman dye test (SFDT)

Serum samples were tested for toxoplasmosis by using vigorous antigen and methylene-blue dying. An antibody titre of 1/16 and over was accepted to be positive (Sabin and Feldman, 1948).

2.3.3. Indirect fluorescence antibody test (IFAT)

Antigen was prepared from *L. infantum* (MON-1) promastigotes. Briefly, promastigot of *L. infantum* (MON-1) was maintained in NNN medium and grown in the RPMI 1640 containing 10% foetal calve serum. Promastigots were harvested from 4 to 6 days old cultures and washed eight times in phosphate buffered saline (PBS) by centrifugation and resuspended at a concentration of 2×10^6 promastigotes/mL in PBS. A volume of 10 μL of these suspension was added to multispot slides. After air drying, slides were stored at −20 °C until used (Taylan et al., 2003).

The IFAT was performed according to De Korte et al. (1990). Two-fold serial dilutions of sera were prepared in PBS (1:16 to 1:256), and aliquots of 10–12 μL were added to antigen coated multiwell slides. Positive and negative controls were included in each series of analysed samples. Following incubation in a humid chamber for 30 min at 37 °C, slides were washed. Fluorescent staining was performed using