ABSTRACT

The prevalence of antibodies to *Toxoplasma gondii* was investigated by the Sabin-Feldman Dye test (SFDT) in 72 stray cats from Nigde, Turkey. A total of 55 (76.4%) of the analysed sera had antibodies to *T. gondii*. The seropositivity of *T. gondii* was 77.1% in male and 75.7% in female cats (*P* > 0.05). Faeces of these cats were also examined by zinc sulphate flotation method for the presence of parasite oocysts and eggs of other parasites. Two protozoan parasites were identified as *Isospora* spp. (12.5%) and *Eimeria* spp. (4.1%) in cats. *Toxoplasma gondii* oocysts were not found in any faecal samples analysed. Two parasitic helminth species were observed: *Toxocara cati* (15.2%) and *Toxascaris leonina* (20.8%). These common ascarids were recorded for the first time in cats from Nigde.

Key words: *Toxoplasma gondii*, Cat, Dye Test, Coprology, Turkey.
Introduction

Toxoplasmosis, a zoonotic disease found worldwide, is caused by an obligate intracellular coccidian parasite Toxoplasma gondii (Dubey and Beattie, 1988). Members of the family Felidae (domestic and wild cats) are the definitive host and in their intestinal epithelial cells the sexually mature stage of the parasite is completed. They are the main reservoir of infection (Dubey, 1994, 1998).

Cats usually become infected by ingestion of encysted organisms present in the tissues of intermediate hosts, or by ingestion of oocysts. Viable organisms are released and invade epithelial cells of the small intestine where they undergo sexual cycle. Oocysts, which are shed in great numbers in the faeces for two to three weeks, contaminate the environment. The unsporulated oocysts may sporulate within less than five days depending on environmental conditions, becoming infectious to the other animals and to humans. Sporulated oocysts can survive in the environment (in moist shaded soil or sand) for several months due to their resistance to environmental conditions (Dubey, 1998; Webster, 2001).

Since clinical signs are nonspecific mimicking several other infectious diseases, and isolation and identification of the parasite are time-consuming, hazardous and expensive, the diagnosis of toxoplasmosis in both man and animals is usually based on the results of serological tests (Soulsby, 1986; Dubey and Beattie, 1988).

In Turkey, epidemiology of toxoplasmosis has not been extensively investigated and little is known on the distribution and prevalence of the disease in cats.

The aim of the present study was to determine the prevalence of antibodies against T. gondii and to examine faecal samples of cats from Nigde, Turkey.

Material and methods

Study Area

Nigde is located in the middle of Turkey with an altitude of 1240 m (37°58' N longitude, 34°41' E latitude). Because it has a subtropical climate, the summers are warm and dry and the winters are cold and snowy. Annual average precipitation is 348.8 mm, average temperature is 11.1°C and average relative humidity is 55% in Nigde.

Sampling of cats and blood collection

This study was performed between April and June 2003 in Nigde. Stray cats, which were used to living in close contact with people were captured by giving food and brought to the laboratory in a cage. Blood samples were collected from 72 stray cats by cephalic venopuncture without applying anesthesia. Out of 72 cats, 37 were females and 35 were males all of which were more than one year old. Serum samples were obtained by centrifugation at room temperature (25°C), at 4000 rpm for 10 minutes and were stored at -20°C until analysed.

Sabin-Feldman testing

Serum samples were tested for toxoplasmosis by using vigorous antigen and methylene-blue dying at Ankara Refik Saydam National Institute of Hygiene. Sabin Feldman Dye Test (SFDT) was performed as described previously (Sabin and Feldman, 1948).

The SFDT result was regarded as positive if more than 50% of tachyzoites did not accept the dye (unstained) when examined under the light microscope (x 400). The threshold was stated at 1:16 dilution.
Faecal examination

The cats were put in different numbered stainless steel cages in the animal facility and they were fed with commercial special cat food. Immediately after defecation in the laboratory faecal samples were collected from the same cats, the blood samples of which were taken beforehand, and were stored in plastic sample containers at 4°C until examination. A total of 72 faecal samples were examined microscopically by zinc sulphate flotation (specific gravity 1.18) method for the presence of parasite oocysts and eggs. Positive faecal samples for oocysts were mixed with 2.5% potassium dichromate (\( \text{K}_2\text{Cr}_2\text{O}_7 \)) solution and placed in a thin layer in petri dishes for sporulation of oocysts and these were kept at room temperature (25°C) for 1 week. To ensure good oxygenation during sporulation, the oocyst suspensions were gently shaken daily. The oocysts were identified on the basis of their morphological characteristics. (Dubey and Beattie, 1988). Helminth species were also examined for identification (Soulsby, 1986).

Statistical analysis

The analysis was performed using the “t test”. It was used to evaluate gender related prevalence of T. gondii. SPSS for Windows was used for data analysis. Significance was set at \( P < 0.05 \).

Results and discussion

Out of 72 sera examined, 55 (76.4%) were positive at the titers \( \geq 1:16.29 \) sera samples were positive at 1:16 dilution, 16 samples at 1:64 dilution and 10 sera at 1:256 dilution. Table 1 shows gender-related prevalence obtained using samples from 37 (51.4%) females and 35 (48.6%) males. The overall seroprevalence was 75.7% in females and 77.1% in males. No statistically significant difference was observed between genders (\( P > 0.05 \)).

Eimeria spp. oocysts were found in faecal samples in 3/72 (4.1%) cats, while Isospora spp. oocysts were found in 9/72 (12.5%) cats. Toxoplasma gondii oocysts were not found in any faecal samples analysed.

Two helminth species were found in cats. Of the 26 positive cats, Toxocara cati eggs were present in 11 (15.2%) cats, Toxascaris leonina eggs were present in 15 (20.8%) cats. The parasites observed in faecal samples is summarized in Table 2. Cats, particularly domestic cats, are important in transmission of Toxoplasma to other animals and human beings because they are the only hosts that can excrete the environmentally resistant oocysts in faeces (Dubey and Beattie, 1988).

Although there is generally a high seroprevalence of infection in cats, most surveys show that less than 1% is oocyst shedding. This is to be expected since infected cats generally do not re-shed oocysts following their first exposure to T. gondii (Soulsby, 1986; Dubey, 1998). Since oocysts are small, quite similar to other cat coccidia, and the period of shedding is limited, the chance of detecting T. gondii like oocysts during routine faecal examination is low, consequently epidemiological studies must be performed using serologic tests (Dubey, 1994). One of the most reliable serologic tests is the Sabin-Feldman dye test that is sensitive and so far is one of the most specific tests for toxoplasmosis. Its main disadvantages are its high cost and the human hazard of using live organisms (Dubey and Beattie, 1988).

Toxoplasmosis in cats has been reported by many researchers in several countries of the world. The general prevalence of feline toxoplasmosis in various countries varies between 6 and 78.1% (Deeb et
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Table 1. Distribution of Toxoplasma gondii antibodies determined by SFDT in cats relative to genders.

| Titers | Female (%) | Male (%) | Total (%) |
|--------|------------|----------|-----------|
| <1:16  | 9 (24.3)   | 8 (22.9) | 17 (23.6) |
| 1:16   | 17 (46.0)  | 12 (34.2)| 29 (40.3) |
| 1:64   | 6 (16.2)   | 10 (28.6)| 16 (22.2) |
| 1:256  | 5 (13.5)   | 5 (14.3)| 10 (13.9) |
| TOTAL  | 37 (100.0) | 35 (100.0)| 72 (100.0) |

Table 2. Prevalence of parasites in faecal samples from cats.

| Gender | N. examined | Protozoan oocysts in faeces | Helminth eggs in faeces |
|--------|-------------|-----------------------------|------------------------|
|        |             | Toxoplasma | Isospora spp. (%) | Eimeria spp. (%) | T. cati (%) | T. leonina (%) |
| Female | 37          | 0          | 3 (8.1)            | 2 (5.4)           | 3 (8.1)     | 7 (18.9)       |
| Male   | 35          | 0          | 6 (17.1)           | 1 (2.8)           | 8 (22.8)    | 8 (22.8)       |
| TOTAL  | 72          | 0          | 9 (12.5)           | 3 (4.1)           | 11 (15.2)   | 15 (20.8)      |

It has been observed that the seropositivity rates are profoundly influenced by differences in serological methods performed, in areas where the studies were conducted and in number of samples. There are few reports regarding toxoplasmosis in cats in Turkey (Table 3). The seropositivity rates of anti-Toxoplasma antibody in cats vary from 1.2 to 78%. The prevalence of T. gondii in cats in Nigde province, located in central Anatolia, has not been reported previously. In this survey, the serological positive rate of T. gondii in cats from Nigde of Turkey was 76.4%. This seroprevalence rate found was higher than those obtained by several authors in cats from different regions of the world. Prevalence values in cats of 42% in Sweden (Uggla et al., 1990), 33.3% in Bangladesh (Samad et al., 1997), 6% in Japan (Nogami et al., 1998), 32.3% in Spain (Miro et al., 2004) and 35.4% in Brazil (Pena et al., 2006) were already reported. On the other hand, our result was similar to the 78.1% in Lebanon (Deeb et al., 1985).

The seroprevalence rate in the present study was in accordance with the results obtained in various regions of Turkey by Ozcelik et al. (1991) and Poyraz et al. (1995). On the other hand, our prevalence results is higher than that reported by Ekmen (1970), Inci et al. (1996) and Babur et al. (1998).

These studies have shown that the prevalence of Toxoplasma antibodies in the cat population is quite variable, depending on the method, number of animals studied and the geographic area. Comparison of domestic and stray cats...
has pointed out that the latter may become infected due to the fact that stray cats feed predominantly on garbage that contain infected meat or prey on small living intermediate hosts (Dubey, 1973). No significant difference was observed between genders in our study (P>0.05). This result is in accordance with several other studies (Inci et al., 1996; Babur et al., 1998; Miro et al., 2004; Pena et al., 2006).

Because of the important role of the cat in the epidemiology of toxoplasmosis, various surveys of feline infection based on the presence of oocysts of Toxoplasma in the faeces have been carried out. The prevalence of Toxoplasma oocysts in cats was reported to be 0-18.2% in Australia (Collins et al., 1983; Meloni et al., 1993) and 1.3% in Brazil (Pena et al., 2006) whereas no Toxoplasma oocysts were found in Belgium by Vanparijs et al. (1991) and in Spain by Miro et al. (2004).

In our study, out of 72 cats faecal samples examined, four kinds of parasites were found: namely Eimeria spp. oocysts (4.1%), Isospora spp. oocysts (12.5%), Toxocara cati eggs (15.2%) and Toxascaris leonina eggs (20.8%). The Eimeria are normally found in herbivores and birds and are considered pseudo-parasites when found in cats. Eimeria oocysts appear in feline faeces as a result of either ingesting Eimeria infected prey or coprophagy (Dubey, 2005). This is likely a rodent or avian eimerian pseudoparasites.

In the present study, Toxoplasma oocysts were not observed and this may be related to the short patent period of this parasite (1-2 weeks).

**Conclusions**

This study demonstrates the high seroprevalence rate of infection in cats, indicating that T. gondii is endemic also in Nigde and that quite a number of them live in close contact with people. Therefore, infected cats can create a danger for the infection of other animals and human beings, by contaminating the environment with oocysts; the subtropical climate in Nigde contributes to the survival of oocysts. The data obtained from this study may be useful for reference in further studies of its implications for both animal and human health in the region of central Anatolia.

Table 3. Seroprevalence of *T. gondii* infection in cats in Turkey.

| Author          | Number of tested | Method | Positivity (%) | Positivity (%) according to gender | Positivity (%) according to gender |
|-----------------|------------------|--------|----------------|------------------------------------|-----------------------------------|
| Ekmen           | 77 stray cats    | SFDT   | 23.4           | na                                 | na                                |
| Ozcelik et al.  | 50 stray cats    | IHA    | 78             | na                                 | na                                |
| Poyraz et al.   | 53 house cats    | IHA    | 69.8           | Female: 20/32 (54.5%)               | 1-2: 7/13 (53.8%)                 |
|                 |                  |        |                | Male: 17/21 (80.9%)                | 3-4: 21/28 (75.0%)                |
|                 |                  |        |                |                                   | 5-6: 9/12 (75.0%)                 |
| Inci et al.     | 65 household cats| SFDT   | 43             | Female: 22/50 (44%)                | <1: 6/13 (46.1%)                  |
|                 |                  |        |                | Male: 6/15 (40%)                   | 1-2: 20/45 (44.4%)                |
|                 |                  |        |                |                                   | >2: 2/7 (28.5%)                   |
| Babur et al.    | 36 stray cats    | SFDT   | 55.5           | Female: 11/17 (64.7%)              | 0-1: 3/9 (33.3%)                  |
|                 |                  |        |                | Male: 9/19 (47.3%)                 | >1: 17/27 (62.9%)                 |

SFDT: Sabin-Feldman Dye Test; IHA: Indirect Hemagglutination; na: not available.
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