Seroprevalence of *Toxoplasma gondii* infection in domestic dogs in an area from northwest of Iran: a cross-sectional study using immunodominant surface antigen 1 (SAG1)

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Abstract *Toxoplasma gondii* is an obligate intracellular protozoan parasite that infects humans and animals. *T. gondii* surface antigen 1 (SAG1) is an appropriate antigen with high specificity and sensitivity for the detection of *T. gondii* infection in humans and animal hosts. The aim of this study was to determine the seroprevalence of *T. gondii* infection using SAG1 antigen (P30) in ownership dogs in Meshkin-Shahr district in the northwestern Iran. The sera samples were collected from 171 domestic dogs and tested using indirect ELISA (SAG1 antigen). The data were analyzed using SPSS software version 13. From a total of 171 dogs, 82 (48 %) of them were sero-positive. No statistical significant difference was seen between *T. gondii* infection and gender ($P = 0.995$). The highest seroprevalence of rate was observed in >5 years animals; but

no statistical significant difference was seen between *T. gondii* infection and age ($P = 0.589$). Our findings indicate that *Toxoplasma* seropositivity rate is high in ownership dogs in northwest of Iran. This is probably due to high exposure to contaminated food, soil, or water sources with sporulated *Toxoplasma* oocysts.

Keywords Seroprevalence · *Toxoplasma gondii* · Dogs · ELISA · Iran

Introduction

Toxoplasmosis is one of the common zoonosis infections which has the potential to be transmitted between human and animals. Also it is caused by an obligate intracellular

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protozoan parasite *Toxoplasma gondii* (Montoya and Liesenfeld 2004) that infects humans and warm-blooded animals such as mammals and birds (Dubey 2004).

Felidae are the only known definitive host for *Toxoplasma gondii* and thus they are the most important reservoir of infection (Dubey et al. 2009). The prevalence of this parasite has a wide variation depending on animals, geographic factors, climate and social-cultural habits (Garcia et al. 2006).

The distribution of *T. gondii* in dogs is worldwide, with prevalence rates ranging from 20 to 91 % in different countries (Ali et al. 2003; Azevedo et al. 2005; Dubey et al. 2007; Silva et al. 2002). Clinical toxoplasmosis in dogs is often associated with immunosuppression induced by canine distemper virus infection (Hosseinienejad et al. 2011). Clinical manifestations of toxoplasmosis can be significantly different in each infected dog and include neuro-muscular, gastrointestinal, respiratory or skin disorders but clinical symptoms mainly appears in respiratory which is due to reactivation of concealed infection (Dubey et al. 1989; Hoffmann et al. 2012). However felidae are the only main host for *T. gondii*, dogs can swallow the infected feces of cats and play a role in the mechanical transmission of the of *T. gondii* oocyte to human (Frenkel et al. 2003; Lindsay et al. 1997). In one experiment sporulated oocysts in dogs' feces were observed 2 days after eating of *T. gondii* oocyte and also is believed that infected dogs play role in transmission of parasite to their owners with licking (Schares et al. 2005). One of the most important routes of *Toxoplasma* infection in dogs is eating raw animal meat that may contain tissue cyst (Alvarado-Esquivel et al. 2014). To diagnose canine toxoplasmosis, ELISA test using SAG1 antigen shows a reliable sensitivity and specificity. This method is based on IgG antibody detection (Hosseinienejad et al. 2009).

Toxoplasmosis prevalence in dogs has not been widely studied in Iran (Gharekhani et al. 2014; Hosseinienejad et al. 2011; Khanmohammadi and Ganji 2012). There are a few studies on prevalence of *T. gondii* serum infection using SAG1 antigen in dogs in the world. Also there is no exact data available about prevalence of *T. gondii* in Meshkin-Shahr districts dogs, where is endemic area of visceral leishmaniasis in Iran and has many stray cats.

This study aimed to determine sero-prevalence of toxoplasmosis using SAG1 antigen in ownership dogs of Meshkin-Shahr district in north-western of Iran.

Materials and methods

Study area

Meshkin-Shahr district in the central northern part of the Ardabil province located at an altitude of 1490 m above

sea level between longitudes 47°19' and 48°17' east and latitudes 38°57' and 38°13' north (Fig. 1). The relative humidity alters between 61 and 70 % and the annual rainfall varies between 300 and 385 mm in this area. Forty-two percent of their populations settle in urban areas, 58 % live in rural areas and small population has nomadic living. The study area has moderate mountainous weather. In this area there are too majority domestic dogs with relationship with the human population which used as guard and herd dogs and also lower stray dogs are found (Salahi-Moghaddam et al. 2010).

Animals

A total of 171 ownership dogs from 6 villages of Meshkin-Shahr District were selected by multi-stage cluster sampling from 2009 to 2010. Selected villages relate to 5 geographical zones of the city: north, south, west, east and center and each village provided 17–50 blood samples. The dogs information such as sex and age were taken from the owners and physical examination.

Preparation of the serum samples

All blood samples were prepared from the cephalic vein by venapuncture, poured into 10 ml polypropylene tubes and were labeled with each dog's special code than processed 4–10 h after collection. The collected samples were centrifuged at 800 g for 5–10 min. The sera were kept at −20° C until tested.

Indirect ELISA

For the diagnosis of *T. gondii* infection, sera were tested with an indirect ELISA using an affinity Immunodominant surface antigen 1 (SAG1). SAG1 antigen was obtained from *T. gondii*, RH strain with incorporation of affinity purification method following Hosseinienejad et al. (Hosseinienejad et al. 2009). Optical density (OD) values were measured at 450 nm on an ELISA reader (Labsystems Multiskan[®] plus). An optimal serum dilution of 1:100 was established by checkerboard titration for the purpose of the test. Index values were calculated by the formula; $SIn = (S_n - N) / (P - N)$ where SIn is the individual ELISA index value, S_n is the OD value obtained for the mean of tested samples, N is the OD value obtained for the negative serum, and P represents the OD values obtained for the positive serum. Based on Hosseinienejad et al., study Anti- *T. gondii* antibodies at ≥ 0.790 by ELISA were considered as *T. gondii* positive results (Hosseinienejad et al. 2009).

Fig. 1 Geographical location of Ardebil province and Meshkin-Shahr county, Iran

Ethical approval

This study was approved by the Ethics Committee of Tehran University of Medical Sciences, Iran (No: 88-01-27-8485).

Statistical analysis

Statistical test including Chi squared (X²) and MacNemar were used to compare seroprevalence rate with age and gender of domestic dogs. Statistical analyses were performed using SPSS software version 13 (SPSS Inc, Chicago, IL, USA), with a probability (*P*) value of <0.05 as statistically significant.

Results

All of the dogs were of mixed breed and all of them were kept as guarding dogs and sheep-dogs. From a total of 171 dogs, 82 (48 %) were positive. Of these, 70 (47.9 %) of the males and 12 (48 %) of the females had anti-*T. gondii* antibodies (Table 1). No statistically significant difference was seen between infection and the gender (*P* = 0.995).

In this survey, most of infected dogs were arranged in >5 years old age group (55.6 %) but no significant statistically difference was seen between *T. gondii* infection rate and age groups (*P* = 0.589) (Table 1).

Of the 6 villages of Meshkin-Shahr areas, the highest and lowest infection rate was report in Oorkandi (79.3 %) and Parikhan (24 %), respectively (Fig. 2).

Discussion

Immunodominant surface antigen 1 (SAG1, P30) is considered an important antigen for sero-diagnosis of *T. gondii* infection in both human and animals (Handman et al. 1980). SAG1 antigen has also been shown to be effective in the sero-diagnosis in cats (Huang et al. 2002; Kimbita et al. 2001). This antigen is stage specific antigen that only exists in tachyzoite stage of *T. gondii* (Kasper et al. 1984). Hosseini et al. (2009) showed that ELISA test using SAG1 antigen has a relative sensitivity and specificity of 94.52 and 93.60 %, respectively for diagnosis of *T. gondii* infection in dogs and does not show cross-reactivity with closely related protozoan parasite, *Neospora caninum* (Hosseini et al. 2009). Therefore, in current study

Table 1 Sero-prevalence of canine *T. gondii* infection in dogs in different variables in Meshkin-Shahr District, northwestern Iran

	Age groups (years)			Sex		Total
	<2	2–5	>5	Male	Female	
No. of sample (%)	52 (30.4)	83 (48.5)	36 (21)	146 (85.4)	70 (47.9)	171 (100)
No. of positive (%)	24 (46.2)	38 (45.8)	20 (55.6)	25 (14.6)	12 (48.0)	82 (48.0)

Fig. 2 Frequency of *T. gondii* infection among the domestic dogs in different sampling area, northwestern Iran

ELISA test using SAG1 antigen was selected for diagnosis of *T. gondii* in dogs.

In the present study, the seroprevalence of *T. gondii* in dogs was 48.0 %. A few studies have been performed in Iran to evaluate *T. gondii* infection in dogs. Hosseinienejad et al. showed that seroprevalence of *T. gondii* in dogs in Tehran using an indirect fluorescent antibody test (IFAT) is 22.47 % (Hosseinienejad et al. 2011). In Khanmohammadi survey, using latex agglutination test, 10.1 % of shepherd dogs from Sarab district, northwest Iran had antibodies to *T. gondii* (Khanmohammadi and Ganji 2012). Gharekhani et al. were detected 10.7 % of seropositive dogs using Modified Agglutination Test in western Iran (Gharekhani et al. 2014). It is possible that environmental characteristics changes among the Iran's districts might explain the differences in seroprevalence of *T. gondii* infection in dogs.

Seroprevalence of *T. gondii* found in this study was more than 7.9 % reported from Taiwan by using a latex agglutination test, 32 % from Trinidad and Tobago using a latex agglutination test, 30.9 % of police dogs in Shenyang, northeastern China using MAT, 47.3 % from Brazil using IFAT and 21.6 % pet dogs from Kunming, southwest China using indirect haemagglutination (IHA) and less than 88.5 % reported from Brazil using IFA test and 67.4 % from Sri Lanka using modified agglutination test, 67 % from Senegal using and 67.3 % from Mexico using MAT (Alvarado-Esquivel et al. 2014; Davoust et al. 2014; Duan et al. 2012; Dubey et al. 2007; Lin 1998; Paulan Sde et al. 2013; Santos et al. 2009).

The seroprevalence values among male and female dogs were 47.9 and 48 %, respectively. No statistically significant difference was found between *T. gondii* infection and gender ($P = 0.995$). Similar findings were reported by

other authors (Alvarado-Esquivel et al. 2014; Duan et al. 2012; Hosseinienejad et al. 2011; Nguyen et al. 2012; Santos et al. 2009).

Based on the present study, sero-prevalence in older dogs (above of 5 years old) higher than other groups but significant difference was not detected among the age groups ($P = 0.589$). In previous studies in dogs of Mexico, Korea and China, the seroprevalence of *T. gondii* infection did not increase with age while according surveys in dogs of Trinidad and Tobago and Taiwan, sero-prevalence of *T. gondii* increases with age (Ali et al. 2003; Alvarado-Esquivel et al. 2014; Duan et al. 2012; Lin 1998; Nguyen et al. 2012).

The rural parts of Meshkin-Shahr district are mainly agricultural areas and the people are in a close contact with dogs, which are used for shepherding and guarding, therefore they may be implicated in the mechanical transmission of *T. gondii* to humans.

In comparison of this study with others in Iran and different parts of the world, is realized that dogs in Meshkin-Shahr show a high rate of *T. gondii* infection. High prevalence of this parasite in Meshkin-Shahr probably due to higher exposure to contaminated food, soil, or water sources with sporulated oocysts and also adequate climate, high environmental contamination, eating of infected meat with tissue cyst, predation of rodents that existed in the area, high population of cats and cultural behavior of people.

In conclusion, toxoplasmosis among domestic dogs in Meshkin-Shahr district from northwestern Iran is very high. Early detection and control of toxoplasmosis in cats that live in the studied areas can reduce the incidence of the infection in humans and other intermediate hosts.

Limitation of the present study is the relatively small number of selected domestic dogs and it is not used from random sampling method. A multi-stage cluster sampling is recommended for future investigation.

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