Short Paper

A study on seroprevalence and coproantigen detection of *Toxoplasma gondii* in companion cats in Ahvaz area, southwestern Iran

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(Received 2 Jan 2010; revised version 9 Jun 2010; accepted 12 Jun 2010)

Summary

Cats play crucial roles in the epidemiology of toxoplasmosis. In the present study, a total of 198 companion cats of different ages were examined for serum antibodies against *Toxoplasma gondii* by immunochromatography assay and for oocyst presence in faeces by microscopic examination (flotation method) and immunochromatography assay. The cats were selected between referred cases to the Veterinary Hospital of Ahvaz University, southwestern Iran from December 2006 to November 2009. Classification was made by age, sex, breed, region and season. The studied cats were divided into three groups based on age (<6 months, 6 months–3 years and >3 years) and into five regions based on area (north, east, west, south and central). The results were analyzed by Chi-square analysis, Fischer's exact test and Z test. Forty nine of 198 serum samples (24.75%) had antibodies against *Toxoplasma gondii* (95% CI: 18.7-30.7%). Prevalence was significantly higher in adult cats above 3 years (38.8%) and 6 months–3 years (26.6%) compared with cats less than 6 months (3.8%) (P<0.001). Prevalence was higher in male cats (29.7%) than females (20.6%), in the summer season (26.2%) and west region (27.5), but the difference was not significant between the prevalence of infection relative to host gender (P=0.14), season (P=0.99) and region (P=0.98). Faecal flotation technique and immunochromatography assay was carried out on faecal samples also and *T. gondii* oocysts were not detected in any of the 198 samples. Our study showed that the prevalence of infection (24.75%) is relatively high in serum of companion cats in the Ahvaz district.

Key words: *Toxoplasma gondii*, Prevalence, Immunochromatography, Cat, Ahvaz

Introduction

Toxoplasma gondii is a worldwide parasite that can infect the central nervous system of warm-blooded animals, including people (Montoya and Liesenfeld, 2004). Domestic cats and other felids have been shown as the major reservoir hosts of this infection. It is generally assumed that cats play a major role in transmitting *T. gondii* through faecal contamination of soil, food or water, because they can excrete millions of oocysts in a short period of time (1-2 weeks) (Dubey, 2008). *Toxoplasma gondii* oocysts have been detected in the faeces of less than 1% of cats (Dubey and Beattie, 1988). Carnivorous animals become infected mostly by ingestion of meat containing bradyzoite in tissue cyst (Greene, 2006; Dubey, 2008). *Toxoplasma gondii* infection has become a major public health concern in recent times due to the immunosuppressive effects of the ravaging HIV/AIDS pandemic (Lindstrom *et al*., 2006; Uneke *et al*., 2007). The role of oocysts as potential sources of infection should be considered in future

epidemiological studies (Tenter *et al*., 2009). In Iran, large numbers of cats are found roaming residential streets and increasing the risk of public health for humans and other animals. They can be an important potential source of transition of zoonotic parasites such as *T. gondii*. Previous studies have shown that the prevalence of *T. gondii* antibodies in the cat population is quite variable, from 0 to 100% depending on the method, number of animals studied and the geographic area (Dubey and Beattie, 1988).

Some results of the studies carried out in Iran showed a high prevalence of *Toxoplasma* infection in cats. For example, a seroprevalence study of *Toxoplasma* in Sari revealed the overall prevalence of *T. gondii* IgG antibodies (LAT titre ≥1:1) in 40% of stray cats, with regional variations (Sharif *et al*., 2009). In another study in Tehran, 90% of stray and 36% of household cats were positive for *Toxoplasma* antibody (Haddadzadeh *et al*., 2006). *Toxoplasma* antibodies were found in 49.6% of healthy persons compared with 72.3% in suspected patients in the Ahvaz area (Hoghooghi-Rad and Afraa, 1993). The overall infection rate for *T. gondii* was 32.1% in cats in the Kerman district (Akhtardanesh *et al*., 2010).

Several laboratory methods have been developed to detect antibody in the serum of infected cats such as PCR, ELISA, LAT (latex agglutination test), IHA (indirect hemagglutination assay), IFAT (indirect fluorescent antibody test) and immuno-chromatography (IC). Though these tests are more sensitive and specific, they are expensive and require a specialized laboratory (Zhang *et al*., 2009a).

IC is rapid, simple, sensitive, and specific. It will be a suitable diagnostic tool for detection of the specific antibodies in *T. gondii* infection in cats under field conditions (Xiaohong *et al*., 2004). Quicking Toxoplasma Antibody Rapid Test is a sandwich lateral flow immuno-chromatography assay for the qualitative detection of Toxoplasma antibody in animal's serum (Catalog No. W81042, Biotech Co., Ltd, Shanghai). Specificity and sensitivity for kits of Toxo Ab and Ag Test were found to be high at 98% and 100%, respectively according to the manufacturer's instructions.

Faecal flotation technique was used to detect oocyst in faecal samples as well. Although flotation is a reference method for the detection of *Toxoplasma gondii* oocysts, it has been suggested that an alternative test is also needed because microscopic examination is time consuming and needs an experienced microscopist (Greene, 2006). The objective of the present survey was to investigate the seroprevalence and coproantigenic detection of *T. gondii* in the faeces and serum samples of companion cats in the Ahvaz area, southwestern Iran.

Materials and Methods

Study area and sample population

This study was performed in the Ahvaz area, southwestern Iran, situated at an elevation of 12 meters above sea level and the climate is warm-humid. In the present study, a total of 198 companion cats of different ages were examined for serum antibodies against *Toxoplasma gondii* by immunochromatography assay and for oocyst detection in faeces by microscopic examination (flotation method) and immunochromatography assay. The cats used in this study were cases referred to the Veterinary Hospital of Ahvaz University from December 2006 to November 2009. Most of the cats were apparently healthy and were mostly referred for vaccination and other reasons such as viral infections (*feline panleucopenia* and herpesvirus). They were kept indoors without free access to outside sources in most cases. Classification was made by age, sex, breed, region and season. Information about companion cats was taken from their owners. The studied cats were divided into three groups based on age (group 1: <6 months, group 2: 6 months–3 years and group 3: >3 years) and into five regions based on area (north, east, west, south and central). Most of the studied cats were domestic short hair (DSH).

Laboratory methods

Ketamine (15 mg/kg) and acepromazine (0.15 mg/kg) were injected for sedative effects. Blood samples were collected from the juglar veins and allowed to clot, then centrifuged for 5 min at 1800 × g. Serum

was removed and stored at -20°C until assayed. *Toxoplasma gondii* antibodies were detected with a commercial Rapid test kit (Toxo Ab Test) (Catalog No. W81042, Biotech Co., Ltd, Shanghai).

Rectal contents of 198 cats were collected and subjected to a faecal flotation technique (Greene, 2006). Faeces (1 g) of each animal were emulsified in sucrose solution (specific gravity 1.203), filtered through gauze, and centrifuged in a 15 ml tube at 400 g for 10 min. A drop of the float from the meniscus was examined microscopically at ×400 magnification for the presence of *T. gondii* oocysts.

Also, faecal samples were collected from the studied cats using the sample collection swab pre-wetted with saline solution. The swabs were inserted into the specimen tube containing assay diluents and continued following the manufacturer's instructions (Catalog No. W81021, Biotech Co., Ltd, Shanghai). All data were entered and stored in a computerized database. Age was estimated by dental formulary and owner's information.

Interpretation of the test

A color band (control band) will appear in the left section of the result window to show that the test is working properly. If another color band appears in the right section of the result window, this band is the test band. The presence of only one band within the result window indicates a negative result. The presence of two color bands (T and C) within the result window indicates a positive result (Catalog No. W81021, Biotech Co., Ltd, Shanghai).

Statistical analysis

Cats were grouped by age, sex, breed, season and geographic area to determine whether these factors were associated with *T. gondii* infection, by Chi-square test, Fisher's exact test (breed) and Z test (for confidence interval). Statistical comparisons were carried out using SPSS 16.0 statistical software. Differences were considered significant when $P<0.05$.

Results

Forty nine of 198 serum samples (24.75%) had antibodies against *Toxoplasma gondii* (95% CI for proportion: 18.7-30.7%). Prevalence was significantly higher in adult cats above 3 years (38.8%; 26 out of 67) and 6 months–3 years (26.6%; 21 out of 79) compared with cats less than 6 months (3.8%; 2 out of 52) ($P<0.001$). Of course, the difference was not significant between cats above 3 years and 6 months–3 years ($P=0.16$). Prevalence was higher in male cats (29.7%; 27 out of 91) than females (20.6%; 22 out of 107), in the summer season (26.2%; 11 out of 42) and west region (27.5; 11 out of 40), but the difference was not significant between the prevalence of infection relative to host gender ($P=0.14$), season ($P=0.99$) and region ($P=0.98$). Prevalence in other seasons (winter, spring and autumn) was 24.6% (14 out of 57), 22.9% (11 out of 48) and 25.5% (13 out of 51), respectively. Prevalence in other regions (north, east, south and central) was 22.2% (8 out of 36), 23.5% (8 out of 34), 24.5% (12 out of 49) and 25.6% (10 out of 39), respectively also. One hundred seventy nine out of 198 (90.4%) of the companion cats were DSH breed. *Toxoplasma gondii* oocysts were not detected in any of the 198 faecal samples by faecal flotation technique and immunochromatography assay. The results are summarized in Tables 1 and 2.

Table 1: Prevalence of *Toxoplasma gondii* infection in companion cats of different age and sex in Ahvaz district, Iran by immunochromatography assay, 2006-2009

Sex	Age					
	<6 months		6 months–3 years		>3 years	
	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
Male	23	1	22	11	19	15
Female	27	1	36	10	22	11
Total = 198	50	2	58	21	41	26

Table 2: Prevalence of *Toxoplasma gondii* infection in companion cats of different age and region in Ahvaz district, Iran by immunochromatography assay, 2006-2009

Region	Age					
	<6 months		6 months–3 years		> 3 years	
	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
North	10	0	10	5	8	3
East	11	1	7	3	8	4
West	12	0	11	3	6	8
South	8	1	18	4	11	7
Central	9	0	12	6	8	4
Total	50	2	58	21	41	26

Discussion

The present study, which is the first report on prevalence of *T. gondii* in companion cats in the Ahvaz district, revealed that 24.75% of referred cats were affected by Toxoplasma. From the results of this research, we conclude that a considerable percentage of cats in southwest Iran (Ahvaz) are infected with *T. gondii*. These infected cats may play an important role in the transmission of toxoplasmosis to humans. It seems that climatic conditions in this area (warm and humid) are relatively suitable for the spread and survival of the oocysts. Statistical analysis was calculated in referred cats to the Veterinary Hospital for 3 years, so it is a suitable sample of the cat population in the Ahvaz district.

In our study, prevalence was significantly higher in adult cats above 3 years (38.8%) and 6 months–3 years (26.6%) than cats less than 6 months (3.8%) and these results were similar to those described by Steven *et al*. (2000), Gauss *et al*. (2003), Miro *et al*. (2004), Haddadzadeh *et al*. (2006), Alvarado-Esquivel *et al*. (2007), Craeye *et al*. (2008) and Sharif *et al*. (2009). It shows that high age increases the risk of exposure to *T. gondii*.

A higher seroprevalence was seen in male companion cats than females in our survey, of course the difference was not significant (P=0.14). Haddadzadeh *et al*. (2006) tested 100 serum samples from 50 stray and 50 household cats in Tehran and found no significant differences in the *T. gondii* antibody titres between males and females. Similar findings were reported by Sumner and Ackland (1999), Gauss *et al*. (2003), Smielewska-Loś and Pacoń (2003), Salant and Spira (2004), Pena *et al*. (2006), Haddadzadeh *et al*. (2006), Hooshyar *et al*. (2007) and Sharif *et al*. (2009). Our results showed agreement with the above results.

The prevalence of *T. gondii* has been studied in different cities of Iran, for example, 32.1% in Kerman (Akhtardanesh *et al*., 2010), 40% in Sari (Sharif *et al*., 2009), 86% in Kashan (Hooshyar *et al*., 2007), and 36-90% in Tehran (Haddadzadeh *et al*., 2006).

In previous reports on the prevalence of *T. gondii* in stray cats in the Ahvaz area, 59.4% (60 out of 101) of the serum samples were positive by Direct Agglutination Test. As to gender, 48.5% and 51.5% of serum samples were female and male, respectively. There was no statistically significant difference in the number of positive males and females (Hoghooghi-Rad and Razi Jalali, 1992). It shows that stray cats are more important in infection spread compared with companion cats. Another study in Tabriz showed 36.2% infection in cats (Jamali, 1996).

Other surveys of *T. gondii* prevalence have been recorded, 17.98% in China (Zhang *et al*., 2009b), 8.1-16.1% in Korea (Kim *et al*., 2008), 21% in Mexico (Alvarado-Esquivel *et al*., 2007), 40.3% in Ankara (Ozkan *et al*., 2008), 44.1% in Czech Republic (Sedlak and Bartova, 2006), 87.3% in Brazil (Cavalcante *et al*., 2006), 25.5-51.9% in Spain (Gauss *et al*., 2003; Miro *et al*., 2004), 41% in Washington (Ladiges *et al*., 1982) and 70.2% in Belgium (Dorny *et al*., 2002).

Having an antibody titre to *Toxoplasma* usually means that the main shedding period of oocysts in cats has been finished (Sumner and Ackland, 1999). In our study, most of the household cats (75.25%) were negative, which means they are at risk of exposure to

infection, shedding oocysts, and so have the potential to transmit the infection to their owners.

In the present research, no oocysts of *T. gondii* were found in the faeces of 198 cats by concentration methods and immuno-chromatography, possibly because the oocysts are excreted by cats during a short period of time (1-2 weeks) after primo-infection (Dubey and Beattie, 1988). Our results on antigenic detection of *Toxoplasma* in the studied cats were similar to other studies found in the Pena *et al*. (2006) and Sharif *et al*. (2009) survey in Sari, in which *T. gondii* oocysts were not found in any of the faecal samples analyzed. Only 2 out of 100 smear preparations of intestinal mucosa showed trophozoites of *T. gondii* (Sharif *et al*., 2009).

In our survey, 90.4% of the studied companion cats were DSH. They are usually adopted by owners as kittens or older and may be seropositive for toxoplasmosis before adoption. Therefore, the exact relation between indoor and outdoor breeding type could not be differentiated in the present study. Due to close contact of cats with human and the fact that children play outdoors on the soil, cats can be an important potential source of transmission of zoonotic parasites such as *T. gondi* (Greene, 2006; Dabritz and Conrad, 2009). Prevention efforts should focus on educating cat owners about the importance of collecting cat faeces in litter boxes, spaying owned cats, reducing the numbers of feral cats and promoting rigorous hand hygiene (Meireles *et al*., 2004; Dabritz and Conrad, 2009). Our results will be the basis of further studies that will allow us to deepen our knowledge of the epidemiology of *T. gondii*. Further studies in various areas will be necessary to survey the overall epidemiological status of toxoplasmosis in companion and stray cat populations.

Acknowledgement

We would like to mention our great appreciation of the Research Council of Shahid Chamran University of Ahvaz for the financial support.

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