Short communication

Seroprevalence of *Toxoplasma gondii* Infection in Dogs in Tehran, Iran

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ABSTRACT

**Background:** *Toxoplasma gondii* infects a wide range of animals; felines are definitive hosts and other animals including the dogs are intermediate hosts. The aim of this study was to determine the seroprevalence of *T. gondii* infection in dogs in Tehran, capital of Iran and to investigate possible associated risk factors.

**Methods:** Three hundreds ninety six serum samples were collected during 2007-8 from the dogs. Collected samples were tested using an indirect fluorescent antibody test (IFAT) in dilutions of 1:16 and more. All procedures were carried out in Shahrekord University, Iran. All the data were analyzed using SPSS software, qui square test with confidence interval of 0.95.

**Results:** From evaluated samples, 89 (22.47%) were positive in titers of at least 1:16. further evaluations in other dilutions showed positive results in dilutions of maximum 1:16 , 1:32, 1:64, 1:128 and 1:256 in 38, 29, 15, 2 and 5 dogs respectively. Investigation of the role of risk factors showed no sex predisposition while infection rate was significantly higher in dogs older than one year old. Living places were of significant importance; infection rate was significantly higher in stray or guard dogs in compare with household dogs (*P*<0.05).

**Conclusion:** Relatively high seroprevalence of *T. gondii* infection in dogs in Tehran shows high environmental contamination. It is recommended that the dogs with suspected clinical signs be tested for *T. gondii* infection.

**Keywords:** Toxoplasma gondii, Dogs, Prevalence, Iran

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Introduction

Toxoplasma gondii; one of the most common zoonosis is an apicomplexan parasite that infects a wide range of animals among which felines are definitive and other animals including dogs are intermediate hosts (1). The disease may be a latent infection in clinically normal dogs or with sever clinical signs especially in immunosupressed dogs. Clinical signs of the disease in dogs can vary considerably and include neuromuscular, respiratory or gastrointestinal disorders (2). A role for dogs in the transmission of T. gondii to human has been postulated, based on observations that dogs ingest cat feces and often roll in cat feces and other foul-smelling substances (3). Viable sporulated oocysts were present for 2 days in feces of the dogs fed sporulated T. gondii oocysts. These dogs were seroconverted to T. gondii (4). Recently, Schares et al. have found viable T. gondii oocysts in some of the fecal samples collected from dogs in Germany (5). Toxoplasma gondii infection in dogs is distributed worldwide, with prevalence rates ranging from 20% to 91% in different countries (6-8).

Indirect fluorescent antibody test (IFAT) is a reference test that with acceptable sensitivity and specificity provides valuable information about T. gondii infection and many other infectious diseases in human and animals. It has been used for sero-epidemiological studies previously in Iran as well as other countries (9-15).

To our knowledge, no published data about seroepidemiology of T. gondii infection in dogs in Iran is present. This study was aimed to investigate the serological prevalence of T. gondii infection in Iran and to investigate related risk factors.

Material and Methods

Sample collection
Serum samples were collected from 396 dogs from Tehran Province during 2007-2008 among which 196 were referred to small animal clinic of Tehran University for annual vaccination and check-up and 200 were collected from shelters and stray dogs from Karadj area. All of the dogs were clinically healthy by sampling. Serum samples were stored at -20°C until use. Information about age, sex and breed of the dogs were recorded in a query form (Table 1).

Parasites
RH strain of T. gondii (16) were maintained in Vero cell cultures and purifed as described previously (9). Cell culture derived tachyzoites were used immediately for the preparation of IFAT slides. RH strain of tachyzoites were kindly provided by Friedrich Loeffeler Institute, Germany and multiplied in Vero cell cultures in research institute of animal embryo technology, Shahrekord University, Iran.

Indirect Fluorescent Antibody Test (IFAT)
An IFAT was performed as described (17). Serum samples were diluted using phosphate buffer saline (pH 7.4). Dilutions of 1:16 have been used as starting dilution in IFAT. Positive samples in this dilution were subjected to serial dilutions (two fold dilutions) until being negative. Ten well slides (Biogen, Tehran, Iran) with formalized tachyzoites attached were incubated with diluted dog sera followed by incubation with a fluorescein Isothiocyanate labeled rabbit anti-dog IgG (Sigma-Aldrich, USA). Positive and negative control sera were obtained from dogs previously tested by other serological assays (IFAT and ELISA) (9).
Results

Among analyzed serum samples, 89 (22.47%) were positive in titer of at least 1:16. Further evaluations in other dilutions showed positive results in dilutions of maximum 1:16, 1:32, 1:64, 1:128 and 1:256 in 38, 29, 15, 2 and 5 dogs respectively. To rule-out the possibility of mistakes in data related to ages especially about stray dogs, animals were divided to two age groups; more and less than one year old. From 85 dogs of less than one year old, 7 (8.23%) had anti-T. gondii antibodies in dilutions of at least 1:16 while from 311 dogs of 1 year old and older 82 (26.36%) were sero-positive in this dilution; seroprevalence of T. gondii infection was significantly higher in dogs of the later group (P≤0.05). Although infection rate was slightly more in male dogs, no significant difference was seen between two evaluated sexes (P<0.05). From total of 155 household dogs 9.03% were seroconverted to T. gondii while infection rate was 31.12% in stray or guard dogs. Hence living place was significantly related to the infection rate (P<0.05).

Table 1: Sero-prevalence of canine T. gondii infection regard to age, sex and living status of the dogs evaluated by IFAT on sera collected from Tehran and Karadj

| Age       | Sex       | Living Status |
|-----------|-----------|---------------|
| <1 year   | ≥1 year   | Male          |
| n (%)     | n (%)     | n (%)         |
| Positive  | 7 (8.23)  | 82 (26.36)    |
| Negative  | 78 (91.77)| 229 (73.67)   |

| Age       | Sex       | Living Status |
|-----------|-----------|---------------|
| <1 year   | ≥1 year   | Male          |
| n (%)     | n (%)     | n (%)         |
| Positive  | 50 (23.8) | 39 (20.96)    |
| Negative  | 160 (76.2)| 147 (79.04)   |

| Age       | Sex       | Living Status |
|-----------|-----------|---------------|
| <1 year   | ≥1 year   | Female        |
| n (%)     | n (%)     | n (%)         |
| Positive  | 39 (20.96)| 14 (9.03)     |
| Negative  | 141 (90.97)| 166 (68.88)   |

| Age       | Sex       | Living Status |
|-----------|-----------|---------------|
| <1 year   | ≥1 year   | Household     |
| n (%)     | n (%)     | n (%)         |
| Positive  | 75 (31.12)| 75 (31.12)    |
| Negative  | 307 (77.52)| 307 (77.52)   |

Discussion

Dietary habits of the dogs cause them to be highly predisposed to T. gondii infection because of the possibility to eat infected tissues from intermediate hosts and the close contact with soil containing sporulated T. gondii oocysts (3, 4). Although clinical toxoplasmosis is rare in dogs population, congenital transmission remains of clinical importance in fetuses. Uncompleted immune system in puppies and immunodeficiency due to concomitant infections such as canine distemper virus remain of potential risk factors for T. gondii infections. However clinical signs in dogs are variable and include respiratory, digestive, ocular, neurological and muscular disturbances (2).

A few studies have been performed in Iran to evaluate T. gondii infection in animals including the dogs. T. gondii infection was estimated as 14.2% in these studies (18, 19). Seroprevalence rate of T. gondii in dogs has been reported from 19.6 to 91% from different countries and this shows worldwide distribution of this protozoan parasite (6, 8,
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Seroprevalence of 22.47% for *T. gondii* found in this study was more than 7.9% reported from Taiwan and 21.3% from Brazil and less than 30% from Sweden and 32% from Trinidad and Tobago (20-23).

Our finding of higher seroprevalence of *T. gondii* in older dogs is in agreement with other investigators and has been related to a higher chance for exposure to *T. gondii* over time and increasing the susceptibility in older dogs (24, 25).

Higher infection rate in stray and guard dogs in comparison with household dogs are probably due to higher exposure to contaminated food, soil, or water sources with sporulated oocysts. The sex of dogs was not significantly associated with the infection rate. This is agreement with other studies (22, 25).

Relatively high seroprevalence of *T. gondii* infection in dogs in Iran can be due to high environmental contamination. Dogs with suspected clinical signs should be tested for toxoplasmosis.

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### References

1. Frenkel JK. Pursuing Toxoplasma. J Infect Dis. 1970; 122 (6): 553-559.
2. Dubey JP, Lappin M. Toxoplasmosis and Neosporosis. In: Green C. editor. Infectious diseases of the dog and cat. New York; 2006 P. 2 754-2775.
3. Frenkel JK, Lindsay DS, Parker BB, Dobesh M. Dogs as possible mechanical carriers of Toxoplasma, and their fur as a source of infection of young children. Int J Infect Dis. 2003; 7 (4): 292-293.
4. Lindsay DS, Dubey JP, Butler JM, Blagburn BL. Mechanical transmission of *Toxoplasma gondii* oocysts by dogs. Vet Parasitol. 1997; 73 (1-2): 27-33.
5. Schares G, Pantchev N, Barutzki D et al. Oocysts of *Neospora caninum*, *Hammomdia heydorni*, *Toxoplasma gondii* and *Hammondia hammondi* in faeces collected from dogs in Germany. Int J Parasitol. 2005; 35 (14): 1525-1537.
6. Uggla A, Mattson S, Juntti N. Prevalence of antibodies to *Toxoplasma gondii* in cats, dogs and horses in Sweden. Acta Vet Scand. 1990; 31 (2): 219-222.
7. Wanha K, Edelhofer R, Gabler-Eduardo C, Prosl H. Prevalence of antibodies against *Neospora caninum* and *Toxoplasma gondii* in dogs and foxes in Austria. Vet Parasitol. 2005; 128 (3-4): 189-193.
8. Silva NM, Lourenco EV, Silva DA, Mineo JR. Optimisation of cut-off titres in Toxoplasma gondii specific ELISA and IFAT in dog sera using immunoreactivity to SAG-1 antigen as a molecular marker of infection. Vet J. 2002; 163 (1): 94-98.
9. Hosseininejad M, Azizi HR, Hosseini F, Schares G. Development of an indirect ELISA test using a purified tachyzoite surface antigen SAG1 for sero-diagnosis of canine *Toxoplasma gondii* infection. Vet Parasitol. 2009; 164 (2-4): 315-319.
10. Hosseininejad M, Hosseini F, Mahzounieh M et al. Seroprevalence of *Neospora caninum* infection in dogs in Chaharmahal-va-Bakhtiari Province, Iran. Comparative Clinical Pathology. 2010; 19 (3): 269-270.
11. Hosseininejad M, Hosseini F, Mosharraf M, Shahbaz S, Mahzounieh M, Schares G. Development of an indirect ELISA test us-
ing an affinity purified surface antigen (P38) for sero-diagnosis of canine Neospora caninum infection. Vet Parasitol. 2010; 3:337-42.

12. Hosseininejad M, Pirali-Kheirabadi K, Hosseini F. Seroprevalence of Neospora caninum Infection in Camels (Camelus dromedarius) in Isfahan Province, Center of Iran. Iranian J Parasitol. 2009; 4 (4): 61-64.

13. Malmasi A, Hosseininejad M, Haddadzadeh H, Badii A, Bahonar A. Serologic study of anti-Neospora caninum antibodies in household dogs and dogs living in dairy and beef cattle farms in Tehran, Iran. Parasitol Res. 2007; 100 (5): 1143-1145.

14. Pirali-Kheirabadi K, Mahzounieh M, Hosseininejad M, Teimori J, Taheri M. Calibration of an indirect fluorescent antibody test (IFAT), using Anti-camel IGG-FITC conjugated antibody, produced under laboratory conditions in rabbit. J Camel Prac Res. 2008; 15 (1): 21-23.

15. Hosseininejad M, Pirali-Kheirabadi K, Ebrahimi A, Hosseini F. Toxoplasma gondii infection in camels (Camelus dromedarius): a serologic assay in Iran. J Camel Prac Res. 2010; 17 (1): 35-36.

16. Sabin A. Toxoplasmic encephalitis in children. J Am Vet Med Assoc. 1941; 116 801-814.

17. Camargo ME. Improved technique of indirect immunofluorescence for serological diagnosis of toxoplasmosis. Rev Inst Med Trop Sao Paulo. 1964; 12 117-118.

18. Ghorbani M, Hafizi A, Shegerfcar MT, Rezaian M, Nadim A, Anwar M, Afshar A. Animal toxoplasmosis in Iran. J Trop Med Hyg. 1983; 86 (2): 73-76.

19. Shad-Del F. Sero-prevalence of Toxoplasma infection in human and dog population in Shiraz. Appl Anim Res. 1993; 3 83-89.

20. Bjorkman C, Lunden A, Uggla A. Prevalence of antibodies to Neospora caninum and Toxoplasma gondii in Swedish dogs. Acta Vet Scand. 1994; 35 (4): 445-447.

21. Ali CN, Harris JA, Watkins JD, Adesiyun AA. Seroepidemiology of Toxoplasma gondii in dogs in Trinidad and Tobago. Vet Parasitol. 2003; 113 (3-4): 179-187.

22. Lin DS. Seroprevalences to Toxoplasma gondii in privately-owned dogs in Taiwan. Prev Vet Med. 1998; 35 (1): 21-27.

23. De Souza SLP, Gennari SM, Yai LEO, D’Auria SRN, Cardoso SMS, Junior JSG, Dubey JP. Occurrence of Toxoplasma gondii antibodies in sera from dogs of the urban and rural areas from Brazil. Rev Bras Parasitol Vet. 2003; 12 (1): 1-3.

24. Canon-Franco WA, Bergamaschi DP, Labruna MB, Camargo LM, Silva JC, Pinter A, Gennari SM. Occurrence of anti-Toxoplasma gondii antibodies in dogs in the urban area of Monte Negro, Rondonia, Brazil. Vet Res Commun. 2004; 28 (2): 113-118.

25. Azevedo SS, Batista CS, Vasconcellos SA, Aguilar DM, Ragozo AM, Rodrigues AA, Alves CJ, Gennari SM. Seroepidemiology of Toxoplasma gondii and Neospora caninum in dogs from the state of Paraíba, Northeast region of Brazil. Res Vet Sci. 2005; 79 (1): 51-56.