A serological and parasitological study of *Toxoplasma gondii* infection in stray cats of Mashhad, Khorasan Razavi province, Iran

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**Abstract**

The aim of the present study was to determine seroprevalence of *Toxoplasma gondii* infection in stray cats and correlation with oocyst shedding and IFN-γ concentration. From April to August 2016, one hundred fifty-nine stray cats were captured from various localities in Mashhad area. The blood and fecal samples were collected from each cat. The serum samples were examined to detect antibodies against *T. gondii* infection by ELISA assay and the fecal samples were microscopically examined for *T. gondii* oocyst detection. The concentration changes of IFN-γ in serum samples of seropositive and seronegative cats were measured using ELISA kit. The results showed that 59.12% (94/159) of cats had antibodies against *T. gondii* infection. The seroprevalence of *T. gondii* infection in the adult cats above three years olds was higher than other groups. Regarding gender, month and region factors, the difference of seroprevalence of *T. gondii* infection was not significant. In this study, the *Toxoplasma/Hammondia* like oocyst (THLO) were detected in 2.56% (4/156) in fecal samples of one seropositive and three seronegative cats. Results also showed that the mean value for IFN-γ concentration in the seropositive cats was significantly higher than that of the seronegative cats. Based on the results, the high percentages of stray cats were infected with *T. gondii* in this area. The IFN-γ concentration of seropositive cats was higher than that of the seronegative cats.
Introduction

Toxoplasma gondii, one of the most common parasitic infections of man and other warm-blooded animals, is an obligate intracellular parasite and a member of the Apicomplexa phylum. The Felidae family play prominent roles in the epidemiology of T. gondii infection because they repel millions of oocysts in a short period of time, 1 to 2 weeks, in feces and hence, pollute soil, food and/or water. Oocysts of T. gondii have been detected in the feces of less than 1.00% of cats. Based on serological studies, about one third of the world’s population has been exposed to this widespread zoonotic agent. It has caused a major public health concern due to the immunosuppressive effects of HIV/AIDS.

Toxoplasma gondii infection induces a powerful IFN-γ driven cell-mediated immune response in the mammalian hosts. IFN-γ plays an important role in alternation of tachyzoites to bradyzoites and blockage reactivation tachyzoites. This is a necessary response to elimination of acute infection and control of a chronic, latent infection in the CNS. Recently, some studies have showed that ELISA-based on IFN-γ assay could be used as useful diagnostic tool for acute and chronic T. gondii infection. Several diagnostic methods such as serological tests, fecal flotation technique and PCR are being used for determining T. gondii infection in cats. In Iran, large numbers of cats are found roaming in streets and can be an important potential source of transition of zoonotic diseases such as T. gondii infection. The results of the epidemiological studies showed a high prevalence of Toxoplasma infection in cats of Iran.

There is a poor literature regarding prevalence of T. gondii infection in cat in Mashhad, Iran. The aim of the present survey was to determine the seroprevalence of T. gondii infection in stray cats in the Mashhad and the relationship of seropositive status of cat with oocyst shedding and the serum IFN-γ concentration.

Materials and Methods

Study area. The study was performed from April 2016 to August 2016 in Mashhad, the capital city of Khorasan Razavi province, located in the northeastern part of Iran at 36° 18’ 38.5164” N and 59° 35’ 58.0452” E, with an area of more than 328 km². The climate is the northern temperature with a semi-arid climate with cold winters and moderate summer.

Ethics and animal experimentation. All animal experiments of this study, No. 3.40334, were performed in strict accordance with the guidelines approved by the Animal Ethics Committee of the University.

Sample size and sampling. The prevalence of T. gondii infection in stray cats was estimated 1.20% and 89.20% in the various regions of Iran. Based on expected proportion at 10.00%, the desired sample size was 159 stray cats, using a 95.00% level confidence and 5.00% desired absolute precision. In this study, Mashhad was divided into four regions, The north, south, east, and west, and the stray cats were captured by the traps. The trapped cats were transported to the Small Animal Clinic, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran. The stray cats with different ages and genders initially were physically examined for any clinical signs by a veterinarian. Then the blood and fecal samples were collected from cats. The blood samples in a plain test tube were centrifuged for 5 min at 800 g after clotting at room temperature for 2 hr. The serum was removed and stored at −20 °C till ELISA assay.

Parasitological method. Fecal samples of 159 cats were examined by fecal flotation technique methods. Briefly, 1.00 g of fecal sample of each stray cats 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. The supernatant of solution was taken and examined microscopically for presence of T. gondii oocysts.

Toxoplasma gondii antibodies assay. Toxoplasma gondii antibodies were detected through indirect ELISA using a commercially available kit (ID.vet Innovative Diagnostics, Grabels, France) according to the manufacturer’s instructions. Briefly, 90.00 μL dilution buffer 2 was added to each well of microplate, followed by 10.00 μL of the negative control in wells A1 and B1, and 10.00 μL of the positive control in wells C1 and D1. The serum samples were thawed and 10.00 μL were dispensed into the remaining wells. Microplates were then incubated for 45 min at room temperature. The wells were washed thrice with 300 μL wash solution, then 100 μL of conjugate were added to wells. Microplate were then incubated for 45 min at room temperature. The wells were washed thrice with 300 μL wash solution, 100 μL substrate solution was added, followed by incubation in the dark for 15 min at room temperature. The reaction was stopped by adding 100 μL stop solution. The optical density (OD) of the samples and controls were measured at 450 nm and recorded using a microplate reader (ELx800 absorbance reader; BioTeK, Winooski, USA). The test was considered valid if the mean OD values of the positive control was greater than 0.350 (ODpc > 0.350), and if the ratio of the OD values of the positive and negative controls was greater than 3.5 (ODpc/ODnc > 3.5). The sample/positive (S/P) percentage was computed for each sample. The results for each sample were labeled as either negative (S/P < 40.00%), doubtful (40.00% < S/P < 50.00%), or positive (S/P > 50.00%).
in this study, IFN-γ concentrations were compared in two equal seropositive and seronegative groups, 41 samples. The statistical analyses showed a significant correlation between serology results and IFN-γ concentrations in sampled cats (R= – 0.29, p < 0.05; Table 2). Toxoplasma/Hammondia like oocyst (THLO) were detected in 2.56% (4/159) of fecal samples in the stray cats using fecal flotation technique (Fig. 1). In this study, three of the cats were seronegative.

Discussion

The present study was the first report on seroprevalence of T. gondii infection in stray cats in the Mashhad, Iran. The results showed that 59.12% of sampled cats were infected with T. gondii. Different serological methods have been used in epidemiological studies of feline toxoplasmosis in Iran.15

Based on the previous studies, the seroprevalence of T. gondii infection in cats was reported with 89.00 to 90.00% in Tehran (Iran),11 86.00% in Kashan (Iran),13 24.70% and 59.40% in Ahvaz (Iran),12,17 85.00% in Gorgan (Iran),10 44.20% in Kerman (Iran),14 35.30% in Urmia (Iran),19 32.00% in Isfahan (Iran),20 40.00% in Sari (Iran),10 90.00% in Saudi Arabia,21 66.00% in Iraq 22 and 73.90% in India,23 57.80% in China,24 34.40% in Turkey,25 40.00 and 58.00 in Brazil,26,27 45.20 % in Colombia,28 44.70% in Portugal,29 40.70% in Italy,30 and 91.60% in Ethiopia.31 It seems that variations in reports of the sero-prevalence of T. gondii infection in various regions of Iran and other countries might be due to the differences in type of cat population, the weather, the sampling time and the used serologic methods.

Many studies show that stray cats generally have higher seropositivity rates and are more capacity to get infection.19,26 Lifestyle of stray cats affect their daily contact with the possible sources of contamination from intermediate hosts like raw or undercooked meat of

Results

Out of 159 serum samples of stray cats, 94 (59.12%) had antibodies against T. gondii. The Seroprevalence of T. gondii was significantly higher in adult cats above three years of age than compared to other age groups (p < 0.001). There was no significant difference between the seroprevalence of T. gondii infection in different months and in different sex and ages groups in sampled cats (p > 0.05), (Table 1).

| Risk factors | variables | Negative | Positive (%) | Total |
|--------------|-----------|----------|--------------|-------|
| Age          | < 6 months | 23       | 6 (20.68)    | 29    |
|              | 6 months-3 years | 31   | 21 (40.38) | 52    |
|              | > 3 years   | 11       | 67 (85.89)  | 78    |
| Gender       | Male       | 18       | 43 (70.49)  | 61    |
|              | Female     | 47       | 51 (52.04)  | 98    |
| Sampling time (month) | April | 19       | 17 (47.22)  | 36    |
|              | May        | 8        | 21 (72.41)  | 29    |
|              | June       | 2        | 11 (84.62)  | 13    |
|              | July       | 19       | 17 (44.74)  | 38    |
|              | August     | 15       | 28 (65.12)  | 43    |
| Region       | North      | 13       | 7 (35.00)   | 20    |
|              | East       | 17       | 33 (66.00)  | 50    |
|              | West       | 13       | 28 (65.12)  | 43    |
|              | South      | 22       | 24 (52.17)  | 46    |
| Total        |            | 65       | 94 (59.11)  | 159   |

Fig. 1. The Toxoplasma/Hammondia like oocyst (THLO) in fecal sample of cat.
domestic animals, mice, birds, and reptiles which may harbor *Toxoplasma* cysts. In the present study the seroprevalence of *T. gondii* infection in adult cats above three years was higher than that of the young cats. The similar results were reported by in Iran and other countries. It seems that elderly cats have more chance to get *T. gondii* infection. Unlike to current study, the higher seroprevalence of *T. gondii* infection was reported in stray cats in Iraq and Italy.

In the present study, the difference in the frequency of *T. gondii* infection in male and female cats were not significant. The results of the present study were in agreement with the studies that conducted in Iran and other countries; however, disagreed with some other the studies, indicating that the frequency of *T. gondii* infection were significantly gender-related in stray cat in Iran and Iraq and Italy.

According to the sampling period, no significant influence of month was evidenced in the present study. The results were in agreement with other studies that showed the seropositivity of sampled cat were the same in different months. The findings of the present study were contrary to other studies which found the increased infection rate occurring in the spring/summer.

In this study, IFN-γ concentration was compared to seropositive and seronegative groups by cat IFN-γ ELISA. Results showed that IFN-γ concentration mean value in positive group was higher than that of seronegative group. Our results confirmed that the Interferon-gamma release assay (IGRA) in serum samples could be used to detect *T. gondii* infection in cats. Some studies have shown that the IRGA is a sensitive test to detect *T. gondii* infection in early stage of disease in human and animal.

The *Toxoplasma/Hammondia* like oocyst (THLO) were found in four stray cats (2.56%) in our study. Similar results were reported in the previous studies in Iran and Ethiopia, however, some studies did not detect any *Toxoplasma* oocysts in fecal samples of cats. The shedding of *Toxoplasma* oocysts depends on the age, immune status of cat and climatic condition in each area.

In this study, 75.00% of the positive oocysts shedding cats were seronegative. Two reasons might have caused these results: 1) these cats may be infected only with *Hammondia* spp and 2) The sporogony stage of *Toxoplasma* of in the intestinal tissue has caused poor immune system stimulation with low antibody titer against *Toxoplasma* infection and was non-detectable by ELISA.

In conclusion, the high seroprevalence of *T. gondii* infection was detected among stray cats in Mashhad. The *Toxoplasma/Hammondia* like oocysts (THLO) were microscopically observed in fecal samples of a few stray cats. Furthermore, Interferon-gamma release assay (IRGA) showed that it could be used as sensitive diagnostic method for detection of *T. gondii* in cat.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Dubey JP, Beattie CP. Toxoplasmosis of animals and man. Boca Raton, USA: CRC Press 1968; 8-64.
2. Dubey JP, Lappine MR. Toxoplasmosis and neosporosis. In: Greene CE (Ed). Infectious diseases of the dog and cat. 4th ed. Philadelphia, USA: Saunders 2012; 806-827.
3. Dubey JP. The history of *Toxoplasma gondii* – the first 100 years. J Eukaryot Microbiol 2008; 55(6): 467-475.
4. Lindstrom I, Kaddu-Mulindwa DH, Kironde F, et al. Prevalence of latent and reactivated *Toxoplasma gondii* parasites in HIV-patients from Uganda. Acta Tropica 2006; 100(3): 218-222.
5. Bohn W, Heesemann J, Gross U. Induction of bradyzoite-specific *Toxoplasma gondii* antigens in gamma interferon-treated mouse macrophages. Infect Immun 1993; 61(3): 1141-1145.
6. Suzuki Y, Orellana MA, Schreiber RD, et al. Interferon-gamma: The major mediator of resistance against...
Toxoplasma gondii. Science 1988; 240(4851): 516-518.
7. Mahmoudi S, Mamishi S, Suro X, et al. Early detection of Toxoplasma gondii infection by using an interferon gamma release assay. Exp Parasitol 2017; 172: 39-43.
8. Yin Q, El-Ashram S, Liu XY, et al. Early detection of Toxoplasma gondii-infected cats by interferon-gamma release assay. Exp Parasitol 2015; 157: 145-149.
9. Yin Q, El-Ashram S, Liu H, et al. Interferon-gamma release assay: An effective tool to detect early Toxoplasma gondii infection in mice. PLoS One 2015; 10(9): e0137808.
10. Shirif M, Daryani A, Nasrolahi M, et al. Prevalence of Toxoplasma gondii antibodies in stray cats in Sari, northern Iran. Trop Anim Health Prod 2009; 41(2): 183-187.
11. Haddadzadeh H, Khazrainia P, Aslani M, et al. Seroprevalence of Toxoplasma gondii infection in stray and household cats in Tehran. Vet Parasitol 2006; 138(3-4): 211-216.
12. Hoghgoohi-Rad N, Afraa M. Prevalence of toxoplasmosis in humans and domestic animals in Ahwaz, capital of Khoozestan Province, south-west Iran. J Trop Med Hyg 1993; 96(3): 163-168.
13. Hooshyar H, Rostamkhani P, Talari S, et al. Toxoplasma gondii infection in stray cats. Iranian J Parasitol 2007; 2(1): 18-22.
14. Akhtardanesh B, Ziaali N, Sharifi H, et al. Feline immunodeficiency virus, feline leukemia virus and Toxoplasma gondii in stray and household cats in Kerman, Iran: Seroprevalence and correlation with clinical and laboratory findings. Res Vet Sci 2010; 89(2): 306-310.
15. Rahimi MT, Daryani A, Sarvi S, et al. Cats and Toxoplasma gondii: A systematic review and meta-analysis in Iran. Onderstepoort J Vet Res 2015; 82(1): 823-832.
16. Little SE, Lindsay DS. Laboratory diagnosis of protozoal infections In: Greene CE (Ed). Infectious diseases of the dog and cat. 4th ed. Philadelphia, USA: Saunders 2012; 711-717.
17. Mosallanejad B, Avizeh R, Razi-Jalali MH, et al. A study on seroprevalence and coproantigen detection of Toxo- plasma gondii in companion cats in Ahvaz area, south-western Iran. Iranian J Vet Res 2011; 12(2): 139-144.
18. Namroodi S, Shariat Bahadori E. Analysis of feral cats role in dissemination of Toxoplasma gondii infection in rural area, Golestan province, north-east of Iran. Int J Epidemiol Res 2015; 2(4): 190-196.
19. Raeghi S, Sedeghi S. Prevalence of Toxoplasma gondii antibodies in cats in Urmia, northwest of Iran. J Anim Plant Sci 2011; 21(2): 132-134.
20. Saljoghiyan H. Survey on seroprevalence of toxoplasmosis in stray cats from Isfahan, Iran. In Proceedings: Congress of National Veterinary Pathobiology. Garmsar, Iran 2011; 77.
21. Hamdan I, Mohammed Al. Seroprevalence of Toxoplasma gondii infection in cats, dogs and ruminant animals in Al-Ahsa area in Saudi Arabia. Res J Med Sci 2011; 5(4): 190-192.
22. Dhamraa R, Alwan JM. Seropathological diagnosis of Toxoplasma gondii in stray cats in Baghdad province. Iraq J Vet Med 2014; 38(2): 92-98.
23. Dubey JP, Moura L, Majumdar D, et al. Isolation and characterization of viable Toxoplasma gondii isolates revealed possible high frequency of mixed infection in feral cats (Felis domesticus) from St Kitts, West Indies. Parasitology 2009; 136(6): 589-594.
24. Qian W, Wang H, Su C, et al. Isolation and characterization of Toxoplasma gondii strains from stray cats revealed a single genotype in Beijing, China. Vet Parasitol 2012; 187(3-4): 408-413.
25. Can H, Doskaya M, Ajzenberg D, et al. Genetic characterization of Toxoplasma gondii isolates and toxoplasmosis seroprevalence in stray cats of Izmir, Turkey. PLoS One 2014; 9(8): e104930.
26. Meireles LR, Galisteo AJ, Pompeu E, et al. Toxoplasma gondii spreading in an urban area evaluated by seroprevalence in free-living cats and dogs. Trop Med Int Health. 2004; 9(8): 876-881.
27. Dubey JP, Navarro IT, Sreekumar C, et al. Toxoplasma gondii infections in cats from Parana, Brazil: Seroprevalence, tissue distribution, and biologic and genetic characterization of isolates. J Parasitol 2004; 90(4): 721-726.
28. Dubey JP, Su C, Corte JA, et al. Prevalence of Toxoplasma gondii in cats from Colombia, South America and genetic characterization of T. gondii isolates. Vet Parasitol 2006; 141(1-2): 42-47.
29. Waap H, Cardoso R, Leitao A, et al. In vitro isolation and seroprevalence of Toxoplasma gondii in stray cats and pigeons in Lisbon, Portugal. Vet Parasitol 2012; 187(3-4): 542-547.
30. Papini R, Shrama C, Rosa B, et al. Serological survey of Toxoplasma gondii infections in stray cats from Italy. Revue Med. Vet 2006; 157(4): 193-196.
31. Dubey JP, Darrington C, Tiao N, et al. Isolation of viable Toxoplasma gondii from tissues and feces of cats from Addis Ababa, Ethiopia. J Parasitol 2013; 99(1): 56-58.
32. Sumner B, Ackland ML. Toxoplasma gondii antibody in domestic cats in Melbourne. Aust Vet J 1999; 77(7): 447-449.
33. Razmi GR. Prevalence of feline coccidia in Khurasan province of Iran. J Appl Anim Res 2000; 17: 301-303.
34. Dubey JP, Zhu XQ, Sundar N, et al. Genetic and biologic characterization of Toxoplasma gondii isolates of cats from China. Vet Parasitol 2007; 145(3-4): 352-356.
35. Advincula JK, Iewida SY, Cabanacan-Salibay C. Serologic detection of Toxoplasma gondii infection in stray and household cats and its hematologic evaluation. Sci Med 2010; 20(1): 76-82.