ORIGINAL ARTICLE

Veterinary Research Forum. 2019; 10 (2) 119 - 123
doi: 10.30466/vrf.2019.71293.1975
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

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

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Article Info

Article history:

Received: 05 September 2017
Accepted: 21 February 2018
Available online: 15 June 2019

Key words:

ELISA
Interferon gamma
Prevalence
Stray cat
Toxoplasma

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.

مطالعه سرولوژی و انگل شناسی آلودگی *توکسوپلاسما گوندیی* در گربه های ولگرد مشهد، استان خراسان رضوی، ایران

چکیده

توکسوپلاسموز یکی از بیماری مهم مشترک انسان و حیوان می باشد. هدف این مطالعه تعیین میزان شیوع سرمی آلودگی توکسوپلاسما در گربه های ولگرد شهرستان مشهد و ارتباط ان با دفع اووسیست و غلظت سرمی اینترفرون گاما می باشد. در این مطالعه از فروردین تا شهریور ماه ۱۳۹۵ تعداد ۱۵۹ قلاده گربه ولگرد در نقاط مختلف شهرستان مشهد گرفته شد و از هر گربه نمونه خون و مدفوع جمع آوری شد. نمونه های سرمی خون جهت یافتن آنتی بادی علیه توکسوپلاسما با روش الایزا آزمایش شدند و نمونه های مدفوع جهت مشاهده اووسیست های توکسوپلاسما مورد آزمایش میکروسکوپی قرار گرفتند. علاوه براین غلظت سرمی اینترفرون گاما در گربه های واجد آنتی بادی و فاقد آنتی بادی علیه توکسوپلاسما با روش الایزا اندازه گیری و مورد مقایسه قرار گرفتند. نتایج بدست آمده نشان داد که ۵۹/۱۲ درصد (۹۴ از ۱۵۹) گربه ها واجد آنتی بادی علیه توکسوپلاسما بودند. در این مطالعه شیوع سرمی آنتی بادی علیه توکسوپلاسما در گربه های بالای سه سال بیشتر از سایر گروه های سنی بود. در این بررسی اختلاف معنی داری بین آلودگی سرمی و فاکتورهای جنس ، ماه و محل نمونه برداری به دست نیامد. در این مطالعه، در ۲/۵۶ درصد (۴ از ۱۵۶) از نمونه های مدفوع، اووسیست های مشابه تک یاخته های *توکسوپلاسما/هاموندیا* مشاهده شد. تمام این گربه ها بجز یک گربه، همه سرم منفی بودند. نتایج نشان داد که میانگین غلظت سرمی اینترفرون گاما در گربه های سرم مثبت به میزان بسیار معنی داری نسبت به گروه سرم منفی بیشتر می باشد. براساس نتایج بدست آمده نتیجه گیری می شود که درصد بسیار بالایی از گربه های ولگرد شهرستان مشهد آلوده به توکسوپلاسما بود. غلظت سرمی اینترفرون گاما در گربه های سرم مثبت به میزان معنی داری بیشتر از گربه های سرم منفی بودند.

واژه های کلیدی: الایزا، اینترفرون گاما، توکسوپلاسما، گربه ولگرد ، میزان شیوع

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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.[1] 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[2] 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.[3] Based on serological studies, about one third of the world's population has been exposed to this widespread zoonotic agent.[1] It has caused a major public health concern due to the immuno-suppressive effects of HIV/AIDS.[4]

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.[5] This is a necessary response to elimination of acute infection and control of a chronic, latent infection in the CNS.[6] 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.[7-9] Several diagnostic methods such as serological testes, 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.[10-15]

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 km^2. 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.[15] 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.[16] 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 ($OD_{PC} > 0.350$), and if the ratio of the OD values of the positive and negative controls was greater than 3.5 ($OD_{PC}/OD_{NC} > 3.5$). 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%).

IFN-γ concentration assay. The concentration of IFN-γ in the cat's serum samples were measured using a commercially available ELISA kit (Shanghai Korain Biotech Co. Ltd, Shanghai, China). This kit uses enzyme-linked

immune sorbent assay based on the biotin double antibody sandwich technology to assay the cat IFN-γ. The procedure of this test was done according to the manufacturer's recommendations. The OD of each well was read at 450 nm in the ELISA plate reader (BioTek). The standard curve was drawn based on the standard concentrations and the corresponding OD values were calculated. Then according to the OD value of samples, the concentration of the corresponding sample was calculated.

Statistical analysis. Cats were grouped based on age, sex, sampling month and geographic area and differences of seroprevalence *T. gondii* infection between among groups were analyzed by Chi-square test. The normality and differences of IFN-γ concentrations between seropositive and seronegative groups were determined using kolmogorov-smirnov test and Student *t*-test, respectively. The correlation, Coefficient R2, among the serum test, stool exam and levels of IFN-γ were analyzed with the Pearson correlation coefficient. The statistical analyses were carried out using SPSS software (version 21.0; IBM Corp., Chicago, USA). Differences were considered significant when p value < 0.05.

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).

Table 1. Seroprevalence of Toxoplasma gondii infection in stray cats by different risk factors.

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

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.

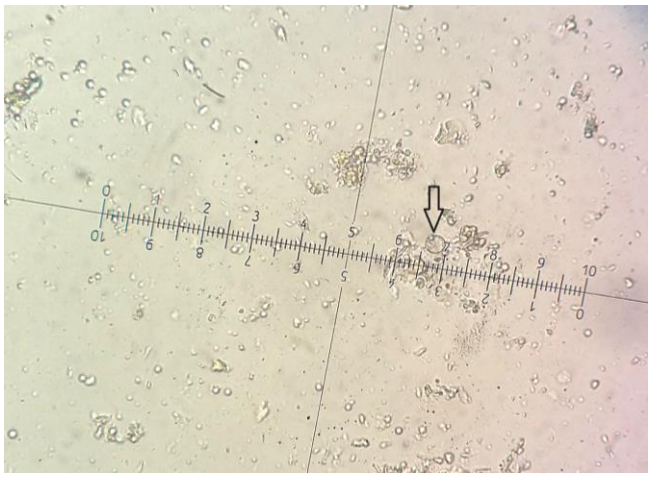

Fig. 1. The *Toxoplasma/Hammondia* like oocyst (THLO) in fecal sample of cat.

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),[18] 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

Table 2. The correlation between seroprevalence of *Toxoplasma gondii* infection, oocyst shedding and IFN-γ value in sampled cats.

Variables		Seroprevalence of *T. gondii*	IFN-γ value	Oocyst shedding
Seroprevalence of *T. gondii*	Pearson Correlation	1	0.298*	0.113
	Sig. (2-tailed)	-	0.007	0.311
	No.	82	82	82
IFN-γ value	Pearson Correlation	0.298*	1	0.120
	Sig. (2-tailed)	0.007	-	0.282
	No.	82	82	82
Oocyst shedding	Pearson Correlation	0.113	0.120	1
	Sig. (2-tailed)	0.311	0.282	-
	No.	82	82	82

* Correlation is significant at the 0.01 level (2-tailed).

domestic animals, mice, birds, and reptiles which may harbor *Toxoplasma* cysts.[27] 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.[10,11,14,17,25] and other countries.[28,29] 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[22] and Italy.[30]

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,[10,11,13,14,17,19] and other countries,[29,32] however, disagreed with some other the studies, indicating that the frequency of *T. gondii* infection were significantly gender-related in stray cat in Iran,[18] Iraq,[22] and Italy.[30]

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.[10,18,29] The findings of the present study were contrary to other studies which found the increased infection rate occurring in the spring/summer.[30]

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.[7-9]

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[19,33] and Ethiopia,[31] however, some studies did not detect any *Toxoplasma* oocysts in fecal samples of cats.[17,24,28,34,35] 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.

Acknowledgments

We would like to thank Mr. Amin Bakhshani for his help during sampling. The research leading to these results was funded by a grant (No. 3.40334) from the Research Council of Ferdowsi University of Mashhad, Mashhad, Iran.

Conflict of interest

The authors declare that there is no conflict of interest.

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