



## ORIGINAL ARTICLE

# Serologic study of Toxoplasmosis in Clinically Healthy and FeLV/FIV infected household cats in Tehran, Iran

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### ABSTRACT

The aim of the present study was to determine *Toxoplasma gondii* seroprevalence in a population of household cats in Tehran, Iran and to identify the influence of some risk factors on *T.gondii* seropositivity, especially infection with Feline Leukemia Virus (FeLV) and Feline Immunodeficiency Virus (FIV). Serum samples of 84 household cats referred to private small animal clinics in different regions of Tehran (Since February 2013 to January 2014) were assessed by ELISA method and the overall infection rate for *T. gondii* was 19.05%. Results of the multivariate logistic regression analysis showed higher incidence of seropositivity in adult cats and in cats fed by raw meat ( $P < 0.05$ ). Overall seroprevalence for FeLV and FIV by immunochromatography assay (ICGA) was 2.38% and 16.67%, respectively. There was no statistically significant correlation between infection with FeLV/FIV and seropositivity for *T.gondii*.

**Keywords:** *Toxoplasma gondii*, Seroprevalence, ELISA, FeLV, FIV, Cat, Tehran

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### INTRODUCTION

*Toxoplasma gondii* is an obligate intracellular coccidian parasite that infects virtually all species of warm-blooded animals, including human beings [1, 2]. Cats play a crucial role in the epidemiology of toxoplasmosis due to enteroepithelial life cycle that is found only in these definitive hosts but because of the way cats defecate, bury their feces, and keep their hair coats clean, transmission of *T.gondii* oocysts to humans by touching and caring for a pet cat is very unlikely and ingestion of raw or undercooked meat is an important source of toxoplasmosis in humans [1, 3]. Cats excrete around 20 million oocysts between 3 and 18 days after infection [4]. Clinically unapparent toxoplasmosis is manifested by the presence of IgG antibodies, but the opportunistic character of the infection could appear in immunosuppression. FIV and FeLV related immunosuppression may be a risk factor for active toxoplasmosis in cats that increase the risks of human toxoplasmosis [5, 6]. Other factors might have influence on seropositivity for *T.gondii* is age, sex and breed, lifestyle, type of feeding and sexual status. Enzyme-linked immune-sorbent assay (ELISA) methods are generally most reliable for determining toxoplasma specific antibody titers. Positive IgG and IgM antibody titers document previous or current infection, respectively [3]. ELISA has been adapted for the detection of IgM, IgG, and IgA class antibodies against *T. gondii* in feline sera. ELISA methods are as sensitive as indirect fluorescent antibody and more sensitive compared with latex agglutination test or indirect hemagglutination test [1]. Whereas cats are one of the most popular companion animals in Iran, this study has conducted to evaluate seroprevalence of *T.gondii* in a population of household cats in Tehran, Iran and detecting its relation to described presumptive risk factors, especially seropositivity for FeLV/FIV.

### MATERIALS AND METHODS

Between February 2013 through January 2014, 84 client-owned cats referred to private small animal clinics in different regions of Tehran were randomly selected with no limitation for age, gender and lifestyle. Informed consent was obtained from each cat owner prior to the study. A detailed questionnaire was completed for each animal. Investigated parameters included putative risk factors such as age,

gender, breed, type of feeding, sexual intactness and housing conditions (only indoors or outdoors; single or multicas household; to be in contact with other cats or not). Blood samples (1 ml) were drawn from cephalic vein. Blood samples were left for about an hour to clot. The clotted blood was then separated with a fine loop immediately and was centrifuged at 3500 rpm for 10 minutes. The separated serum samples were stored at -20°C until further analysis. All sera were tested for anti *T.gondii* antibody by using a commercial indirect ELISA kit (ID Screen® Toxoplasmosis Indirect Multi-species, IDvetCompany, Montpellier, France) with the estimated sensitivity ranged between 95.7% and 97.1% and the specificity between 97.3% and 97.6 %. [7]. FeLV/FIV immunochromatography assay was carried out with a commercial kit (Speed Duo® FeLV/FIV, BVT Company, La Seyne sur Mer, France) for detecting p27 antigen of FeLV and anti-Gp40 antibody for FIV according to the manufacturer's instructions. Samples with positive results were retested after a minimum of 30 days according to the guidelines of the American Association of Feline Practitioners for feline retrovirus management and only considered truly positive if they tested positive for the second time [8]. The sensitivity and specificity of the kit in comparison to viral isolation was recorded as 89.1% and 97.7%, respectively. In statistical analysis, presence of anti *T. gondii* antibodies was set as an outcome variable and the independent variables were positive immunochromatography test, age, gender, breed, type of feeding, sexual intactness and housing conditions. Cats were grouped by these presumptive risk factors to determine whether these factors were associated with *T. gondii* seropositivity, by Chi-square test and multivariate logistic regression analysis. Statistical comparisons were carried out using SPSS software for windows (Release 18.0 standard version, SPSS Inc., Chicago, Illinois). The differences were considered statistically significant when  $P < 0.05$  (CI = 95%).

## RESULTS AND DISCUSSION

16 of 84 serum samples had antibodies against *Toxoplasma gondii* and the overall infection rate was 19.05%. Prevalence was significantly higher in adult cats (above 1 year), 13 of 16 seropositive sera (81.25%) belong to adult cats ( $P < 0.03$ ). Also, cats fed by raw meat had more seropositive results in ELISA test than cats fed by commercial or cooked food and the difference between these two groups was statistically significant ( $P < 0.001$ ). Overall seroprevalence for FeLV and FIV by immunochromatography assay (ICGA) was 2.38% and 16.67%, respectively and no cat was positive for both viruses, simultaneously. There was no statistically significant correlation between infection with FeLV/FIV and seropositivity for *T.gondii*, as same as other putative risk factors. Signalment, clinical signs and other data related to *T.gondii* seropositive cats are summarized in Table 1.

**Table 1: Signalment, FeLV and FIV infection status and other data related to *T. gondii* infected cats**

Age	Gender	Breed	FeLV	FIV	Single Or Multicat	Feeding by raw meat	Access to outdoor	Neutered	Health Status
7 Y	F	DSH	-	-	Multi	+	+	+	Gingivitis, Stomatitis
7 Y	F	DSH	-	-	Single	-	-	+	Clinically healthy
6 Mo	M	Persian	-	-	Single	+	-	-	Fever
7 Mo	F	DSH	-	-	Single	+	+	-	Gingivitis, Stomatitis
7 Y	M	DSH	-	-	Multi	+	+	-	Gingivitis, Stomatitis Abscess
5 Y	F	DSH	-	-	Multi	+	+	+	Gingivitis, Stomatitis, Abscess
4.5 Y	M	DSH	+	-	Single	+	+	-	Gingivitis, Stomatitis Abscess, Lymphadenopathy
10 Y	F	DSH	-	-	Multi	+	+	+	Gingivitis, Stomatitis Cachexia, Pale Mucous Membrane
2 Mo	F	DSH	-	-	Multi	+	-	-	Cachexia, Pale Mucous Membrane
10 Y	M	DSH	-	+	Multi	+	-	+	Gingivitis, Stomatitis Abscess, Lymphadenopathy
1.5 Y	M	DSH	-	-	Multi	+	+	-	Gingivitis, Stomatitis Pale Mucous Membrane
8 Y	M	DSH	-	+	Single	+	+	-	Cachexia, Pale Mucous Membrane
6 Y	F	DSH	-	+	Multi	+	+	+	Clinically healthy
4 Y	F	DSH	-	-	Multi	+	-	-	Clinically healthy
1 Y	F	DSH	-	-	Multi	+	+	-	Clinically healthy
1.5 Y	F	DSH	-	-	Single	+	-	-	Fever

**M = Male, F = Female, Y = Year, Mo = Month, DSH = Domestic Shorthair**

The overall prevalence of *T. gondii* infection in a population of household cats in Tehran, Iran was 19.05%. The seroprevalence of *T. gondii* has been studied in different cities of Iran previously and the overall seropositivity was clearly higher than that estimated in our study. Some reported seroprevalences were, 32.1% in Kerman [9], 40% in Sari [10], 86% in Kashan [11], 36-90% in Tehran [12] 35.5% in Urumia [13] and 24.75% in Ahvaz [14]. It seems that this considerable difference is related to the study population that has been selected. In all these previous studies that have been done in Iran, study populations included stray cats or both stray and household cats that lead to increased overall rate of seropositivity for *T. gondii*. In contrast, in our study, all population was household cats as seen in the studies in South Korea in 2013 and in China in 2011 that the prevalence among household cats was only 2.2% and 15.6%, respectively [2, 15]. On the other hand, it should be considered that this prevalence (19.05%) is almost high for household cats. It may have some probable reasons: 1) Domestic short hair cats, which are estimated to be more than 95% of pet cats in Iran, usually adopted by owners as kittens or older, may have been seropositive for toxoplasmosis before adoption. 2) Many of these household cats are not indoor pets during their lives and have access to outdoor, so they are able to access to contaminated areas, *T. gondii* infected intermediate or definitive hosts and also garbage. As seen in our study, 10 out of 16 *T. gondii* seropositive cats have access to outdoor. 3) Feeding household cats by raw or uncooked meat is an important source of infection and this fact is consistent with our results, 15 out of 16 *T. gondii* infected cats fed by raw meat. Other surveys of *T. gondii* seroprevalence have been recorded in different countries, 17.98% in China [16], 11% in Bangkok, Thailand [17], 40.3% in Turkey [18], 44.1% in Czech Republic [19], 87.3% in Brazil [20], 25.5-36.9% in Spain [21], 5.4% in Japan [22] and 70.2% in Belgium [23] respectively. These differences might be due to differences in *T. gondii* infection rates, time and season of sampling and differences of sensitivities and specificities of used tests. The results indicated that prevalence of antibodies varied with ages, and *T. gondii* seroprevalence in older animals (more than 1 year) was generally higher than young cats (younger than 1 year old), and the difference was statistically significant ( $P < 0.001$ ) which is consistent with other reports [7, 9, 10, 12, 14, 20, 21, 22, 24]. Feeding by raw or undercooked meat was another statistically significant risk factor for *T. gondii* infection ( $P < 0.001$ ) which agrees with some other studies [9, 13]. As mentioned before, Domestic short hair cats, which are estimated to be more than 95% of pet cats in Iran, usually adopted by owners as kittens or older, may have been seropositive for toxoplasmosis before adoption. Therefore, the exact relation between indoor and outdoor breeding type could not be differentiated easily in our study [9]. There was no significant correlation between FeLV/FIV seropositivity and seroprevalence for *T. gondii* that is consistent with other reports just about FeLV, but not FIV [9, 23]. It seems that one of the most important reasons for such miscorrelation was due to very low prevalence of Feline Leukemia Virus infection rate in Tehran, as seen in the results of our previous study in 2011 [25]. Furthermore, no significant difference between seroprevalences in male and female cats and also outdoor access was noted, which agrees with data obtained from other studies [9, 10, 22, and 23].

Our study demonstrated that the positive rate of *T. gondii* infection in household cats is almost high and that this relatively high prevalence might be a potential risk for public health, although there was no correlation between FeLV/FIV seropositivity and *Toxoplasma gondii* infection. Prevention efforts should focus on educating cat owners about the importance of collecting cat feces in litter boxes, spaying owned cats, restricting their access to outdoor and feeding household cats by cooked food or commercial cat food instead of raw meat. Our results will be the basis for 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.

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