



Research Article

Seroprevalence and Risk Factor Analysis of *Toxoplasma gondii* Infection in Pet Dogs of Peshawar, Khyber Pakhtunkhwa, Pakistan

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Authors' Contributions

MAK conducted the experiment and wrote the manuscript. FG helped in experiment. ZA did statistical analysis. RU and MA revised the manuscript. SA helped in sampling collection. AK designed the experiment. Kalimullah supervised the research.

Keywords

ELISA, *T. gondii*, Dogs, Prevalence, Risk factors

Abstract | Toxoplasmosis is a parasitic disease caused by *T. gondii*, which infects warm-blooded animals worldwide. This study was conducted with the main objectives, determination of seroprevalence of *T. gondii* infection in pet dogs. To evaluate the main associated risk factors related with *T. gondii* exposure to this region. A total of a hundred number of blood samples (72 males and 28 females) were collected from Peshawar, Pakistan. The serum samples were analyzed for IgG and IgM by using enzyme-linked immunosorbent assay (ELISA), and statistical analysis carried out. Out of these tested samples, 20% were positive for both IgG and IgM against *T. gondii*. The positive prevalence of *T. gondii* was 11.11% in male dogs and 42.85% in female dogs. Further details of results are given in below in main data. The finding results indicate that the seroprevalence of *T. gondii* is high and it is a posing concern to public health, but comparatively, our study shows a less prevalence rate as compared to other cities of Pakistan. Preventive measure must be taken to control the *T. gondii* infection.

Novelty Statement | This is first study that has been conducted in the whole province of Khyber Pakhtunkhwa for the safety of dog owners in Peshawar.

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Introduction

A single-cell protozoan parasite called *T. gondii* was found more than 100 years ago in the North African comb rat. It has been reported that *T. gondii* infection is

found in many animals such as birds, mammals and even it also infects human as this parasite is zoonotic by nature and transmits from animals (Nicolle *et al.*, 1908; Hill *et al.*, 2005).

Abbreviations

Indirect hemagglutination assay (IHA), Enzyme linked immunosorbent assay (ELISA) Indirect fluorescence antibody test (IFAT), *Toxoplasma gondii* (*T. gondii*), IgG Immunoglobulin G, IgM, Immunoglobulin M.

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T. gondii infection cause fetal disease and abortion. About one third human population in the world is effected by *T. gondii* and only a small percentage of people show clinical symptoms (Tenter *et al.*, 2000; Abu-Madi *et al.*, 2008). If a pregnant woman is infected by *T. gondii* infection it leads to serious diseases like blindness, encephalitis, abortion, etc. (Lindsay *et al.*, 1997). Morbidity and death can be caused by *T. gondii* in immunodeficient people (e.g. HIV/AIDS patients) (Belanger *et al.*, 1999; Montoy *et al.*, 2004). Pet dogs are habitually considered to be the closest friends of humans. Several studies showed the shedding of *T. gondii* by dogs. Though, it is originated that dogs ingest *T. gondii* oocysts via food and water that pass through intestinal tract and finally excreted in feces (Lindsay *et al.*, 1997). It has been found that the presence of pet dogs in houses is the main risk factor for *T. gondii* infection in humans (Sroka *et al.*, 2010).

Dogs can enable the transmission of toxoplasmosis to human exposure to oocysts present in the environment by rolling in contaminated cat feces or ingesting oocysts contaminated food (Frenkel *et al.*, 1995). The study over *T. gondii* in pet dogs has been conducted widely (Kamani *et al.*, 2010). In dogs, the clinical symptoms of *T. gondii* are diarrhea, ataxia and respiratory impairment (Ahmed *et al.*, 2014). The disease can be confirmed by signs and symptoms, but the best way is confirmation tests like indirect hemagglutination assay (IHA), enzyme-linked immunosorbent assay (ELISA) and indirect fluorescence antibody test IFAT (Carlier *et al.*, 1980; Watson *et al.*, 1982). Dogs are considered to be the most favorite pets and thus play a significant role in people's daily life (Ahmed *et al.*, 2014) and this brings many benefits like mental and physical health (Jadoon *et al.*, 2009). This is an obligate parasite and has a huge impact on human and animals' life. In many countries, the number of dogs and other pets has increased quietly. This is why, it is very important to expose the data regarding this parasite to create awareness (Lui *et al.*, 2013). In the present study, ELISA was used. The present study was conducted with the aim that the determination of seroprevalence of *T. gondii* infection in pet dogs. The present findings provide a piece of evidence that the dog's owners are significantly posed to the risk of *T. gondii* infection.

Materials and Methods

Ethical approval

This study was approved by the ethical committee of the Department of Zoology, Kohat University of Science and Technology Pakistan and ethical committee of the Veterinary Research Institute Peshawar, Pakistan.

Study area

The present study was carried out in Peshawar, the capital of Khyber Pakhtunkhwa Pakistan. Peshawar lies

between Latitudes 33° 54' to 34° 4' N and Longitudes 71° 23' to 71° 43' E. The total area of this district is 1,257 square km. The total population of Peshawar is 3,307,798 Million (Aslam *et al.*, 2006).

Sampling of pet dogs

A total hundred serum samples were collected from leg veins of dogs between June and October. Around 3-4 ml of blood samples were collected in non-EDTA tubes (Gel Tubes). The collected samples placed at room temperature for clotting. After clotting, the blood samples were centrifuged at 3000rpm for 15 minutes. The separated sera were stored at -20°C for further analysis of anti-*T. gondii*-specific IgG and IgM antibodies.

Serological examination

An indirect enzyme-linked immunosorbent assay (ELISA) was used for the detection of IgG and IgM antibodies against *T. gondii* in sera by using a commercial kit (ID Screen *Toxoplasmosis* Indirect® ID-VET Company, France).

Statistical analysis

Differences in seroprevalence of *T. gondii* infected pet dogs between male and female dogs, different age groups, breed wise differences, area wise differences were analyzed using One-way ANOVA test in SPSS. The P value < 0.05 was considered statistically significant.

Results

Seroprevalence in dogs

The study was conducted to investigate the seroprevalence of *T. gondii* and to find out the risk factors linked with this protozoan parasite in dogs. The overall seroprevalence IgG and IgM in dogs showed 20% (20/100) given below in Table 1. IgG antibodies were found in 14 samples. The IgG prevalence was 14% (14/100) and IgM prevalence was 6% (6/100). One dog was infected with both *T. gondii* IgG and IgM. The prevalence of IgG and IgM were analyzed through Chi square test and considered statistically significant. The P value was <0.05.

Table 1: The prevalence rate of total number of samples.

Total number of dogs	ELISA test	Prevalence	Percentage (%)	P value
100	IgG	14	14%	<0.05
100	IgM	6	6%	

Prevalence rate

Prevalence rate is determined with the help of the following formula:

$$\text{Prevalence Rate} = \frac{\text{No. of positive samples}}{\text{No. of total samples examined}} \times 100$$

Prevalence rate of sample for IgG= $14/100 \times 100 = 14\%$
 Prevalence rate of sample for IgM= $6/100 \times 100 = 6\%$

Gender wise seroprevalence

The serum samples were collected according to the questionnaire in which male and female dogs were mentioned accordingly. The serum samples collected from male dogs were 72 but still, it doesn't show high seroprevalence of IgG and IgM antibodies. Because according to the literature, these cases mostly occurred in female dogs. The detected IgG in male dogs was 6, which shows 8.33% seroprevalence. The IgM was detected 2 in two male dogs and its prevalence rate was 2.77%. The prevalence of both IgG and IgM in the male dogs was 11.11%. The serum samples collected from female dogs were 28 in which 8 samples were seropositive for IgG and 4 were IgM positive. The prevalence rate of IgG was 28.57% and 14.28% for IgM. The seroprevalence rate for both IgG and IgM were 42.85% which was much higher than male dogs that were 11.11%. The P-value tested through Chi square test and was >0.05 and it was considered statistically not significant. All the data regarding gender-wise prevalence is given in Table 2.

Area wise seroprevalence

The dogs were divided into two groups on the basis of the area. i.e. Rural and Urban mentioned in Table 3. To correlate the prevalence rate of *T. gondii* infection with the residential status of dogs than it was observed that the prevalence rate was higher in urban areas as compared to rural areas. The number of samples indicated that the peoples of the rural area keep more dogs at their homes as compared to urban areas. The samples collected from the urban areas were 36 in which 5 were IgG positive shows a 16.66% prevalence rate. The IgM detected from the urban area was 2 which shows 5.55% seroprevalence. The overall IgG and IgM seroprevalence rate in the urban areas was 22.22%. The samples collected from the rural areas were 64. Out of these 9 were detected for IgG seropositive and 4 were IgM positive. The prevalence rate of IgG in rural dogs was 14.06% and IgM prevalence was 5.25%. The overall prevalence of IgG and IgM in both rural and urban was 20%. The P-value was < 0.05 analyzed by using chi square test and it was considered statistically significant.

Age wise prevalence

To find out the prevalence of *T. gondii* infection with the age groups, dogs were divided into three groups regarding age. (A) (B) and (C). In group (A) the dogs were included that was of one year or less than one year (0-1). 42 dogs were included in the group (A) out of which 4 were positive regarding IgG and 2 were positive regarding IgM. IgG show 9.52% seroprevalence and IgM show 4.76% seroprevalence. The IgG prevalence percentage was a little high in the group (A) as compared to IgM. The overall prevalence rate of IgG and IgM was 14.28%. In group (B),

the dogs included whose age was between 1 to 3 years. (1-3). 40 numbers of samples were included in the group (B) out of which 6 were detected as IgG positive which shows a 15% prevalence rate. 2 were IgM positive which prevalence rate was 5%. The overall prevalence of IgG and IgM was 20%. The number of samples included in the group (C) was 18. The age of dogs included in this group was between 3 to 5 years. The IgG positivity in this group was 4 which shows 22.22% and IgM positive were 2 which shows an 11.11% seroprevalence rate. The number of IgG was higher than the number of IgM in the group (C). The overall prevalence was 33.33% in this group. The age-wise prevalence of IgG was 14% and IgM was 6%. Combine IgG and IgM seroprevalence was 20%. Age wise P-value was >0.05 which was analyzed through Chi square test and it was considered statistically non significant. This data is given in Table 4. The aged Dogs show more prevalence rate as compared to young ones; this may be because of a weak of the immune system due to aging.

Breed wise seroprevalence

The samples collected from different breeds of dogs like a bull terrier, bulldogs, libra dog, German shepherd, and pointer. The seroprevalence rate was much higher in Libra dog. Samples collected from Bullterrier were 17 which shows 0.00% seroprevalence regarding IgG and IgM. 22 samples were collected from Bulldog in which the seroprevalence of IgG was 0. The antibody against IgM was detected in 2 dogs which show 9.09% seroprevalence. The number of samples conducted from German shepherd was 34 in which 4 dogs were seropositive for IgG and show 11.76% seroprevalence. The IgM conducted from German shepherd was 4 and show 11.76% of seroprevalence. The serum samples conducted from breed pointer were 13 in which 2 were IgG positive while IgM was not detected. The prevalence rate of IgG in pointer dog was 15.38% and 0.00% IgM. 14 serum samples collected from Libra dog in which 8 samples were IgG positive and no IgM was detected. Libra dog show more seroprevalence regarding IgG as compared to other breeds of dogs and the prevalence rate was 57.14%. The overall seroprevalence of both IgG and IgM was 20%. The P value was > 0.05 which was tested through Chi square test and it was statistically non-significant given in Table 5.

Risk factor associated with *T. gondii*

In dogs, area, gender, and age was a significant contributing factor regarding *T. gondii* infection. The young dogs show a very less prevalence rate regarding IgG and IgM. This is probably due to a strong immune system. The aged dogs whose age was 5 or more than 5 years show high prevalence rate as compared to younger dogs. The prevalence rate of IgG was 22.22% and IgM was 11.11% in old aged dogs. Gender wise the prevalence rate was very high in female dogs in a smaller number of serum samples. In 28 serum samples, the prevalence rate in the female

Table 2: Gender wise seroprevalence of *T. gondii* infection in dogs.

Gender	Sample size	IgG positive	IgG % positive	IgM positive	IgM %	Overall %	P Value
Male	72	6	8.33	2	2.77	11.11	>0.05
Female	28	8	28.57	4	14.28	42.85	
Total	100	14	14	6	6	20	

Table 3: Area wise seroprevalence of *T. gondii* infection in dogs.

Area	Sample size	IgG positive	IgG positive %	IgM positive	IgM positive %	Overall %	P value
Urban	36	5	16.66	2	5.55	22.22	< 0.05
Rural	64	9	14.06	4	5.25	20.31	
Total	100	14	14	6	6	20	

Table 4: Age wise seroprevalence of *T. gondii* infection in dogs.

Age	Sample size	IgG positive	IgG positive %	IgM positive	IgM positive %	Overall %	P Value
0-1 (Year)	42	4	9.52	2	4.76	14.28	>0.05
1-3 (Years)	40	6	15	2	5	20	
3-5 (Years)	18	4	22.22	2	11.11	33.33	
Total	100	14	14	6	6	20	

Table 5: Breed wise seroprevalence of *T. gondii* infection in dogs.

Breed	Sample size	IgG positive	IgG positive%	IgM positive	IgM positive%	Overall %	P Value
Bull terrier	17	0	0.00	0	0.00	0.00	>0.05
Bull dog	22	0	0.00	2	9.09	9.09	
German shepherd	34	4	11.76	4	11.76	23.52	
Pointer	13	2	15.38	0	0	15.38	
Libra dog	14	8	57.14	0	0.00	57.14	
Total	100	14	14	6	6	20	

dogs was 42.85% regarding IgG and IgM respectively. The seroprevalence was found more positive in those dogs who have frequent access to outdoor. Those dogs who have frequent access to outdoor without an owner have more chances of *T. gondii* infection because they can easily consume cysts contaminated food or drink. According to the literature, cats are a natural pool of *T. gondii* and from these infected cats, the cysts of *T. gondii* are shed into the environment and contaminate the soil. From here the infection is transfer into pet dogs. As dogs are considered close friends in our society and some peoples keep dogs at their homes. Further, this infection is transfer into a human.

Discussion

There is a severe lack of awareness about the extension and influence of *toxoplasmosis* infection and its consequences are in Pakistan because of parasitology laboratories that are not developed in Peshawar. This was the first study on the subject in Peshawar and even in Khyber Pakhtunkhwa. The study was conducted to investigate the seroprevalence of *T. gondii* and to find out the risk factors linked with this protozoan parasite in dogs. The

current study is conducted by using a cellular tool Enzyme-linked immunosorbent assay (ELISA). Results revealed that the overall seroprevalence of IgG and IgM in dogs was 20%. Many other studies conducted by using ELISA and PCR but, our study is related to prevalence. So, we did not use PCR. IgG antibodies were found in 16 dogs. The IgG prevalence was 14% followed by IgM prevalence was 6%. One dog was infected with both *T. gondii* IgG and IgM while the other 19 seropositive dogs were infected either with IgG or IgM. So, the overall prevalence percentage was 20%. The seroprevalence of *T. gondii* is conducted by many authors around the world. A study conducted by [Ahmed *et al.* \(2014\)](#) in Potohar, Pakistan showed a high prevalence rate that is (28.43%), and (48.66%) in Lahore, Pakistan, conducted by [Jadoon *et al.*, 2009](#). If we compare our study, our prevalence rate is 20%. It means prevalence rate of our study is less than the other cities of Pakistan. The prevalence of IgM antibodies of *T. gondii* is found less as compared to IgG antibodies in both studies. A study conducted in Iran over seroprevalence of IgG and IgM. The prevalence rate of both IgG and IgM was 31.2% in stray dogs and 9.03% in pet dogs ([Hosseini *et al.*, 2011](#)). While another study in Iran shows the higher prevalence rate that is (77.7%) in stray dogs and (40%) in

pet dogs (Shadfar *et al.*, 2012). Another study conducted in P. R. China shows a high proportion of prevalence rates (30.9%) in police dogs, (Liu *et al.*, 2013); (30.0%) prevalence in Portugal (Lopes *et al.*, 2011); 52/309 dogs were seropositive (16.8%) in Colombia (Dubey *et al.*, 2007); (40.3%) in both, urban and rural areas in China (Yan *et al.*, 2012); (67.8%) in Sri Lanka (Dubey *et al.*, 2007). A study conducted in Bangkok metropolitan area in dogs. The results of this study revealed that 40/427 (9.4%) dogs were seropositive to *Toxoplasma gondii* infection (Jittapalapong *et al.*, 2007). A study conducted in South China shows a prevalence rate of 611 dogs. 132/611 (21.6%) was seropositive for *T. gondii* (Duan *et al.*, 2012). Another same as a study conducted in Manila in which (24/158) (15.2%) was positive regarding *T. gondii* (Guy *et al.*, 2016). So, it is conducted that, our results if compared with other studies in Pakistan or with other countries. Our study showed the less seroprevalence rate of *T. gondii* in dogs. But we suggested that this study is not enough for awareness of *T. gondii* infection for all those, who keep dogs and other pets at house. There is need of molecular level studies for further considerations. Studies must be conducted regarding environment i.e. prevalence and molecular studied for soil contamination by *T. gondii*. Because in some cases, the dogs get infection of toxoplasma form soil which is contaminated by cat feces.

Conclusion

Infection by *T. gondii* is very common worldwide and the positive prevalence regarding age, gender, region is differing from each other. Due to the high level of consequences caused by this parasite in peoples, control measures and education should be given to minimize human exposure to *T. gondii* infection. Till now, no proper antibiotics have been developed. The application of green Nanomaterials may be a good inhibitory component. Our study provides a strong indication, that a relationship between owner and pets may increase a chance of *T. gondii* infection in their owners. Controlling measurements must be taken to decrease the chances of *T. gondii* infection and further investigation is still required.

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Ethical approval

This study was approved by the ethical committee of the Department of Zoology, Kohat University of Science

and Technology Pakistan and ethical committee of the Veterinary Research Institute Peshawar, Pakistan.

Conflict of interest

The authors have declared no conflict of interest.

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