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

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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.

Keywords | ELISA, T. gondii, Dogs, Prevalence, Risk factors

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 in 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., 1997; 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 (Stroka 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 the most favorite pets and thud 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.

**Sero logical 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**

**Sero prevalence 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.

| 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 Percentage of *T. gondii* in Pet Dogs of Peshawar

**Prevalence rate of sample for IgG** = 14/100 x 100 = 14%

**Prevalence rate of sample for IgM** = 6/100 x 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 week 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        |         |

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 (Hosseininejad 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 dogs. There is need of molecular infection for all those, who keep dogs and other pets at house. There is need of molecular 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 antibiotics have been developed. The application of green human exposure to T. gondii (15.2%) was positive regarding same as a study conducted in Manila in which (24/158) study showed the less seroprevalence rate of T. gondii with other studies in Pakistan or with other countries. Our 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.

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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.

Reference

Abu-Madi, M.A., Al-Molawi, N. and Behnke, J.M., 2008. Seroprevalence and epidemiological correlates of Toxoplasma gondii infections among patients referred for hospital-based serological testing in Doha, Qatar. Parasit. Vectors, 1: 39. https://doi.org/10.1186/1756-3305-1-39

Ahmed, B.A., Gaafar, S.M., Weirich, W.E. and Kanitz, C.L., 1983. Relationship of Toxoplasma infections to other diseases in dogs. Vet. Parasitol., 12:199-203. https://doi.org/10.1016/0304-4017(83)90008-0

Ahmad, N., Ahmed, H., Irum, S. and Qayyum, M., 2014. Seroprevalence of IgG and IgM antibodies and associated risk factors for toxoplasmosis in cats and dogs from sub-tropical arid parts of Pakistan. Trop. Biomed., 31: 777-784.

Aslam, M., Hussain, M., Ashraf, M. and Afridi, A.G.K., 2006. Geological Map of North West Frontier Province. Geological Survey of Pakistan.

Belanger, F., Derouin, F., Grangeot-Keros, L. and Meyer, L., 1999. HEMOCO and SEROCO Study Groups. Incidence and risk factors of toxoplasmosis in a cohort of human immunodeficiency virus-infected patients: 1988–1995. Clin. Infect. Dis., 28: 575-581. https://doi.org/10.1086/515147

Carlier, Y., Bout, D., Dessaint, J.P., Capron, A., Van Knapen, F., Ruitenberg, E.J., Bergquist, R. and Huldt, G., 1980. Evaluation of the enzyme-linked immunosorbent assay (ELISA) and other serological tests for the diagnosis of toxoplasmosis. Bull. WHO., 58: 99.

Dantas-Torres, F., 2014. Otranto D. Dogs, cats, parasites, and humans in Brazil: opening the black box. Parasites Vectors, 7: 22. https://doi.org/10.1186/1756-3305-7-22

Duan, G., Tian, Y.M., Li, B.F., Yang, J.F., Liu, Z.L., Yuan, F.Z., Zhu, X.Q. and Zou, F.C., 2012. Seroprevalence of Toxoplasma gondii infection in pet dogs in Kunming, Southwest China. Parasites Vectors, 5:118. https://doi.org/10.1186/1756-3305-5-118

Dubey, J.P., 2010. Toxoplasmosis of animals and humans. CRC Press Inc. Second edition. Boca Raton, New York. pp. 1-313.

Dubey, J.P., Cortes-Vecino, J.A., Vargas-Duarte, J.J., Sundar, N., Velurugan, G.V., Bandini, L.M., Polo, L.J., Zambrano, L., Mora, L.E., Kwok, O.C. and Smith, T., 2007. Prevalence of Toxoplasma gondii in dogs from Colombia, South America.
and genetic characterization of T. gondii isolates. *Vet. Parasitol.*, 145: 45-50. https://doi.org/10.1016/j.vetpar.2006.12.001

Dubey, J.P., Rajpakse, R.P., Wijesundera, R.R., Sundar, N., Velmurugan, G.V., Kwok, O.C. and Su, C., 2007. Prevalence of *Toxoplasma gondii* in dogs from Sri Lanka and genetic characterization of the parasite isolates. *Vet. Parasitol.*, 146: 341-346. https://doi.org/10.1016/j.vetpar.2007.03.009

Frenkel, J.K., Hassanine, K.M., Hassanine, R.S., Brown, E., Thulliez, P. and Quintero-Nunez, R., 1995. Transmission of *Toxoplasma gondii* in Panama City, Panama: a five-year prospective cohort study of children, cats, rodents, birds, and soil. *Am. J. Trop. Med. Hyg.*, 53: 458-468. https://doi.org/10.4269/ajtmh.1995.53.53.458

Frenkel, J.K., Lindsay, D.S., Parker, B.B. and Dobesh, M., 2003. Dogs as possible mechanical carriers of Toxoplasma, and their fur as a source of infection of young children. *Int. J. Infect. Dis.*, 7: 292-293. https://doi.org/10.1016/S1201-9712(03)00112-3

Guy, L.M. and Penuliar, G.M., 2016. Seroprevalence and risk factor analysis of *Toxoplasma gondii* Among Stray and Domesticated Dogs (Canis familiaris) in Antipolo and Metro Manila. *Philippine J. Sci.*, 145: 49-55.

Hill, D.E., Chirukandoth, S. and Dubey, J.P., 2005. Biology and epidemiology of *Toxoplasma gondii* in man and animals. *Anim. Hlth. Res. Rev.*, 6: 41-61. https://doi.org/10.1079/AHR2005100

Hosseinejad, M., Malmasi, A., Hosseini, F., Selk-Ghaffari, M., Khorrami, N., Mohebali, M., Shojaee, S., Mirani, A., Azizzadeh, M., Mirshokraei, P. and Aliari, A., 2011. Seroprevalence of *Toxoplasma gondii* infection in dogs in Tehran, Iran. Iranian J. Parasitol., 6: 81.

Jadoon, A., Akhtar, T., Maqbool, A., Anjum, A.A. and Ajmal, A., 2009. Seroprevalence of *Toxoplasma gondii* in Canines. *J. Anim. Plant Sci.*, 19: 179-181.

Jittapalapong, S., Nimsupan, B., Pinyopanuwat, N., Chimnoi, W., Kabeya, H. and Maruyama, S., 2007. Seroprevalence of *Toxoplasma gondii* antibodies in stray cats and dogs in the Bangkok metropolitan area, Thailand. *Vet. Parasitol.*, 145: 138-141. https://doi.org/10.1016/j.vetpar.2006.10.021

Kamani, J., Mani, A.U., Kunshe, H.A., Dogo, G.I., Yidawi, J.P., Pauline, D.K., Nnabuife, H.E., Ioan, P. and Egwu, G.O., 2010. Serosurvey for *Toxoplasma gondii* in dogs in Maiduguri, Borno State, Nigeria. *J. Infect. Dev. Countries*, 4: 15-18. https://doi.org/10.3855/jidc.428

Lindsay, D.S., Dubey, J.P., Butler, J.M. and Blagburn, B.L., 1997. Mechanical transmission of *Toxoplasma gondii* oocysts by dogs. *Vet. Parasitol.*, 73: 27-33. https://doi.org/10.1016/S0304-4017(97)00048-4

Liu, Q.X., Wang, S., Wang, L.Q., Xing, J., Gao, W.J., Liu, G.F., Zhao, B., Zhang, H.B. and Gao, L.H., 2014. Seroprevalence of *Toxoplasma gondii* infection in dogs and cats in Zhenjiang City, Eastern China. *Asian Pac. J. Trop. Biomed.*, 4: 725-728. https://doi.org/10.1016/S0140-6736(04)16412-X

Lopes, A.P., Santos, H., Neto, F., Rodrigues, M., Kwok, O.C.H., Dubey, J.P. and Cardoso, L., 2011. Prevalence of antibodies to *Toxoplasma gondii* in dogs from northeastern Portugal. *J. Parasitol.*, pp. 418-420. https://doi.org/10.1645/GE-2691.1

Lu, A.T., Gao, Y. and Du, S., 2010. Survey on cats and dogs infected with *Toxoplasma gondii* at part area of Inner Mongolia. *Anim. Husb. Feed Sci.*, 31: 155-156.

McConnell, A.R., Brown, C.M., Shoda, T.M., Stayton, L.E. and Martin, C.E., 2011. Friends with benefits: on the positive consequences of pet ownership. *J. Pers. Soc. Psychol.*, 101: 1239. https://doi.org/10.1037/a0024506

Montoya, J.G. and Liesenfeld, O., 2004. Toxoplasmosis. *Lancet* 363, 1965–1976. Cross Ref, PubMed, CAS, Web of Science® Times Cited. pp. 807. https://doi.org/10.1016/S0140-6736(04)16412-X

Nicolle, C., 1908. Sur une infection a corps de Leishman (on organismes voisins) du gondi. *CR Acad Sci.*, pp. 147-736.

Rengifo-Herrera, C., Pile, E., García, A., Pérez, A., Pérez, D., Nguyen, F.K., de la Guardia, V., Mcleod, R. and Caballero, Z., 2017. Seroprevalence of *Toxoplasma gondii* in domestic pets from metropolitan regions of Panama. *Parasite*, pp. 24. https://doi.org/10.1051/parasite/2017009

Shadfar, S., Shabestari, A., Zendeh, M.B., Gasemi, B. and Zamzam, S.H., 2012. Evaluation of *Toxoplasma gondii* IgG antibodies in stray and household dogs by ELISA. *Glob. Vét.*, 9: 117-122.

Simşek, S., Urtik, A.E., Babür, C. and Köroğlu, E., 2006. Seroprevalence of *Toxoplasma gondii* in dogs in the province of Kocaeli. *Turkiye Parazitoloji Dergisi*, 30: 171-174.

Sroka, J., Wójcik-Fatla, A., Szymanska, J., Dutkiewicz, J., Zając, V. and Zwolinski, J., 2010. The occurrence of *Toxoplasma gondii* infection in people and animals from rural environment of Lublin region–estimate of potential role of water as a source of infection. *Ann. Agric. Environ. Med.*, 17: 125-132.

Tenter, A.M., Heckeroth, A.R. and Weiss, L.M., 2002. Erratum- *Toxoplasma gondii*: From animals to humans. *Int. J. Parasitol.*, 31: 217-220. https://doi.org/10.1016/S0020-7519(01)00125-4

Watson, A.D., Farrow, B.R. and McDonald, P.J., 1982. Prevalence of *Toxoplasma gondii* antibodies in pet dogs from northeastern Portugal. *J. Parasitol.*, pp. 418-420. https://doi.org/10.1645/GE-2691.1
dogs and cats. *Aust. Vet. J.*, 58: 213-214. https://doi.org/10.1111/j.1751-0813.1982.tb00673.x

Wu, S.M., Huang, S.Y., Fu, B.Q., Liu, G.Y., Chen, J.X., Chen, M.X., Yuan, Z.G., Zhou, D.H., Weng, Y.B., Zhu, X.Q. and Ye, D.H., 2011. Seroprevalence of *Toxoplasma gondii* infection in pet dogs in Lanzhou, Northwest China. *Parasites Vectors*, 4: 64. https://doi.org/10.1186/1756-3305-4-64

Yan, C., Fu, L.L., Yue, C.L., Tang, R.X., Liu, Y.S., Lv, L., Shi, N., Zeng, P., Zhang, P., Wang, D.H. and Zhou, D.H., 2012. Stray dogs as indicators of *Toxoplasma gondii* distributed in the environment: the first report across an urban-rural gradient in China. *Parasites Vectors*, 5: 5. https://doi.org/10.1186/1756-3305-5-5