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ELISA-BASED DETECTION AND PREVALENCE OF ANTI-*TOXOPLASMA GONDII* ANTIBODIES IN CLINICALLY HEALTHY PET DOGS FROM SOUTHERN PUNJAB, PAKISTAN

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Abstract

Toxoplasma gondii is an intracellular zoonotic parasite capable of infecting all warm-blooded animals, including humans, dogs and birds. Its infectivity may result in serious health problems, particularly in immunocompromised patients and pregnant women. Dogs may serve as intermediate hosts, potentially reflecting environmental exposure to T. gondii. The present cross-sectional study was conducted to determine the seroprevalence of T. gondii and identify associated risk factors in 184 clinically healthy pet dogs from southern Punjab, Pakistan. Serum samples were screened for anti-Toxoplasma gondii antibodies using a commercial ELISA ID Screen® Toxoplasmosis Indirect Multi-Specie (TOXO-MS, ID Vet, Grables, France). The overall seroprevalence was 16.84% (31/184). Female dogs exhibited a higher seropositivity rate 18.55% that of male pet dogs 14.94%, with a non-significant ($P \geq 0.05$) difference of prevalence rate among two genders. Statistically, no significant ($P \geq 0.05$) difference of seropositivity was found between age groups and

seropositivity as dogs. However, animals aged >2 years were more seropositive than those aged 1–2 years and 1 month–11 months of age. Among the different breeds, Bulldogs have had the highest seroprevalence 23.07% (6/26), followed by Bully dogs 22.22% (2/9) Katha 20%, Labrador 17.85%, Gultair 15.78%, German Shepherd 15.38%, Bull Terrier 12.5% and Rottweiler 11.11%, while none of the beauty dog was detected as positive to T. gondii. However, the differences in prevalence were not statistically significant (P ≥ 0.05). Feeding practices and housing type were found to be significantly (P ≤ 0.05) associated with Toxoplasma gondii positivity while vaccination status, gender of the owner, and exposure to other pets were not significantly (P ≥ 0.05) associated with positivity. These findings demonstrate the distribution of T. gondii among pet dogs from southern Punjab, Pakistan and highlight the importance of canine exposure in understanding the epidemiology and environmental transmission of this zoonotic pathogen.

INTRODUCTION

Toxoplasma gondii is an obligatory intracellular protozoan parasite belonging to the phylum Apicomplexa. It is the causative agent of toxoplasmosis, an important zoonotic disease affecting humans as well as a wide range of domestic and wild animals. Cats can serve as the final host, while dogs, humans and other mammals act as intermediate host. The parasite has a cosmopolitan distribution that is transmitted primarily through consumption of sporulated oocysts from contaminated food, soil, and water and ingestion of raw or undercooked meat containing tissue cysts (Tenter et al., 2000; Dubey, 2010). Its ability to maintain chronic infection and persistence within the host tissues make it an important public and veterinary health concern. The epidemiological importance of *Toxoplasma gondii* infection is associated with the host range, transmission routes and environmental factors.

Dogs are considered as an important intermediate host of *Toxoplasma gondii* due to their close association with humans and vast exposure to contaminated environments. Dogs can acquire infection through ingestion of contaminated food, water, soil, or infected animal tissues. Their hunting behavior, outdoor access and contact with cat feces may increase their exposure to infective oocysts. Although most infected dogs remain clinically healthy, toxoplasmosis may occasionally cause systemic, neurological, respiratory, and muscular disorders, particularly in young or immunocompromised animals (Lindsay et al., 1997; Dubey, 2010).

Sero-epidemiological studies are important to identify the past exposure to *T. gondii* infection, particularly in clinically healthy animals. The detection of anti-Toxoplasma IgG antibodies indicates previous exposure to the pathogen, without confirming the active infection. Several techniques are available to detect *T. gondii* infection, including IFAT, MAT, latex agglutination, and ELISA (enzyme-linked immunosorbent assay). ELISA is the most commonly used assay due to high sensitivity, specificity, reproducibility, and suitability for screening large numbers of serum samples. Therefore, ELISA testing provides a useful and easy approach for determining the prevalence of *T. gondii* in apparently healthy canine populations (Dubey, 2010; Robert-Gangneux and Dardé, 2012).

The prevalence of canine toxoplasmosis varies considerably in various geographical regions, influenced by several risk factors, including age, housing conditions, outdoor access, contact with cats, and environmental contamination. In Pakistan, previous studies have revealed the occurrence of *Toxoplasma gondii* antibodies in domestic dogs. Ahmad et al. (2014) reported 25.49% seroprevalence among dogs from subtropical arid regions of Pakistan. Similarly, studies performed in Faisalabad and Khyber Pakhtunkhwa have had demonstrated the seroprevalence of toxoplasmosis in dogs (Fatima et al., 2026; Said et al., 2023; Hamidullah et al., 2022; Kalimullah et al., 2020). Furthermore, recent studies from other countries have highlighted the occurrence of *T. gondii* infection in dogs. Marzok et al. (2024) described a prevalence rate of 24% in dogs from Egypt, Sioutas et al. (2024) from Greece and Wen et al. (2025) from China emphasizing the importance of toxoplasmosis in dogs worldwide. However,

epidemiological information regarding prevalence of IgG antibodies in clinically healthy pet dogs in Southern Punjab remains limited.

Therefore, the present study was designed to detect and determine the seroprevalence of IgG antibodies to *T. gondii* in clinically healthy pet dogs from Southern Punjab, Pakistan, using indirect commercial ELISA. The specific objectives were to determine the overall seroprevalence of anti-*Toxoplasma gondii* antibodies and to evaluate the association with potential risk factors, including age, sex, breed, feeding habits, housing conditions, outdoor access, and contact with cats. The present study will provide baseline epidemiological information regarding previous exposure to *Toxoplasma gondii* in pet dogs from the prescribed study area.

2. MATERIALS AND METHODS

2.1 Study Design

The current study was approved by Advance Studies and Research Board (ASRB), Bahauddin Zakariya University, Multan, Pakistan. A cross-sectional study was carried out to examine the sero-prevalence of *T. gondii* in dogs from the south Punjab Province, Pakistan. This study area accounts for approximately 52% of the total area of Punjab which comprised of 3 administrative divisions/zones and eleven districts. It covers about 79,662 km² and has an estimated human population of 11.2 million. The weather conditions are characterized by extremely hot dry summers with temperature ranges from 45-50 °C and relatively mild winters from 15-20 °C. Earlier studies on the prevalence of *T. gondii* in dogs, animals and humans from upper Punjab revealed that the parasite is prevalent and may be an important cause of abortion in this area (Fatima et al., 2026; Said et al., 2023; Nazir et al., 2017).

Blood samples from the 184 apparently healthy pet dogs from South Punjab were collected. The sample size was based on results calculated by a computer software program (Sample Size Calculator; https://www.surveysystem.com/sscalc.htm). The most commonly used dog breeds for this study were Bull dog, German shepherd, Rottweiler, Labrador, Gultair, Bully dogs, Katha, Bullterrier and beauty dogs. The 3 different age groups i.e., 1- 11 months, 1 - 2 two years >2 years and were selected. Approximately 3-5 ml of blood sample was collected from Cephalic vein and transfer into vacutainer without any anticoagulant. From blood sample serum was separated by centrifugation at 3000 rpm for 5min. Sample was collected in properly labeled serum collecting tubes. Samples were stored at -80°C until further processing. A "Convenience Sampling Technique" was used to detect the Anti-*Toxoplasma gondii* antibodies in pet dogs from various pet clinics and pet shows and pet rearing centers.

2.2. Risk factors

Epidemiological risk factors was assessed by getting information to risk factors for *T. gondii* positivity at the of blood sampling. The descriptive epidemiological data was collected by using a well-designed questionnaire. Additional epidemiological information regarding age, sex, dog breed, access to outside, exposure to other pets, area and hunting practice were obtained by interview to the owner. This information was used for statistical analysis (see below).

2.3 Serology

The prevalence of IgG antibodies to *T. gondii* was detected by using a commercially available ELISA Kit, indirect ELISA kit ID Screen® Toxoplasmosis Multi-Specie (TOXO-MS, ID. Vet, Grables, France) as per manufacturer's recommendation with 91.4% sensitivity and 99.4% specificity. The Optical density (OD) value was measured at 450 nm using an automated ELISA plate reader. Positive and negative controls were supplied by the manufacturer with the ELISA kit. Interpretation of serum samples indicating S/P (S/P%) was measured by following formula.

$$\mathrm{S/P(\%)} = \frac{\mathrm{OD}_{\text{sample}} - \mathrm{OD}_{\text{negative control}}}{\mathrm{OD}_{\text{positive control}} - \mathrm{OD}_{\text{negative control}}} \times 100$$

Serum samples with S/P% of less than or equal to 40% were declared as negative.

Serum samples with S/P% between >40% to <50% were counted as doubtful and negative.

Samples with S/P% of greater than or equal to 50% were declared as positive.

2.4. Statistical analysis

The mean of serum samples was calculated with the help of computer software Microsoft Excel 2010 and Pearson. Pearson's Chi square test and Fisher's exact test was used to analyze variations in prevalence rate. Statistical data was analyzed with help of a software SPSS (SPSS for Window, version 21; SPSS, Chicago, IL). Level of significance $P \leq 0.05$ were considered as significant.

3. RESULTS

Thirty-one (16.84%) of the 184 blood samples from apparently healthy pet dogs were found to be positive to Anti-*Toxoplasma gondii*. Female pet dogs were detected as more positive to *Toxoplasma gondii* infection18.55% (18/97) compare to male pet dogs 14.94% (13/87) (Table 1). A non-significant difference ($P \geq 0.05$) was detected in the seroprevalence of *Toxoplasma gondii* antibodies between males and female pet dogs. The observed prevalence rate was not significantly different ($P \geq 0.05$) among different breeds of dogs, the highest percentage prevalence was observed in Bull dogs 23.07% followed by Bully dogs 22.22%, Katha 20%, Labrador 17.85%, Gultair 15.78%, German shepherd/Alsatian 15.38%, Bullterrier 12.5%, Rottweiler 11.11% and 0% in beauty dogs (Table 1). Age wise prevalence was also not statistically significantly ($P \geq 0.05$) different within the three age groups of dogs, maximum prevalence rate to *Toxoplasma gondii* was observed in dogs of > 2 years 20.89% as compare to dogs with age of 1 month -11 months 15.27% and 1 year – 2 years 13.3% (Table 1).

Table 1: Seroprevalence of *Toxoplasma gondii* antibodies in pet dogs according to gender, breed, and age

Risk Factor	Category	Positive (n/N)	Prevalence (%)	95% CI (%)	χ^2	df	P-value
Gender	Male	13/87	14.94	8.95-23.90	0.428	1	0.513
	Female	18/97	18.56	12.07-27.44			
Breed	German Shepherd	12/78	15.38	9.03–24.99	1.821	8	0.986
	Rottweiler	1/9	11.11	1.99–43.50			
	Labrador	5/28	17.86	7.88–35.59			
	Gultair	3/19	15.79	5.52–37.57			
	Katha	1/5	20.00	3.62–62.45			
	Bull terrier	1/8	12.50	2.24–47.09			
	Bull dog	6/26	23.08	11.03–42.05			
	Beauty dog	0/2	0.00	0.00–65.76			
	Bully dog	2/9	22.22	6.32–54.74			
Age	1 m–11m	11/72	15.28	8.75–25.32	1.307	2	0.520
	1–2 years	6/45	13.33	6.26–26.18			
	>2 years	14/67	20.90	12.88–32.07			
Overall	Total	31/184	16.85	12.13–22.92	-	-	-

CI = Confidence interval χ^2 = Pearson chi-square test df = degree of freedom

We found that non-vaccinated 19.76% pet dogs have had higher rate of positivity to *T. gondii* that of vaccinated dogs 14.28% (Table 2). These differences in prevalence based on vaccination status against other diseases were statistically not significant ($P \geq 0.05$). Feeding practices was to be a non-significant ($P \geq 0.05$) risk factor to the prevalence of *T. gondii* in dogs. Dogs that feed on mix food were more positive for the *Toxoplasma gondii* 20.33% as compare to the dogs that feed on homemade food/ raw food 17.56% and commercial food 11.76% (Table 2). The effect of housing was also examined by dividing into three categories such as cage system, free outdoor walk and tie to rope. Dogs that free to outdoor walk were more infected 28.88% than that of cage dogs 15.68% and tied/tie to rope 11.36% with significant ($P \leq 0.05$) difference of prevalence among three categories (Table 2).

The prevalence was not significantly different ($P \geq 0.05$) on the basis of owner's gender i.e. either male or female. Pet dogs of female owners were more infected 17.77% than of male owners 16.54%. We found

that the prevalence was not significantly different (P ≥ 0.05) dogs that exposed to pregnant owner and non-exposed to pregnant owner. Pet dogs that were not exposed to pregnant female owner were more seropositive 17.94% than the dogs with exposure of pregnant female owner 16.66%. High prevalence rate was observed in non-dewormed 26.5% dogs compared to the dewormed 8.91% dogs with a significant level of difference (P ≤ 0.05) of positivity to *Toxoplasma gondii* infection (Table 2).

The prevalence rate of *Toxoplasma gondii* infection was 18.44% in exposed versus 14.81% in non-exposed dogs to cats. These findings were not significantly (P ≥ 0.05) different. Prevalence of antibodies to *T. gondii* was 20% in dogs exposed to livestock population and 13.82% in non-exposed dogs (Table 2). The difference of positivity was noticed as statistically non-significant (P≥0.05).

Table 2: Prevalence of *Toxoplasma gondii* antibodies in pet dogs according to management, feeding, housing, and exposure-related factors

Factor	Category	Positive (n/N)	Prevalence (%)	95% CI (%)	χ^2	df	P-value
Vaccination	Vaccinated	14/98	14.29	8.70–22.56	0.982	1	0.322
	Non-vaccinated	17/86	19.77	12.72–29.40			
Food type	Commercial food	6/51	11.76	5.51–23.38	1.481	2	0.477
	Homemade	13/74	17.57	10.56–27.77			
	Mixed food	12/59	20.34	12.04–32.27			
Housing	Cage system	8/51	15.69	8.17–28.01	6.596	2	0.037*
	Outdoor access	13/45	28.89	17.73–43.37			
	Tied to rope	10/88	11.36	6.29–19.67			
Gender of owner	Male	23/139	16.55	11.29–23.61	0.037	1	0.848
	Female	8/45	17.78	9.29–31.33			
Exposure to pregnant owner	Exposed	1/6	16.67	3.01–56.35	0.006	1	0.939
	Non-exposed	7/39	17.95	8.98–32.67			
Deworming	Yes	9/101	8.91	4.76–16.07	10.068	1	0.002*
	No	22/83	26.51	18.20–36.89			
Exposure to other pets	Exposed	19/103	18.45	12.14–27.02	0.427	1	0.514
	Unexposed	12/81	14.81	8.68–24.13			
Exposure to livestock	Exposed	18/90	20.00	13.04–29.41	1.249	1	0.264
	Unexposed	13/94	13.83	8.26–22.24			
Overall	Total	31/184	16.85	12.13–22.92	–	–	–

CI95%= Confidence interval χ^2 = Pearson chi-square test df = degree of freedom; * indicates statistical significance P ≤ 0.05

4. DISCUSSION

Clinical and sub-clinical form of toxoplasmosis in dogs and other canids have been reported from many countries of the world. The present study revealed an overall prevalence of *T. gondii* of 16.84% (31/184) in apparently pet dogs, indicating considerable exposure to the parasite in the study area. These findings are comparable to previous studies, who reported a prevalence of 18.6% by Modified Agglutination test in China (Sheng Zhijin et al., 2020) and 15.5% in Angola (Lopes et al., 2014), 17.3% to 23.6% in pet dogs by Duan et al., in different area of Kunming, southwest China (Duan et al., 2012).

However, the prevalence was lower than those reported in Egypt, 98.0% (El Behairy et al., 2013) Cambodia 50.5% (Nguyen et al., 2021) Iran 46.67% (Zarra-Nezhada et al., 2017) and northeastern China 30.9%, (Liu et al., 2013). Conversely, it was higher than the 10.81% and 10.9% reported in Lanzhou, China and Bangkok, Thailand respectively (Wu et al., 2011; Jittapalapong et al., 2009). Such variations may be attributed to differences in geographical location, climatic conditions, dog management practices, population characteristics, and diagnostic techniques (Dubey et al., 2020).

In the current study, female dogs (18.55%) showed higher seropositivity to *T. gondii* than males (14.94%). Similarly, older dogs >2 years of age exhibited the highest prevalence of (20.89%), suggesting an increased post-natal exposure to environmental sources of infection with advancing age. Nevertheless, earlier studies have reported inconsistent associations between seropositivity, age, and sex. Dini et al. (2024) also reported that sex of animal and age were not significantly ($P \geq 0.05$) associated with seropositivity of dogs. Similarly, Zarra-Nezhada et al., (2017) found higher positivity rate of 56% in females than males 39% in Iran.

Housing practices and deworming status were found to be significantly ($P \leq 0.05$) associated with seropositivity, with dogs having outdoor access exhibiting the highest prevalence rate of 28.88%, compared with caged 15.68% and tied dogs 11.36%. These results may reflect increased exposure to contaminated soil, sporulated oocysts, infected prey, and contaminated food or water. These findings are supported by Dini et al. (2024), who reported approximately fivefold higher rate of seropositivity in dogs living outdoors. Correspondingly, Said et al. (2023) also reported a higher rate of seroprevalence in dogs from rural areas of Pakistan.

Vaccination status and feeding practices was not significantly corelated to *T. gondii*. positivity in dogs. We found that non-vaccinated dogs 19.76%, dogs receiving mixed diets 20.33% and non-dewormed dogs 26.50%, exhibited higher seropositivity. However, it should be interpreted cautiously because it may reflect differences in managemental practices rather than direct causal associations. The higher positivity in dogs exposed to other pets 18.44% and livestock population 20.0% further suggesting a possible exposure to contaminated environments. Dini et al. (2024) similarly identified that outdoor access and pet exposure as significant risk factors for canine *T. gondii* positivity. The results of current study was also in agreement with the previously reported studies by other researchers and providing a clue of horizontal transmission within different species of animals (Lopes et al., 2014; Dubey et al., 2010).

Bulldogs exhibited the highest seroprevalence of 23.07%, followed by Bully dogs 22.22%, Katha dogs 20.0%, Labrador 17.85%, Gultair 15.78%, German shepherd/Alsatian 15.38%, Bullterrier 12.5%, Rottweiler 11.11% and 0% in beauty dogs. In a previous study Kamani et al., revealed that Alsatians dogs and their close breeds detected less seropositive in Nigeria (Kamani et al., 2010).

CONCLUSION

Overall, the findings demonstrate that canine exposure to *Toxoplasma gondii* is influenced by environmental and managemental risk factors. Recent evidence from Pakistan further supports its distribution, as Fatima et al. (2026) molecularly detected *T. gondii* in 16.7% of dogs from Punjab. Similarly, Said et al. (2023) reported considerable canine sero-prevalence from Khyber Pakhtunkhwa, Pakistan. These findings highlight the importance of regular surveillance, improved hygiene and managemental practices. However, further studies involving larger scale dog populations, molecular detection, and parasite genotyping are required to improve the understanding of epidemiology and transmission dynamics of *Toxoplasma gondii* in Pakistan.

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Informed Consent

Written consent for the publication of the study findings was obtained from all the authors involved.

Conflict of interest

The authors declared that they have no conflict of interest.

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