Biological and Clinical Sciences Research Journal
eISSN: 2708-2261; pISSN: 2958-4728
www.bcsrj.com
DOI: https://doi.org/10.54112/bcsrj.v6i4.1624
*Biol. Clin. Sci. Res. J., Volume 6(4), **2025**: 1624*
Original Research Article

ELISA-Based Seroprevalence and Risk Factors for Toxocara Canis and Dirofilaria Immitis in Dogs in Khyber Pakhtunkhwa, Pakistan

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(Received, 24th November 2024, Accepted 22nd April 2025, Published 30th April 2025)

Abstract: *Parasites are responsible for several important and widespread zoonotic diseases transmitted through companionship between animals and humans. Zoonotic parasitic infections impact human and animal health and food safety and have significant economic implications. Limited data on the seroprevalence of Toxocara canis and Dirofilaria immitis infections in household dogs in Khyber Pakhtunkhwa, Pakistan.* ***Objective:*** *To determine the seroprevalence of Toxocara canis and Dirofilaria immitis in household dogs using a serological technique and to assess the associated risk factors.* ***Methods:*** *This cross-sectional study included 405 dog blood samples collected from veterinary facilities and private pet clinics across Khyber Pakhtunkhwa. An ELISA test was used to detect anti-Toxocara and anti-Dirofilaria IgG antibodies. Data regarding gender, age, area (rural/urban), and deworming status were gathered from owners through questionnaires. Chi-square tests were used to determine associations between seroprevalence and potential risk factors, with a significance level set at $p < 0.05$.* ***Results:*** *The overall seroprevalence was 22.71% (92/405; 95% CI: 18.9%–27.0%) for Toxocara canis and 2.22% (9/405; 95% CI: 1.1%–4.1%) for Dirofilaria immitis. For Toxocara canis, area (rural vs. urban, $p < 0.05$) and deworming status ($p < 0.05$) were significantly associated with infection. Age and gender were not significantly associated with Toxocara canis infection ($p > 0.05$). None of the investigated risk factors showed a statistically significant association with Dirofilaria immitis seroprevalence ($p > 0.05$).* ***Conclusion:*** *The study highlights a substantial seroprevalence of Toxocara canis and a lower prevalence of Dirofilaria immitis among household dogs in Khyber Pakhtunkhwa. Risk factors such as living in rural areas and a lack of deworming were associated with higher Toxocara infection rates. Effective control requires a comprehensive "One Health" strategy collaboratively addressing animal and human health.*

Keywords: Toxocariasis, Dirofilariasis, Zoonoses, Dogs, Serologic Tests

[***How to Cite:*** Said A, Saqib MN, Aziz T, Sadeeq M, Shah SKA, Faisal S, Khan FN. Elisa-based seroprevalence and risk factors for Toxocara canis and Dirofilaria immitis in dogs in Khyber Pakhtunkhwa, Pakistan. *Biol. Clin. Sci. Res. J.*, **2025;** 6(4): 125-128. doi: https://doi.org/10.54112/bcsrj.v6i4.1624

Introduction

Parasitic zoonoses are a significant concern for both public and animal health, particularly due to their transmission through close companionship between humans and domestic animals such as dogs and cats. Zoonotic parasitic infections negatively impact human health, food security, and impose a substantial economic burden worldwide (1). Individuals working closely with animals, including veterinarians, butchers, and animal handlers, are at heightened occupational risk (2,3). The global emergence and re-emergence of parasitic zoonoses have been well-documented, particularly in low- and middle-income countries where hygiene and veterinary infrastructure are often suboptimal (4,5).

Toxocara canis (T. canis), a prevalent intestinal nematode of dogs and other canids, sheds durable eggs into the environment, contaminating soil, water, plants, and food (6). Puppies are particularly susceptible to patent infections, increasing environmental contamination (7). Human infection occurs via accidental ingestion of embryonated eggs from contaminated sources, leading to toxocariasis—a significant public health concern in tropical and developing regions (8,9). Definitive hosts such as dogs, cats, and wild canids harbor the adult parasites, while the infective eggs remain viable in the environment for extended periods (10). Human infection is incidental and results in visceral larval migrans (VLM), ocular toxocariasis, or neurotoxocariasis, depending on the migration site of larvae (11,12). Symptoms range from asymptomatic infections to severe neurological, ocular, and pulmonary manifestations (12,13). Diagnosis largely relies on serological techniques such as enzyme-linked immunosorbent assay (ELISA), detecting Toxocara excretory-secretory (TES) antigens (14). Although Western blotting remains the gold standard, it is less commonly employed due to its complexity and cost (14,15).

Similarly, Dirofilaria immitis (D. immitis), the causative agent of dirofilariasis, poses a serious veterinary and emerging zoonotic threat. Transmission primarily occurs through mosquito vectors, with infection rates correlating with mosquito activity and environmental factors (16,17). In dogs and cats, D. immitis leads to cardiopulmonary disease, and in humans, pulmonary dirofilariasis manifests as lung nodules often mimicking tumors (17,18). Diagnosis in animals involves the identification of microfilariae through microscopy (e.g., Knott's test) or detecting circulating antigens via ELISA or immunochromatographic tests (19,20).

Given the substantial zoonotic risk and growing prevalence of these parasites, particularly in regions with close human-animal interaction, comprehensive epidemiological assessments are imperative. Estimating the seroprevalence of T. canis and D. immitis in household pets is essential to designing effective preventive and control strategies tailored to the local context. Therefore, this study aims to determine the seroprevalence of T. canis and D. immitis in domestic dogs using

sensitive immunodiagnostic methods to contribute towards improved zoonotic disease surveillance and public health protection.

Methodology

Khyber Pakhtunkhwa (KPK) is one of Pakistan's four administrative provinces. It is situated in the western part of the country and shares a border with Afghanistan. Geographically speaking, it is the smallest of the four provinces, but in terms of population and economic output, it ranks third in Pakistan. An equal number of dogs were collected amongst the three study districts (Mardan, Swabi, and Nowshera) (Figure 1).

The following formula was used to determine sample size. (Daniel & Cross, 2018).

$$n = \frac{Z^2 P(1-P)}{d^2}$$

N = sample size, Z = confidence level, P = expected prevalence, and d = precision.

If no prevalence study is conducted, then Z=1.96 is the 95% confidence limit. The prevalence is assumed to be 50%, and then P = Estimated prevalence (e.g., 0.5 for 50%) and Desired precision = d = (e.g., 0.05 for ± 5%) (Thursfield, 2018).

$$n = \frac{1.96^2 \times 0.5(1-0.5)}{0.05^2}$$

$$n = 384$$

According to the formula, the sample size calculated was 384. This study collected 405 samples from dogs between September 2019 and September 2020. Before the study, the district director of livestock was permitted to inform all veterinary doctors and assistants in the study area. To that end, the study included client-owned dogs who came to the Civil Veterinary Hospital in each district for vaccination and routine check-ups were included in this study. Each dog owner who provided a sample of their dog was spoken to about the present research topic and asked to sign an informed consent form that included all the written information about the study. A veterinary doctor or assistant assisted in collecting blood samples from dogs. Stray dogs were excluded from this study.

A total of 400 blood samples were collected from dogs and cats. A 3 mL blood sample was collected and transferred to a gel tube. Blood samples were conveyed to the laboratory, kept overnight at 4°C, and centrifuged at 2000 rpm for 4 min. The collected sera were stored at -20°C until serological testing. Serum was tested using DiroCHEK® Canine Heartworm Antigen Test Kit (Synbiotics Corporation, San Diego, USA) and *Toxocara* vet ELISA kit (Demeditec Diagnostics GmbH, Germany) for detection of IgG antibodies against D. immitis and T. canis, respectively (Scavo et al., 2022).

The proportion of *T. canis* seropositive in dogs and possible risk factors was evaluated using chi-square, Fisher's exact test. Statistical significance was defined as a p-value of 0.05. STATA version 17 was used for all data analysis (Stata Corporation, College Station, Texas, USA).

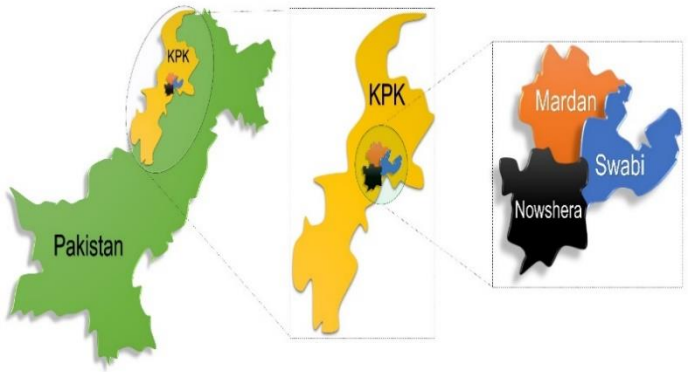

Figure 1. Map of Pakistan (green), indicating the location of the province of Khyber Pakhtunkhwa (KPK) (yellow), containing the three study districts: Nowshera (black), Swabi (blue), and Mardan (orange).

Results

Overall seroprevalence of *T. canis* in the three districts was 22.71% (92/405, 95% CI: 0.189%-0.270%) in dogs. The seroprevalence of *T. canis* in districts Mardan, Swabi and Nowshera 26.66% (36/135, 95% CI: 0.199%-0.347%), 20% (27/135, 95% CI: 0.141%-0.275%), and 21.48% (29/135, 95% CI: 0.153%-0.291%) in dogs respectively. There was no significant difference in the seroprevalence of *T. canis* between the three districts (P> .05) (Table 1). Table 2 shows the seroprevalence of *T. canis* in dogs with risk factors. Comparing the total seropositive (92 subjects) with seronegative populations (313 subjects), the chi-square analysis confirmed that area (rural vs urban) ($p < .05$) and deworming were risk factors associated with *T. canis* infection in our study. All other risk factors, age and gender, were not statistically significant (P> .05) in this study (Table 2).

The overall seroprevalence of *D. immitis* in the three districts was 2.22% (09/405, 95% CI: 0.011%-0.041%) in dogs. The seroprevalence of *D. immitis* in district Mardan, Swabi, and Nowshera was 3.70% (5/135, 95% CI: 0.015%-0.083%), 2.22% (3/135, 95% CI: 0.007%-0.063%), and 0.74% (1/135, 95% CI: 0.001%-0.040%) in dogs, respectively. There was no significant difference in seroprevalence of *D. immitis* between the three districts (P> .05) (Table 3). Comparing the total seropositive (9 subjects) with seronegative populations (396 subjects), the chi-square analysis confirmed that all risk factors, age, gender, area (rural vs urban), and deworming, were not statistically significant (P> .05) in this study (Table 4).

Table 1. District-wise seroprevalence of *Toxocara canis* in dogs

Districts	No of Samples	Positive Samples	Prevalence (%)	95% CI	P-value
Mardan	135	36	26.66	0.199- 0.347	0.5
Swabi	135	27	20	0.141- 0.275	
Nowshera	135	29	21.48	0.153- 0.291	
Total	405	92	22.71	0.189- 0.270	

Table 2. Risk Factors of *Toxocara canis* in Dogs

S. No	Risk Factor	Category	Negative	Positive	Percentage (%)	95% CI	P-Value (Chi-Square)
1	Age (Years)	1–2	126	23	18.25	0.124–0.259	0.5
		3–4	181	44	24.30	0.186–0.310	
		>4	98	25	25.51	0.179–0.349	
2	Gender	Male	215	53	24.65	0.193–0.308	0.4
		Female	190	39	20.52	0.154–0.268	
3	Area	Rural	275	73	26.54	0.216–0.320	0.03
		Urban	130	19	14.61	0.095–0.217	
4	Deworming	Yes	151	11	7.28	0.041–0.125	0.00001
		No	254	81	31.88	0.264–0.378	

Table 3. District-wise seroprevalence of *Dirofilaria immitis* in dogs

Districts	No of Samples	Dogs Positive Samples	Prevalence (%)	95% CI	P-value
Mardan	135	5	3.70	0.015-0.083	0.2
Swabi	135	3	2.22	0.007-0.063	
Nowshera	135	1	0.74	0.001-0.040	
Total	405	9	2.22	0.011-0.041	

Table 4. Risk Factors of *Dirofilaria immitis* in Dogs

S. No	Risk Factor	Category	Total	Positive	Percentage (%)	95% CI	P-Value (Chi-Square)
1	Age (Years)	1–2	126	3	2.38	0.008–0.067	0.6
		3–4	181	6	3.31	0.015–0.070	
		>4	98	0	—	0–0.037	
2	Gender	Male	215	5	2.32	0.01–0.053	0.8
		Female	190	4	2.10	0.008–0.052	
3	Area	Rural	275	7	2.54	0.012–0.051	0.5
		Urban	130	2	1.53	0.004–0.054	
4	Deworming	Yes	151	3	1.98	0.006–0.056	0.8
		No	254	6	2.36	0.001–0.008	

Discussion

In Pakistan, canine toxocariasis is commonly diagnosed by identifying Toxocara canis eggs in dog feces through conventional microscopic examination (21). In the present study, indirect ELISA allowed for the detection of IgG antibodies against T. canis, providing a more sensitive exposure evaluation. The seroprevalence of T. canis among dogs was 22.71%, aligning with findings from intermediate-income regions where a prevalence of 22% was reported (22). However, lower seroprevalence compared to regions such as Iran (71%) (23), Argentina (86.95%) (24), and Brazil (82.7%) (25) suggests significant regional variation likely influenced by climatic, socioeconomic, and management factors.

It is crucial to interpret ELISA results cautiously, as IgG antibodies only reflect exposure history and do not necessarily confirm active infection (26). Persistent IgG antibodies can remain detectable even after the infection has resolved, limiting the ability to differentiate between past and current infections (26).

Age-wise analysis revealed that seroprevalence increased with age, with dogs older than four years showing the highest prevalence (25.51%). This trend suggests cumulative exposure over time, consistent with previous studies where older dogs demonstrated higher seropositivity rates (23,27). Adult dogs are more likely to develop chronic infections, and larval stages of T. canis may persist within host tissues, contributing to ongoing seropositivity (28). Gender did not significantly influence seroprevalence, aligning with previous studies where no major differences between male and female dogs were observed (23,26). However, some studies reported slightly higher male infection rates, attributed to greater roaming behavior and increased environmental exposure (23,29).

Dogs from rural areas demonstrated significantly higher seroprevalence compared to urban dogs ($p=0.03$), a finding consistent with prior research (30). Poor sanitation, lack of organized veterinary care, and environmental contamination in rural regions contribute to greater parasite transmission. Deworming status was also identified as a significant risk factor; dogs not dewormed showed higher infection rates, emphasizing the critical role of preventive veterinary care in reducing parasite burden (30).

Comparatively, serological techniques such as ELISA offer higher sensitivity than traditional fecal examinations; however, limitations in distinguishing between active and past infections persist (26,31).

Regarding Dirofilaria immitis, a vector-borne nematode transmitted by mosquitoes, the overall seroprevalence observed in this study was 2.22%, lower than previously reported figures for Pakistan (4.5%) and other parts of Asia (12.07%) (32,33). Regional variations may influence these differences in mosquito population density, climate, vector competence, and preventive practices. Factors like vector seasonality, environmental temperature, and rainfall patterns directly impact heartworm transmission dynamics (32).

Interestingly, none of the sampled cats tested positive for D. immitis, which may reflect lower susceptibility or differences in environmental exposure compared to dogs. Variations in seroprevalence also depend on the diagnostic methods employed, with necropsy and antigen detection providing higher detection rates than ELISA (34).

Age and gender did not significantly affect heartworm seroprevalence, which is consistent with previous findings (32,35). These results suggest that heartworm exposure depends more on environmental and vector-related factors than intrinsic host characteristics.

Overall, the findings underscore the need for routine serological surveillance, regular deworming programs, and mosquito control measures to reduce the burden of T. canis and D. immitis infections in canine populations in Pakistan.

Conclusion

Toxocara and *Dirofilaria* control is challenging because they affect more than humans and call for a one-health strategy. Vertical transmission is frequent in dogs and cats; domestic animal carrier rates directly impact the acquisition risk, and there is no vaccination. Therefore, cooperation across different professions is essential for effective control. The primary healthcare provider's role is crucial in educating patients on preventing this parasitic illness because *Toxocara* and *Dirofilaria* are neglected tropical infections that need research and publicity.

Declarations

Data Availability statement

All data generated or analysed during the study are included in the manuscript.

Ethics approval and consent to participate

Approved by the department concerned.

Consent for publication

Approved

Funding

Not applicable

Conflict of interest

The authors declared the absence of a conflict of interest.

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All authors reviewed the results and approved the final version of the manuscript. They are also accountable for the integrity of the study.

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