

Zoonotic Parasites in Stray Dogs and Their Public Health Implications

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ABSTRACT

Background: Stray dogs are important reservoirs of zoonotic intestinal parasites, particularly in urban settings where close human–animal contact, poor waste disposal, and environmental contamination may facilitate transmission. Limited field-based data are available from Pakistan regarding the parasitic burden of stray dogs in public and healthcare-adjacent environments. **Objective:** This study aimed to determine the prevalence and distribution of intestinal parasites among stray dogs in and around a tertiary care hospital setting in Punjab, Pakistan, with emphasis on parasites of zoonotic importance. **Methods:** A cross-sectional field and laboratory-based study was conducted over six months. Fresh fecal samples were collected from 210 stray dogs from hospital surroundings, residential streets, public parks, and waste-disposal areas. Samples were examined using macroscopic assessment, direct wet mount microscopy, zinc sulfate flotation, formalin–ether sedimentation, and modified McMaster egg-counting techniques. Parasites were identified morphologically, and helminth parasite load was categorized as low, moderate, or high. Data were analyzed using descriptive statistics, and associations with age group and sampling location were assessed using chi-square testing where applicable. **Results:** Overall, 134 of 210 fecal samples were positive for one or more intestinal parasites, giving a prevalence of 63.8%. Mono-parasitic infections were detected in 89 positive samples (66.4%), while mixed infections were observed in 45 positive samples (33.6%). The most frequently identified parasites were *Toxocara* spp. 46 (21.9%) and *Ancylostoma* spp. 39 (18.6%), followed by *Giardia duodenalis* 24 (11.4%), *Dipylidium caninum* 21 (10.0%), *Trichuris vulpis* 18 (8.6%), *Cryptosporidium* spp. 17 (8.1%), *Taenia* spp. 13 (6.2%), and *Strongyloides* spp. 11 (5.2%). *Toxocara* spp. was more frequent among juveniles, while the highest location-specific prevalence was observed at waste-disposal sites (72.4%). **Conclusion:** Stray dogs in the study area harbored a high burden of intestinal parasites, including several species of zoonotic relevance. These findings highlight the need for integrated One Health interventions, including stray-dog management, deworming strategies, improved waste disposal, environmental sanitation, and community awareness to reduce potential zoonotic exposure. **Keywords:** Stray dogs; zoonotic parasites; *Toxocara* spp.; *Ancylostoma* spp.; intestinal parasites; public health; One Health; Pakistan.

INTRODUCTION

Zoonotic intestinal parasites transmitted by free-roaming dogs remain an important public health concern in urban and peri-urban settings, particularly in regions where animal population control, waste management, and environmental sanitation are limited. Stray dogs can serve as reservoirs for several helminthic and protozoal parasites of human significance, including *Toxocara* spp., *Ancylostoma* spp.,

Giardia duodenalis, and *Cryptosporidium* spp. These parasites may be shed through feces and contaminate soil, water, and public spaces, creating potential routes of indirect human exposure through contact with infective eggs, larvae, cysts, or oocysts (1–5). The risk is especially relevant for children, immunocompromised individuals, municipal workers, veterinary personnel, and communities living in close proximity to contaminated environments (6–8).

Among canine intestinal parasites, *Toxocara* spp. and *Ancylostoma* spp. are of particular zoonotic importance. Human exposure to *Toxocara* eggs may result in visceral or ocular larva migrans, while canine hookworms are associated with cutaneous larva migrans and other clinical manifestations in exposed populations (8–10). Protozoal parasites such as *Giardia duodenalis* and *Cryptosporidium* spp. also have public health relevance because of their environmental persistence and potential contribution to waterborne or fecal–oral transmission, particularly in settings where sanitation infrastructure is inadequate (15–17). The survival of infective parasitic stages in the environment is influenced by climate, humidity, waste accumulation, and the density of free-roaming dogs, all of which may facilitate sustained transmission in densely populated communities (6,18).

In Pakistan, especially in Punjab, rapid urbanization, inadequate waste disposal, and the presence of large stray-dog populations may increase the risk of environmental contamination with zoonotic parasites. However, local surveillance data remain limited and fragmented. Available studies from Pakistan have reported gastrointestinal parasites in dogs, but many are restricted by clinic-based sampling, limited geographic coverage, or incomplete assessment of public spaces where human exposure may occur (12,13). Field-based studies examining stray-dog fecal contamination around hospitals, residential areas, parks, and waste-disposal sites are particularly important because these environments are shared by vulnerable populations and may represent practical targets for public health intervention.

Estimating the burden and distribution of intestinal parasites in stray dogs is necessary for designing evidence-based control measures. Conventional parasitological techniques, including direct wet mount examination, flotation, sedimentation, and McMaster egg-counting methods, remain useful in resource-limited field settings for detecting common helminthic and protozoal infections and estimating parasite burden. When combined with basic demographic and environmental information, these methods can help identify high-risk parasite patterns and locations requiring targeted One Health interventions involving veterinary, municipal, environmental, and public health authorities (19–21).

Therefore, this study aimed to determine the prevalence and distribution of intestinal parasites among stray dogs in and around a tertiary care hospital setting in Punjab, Pakistan. The study further assessed helminth parasite load and explored variation in infection patterns according to apparent age group and sampling location, with emphasis on parasites of recognized zoonotic importance.

MATERIALS AND METHODS

This cross-sectional field and laboratory-based study was conducted over a six-month period in and around a tertiary care teaching hospital in Punjab, Pakistan. The study was designed to estimate the prevalence of intestinal parasites among stray dogs and to assess the distribution of parasites of potential zoonotic importance across different environmental locations. Sampling sites included hospital surroundings, nearby residential streets, public parks, and waste-disposal areas.

The study population consisted of free-roaming stray dogs without identifiable ownership. Freshly voided fecal samples were collected from areas where stray dogs were observed. Dogs of either sex and different apparent age groups were considered eligible when fecal material could be collected safely without direct animal handling. Animals that showed aggressive behavior or could not be approached safely were excluded from field observation. A non-probability convenience sampling technique was used because of the unpredictable movement patterns of stray dogs and the logistical limitations associated with field sampling. The sample size was estimated using an expected prevalence of 50% to

maximize sample size, with a 95% confidence level and a 5% margin of error. Based on these assumptions, a minimum sample size of approximately 200 fecal samples was required, and 210 samples were ultimately included in the final analysis.

Fresh fecal samples were collected during early morning field visits to reduce degradation of parasitic stages and minimize the possibility of prolonged environmental exposure. A sample was considered fresh on the basis of physical characteristics, including moisture, consistency, and absence of insect activity. Approximately 10–15 g of fecal material was collected using disposable gloves and sterile wooden spatulas and transferred into sterile, screw-capped plastic containers. Each container was labeled with a unique sample identification code, date of collection, and sampling location. No personal or identifiable human data were collected. Samples were transported in insulated cool boxes to the parasitology laboratory within 4–6 hours of collection. When immediate processing was not possible, samples were stored at 4°C for no longer than 24 hours to preserve parasitic morphology.

Each fecal sample was first examined macroscopically for color, consistency, presence of mucus or blood, and visible parasitic forms. Stool consistency was categorized as formed, semi-formed, or diarrheic. Direct microscopic examination was then performed by preparing saline and iodine wet mounts from a small portion of fecal material. Slides were examined under light microscopy at 10× and 40× magnification for helminth eggs, larvae, protozoan cysts, and trophozoites.

A zinc sulfate flotation technique was used to improve detection of helminth eggs and protozoan cysts. Approximately 2 g of feces was mixed with flotation solution, strained to remove debris, and centrifuged at 1500 rpm for 5 minutes. A coverslip was placed on the surface meniscus and then examined microscopically after 10 minutes. Formalin–ether sedimentation was also performed to enhance the detection of heavier parasitic ova and larvae that may not be recovered efficiently through flotation. The sediment was examined under light microscopy using standard parasitological procedures.

For samples positive for helminths, parasite burden was estimated using a modified McMaster egg-counting technique and expressed as eggs per gram of feces. Parasite load was categorized as low, moderate, or high according to standard veterinary parasitology thresholds used for the identified helminth groups. Parasites were identified morphologically on the basis of egg or cyst size, shape, shell characteristics, internal structure, and staining appearance using standard parasitological identification criteria. Particular attention was given to parasites of known zoonotic significance, including *Toxocara* spp., *Ancylostoma* spp., *Giardia duodenalis*, and *Cryptosporidium* spp.

Field and laboratory data were recorded on structured data collection sheets and entered into a computerized database for analysis. The main outcome variable was the presence of one or more intestinal parasites in each fecal sample. Additional variables included parasite species, type of infection, parasite load category for helminth-positive samples, apparent age group of the dog, sex when confidently determinable, and sampling location. Infection was classified as mono-parasitic when one parasite species was identified and mixed infection when two or more parasite species were detected in the same sample.

Data were analyzed using SPSS version 25.0. Descriptive statistics were calculated as frequencies and percentages for categorical variables. Overall parasite prevalence was calculated by dividing the number of parasite-positive samples by the total number of examined samples. Species-specific prevalence was calculated using the total sample size as the denominator, while mixed infection patterns were described among parasite-positive samples. Associations between parasite prevalence and categorical variables, including apparent age group and sampling location, were assessed using the chi-square test where cell counts were adequate. A p-value of less than 0.05 was considered statistically significant.

Quality-control measures were applied throughout laboratory processing. Reagents were prepared according to standard protocols, equipment was checked before use, and laboratory personnel followed

biosafety procedures, including use of gloves, protective clothing, and safe disposal of biological waste. To reduce observer bias, 10% of samples were randomly selected for repeat examination by a second experienced parasitologist. Any discrepancies in parasite identification were resolved through joint microscopic review.

The study involved collection of stray-dog fecal samples from public environments and did not involve experimental procedures, invasive animal handling, or collection of identifiable human information. Field collection and laboratory handling were conducted with appropriate biosafety precautions. Administrative permission and ethical clearance should be reported here if obtained from the relevant institutional or municipal authority.

RESULTS

A total of 210 fresh fecal samples collected from stray dogs were included in the final analysis. Samples were obtained from four environmental locations: residential streets accounted for 72 samples (34.3%), hospital surroundings for 60 samples (28.6%), public parks for 40 samples (19.0%), and waste-disposal areas for 38 samples (18.1%). Based on field observation, 130 dogs (61.9%) were classified as adults and 80 (38.1%) as juveniles. Sex could be confidently determined in 167 dogs, including 93 males (55.7%) and 74 females (44.3%); sex was not confidently determined in 43 dogs.

Overall, 134 of 210 fecal samples were positive for one or more intestinal parasites, giving an overall prevalence of 63.8%. Seventy-six samples (36.2%) were negative on parasitological examination. Among all samples examined, 89 (42.4%) showed single-parasite infection and 45 (21.4%) showed mixed infection. When restricted to parasite-positive samples, mono-parasitic infection accounted for 89 of 134 positive samples (66.4%), while mixed infection was present in 45 of 134 positive samples (33.6%). Dual infections were observed in 36 positive samples (26.9%), whereas three or more parasites were identified in 9 positive samples (6.7%).

Table 1. Baseline Characteristics of Examined Stray-Dog Fecal Samples

Variable	Category	n	%
Total samples examined	—	210	100.0
Sampling location	Hospital surroundings	60	28.6
	Residential streets	72	34.3
	Public parks	40	19.0
	Waste-disposal areas	38	18.1
Apparent age group	Juvenile	80	38.1
	Adult	130	61.9
Sex determination	Sex confidently determined	167	79.5
	Sex not confidently determined	43	20.5
Sex among determinable dogs	Male	93	55.7*
	Female	74	44.3*

*Percentage calculated using dogs with confidently determined sex as denominator, n = 167.

Table 2. Overall Prevalence and Infection Pattern of Intestinal Parasites

Infection status / pattern	n	%
Parasite-positive samples	134	63.8
Parasite-negative samples	76	36.2
Total examined samples	210	100.0
Single-parasite infection among all samples	89	42.4
Mixed infection among all samples	45	21.4
Single-parasite infection among positive samples	89/134	66.4
Two-parasite infection among positive samples	36/134	26.9
Three or more parasites among positive samples	9/134	6.7
Total positive samples	134/134	100.0

Microscopic examination identified eight intestinal parasite taxa, including six helminths and two protozoan parasites. *Toxocara* spp. was the most frequently detected parasite, identified in 46 samples

(21.9%), followed by *Ancylostoma* spp. in 39 samples (18.6%). Other helminths included *Dipylidium caninum* in 21 samples (10.0%), *Trichuris vulpis* in 18 samples (8.6%), *Taenia* spp. in 13 samples (6.2%), and *Strongyloides* spp. in 11 samples (5.2%). Among protozoa, *Giardia duodenalis* was detected in 24 samples (11.4%), while *Cryptosporidium* spp. was detected in 17 samples (8.1%). Because mixed infections were present, species-specific detections exceeded the number of parasite-positive samples.

Table 3. Species-Specific Distribution of Intestinal Parasites Detected in Stray Dogs

Parasite species	Parasite type	Positive samples, n	Prevalence among total samples, %
<i>Toxocara</i> spp.	Helminth	46	21.9
<i>Ancylostoma</i> spp.	Helminth	39	18.6
<i>Dipylidium caninum</i>	Helminth	21	10.0
<i>Trichuris vulpis</i>	Helminth	18	8.6
<i>Taenia</i> spp.	Helminth	13	6.2
<i>Strongyloides</i> spp.	Helminth	11	5.2
<i>Giardia duodenalis</i>	Protozoa	24	11.4
<i>Cryptosporidium</i> spp.	Protozoa	17	8.1

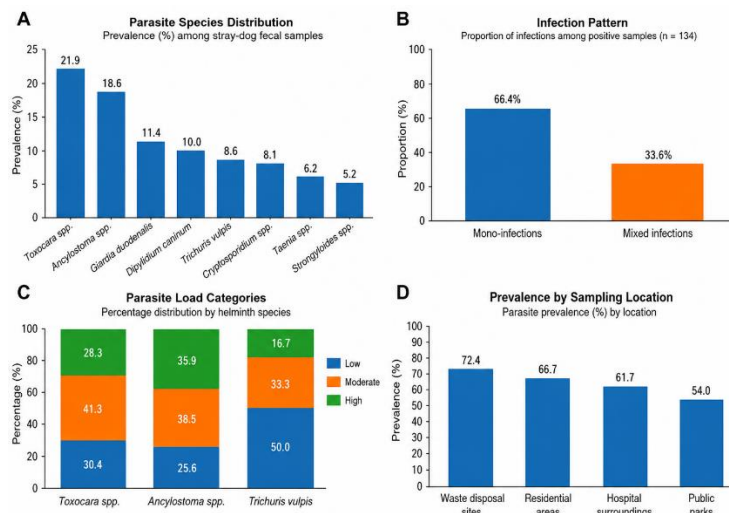


Figure 1 Composite summary of intestinal parasitism in stray dogs. (A) Species-specific prevalence of intestinal parasites detected in 210 fecal samples collected from stray dogs, showing *Toxocara* spp. (21.9%) and *Ancylostoma* spp. (18.6%) as the most frequently identified parasites. (B) Distribution of infection patterns among parasite-positive samples (n = 134), demonstrating that mono-infections accounted for 66.4% and mixed infections for 33.6%. (C) Distribution of low, moderate, and high parasite-load categories among the major helminth species, based on McMaster egg-counting results. (D) Prevalence of parasite-positive samples by sampling location, with the highest prevalence observed at waste-disposal sites (72.4%), followed by residential areas (66.7%), hospital surroundings (61.7%), and public parks (54.0%).

A total of 134 of 210 fecal samples (63.8%) were positive for one or more intestinal parasites. As shown in Figure 1, *Toxocara* spp. and *Ancylostoma* spp. were the most prevalent parasites, while mono-infections were more common than mixed infections among positive samples. Parasite-load assessment showed moderate-to-high burdens particularly among *Toxocara* spp. and *Ancylostoma* spp., and location-wise analysis demonstrated the greatest prevalence of infection in samples collected from waste-disposal sites.

Among helminth-positive samples, parasite load was categorized as low, moderate, or high using the McMaster egg-counting technique. For *Toxocara* spp., low parasite load was recorded in 30.4%, moderate load in 41.3%, and high load in 28.3% of positive detections. For *Ancylostoma* spp., low, moderate, and high parasite-load categories were recorded in 25.6%, 38.5%, and 35.9%, respectively. For *Trichuris* spp., low parasite load was recorded in 50.0%, moderate load in 33.3%, and high load in 16.7%.

Table 4. Helminth Parasite-Load Distribution by Species

Parasite species	Low load, %	Moderate load, %	High load, %
Toxocara spp.	30.4	41.3	28.3
Ancylostoma spp.	25.6	38.5	35.9
Trichuris spp.	50.0	33.3	16.7

Age-stratified analysis showed that *Toxocara* spp. was detected more frequently among juvenile dogs than adult dogs, with prevalence values of 29.6% and 17.2%, respectively. This difference was reported as statistically significant at $p < 0.05$. *Ancylostoma* spp. was detected in 14.1% of juveniles and 21.3% of adults, while *Giardia* spp. was detected in 12.7% of juveniles and 10.5% of adults. These differences were reported as not statistically significant. Exact p-values and raw age-specific counts were not available in the manuscript and should be added if accessible from the dataset.

Table 5. Parasite Prevalence by Apparent Age Group

Parasite species	Juveniles, %	Adults, %	Reported p-value
Toxocara spp.	29.6	17.2	<0.05
Ancylostoma spp.	14.1	21.3	>0.05
Giardia spp.	12.7	10.5	>0.05

Environmental distribution of positive samples varied across sampling locations. The highest parasite prevalence was recorded in samples collected from waste-disposal areas, where 72.4% of samples were positive. This was followed by residential streets at 66.7%, hospital surroundings at 61.7%, and public parks at 54.0%. Raw counts by sampling location should be reported alongside these percentages in the final manuscript to improve transparency and allow independent verification.

Table 6. Parasite Prevalence by Sampling Location

Sampling location	Parasite prevalence, %
Waste-disposal areas	72.4
Residential streets	66.7
Hospital surroundings	61.7
Public parks	54.0

The results showed a high prevalence of intestinal parasitism among stray dogs, with helminths more frequently represented than protozoa. *Toxocara* spp. and *Ancylostoma* spp. were the leading zoonotic helminths, while *Giardia duodenalis* and *Cryptosporidium* spp. were the identified protozoan parasites. Mixed infections were present in approximately one-third of positive samples, and moderate-to-high helminth parasite loads were recorded particularly for *Toxocara* spp. and *Ancylostoma* spp.

DISCUSSION

This cross-sectional field and laboratory-based study demonstrated a high burden of intestinal parasitism among stray dogs in and around a tertiary care hospital setting in Punjab, Pakistan. Overall, 134 of 210 fecal samples were positive for one or more intestinal parasites, yielding a prevalence of 63.8%. The detection of multiple helminthic and protozoal parasites, including *Toxocara* spp., *Ancylostoma* spp., *Giardia duodenalis*, and *Cryptosporidium* spp., indicates that stray dogs in the study environment may contribute to fecal contamination of shared public spaces. Although this study did not assess human infection directly, the presence of parasites with recognized zoonotic relevance supports the need for integrated surveillance and control measures in areas where stray dogs, patients, hospital visitors, residents, and municipal workers may share the same environment (1–5).

The predominance of *Toxocara* spp. and *Ancylostoma* spp. was one of the principal findings of the study. *Toxocara* spp. was detected in 46 samples (21.9%), making it the most frequently identified parasite, while *Ancylostoma* spp. was detected in 39 samples (18.6%). These findings are epidemiologically important because both parasite groups are well-recognized canine helminths with zoonotic potential. Human exposure to embryonated *Toxocara* eggs may lead to visceral or ocular larva migrans, whereas canine hookworms are associated with cutaneous larva migrans and, in some circumstances, other clinical manifestations (6–9). The relatively high detection of these parasites in the present study is consistent with previous reports indicating that stray and free-roaming dogs can serve as important

reservoirs for environmental contamination with zoonotic helminths, particularly in settings where routine deworming, population control, and sanitation measures are limited (2,4,10,11).

The age-related distribution of parasites showed that *Toxocara* spp. was more frequent among juvenile dogs than adults, with reported prevalence values of 29.6% and 17.2%, respectively. This pattern is biologically plausible because *Toxocara canis* can be transmitted through transplacental and transmammary routes, resulting in early infection among puppies and juvenile dogs (7,12). In contrast, *Ancylostoma* spp. was more frequently detected among adult dogs, although the difference was not statistically significant in the reported analysis. This may reflect cumulative environmental exposure, repeated contact with contaminated soil, and sustained roaming behavior among adult stray dogs. However, because raw age-stratified counts and exact p-values were not provided for all subgroup comparisons, these patterns should be interpreted descriptively and verified through complete statistical reporting in the final manuscript.

Protozoan parasites were also identified, with *Giardia duodenalis* detected in 24 samples (11.4%) and *Cryptosporidium* spp. in 17 samples (8.1%). Although their prevalence was lower than that of the leading helminths, their detection remains relevant from a public health perspective because both organisms can persist in the environment and may be transmitted through fecal–oral and waterborne routes (13–15). In resource-limited urban settings, poor waste disposal, contaminated water sources, and inadequate hygiene infrastructure may increase the potential for environmental exposure. The present study relied on microscopy-based identification, which is practical in field laboratory settings but has limited ability to differentiate zoonotic assemblages or species. Therefore, future studies incorporating molecular characterization would be useful to determine whether the detected protozoa represent strains of direct human public health significance.

Mixed infections were observed in 45 of 134 positive samples, representing 33.6% of parasite-positive samples. This finding suggests that a considerable proportion of infected dogs were exposed to environments contaminated with multiple parasite species. Mixed parasitism is common among stray dogs because they often lack veterinary care, feed on waste, roam across contaminated areas, and repeatedly encounter infectious stages in soil and fecal material (10,11). In the present study, the occurrence of mixed infections is important because it may increase the overall shedding burden into the environment. However, because the study design was cross-sectional, it cannot determine whether mixed infections resulted from specific environmental sources, host factors, nutritional status, or repeated exposure over time.

Parasite-load assessment using the McMaster technique added useful quantitative information to the study. Moderate-to-high parasite-load categories were common among *Toxocara* spp. and *Ancylostoma* spp., with high-load proportions of 28.3% and 35.9%, respectively. Higher egg output can increase environmental contamination and may raise the likelihood of contact with infective stages in public spaces, particularly where feces remain unmanaged (10). Nevertheless, parasite-load categories should be interpreted cautiously unless the manuscript clearly defines the egg-per-gram thresholds used to classify low, moderate, and high burdens. Including these thresholds in the Methods section would improve reproducibility and allow comparison with other veterinary parasitology studies.

The environmental distribution of positive samples showed the highest parasite prevalence at waste-disposal sites, followed by residential areas, hospital surroundings, and public parks. Waste-disposal sites may attract stray dogs because of food availability and shelter, while organic waste and moist soil may support persistence of helminth eggs and larvae. The detection of a high infection proportion near hospital surroundings is also relevant because healthcare facilities are accessed by patients, attendants, staff, and immunocompromised individuals. However, these findings should not be interpreted as evidence of confirmed transmission to humans because neither environmental soil samples nor human samples were examined. The results instead identify locations where targeted sanitation, waste management, and animal control measures may be most appropriate.

The findings support the value of a One Health approach to the control of zoonotic parasitic infections. Stray-dog parasitism is not solely a veterinary issue; it is linked to municipal waste management, environmental hygiene, hospital sanitation, community behavior, and public health surveillance. Integrated strategies may include humane stray-dog population management, regular deworming where feasible, improved waste containment, removal of fecal contamination from public spaces, community awareness programs, and coordination between veterinary, municipal, environmental, and healthcare authorities (16,17). Such interventions are particularly important in settings where large stray-dog populations live near hospitals, schools, markets, and residential communities.

This study has several limitations. First, convenience sampling was used because of the unpredictable movement of stray dogs, which limits generalizability to all stray dogs in Punjab. Second, the study relied on field observation for apparent age and sex classification, which may introduce misclassification. Third, microscopy-based methods, although practical and widely used, may underestimate low-intensity infections and cannot reliably determine molecular species or zoonotic assemblages for some protozoa. Fourth, the study did not include environmental soil or water samples, nor did it evaluate human infection, so public health implications should be framed as potential risk rather than confirmed transmission. Finally, subgroup analyses require fuller reporting of raw counts, exact p-values, and denominators to strengthen statistical transparency.

Despite these limitations, the study provides useful baseline evidence on intestinal parasites among stray dogs in a high-contact urban environment. The high overall prevalence, predominance of zoonotic helminths, presence of protozoal parasites, occurrence of mixed infections, and increased prevalence near waste-disposal areas collectively indicate a need for routine surveillance and targeted control measures. Future research should use probability-based or spatially structured sampling, include seasonal assessment, apply molecular diagnostic tools, and incorporate environmental sampling to better define transmission pathways and guide evidence-based interventions.

CONCLUSION

This study found a high prevalence of intestinal parasites among stray dogs sampled in and around a tertiary care hospital setting in Punjab, Pakistan, with 134 of 210 fecal samples testing positive. *Toxocara* spp. and *Ancylostoma* spp. were the most frequently detected helminths, while *Giardia duodenalis* and *Cryptosporidium* spp. were also identified. Mixed infections and moderate-to-high helminth parasite loads were common, and the highest prevalence was observed near waste-disposal sites. These findings indicate that stray dogs may contribute to environmental contamination with parasites of zoonotic relevance. Integrated One Health interventions involving stray-dog management, deworming, improved waste disposal, environmental sanitation, and public awareness are needed to reduce potential zoonotic exposure in shared urban environments.

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Graphical Abstract: Intestinal Parasitism in Stray Dogs

