

Coprovalence of Canine Gastrointestinal Parasitic Infections with Emphasis on *Echinococcus granulosus* in Stray Dogs

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ABSTRACT

Present study was undertaken to explore the prevalence of gastrointestinal parasites in the stray dogs of Ludhiana district of Punjab state, India. A total of 240 faecal samples were collected from stray dogs residing in or near urban areas, near animal post-mortem hall/ slaughter houses and from canine birth control center. Conventional parasitological examination of faecal samples revealed 106(44.16%) samples positive for parasitic eggs/ova. Single parasitic infections (30.83%) were found to be more common than multiple parasitic infections (13.75%). Among different type of enteric parasites, coprovalence of hookworms was found to be highest (12%) followed by coccidian/ *Isospora* oocysts (7.9%), mixed infection of hookworm + *Toxocara* (6.0%), *Toxocara* (5.8%), mixed infection of hookworm+ *Trichuris* + *Coccidia* (4.1%), *Trichuris* (2.9%), hookworm + *Coccidia* (2.9%), taeniid eggs (1.6%) and least prevalence of *Dipylidium caninum* (0.8%) was recorded in the present study. The prevalence of *Taeniid* eggs were higher in stray dogs near post-mortem hall (6.6%) followed by dogs in urban areas (1.7 %) and least in dogs from birth control center (0.8%). Coproantigen ELISA was performed on 92 faecal samples, which revealed prevalence of 19.5% of *E. granulosus* in stray dogs. Moreover, the prevalence of *Echinococcus granulosus* was significantly ($p=0.0138$) higher in dogs near post-mortem halls and slaughter houses compared to other locations. The high prevalence of zoonotic GI parasites among stray dogs indicates a potential threat to human health. *Echinococcus* eggs can remain viable in the environment for several months to over a year, hence, it is essential to implement effective measures to prevent the cystic echinococcosis in both animals and humans. There is an urgent need to implement regular anthelmintic treatment programs (possibly integrated with rabies vaccination campaigns) to control the spread of zoonotic helminthic parasitic infections from stray dogs to the people.

Key words: Dogs, *Echinococcus granulosus*, Gastrointestinal parasitic infections, Zoonosis.

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INTRODUCTION

Estimated stray/street dog population of India was 62 million (End pet homelessness 2020) and estimated pet dog population was over 31 million by end of 2023 (Minhas, 2022). Gastrointestinal parasites are very common in dogs in Indian sub-continent due to favourable warm and humid weather. The common gastrointestinal parasites that infect dogs in India include *Toxocara canis*, *Ancylostoma* spp. (hookworms), *Echinococcus* spp., *Dipylidium caninum*, *Giardia* spp., *Cryptosporidium* spp., *Toxoplasma gondii*, *Sarcocystis* spp. and *Cystoisospora* spp. These parasites are responsible for severe health issues like diarrhea, poor growth, weight loss and even death if present in higher intensity. One of the most common modes of transmission of these gastrointestinal diseases in dogs is faecal-oral route. Dogs become infected by ingesting parasitic eggs/ova from contaminated food, water, soils contaminated with dog excreta or secretions or consumption of raw offal's of small ruminants, skin penetration of the infective larvae or even by transplacental transmission and even by ingesting the infected intermediate host. The prevalence of various species of parasites present in dogs may depend upon its geographical distribution, areas

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where maximum population of dogs reside and constant changes in climatic conditions (Dubie *et al.*, 2023).

Many of these gastrointestinal parasites are of zoonotic importance. Important zoonotic GI parasitic diseases

of dogs which occur in India include echinococcosis, giardiasis, cryptosporidiosis, blastocystosis, cutaneous larva migrans and visceral larva migrans. The genus *Echinococcus* consists of four species which are presently recognized as *Echinococcus granulosus*, *E. multilocularis*, *E. oligarthus* and *Echinococcus vogeli* (Thompson and McManus, 2001). Out of all these parasitic infections, cystic *echinococcus* caused by *Echinococcus granulosus* tapeworm (hydatid disease) is a life-threatening zoonotic disease with worldwide distribution. Further, *E. granulosus* has been divided into 10 different strains (G1 to G10), which have a degree of host adaptation and may be maintained in distinct cycles (CFSPH, 2011). A study on molecular epidemiology of echinococcosis in food producing animals revealed that the buffalo strain (G3) and common sheep strain (G1) are cycling among livestock in north India (Punjab) and that these strains are highly adapted to cattle, buffalo, sheep, goats and pigs (Singh *et al.*, 2012). *Echinococcus* eggs are typically spherical and measure between 30 and 50 μm in diameter with thick, impermeable shell called the embryophore, which protects the hexacanth embryo (oncosphere) inside. The site of infection caused by adult worm is the small intestine of dogs, which become infected after eating offal of the ruminants containing hydatid cysts. Infected dogs release eggs into the environment that can be accidentally ingested by domestic animals or even by humans to further completion of life cycle by the parasite (Mihai-Octav *et al.*, 2024).

Thus, surveillance of parasites and parasitic diseases should be a constant task in order to develop control strategies to prevent their role as vectors for a variety of human and animal pathogens. For various control measurements, reliable methods are needed to monitor the infection in the canine population, although the rate of controlling these diseases is highly variable and has become one of the most important aspects for appropriate diagnosis and control of these infections. Various conventional parasitological techniques have been used mainly in routine diagnosis due to their low cost which are practical and direct in order to detect the parasites in the faecal samples. However, there are certain limitations of conventional parasitological techniques especially for detection of *Echinococcus* eggs from faecal samples. Diagnosis of *Echinococcus* eggs in faecal samples by copromicroscopy is quite challenging as *Taenia* spp. eggs are morphologically similar. The infection of *Echinococcus* and *Taenia* spp. are collectively diagnosed as *Taeniid* eggs. Various other techniques, *viz.* ELISA and PCR may be used for precise diagnosis of these infections. Coproantigens of *E. granulosus* using various immunological assays one of the effective methods which is highly stable on variable environmental conditions for up to 6 days and after storage at -20°C for 1 year without significantly affecting test results (Jenkins *et al.*, 2000). Keeping in view the importance of GIT parasitic diseases in dogs, study was envisaged to explore the prevalence of GIT parasites in stray dogs of Ludhiana

district with emphasis on immunological diagnosis of echinococcosis.

MATERIALS AND METHODS

Sample Collection and Examination

A total of 240 faecal samples were collected from stray dogs living near residences (n=94), near animal post-mortem hall/slaughter houses (n=30) and from animal birth control centers/dog shelters (n=116), Ludhiana, Punjab (India). The freshly laid faecal samples were collected from the dogs stored in clean and sealed screw cap storage containers.

All the faecal samples were processed for detection of parasitic eggs/ova/cysts by conventional parasitological examination (standard faecal flotation and sedimentation techniques; Soulsby, 1982). Proper hygienic measures were maintained during collection and processing of the faecal samples. Thereafter, faecal samples were frozen at -80°C for 7 days in order to partially inactivate infective stages of the parasites and then stored at -20°C until use (Eckert *et al.*, 2001). Samples found positive for coccidia oocysts were processed for sporulation using 2.5% Potassium dichromate to identify the species of coccidian parasite (Soulsby, 1982).

Further, 92 samples (including 4 taeniid eggs positive samples with single infection) were processed for immunological diagnosis of *Echinococcus granulosus*. Canine *Echinococcus* Antigen (Eg) ELISA Kit (Elbscience) was used for further process. Immunodiagnostic sandwich ELISA was performed as per manufacturer's instructions. Briefly, a capture antibody specific to the target antigen is first added to the wells of a microplate, where it binds to the surface. The sample containing the target antigen is then added if *echinococcus* antigen is present, it binds to the capture antibody, while unbound components are washed away. Next, a detection antibody, also specific to the target antigen is added, followed by another wash to remove any unbound detection antibodies. An enzyme-linked secondary antibody is then introduced, which binds to the detection antibody, forming a complete "sandwich" complex. A substrate is subsequently added, where the enzyme converts and results into a visible color change (yellow). Finally, a plate reader measures the color intensity, which correlates with the concentration of the detection antibody which is specific to the target antigen present in the test samples.

Finally, the interpretation of results was carried out as follows: Cut-off = OD Average negative $\times 2.1$ (when OD Average negative < 0.15 , calculate at 0.15, OD Average negative ≥ 0.15 , calculate at the actual value). Positive result: OD Average sample \geq Cut-off, and Negative result: OD Average sample $<$ Cut-off. The samples were processed as per the instructions and protocols given in the ELISA Kit (Elbscience).

Statistical Analysis

Chi-square (χ^2) test was applied on the data for the statistical analysis using online statistical tools like QuickCalcs (<https://www.graphpad.com/quickcalcs/>).



RESULTS AND DISCUSSION

Out of total 240 faecal samples collected from different areas of Ludhiana, Punjab, 106 (44.16%) samples were found to be positive for gastrointestinal parasitic infection. Both single parasitic infection (74, 30.83%) and mixed parasitic infection (32, 13.33%) were detected in the faeces of dogs. The overall prevalence of hookworm eggs was found to be highest being 12.08% followed by *Coccidia / Isospora* (7.91%), Hookworm and *Toxocara* (6.25%), *Toxocara* (5.83%), Hookworm+ *Trichuris* and *Coccidia* (4.16%), *Trichuris* (2.50%), Hookworm + *Coccidia* (2.91%), taeniid eggs (1.66%) and least prevalence of *Dipylidium caninum* (0.83%) was recorded.

Among different category of dogs, maximum prevalence of gastrointestinal (GIT) parasitic infection was found in stray dogs living near post-mortem hall/ slaughter houses (26/30: 86.6%) followed by the stray dogs living near residences/ Urban areas (41/94: 43.6%) and then by the stray dogs from animal birth control centers/dog shelters (39/116: 33.6%). Thus, the study determines that the infection of helminths varies differently according to different areas from where the faecal samples of stray dogs were collected.

The findings of the present study indicate a high prevalence of GIT parasitic infections in stray dogs from the Ludhiana district, with hookworm eggs being the most frequently identified. Given that stray dogs are seldom subjected to deworming, the transmission of GIT parasitic infections among this population is prevalent. Their unrestricted foraging behaviours, coupled with the lack of deworming protocols, further exacerbate the high incidence of these infections. Among various cohorts of stray dogs, those inhabiting areas near post-mortem facilities or slaughter-houses exhibited the highest rates of infection. This is likely due to their easy access to carcasses and offal, which increases the probability of ingesting the metacystode larvae of taeniid parasites. As definitive hosts, these dogs excrete parasitic eggs in their feces. Additional parasitic eggs/ova identified in the fecal samples included *Toxocara* spp., *Dipylidium caninum*, and coccidia (*Isospora* spp.). The presence of hookworm eggs, *Toxocara* eggs, and taeniid eggs (possibly *Echinococcus* spp.) suggests a significant zoonotic risk, highlighting the potential role of stray dogs in transmitting these infections to humans. Similar observations were reported by Traub *et al.* (2004), who documented a high prevalence of *Ancylostomum caninum* (55.6%) and *Toxocara canis* (51.9%), likely linked to environmental contamination, inadequate sanitation, overcrowding, and restricted access to veterinary care in socioeconomically disadvantaged areas. Their study further revealed that nearly all (99%) dogs harbored at least one zoonotic GIT parasite.

Additionally, a study by De *et al.* (2017) reported a notable prevalence of hookworm (*Ancylostoma caninum*) eggs with 13.20% prevalence in dogs from Ludhiana, India. The highest prevalence was observed during the autumn season, when temperatures ranged between 23°C and 30°C, conditions that favour the development of the parasite's pre-infective

stages. Variations in prevalence may also be influenced by geographical location and seasonal changes. In the present study, a higher prevalence of taeniid eggs recorded in samples collected from areas near the post-mortem hall/slaughter houses. These findings are comparable to those of Hadi and Faraj (2016), who reported a 29.1% prevalence of taeniid eggs in stray dogs near a post-mortem hall in Baghdad, using wet mount and sedimentation techniques. The elevated levels of infection in such areas may be attributed to easy access to infected offal from slaughtered animals or to improperly buried carcasses from post-mortem cases, which stray dogs may dig up and scavenge, thereby increasing their risk of infection.

A higher prevalence of Taeniid eggs (6.6%) was observed in dogs living near the post-mortem hall, compared to those residing near residential areas or in animal birth control centers (Table 1). However, distinguishing between the eggs of *Taenia* spp. and *Echinococcus* spp. is challenging. Therefore, to confirm and differentiate *Echinococcus granulosus* infection from *Taenia* species in dogs, an *E. granulosus* antigen ELISA was conducted as part of the study. Ninety-two samples (including four positive samples for taeniid eggs) were processed for immunological diagnosis of *Echinococcus granulosus* by using *Canine Echinococcus* Antigen (Eg) ELISA. Results indicated 18 samples (19.5%) positive for *E. granulosus* antigen. Among different categories of dogs, significantly higher prevalence (44.4%) of infection was found in dogs living near post-mortem halls/slaughter houses as compared to dogs living near urban areas/residences (19%) and dogs living in animal birth control centers/dog shelters (9.3%)(Table 2). Moreover, the prevalence of *Echinococcus granulosus* in stray dogs was found to be significantly higher (Chi-square = 6.0609; p = 0.0138) in dogs near postmortem halls and slaughter houses compared to other locations. Possible reason for significantly higher prevalence could be due to free access of carcass/offal's of intermediate hosts (ruminants). Least prevalence among dogs from dog shelters/birth control centers may be due to deworming of some of dogs in these centers. Thus, the high population of stray dogs near urban areas and near post-mortem hall are responsible for disseminating the *echinococcosis* infection to other ruminants and to human population in the region. All the samples positive for taeniid eggs (4) were found positive for *E. granulosus* antigen indicating potential presence of this zoonotic parasite in the region.

Copro-ELISA is a widely used technique for field surveys and field diagnosis of cystic echinococcosis (CE) in dogs and has been applied effectively in previous studies (Svobodova and Lenska, 2002). This antigen ELISA test helped us to identify the *Echinococcus* spp. infection in faecal samples which may not be detected by conventional parasitological examination. Recent study on prevalence of *Echinococcus* spp. infection done in Kashmir valley, where the dog's faecal sample were collected from different districts which can be related to the present study where use of Copro-ELISA technique was considered as a more reliable diagnostic

Table 1: Coprevalence of enteric parasitic infections in stray dogs from different locations of Ludhiana district, Punjab

Location	Samples Examined	Type of infection Positive samples	Qualitative analysis		
			Parasitic eggs /ova/cysts	Samples Positive	% prevalence
I (Stray dogs near residences /urban area)	94	Single infections (28)	Hookworm	14	14.8
			Taeniid	1	1.7
			<i>D. caninum</i>	--	--
			<i>Trichuris</i>	2	2.1
			<i>Toxocara</i>	5	5.3
		Multiple infections (13)	Coccidia (<i>Isospora</i>)	6	6.4
			Hook worm + <i>Toxocara</i>	6	6.4
			Hook worm + <i>Coccidia</i>	2	13.3
			Hookworm + <i>Trichuris</i> + Coccidia	5	2.1
			II (Stray dogs near Postmortem hall/ slaughter houses)	30	Single infections (18)
Taeniid	2	6.6			
<i>D. caninum</i>	1	3.3			
<i>Trichuris</i>	1	3.3			
<i>Toxocara</i>	3	10.0			
Multiple infections (8)	Coccidia (<i>Isospora</i>)	5			16.6
	Hookworm + <i>Toxocara</i>	4			13.3
	Hookworm + <i>Coccidia</i>	3			10.0
	Hookworm + <i>Trichuris</i> + Coccidia	1			3.3
	III (Stray dogs from Animal Birth control centers/dog shelters)	116			Single infections (28)
Taeniid			1	0.8	
<i>D. caninum</i>			1	0.8	
<i>Trichuris</i>			3	2.5	
<i>Toxocara</i>			6	5.2	
Multiple infections (11)			Coccidia (<i>Isospora</i>)	8	6.8
			Hookworm + <i>Toxocara</i>	5	4.3
			Hookworm + <i>Coccidia</i>	2	1.7
			Hookworm + <i>Trichuris</i> + Coccidia	4	3.4
			Total number of faecal samples	240	Single infections (30.83%)
Mixed infections (13.33%)	Mixed infection of hookworm + <i>Toxocara</i> (6.25%), hookworm+ <i>Trichuris</i> + Coccidia (4.16%), hook- worm + Coccidia (2.91%)				

Table 2: Occurrence of *Echinococcus granulosus* in stray dogs based on the Copro-antigen ELISA

Location	Samples Examined	Positive Samples	% prevalence
I (Stray dogs near residences /urban areas)	31	6	19.0
II (Stray dogs near post-mortem hall/slaughter houses)	18	8	44.4
III (Stray dogs from animal birth control centers/ dog shelters)	43	4	9.3
Total	92	18	19.5

Chi square = 6.0609: p=0.0138, p<0.05.



method identified the overall coproantigen prevalence for *Echinococcus* species as 10.1 % (Abas *et al.*, 2022).

Even after several control measures, CE causes significant morbidity throughout the world. The human CE is endemic in India with highest prevalence in Andhra Pradesh, Tamil Nadu, and Kashmir (Parikh, 2012). In India, the annual incidence of human CE varies from 1 to 200 per 100,000 persons and disease primarily affect different body organs and secondarily during the disease process with immense morbidity (Hemachander *et al.*, 2008). However, Das *et al.* (2023) observed significant decline in seropositivity rate of CE in human population during last 8 years (61.4% in 2013 to 33.8% in 2020) which may be attributed to improved socioeconomic status and better implementation of health programs.

Echinococcus eggs can remain viable in the environment for several months to over a year, especially in cool, moist conditions. However, high temperatures and desiccation are the main factors that cause their inactivation. Adverse weather conditions like rain and wind, as well as invertebrates and birds, play a role in dispersing *Echinococcus* eggs, which may increase human exposure (Barosi and Umhang, 2024). Hence, it is essential to implement effective measures to prevent the cystic echinococcosis in both animals and humans.

CONCLUSION

Findings of this study reveal a high prevalence of gastrointestinal (GIT) parasites among stray dogs across various locations in Ludhiana district. The detection of zoonotic parasites such as hookworms, *Toxocara canis*, and *Echinococcus granulosus* highlights the potential risk these animals pose in transmitting parasitic infections to the human population. The *E. granulosus* antigen Copro-ELISA proves to be a valuable diagnostic tool for detecting echinococcosis in dogs. The results from the Copro-ELISA assay reveal a markedly higher prevalence of *Echinococcus granulosus* in dogs residing near post-mortem halls and slaughter houses, in comparison to other locations. This finding raises significant concerns regarding the implementation of effective prevention strategies for cystic echinococcosis in human populations. Hence, to prevent spread of gastrointestinal zoonotic parasitic diseases, there is an urgent need to implement regular anthelmintic treatment programs for stray dogs (potentially alongside rabies vaccination campaigns) to mitigate the threat of zoonotic parasite transmission in the region.

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