

Full Length Article

Evaluation of Stray Dogs as Sentinels of Zoonotic Parasites in Khyber Pakhtunkhwa Pakistan

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Abstract

Stray dogs act as reservoir for numerous zoonotic diseases and pose a potential public health threat to Pakistani population. Our aim was to elucidate them as sentinels of zoonotic parasites in Khyber Pakhtunkhwa Province of Pakistan. A total of 1195 faecal samples collected from 11 districts, for a period of 4 years (2020 to 2023) were tested for the presence of parasite eggs/larvae through microscopy using standard floatation/sedimentation techniques. Data for differences was analyzed through chi square test and for association was tested by logistic and Poisson regression at significance level of $P \leq 0.05$ using SPSS software. Ten different helminths including eight zoonotic parasites were detected in 291(24.4%) samples. Most common parasites found were *Ancylostoma caninum* (7.7%) and *Toxocara canis* (7.6%) while *Trichuris vulpis* and *Physaloptera* were least prevalent (0.3%). With no consistent regional trends among districts, prevalence was highest in district Mardan (37.3%) followed by Dir Lower (36.2%) and lowest in Dir Upper (16.2%). Most of the districts had prevalence range between 18% and 25%. *Echinococcus/Taenia* spp. was found the most widespread parasite detected in all studied districts, while *Physaloptera* was found only in two districts. Most (18.2%) of the positive samples were infected with single type while only 6.2% were infected with two or more types of parasites. In general, significant differences were observed in prevalence of parasite in different seasons where highest prevalence was recorded in winter, regional distribution with southern districts having highest prevalence and prevalence decreased with conceding years. It is concluded that stray dogs act as reservoir for zoonotic parasites in the study area contaminating the environment and posing a potential risk for human transmission. Further studies on molecular basis are recommended to find out their genotypes and device control measures.

Key words: Epidemiology; Stray dogs; Zoonotic helminths; Coprological detection; Pakistan

Introduction

Despite providing numerous benefits to mankind, dogs harbour parasites many of which are zoonotic and pose a potential public health risk (Sowemimo and Ayanniyi 2017). More than 60 zoonotic parasites have been reported from dogs (Gharekhani 2014). About 75% of dogs in the world are stray dogs which roam and reproduce freely (Alvarado-Esquivel *et al.* 2015). They defecate in public areas and contaminate the environment with parasite eggs/larvae (Traversa *et al.* 2014). Helminths are among the most common parasites detected in gastrointestinal tracts of dogs worldwide. The commonly reported helminths in dogs are Hookworms (*Ancylostoma caninum*, *Uncinaria stenocephala*), Ascarids (*Toxocara canis*, *Toxascaris leonina*), whipworm (*Trichuris vulpis*), flea tapeworm (*Dipylidium caninum*), *Strongyloides stercoralis* and *Echinococcus* species. Among these, *Echinococcus*

granulosus, *Ancylostoma caninum* and *Toxocara canis* are of much zoonotic importance because of cosmopolitan distribution and infecting vital organs such as eyes, liver, lungs and skin (Idika *et al.* 2017; Raza *et al.* 2018).

In Pakistan, stray dogs roam freely in public places and get no veterinary care. According to the comprehensive Disaster Response Services Benji Project Animal Sanctuary Islamabad, there are about three million stray dogs in Pakistan and about fifteen thousand stray dogs only in Peshawar (Arab News 2024). They scavenge food stuff containing different stages of parasites and are thus prone to infection. These dogs contaminate the environment with faeces containing eggs or cysts which can be transmitted to humans through different sources. Moreover, direct contact with animals, agricultural practices and poor hygienic conditions make people in Pakistan prone to infections (Ahmed *et al.* 2017).

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For establishment of effective control and prevention strategies, knowledge about the biology of parasites circulating in the area, monitoring the presence and risk factors associated with humans, reservoir hosts and vectors is important (Ortega-Pacheco *et al.* 2015). Studies regarding the zoonotic parasites of dogs in Pakistan are scarce and are localized with smaller sample size (Saleh and Ahmad 1965; Maqbool *et al.* 1998; Chattha *et al.* 2009; Shabbir *et al.* 2010; Ahmad *et al.* 2011; Farooqi *et al.* 2014; Rehman *et al.* 2017; Ashraf *et al.* 2019; Khan *et al.* 2019). Having limited previous studies and those having limitations along the amplitude of the problem, we aimed to investigate the untreated and neglected stray dogs as a transmission source of zoonotic diseases. By assessing the prevalence, diversity and epidemiology of gastrointestinal helminths will help to recognize zoonotic diseases as an important human health related threat and will also serve as eye opener for the policy makers towards the gravity of this important health related issue.

Materials and Methods

This study was vastly conducted in Khyber Pakhtunkhwa (KP) province of Pakistan (Fig. 1). The province is located in northwest of Pakistan at geographic coordinates of 34.9526°N, 72.3311°E having area of 101741 square kilometers (Government of Khyber Pakhtunkhwa 2024). Geography is mountainous in the north and plain in the south. Temperature rises towards the south (Britannica 2024). Winter season include the months of October till March while summer lasts from April to September. Average annual temperature ranges between 4°C and 18°C during winter and 25°C to 45°C during summer season. Faeces of stray dogs were collected from different locations in 11 districts from 2020 to 2023. Overall, KP was divided into three regions based on location and prevalent environmental conditions. Northern areas included the districts of Dir Upper, Dir Lower, Swat, Buner and Malakand; Central included the districts of Mardan, Swabi, Charsadda and Peshawar while Kohat and Bannu were included in Southern region. Random sample collection method was followed. Each area was visited once in a season to minimize repeated collection of the same samples. The faeces were collected from soil in plastic containers with a screw cap, labelled with specific number and transferred to the laboratory for further study. Necessary information such as date of sample collection and name of locality were noted. In case sample processing was not possible on the same day, these were preserved in refrigerator as described by Thienpont *et al.* (1986).

Faecal examination

Faecal samples were examined using standard direct and indirect techniques. Each sample was first examined by

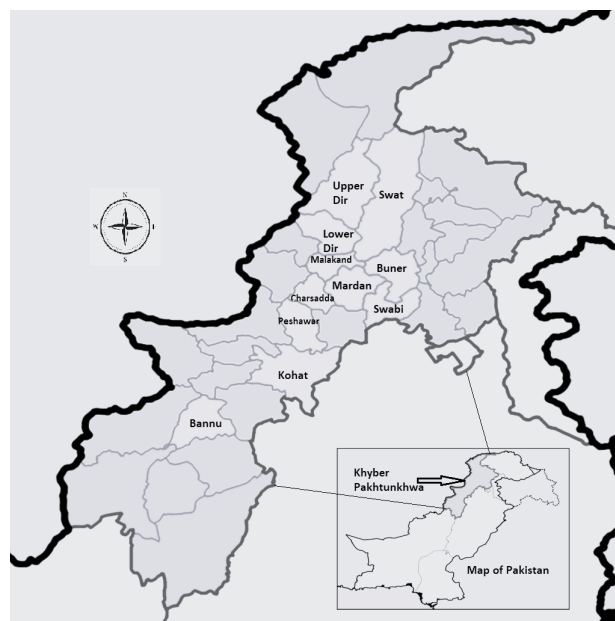


Fig. 1: Map of Khyber Pakhtunkhwa Showing Districts from Where Samples were Collected for Current Research Work during 2020 and 2023

direct microscopic method and then by sedimentation and flotation techniques using saturated sodium chloride and Sheather's sugar solutions (Soulsby 1982; Dryden *et al.* 2005; Zajac *et al.* 2021). The slide was studied systematically at 100X (10X x 10X) and 400X (40X x 10X) magnification using compound light microscope (Dryden *et al.* 2005). The parasite eggs/larvae were identified morphologically using the available keys (Soulsby 1982; Thienpont *et al.* 1986) and online sources.

Statistical analysis

Prevalence of each parasite was calculated as; no. of positive samples/total no. of samples examined. Data was analyzed using different statistical tests at significance level of $P \leq 0.05$ using SPSS software (IBM Corp 2019). Chi square test was used to find the differences in parasite distribution in different seasons and pairwise Chi square test was used for analysis of data in different regions. Parasite prevalence trend in preceding years was tested by poison regression analysis, however due to lesser representation for some species poison was not applicable, hence trend could not be reported for all the species. In order to find out whether a single dog was infected with one type of parasite or more, the positive tested samples were divided into three groups i.e., those infected with single type of parasite (monoparasitism), those infected with two types of parasites (Diparasitism) and those infected with three or more types of parasites (Polyparasitism). Data about type of parasitism was analyzed using binary logistic regression.

Table 1: Prevalence of Gastrointestinal Helminths in Faecal Samples of Stray Dogs in Khyber Pakhtunkhwa Pakistan During the Years 2020-2023

Parasite type	Parasite Species	Number Positive	Prevalence (%)
Nematodes	<i>Ancylostoma caninum</i>	92	7.7
	<i>Toxocara canis</i>	91	7.6
	<i>Strongyloides stercoralis</i>	50	4.2
	<i>Toxascaris leonina</i>	29	2.4
	<i>Uncinaria stenocephala</i>	27	2.3
	<i>Spirocerca lupi</i>	7	0.6
	<i>Physaloptera</i>	4	0.3
	<i>Trichuris vulpis</i>	3	0.3
Total		303	81.1
Cestode	<i>Echinococcus/Taenia</i> spp.	64	5.4
	<i>Dipylidium caninum</i>	7	0.6
Total		71	18.9
Overall		291*	24.4

*The total is not equal to the sum of individual cases because a single sample may contains more than one parasite species

Table 2: Distribution of Gastrointestinal Helminths in Faecal Samples of Stray Dogs in Different Regions and Districts of Khyber Pakhtunkhwa Pakistan during the years 2020-2023

Parasite Species	Northern Region						Central Region				Southern Region				Pair-wise Chi square analysis		
	Dir	Dir	Swat	Buner	Malakand	Total	Mardan	Swabi	Charsadda	Peshawar	Total	Kohat	Bannu	Total	P-value	P-value	P-value
	Upper	Lower	n (%)	n (%)	n (%)	n (%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	Northern	Northern	Central
	n (%)	n (%)													vs. Central	vs. Southern	vs. Southern
<i>Echinococcus/Taenia</i>	15 (5.0)	19 (10.3)	2 (1.4)	5 (8.8)	3 (9.1)	44 (6.1)	4 (4.8)	1 (1.9)	1 (1.2)	7 (5.3)	13 (3.7)	1 (2.1)	6 (7.5)	7 (5.5)	--	--	--
<i>Toxocara</i>	15 (5.3)	22 (11.9)	9 (6.5)	5 (8.8)	0 (0.0)	51 (7.1)	5 (6)	3 (5.7)	11 (13.1)	4 (3.0)	23 (6.5)	5 (10.6)	12 (15.0)	17 (13.4)	0.72	0.01	0.01
<i>Ancylostoma</i>	10 (3.3)	18 (9.7)	7 (5.0)	1 (1.8)	0 (0.0)	36 (5.0)	20 (24.1)	4 (7.5)	6 (7.1)	15 (11.4)	45 (12.8)	5 (10.6)	6 (7.5)	11 (8.7)	0.00	0.10	0.21
<i>Strongyloides</i>	12 (4)	11 (5.9)	5 (3.6)	4 (7.0)	0 (0)	32 (4.5)	3 (3.6)	1 (1.9)	1 (1.2)	3 (2.3)	8 (2.3)	7 (14.9)	3 (3.8)	10 (7.9)	0.07	0.10	0.00
<i>Toxascaris</i>	8 (2.6)	11 (5.9)	2 (1.4)	4 (7.0)	0 (0.0)	25 (3.5)	1 (1.2)	2 (3.8)	0 (0.0)	1 (0.8)	4 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0.02	0.03	0.22
<i>Uncinaria</i>	2 (0.7)	3 (1.6)	3 (2.2)	2 (3.5)	0 (0.0)	10 (1.4)	2 (2.4)	1 (1.9)	1 (1.2)	6 (4.5)	10 (2.8)	3 (6.4)	4 (5.0)	7 (5.5)	0.10	0.00	0.16
<i>Trichuris vulpis</i>	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	1 (1.9)	0 (0.0)	1 (0.8)	2 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	--	--	--
<i>Dipylidium caninum</i>	1 (0.3)	1 (0.5)	0 (0.0)	0 (0.0)	3 (9.1)	5 (0.7)	0 (0.0)	2 (3.8)	0 (0.0)	0 (0.0)	2 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	--	--	--
<i>Physaloptera</i>	2 (0.7)	2 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	4 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	--	--	--
<i>Spirocerca</i>	0 (0.0)	0 (0.0)	1 (0.7)	1 (1.8)	0 (0.0)	2 (0.3)	4 (4.8)	0 (0.0)	0 (0.0)	1 (0.8)	5 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	0.03	0.55	0.17
Overall Prevalence*	49 (16.2)	67 (36.2)	26 (18.7)	13 (22.8)	6 (18.2)	161 (22.5)	31 (37.3)	10 (18.9)	18 (21.4)	28 (21.2)	87 (24.7)	17 (36.2)	26 (32.5)	43 (33.9)			

Data is analyzed through IBM SPSS Statistics 22 and differences in distribution of parasites in different regions is calculated by pairwise chi square test with $P \leq 0.05$ significance value. Northern region: Districts of Dir Upper, Dir Lower, Swat, Buner and Malakand; No. of samples tested: 716; Central region: District Mardan, Swabi, Charsadda and Peshawar; No. of samples tested: 352; Southern Region: District Kohat and Bannu; No. of samples tested: 127; Total No. of samples tested from each district: Dir Upper:302; Dir Lower:185; Swat:139; Buner:57; Malakand:33; Mardan:83; Swabi:53; Charsadda:84; Peshawar:132; Kohat:47; Bannu:80 n: No. of samples positive for that particular parasite. Values in parenthesis show the percentages. The total of positive samples is not equal to the sum of individual cases because a single sample may contains more than one parasite species

Results

Out of 1195 tested samples, 291 (24.4%) were found positive for one or more parasite eggs/larvae. Ten different parasites were detected, most of which (81.1%) were Nematodes and 18.9% were cestodes while no trematodes were found. Most prevalent parasite found was *Ancylostoma caninum* while *Trichuris vulpis* and *Physaloptera* were least prevalent. Prevalence of each parasite was; *Ancylostoma caninum* (7.7%), *Toxocara canis* (7.6%), *Echinococcus/Taenia* spp. (5.4%), *Strongyloides stercoralis* (4.2%), *Toxascaris leonina* (2.4%), *Uncinaria stenocephala* (2.3%), *Dipylidium caninum* (0.6%), *Spirocerca lupi* 0.6%), *Trichuris vulpis* (0.3%) and *Physaloptera* (0.3%) (Table 1).

Regional and district wise distribution and diversity of helminth parasites

Parasite prevalence was highest (33.9%) in Southern districts compared to Central (24.7%) and Northern districts (22.5%). There were significant differences in prevalence of *Toxocara*, *Ancylostoma*, *Strongyloides*, *Toxascaris*,

Uncinaria and *Spirocerca* in different regions (Table 2). Overall, higher prevalence was recorded in Mardan (37.3%), Dir Lower (36.2%) and Kohat (36.2%), while prevalence was lower in Dir Upper (16.2%), Malakand (18.2%), Swat (18.7%) and Swabi (18.9). Higher parasitic diversity was found in Dir Upper, Dir Lower, Swat, Swabi and Peshawar with total of eight types of parasites reported in each district, seven types in district Buner and Mardan, five in Charsadda, Kohat and Bannu while lowest was recorded in district Malakand with only two types of parasites being reported. *Echinococcus/Taenia* spp. was found the most widespread parasite detected in all studied districts while *Physaloptera* was found only in two districts. *Ancylostoma*, *Toxocara*, *Strongyloides* and *Uncinaria* were found in ten, *Toxascaris* in seven, *Dipylidium* and *Spirocerca* in four and *Trichuris* in three districts (Table 2).

Seasonal and trend distribution of gastrointestinal helminth parasites

Temperature had visible influence on the prevalence of parasites. Prevalence was higher in winter season (26.8%) than in summers (20.3%). In relevance to the season,

Table 3: Prevalence of Gastrointestinal Helminths in Faecal Samples of Stray Dogs in Different Seasons and Years in Khyber Pakhtunkhwa Pakistan

Sr. No.	Type of Parasite	Season			Year				Poison Regression Comparisons					
		Winter n (%)	Summer n (%)	P- value	2020 n (%)	2021 n (%)	2022 n (%)	2023 n (%)	2020 P-value	2020 Exp (B)	2021 P-value	2021 Exp (B)	2022 P-value	2022 Exp (B)
01	<i>Echinococcus/Taenia</i>	40 (5.3)	24 (5.4)	0.953	30 (7.8)	7 (4.1)	14 (3.0)	13 (7.5)	0.901	1.042	0.195	0.545	0.017	0.398
02	<i>Toxocara</i>	69 (9.2)	22 (5.0)	0.008	29 (7.6)	8 (4.7)	42 (9.0)	12 (6.9)	0.798	1.092	0.388	0.674	0.431	1.294
03	<i>Ancylostoma</i>	59 (7.9)	33 (7.4)	0.791	24 (6.3)	5 (2.9)	46 (9.8)	17 (9.8)	0.156	0.638	0.017	0.298	0.999	1.000
04	<i>Strongyloides</i>	39 (5.2)	11 (2.5)	0.023	19 (5.0)	4 (2.3)	21 (4.5)	6 (3.5)	0.445	1.430	0.542	0.674	0.578	1.294
05	<i>Toxascaris</i>	20 (2.7)	9 (2.0)	0.490	15 (3.9)	3 (1.8)	5 (1.1)	6 (3.5)	0.801	1.1129	0.335	0.506	0.052	0.308
06	<i>Uncinaria</i>	24 (3.2)	3 (0.7)	0.005	6 (1.6)	0 (0.0)	19 (4.1)	2 (1.2)	0.710	1.355			0.091	3.512
07	<i>Trichuris vulpis</i>	2 (0.3)	1 (0.2)	0.891	1 (0.3)	0 (0.0)	2 (0.4)	0 (0.0)						
08	<i>Dipylidium caninum</i>	4 (0.5)	3 (0.7)	0.754	5 (1.3)	0 (0.0)	2 (0.4)	0 (0.0)	--	--	--	--		
09	<i>Physaloptera</i>	3 (0.4)	1 (0.2)	0.614	4 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.177	--	--	--	1.000	1.016
10	<i>Spirocerca</i>	1 (0.1)	6 (1.4)	0.008	7 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)	0.074	--	--	--	1.000	1.041
Total		201 (26.8)	90 (20.3)		102 (26.6)	22 (12.9)	121 (25.9)	46 (26.6)						

Data is analyzed through IBM SPSS Statistics 22 using chi square test to find the differences in parasites distribution in different seasons and poison regression analysis to find trend in parasite distribution in different years with $P \leq 0.05$ significance; winter: October to March; Summer: April to September; n: No. of samples positive for that particular parasite. Values in parenthesis show percentages. Total no. of samples tested during winter: 751; Total no. of samples tested during summer: 444. Total no. of samples tested during each year: 2020: 383; 2021: 171; 2022: 468; 2023: 173; The total is not equal to the sum of individual cases because a single sample may contains more than one parasite species.

Table 4: Type of Parasitism of Gastrointestinal Helminths in Faecal Samples of Stray Dogs in KP Pakistan

Type of parasitism	Parasite Species	Number Positive	Prevalence (%)
Mono-parasitism	<i>Echinococcus / Taenia species</i>	42	3.5
	<i>Toxocara canis</i>	64	5.4
	<i>Ancylostoma caninum</i>	50	4.2
	<i>Strongyloides stercoralis</i>	20	1.7
	<i>Toxascaris leonina</i>	15	1.3
	<i>Uncinaria stenocephala</i>	12	1.0
	<i>Trichuris vulpis</i>	01	0.1
	<i>Dipylidium caninum</i>	06	0.5
	<i>Physaloptera species</i>	03	0.3
	<i>Spirocerca lupi</i>	04	0.3
Total		217	18.2
Di-Parasitism	<i>Echinococcus/Taenia + Toxocara</i>	05	0.4
	<i>Echinococcus/Taenia + Ancylostoma</i>	03	0.3
	<i>Echinococcus/Taenia + Strongyloides</i>	04	0.3
	<i>Echinococcus/Taenia + Toxascaris</i>	02	0.2
	<i>Echinococcus/Taenia + Uncinaria</i>	03	0.3
	<i>Echinococcus/Taenia + Physaloptera</i>	01	0.1
	<i>Toxocara + Ancylostoma</i>	08	0.7
	<i>Toxocara + Strongyloides</i>	02	0.2
	<i>Toxocara + Toxascaris</i>	05	0.4
	<i>Toxocara + Uncinaria</i>	01	0.1
	<i>Toxocara + Trichuris vulpis</i>	01	0.1
	<i>Ancylostoma + Strongyloides</i>	15	1.3
	<i>Ancylostoma + Toxascaris</i>	04	0.3
	<i>Ancylostoma + Uncinaria</i>	05	0.4
	<i>Ancylostoma + Trichuris vulpis</i>	01	0.1
	<i>Strongyloides + Toxascaris</i>	01	0.1
	<i>Strongyloides + Uncinaria</i>	03	0.3
	<i>Uncinaria + Dipylidium</i>	01	0.1
	<i>Ancylostoma + Spirocerca</i>	01	0.1
Total		66	5.5
Poly-Parasitism	<i>Echinococcus/Taenia + Toxocara + Strongyloides</i>	02	0.2
	<i>Echinococcus/Taenia + Toxocara + Uncinaria</i>	01	0.1
	<i>Toxocara + Ancylostoma + Strongyloides</i>	02	0.2
	<i>Ancylostoma + Strongyloides + Spirocerca</i>	01	0.1
	<i>Ancylostoma + Toxascaris + Uncinaria + Spirocerca</i>	01	0.1
	<i>Echinococcus/Taenia + Ancylostoma + Toxascaris</i>	01	0.1
Total		08	0.7

The total number of parasites may not be equal to the sum of number of individual parasites as a single sample may contains more than one parasite. Mono-parasitism: a single faecal sample infected with one type of parasite; Di-parasitism: a single faecal sample infected with two types of parasites; Poly-parasitism: A single faecal sample infected with three or more types of parasites. Data about type of parasitism was analyzed using Binary logistic regression (Omnibus test P -value = 0.000; Nagelkerke's R^2 value = 1; Odd ratio = 0.322)

statistically significant differences were observed in prevalence and distribution of *Toxocara* ($P = 0.008$), *Strongyloides* ($P = 0.023$), *Uncinaria* ($P = 0.005$) and *Spirocerca* ($P = 0.008$) (Table 3).

In trend analysis we could not see a clear and consistent trend in prevalence of parasites in preceding years. The prevalence was highest in 2020 (26.6%) and 2023 (26.6%), while it was higher in 2022 (25.9%) and

lowest in 2021 (12.9%). Prevalence of *Echinococcus/Taenia*, *Ancylostoma*, *Toxascaris*, *Uncinaria*, *Physaloptera* and *Spirocerca* in different years was statistically significant (Table 3).

Type of parasitism

In analysis for the status and type of parasitism of dogs, majority (18.2%) of the positive samples were infected with single type of parasite while only 5.5% of the samples were infected with two types of parasites. Only fractional percentage (0.7%) of dogs were infected with three or more types. Results of binary logistic regression analysis showed that *P*-value in Omnibus test was less than 0.05 indicating that our model is statistically significant. The Nagelkerke's R^2 value was 1, demonstrating a best fit model and the odd ratio 0.322 showing that the odds of sample being infected with parasite decrease by 67.8% (Table 4).

Parasite associations

Associations among different parasites showed that *Ancylostoma caninum* and *Uncinaria stenocephala*, each was associated with seven other parasites, *Echinococcus/Taenia*, *Toxocara canis*, *Strongyloides stercoralis* and *Toxascaris leonina*, each with six other parasites, *Trichuris vulpis* and *Spirocerca lupi* each with two other parasites while *Dipylidium caninum* and *Physaloptera* with one other type of parasite (Table 4).

Discussion

This is the first comprehensive study conducted on large scale where samples were collected from eleven districts in north, center and south of Khyber Pakhtunkhwa province covering the main populated areas. The only other study conducted in the province is by Khan *et al.* (2019) in the single district Dir Lower. The overall 24.4% prevalence found in this study is nearly similar to that reported in Lahore Pakistan (Farooqi *et al.* 2014), District Dir Lower Pakistan (Khan *et al.* 2019) and India (Moudgil *et al.* 2016). However, prevalence as high as 98% has been reported from Mexico (Alvarado-Esquivel *et al.* 2015) and 86% from Iran (Emamapour *et al.* 2015). Lower prevalence *i.e.*, 20.7% is reported from USA (Stafford *et al.* 2020). This difference can be due to different number of samples tested, detection methods, environmental conditions, type of dogs studied and use of antihelminthic drugs (Gugsa *et al.* 2015).

The ten parasites found is the highest number reported in any study from Pakistan and all except *Spirocerca* and *Physaloptera* are zoonotic. The zoonotic importance of *T. vulpis* is debatable (Traversa 2011), however, recently, its eggs have been detected in 1.3% of human faeces in Malaysia by PCR (Mohd-Shaharuddin *et al.* 2019) which suggests that the zoonotic role of this parasite cannot be neglected. *T. vulpis* has been reported to cause patent

intestinal infections and visceral larva migrans in humans (Hall and Sonnenberg 1956; Sakano *et al.* 1980; Kagei *et al.* 1986; Dunn *et al.* 2002). *Strongyloides*, *Uncinaria*, *Spirocerca* and *Physaloptera* are reported for the first time in dogs from Pakistan. *Strongyloides stercoralis* is a zoonotic parasite reported in dogs from 29 countries with 1.1 % to 26% prevalence (Jaleta *et al.* 2017; Eslahi *et al.* 2022) and is emerging in Europe (Unterkofer *et al.* 2022). It has unique and complex life cycle and eggs are found only in fresh faeces tested immediately after defecation or collected directly from host rectum, therefore not easily detected (Jaleta *et al.* 2017; Page *et al.* 2018). *Uncinaria stenocephala* is also reported from India (Amissah-Reynolds *et al.* 2016), Finland (Pullola *et al.* 2006), Mexico (Alvarado-Esquivel *et al.* 2015), Sub-Saharan Africa (Chidumayo 2018), Sweden (Grandi *et al.* 2021), Greece (Menelaos and Smaragda 2006) and Argentina (Cociancic *et al.* 2018). *S. lupi* is reported from all neighboring countries like China (Jiang and Dai 1988), Bangladesh (Das *et al.* 2011), India (Ramachandran *et al.* 1984), Sri Lanka (Bandaranayaka *et al.* 2019), Iran (Sasani *et al.* 2014) and also from other regions of the world such as Southeast Asia (Aroch *et al.* 2015; Hoa *et al.* 2021), Africa (Greeff *et al.* 2018), Europe (Giannelli *et al.* 2014; Psáder *et al.* 2017) and USA (Pence and Stone 1978). Molecular genotyping of the parasite has also been performed (Rojas *et al.* 2020; Alfaro-Segura *et al.* 2023). The parasite is emerging in many countries and is neglected and underestimated due to limitations of diagnostic methods (Traversa *et al.* 2008). Its eggs are small and difficult to be detected in direct faecal smear or common flotation methods (Markovics and Medinski 1996). *Physaloptera* has been reported from China (Lee 2023), India (Sudan *et al.* 2015), Bangladesh (Raja *et al.* 2014), Iran (Radfar *et al.* 2011; Beiromvand *et al.* 2013; Mehrabani *et al.* 2002), Iraq (Al-Jassim *et al.* 2017; Abdul-Rahman *et al.* 1988). America (Canto *et al.* 2011; Nagamori *et al.* 2020; Soderman and Harkin 2021) and Africa (Tamerat *et al.* 2015; Amissah-Reynolds *et al.* 2016). Infection with *Physaloptera* is difficult to diagnose due to a smaller number of worms present, infection caused by either male or female, inability to produce eggs, or ova with higher density than solutions commonly employed for qualitative faecal flotation (Soderman and Harkin 2021). Most common parasites found were *Ancylostoma* and *Toxocara* spp. which are also reported the most prevalent parasites from Portugal (Silva *et al.* 2020) and Nigeria (Idika *et al.* 2017). Both these parasites have higher fecundity producing large number of eggs and infective larvae (Idika *et al.* 2017). *Toxocara* eggs have hard outer shell and can survive wide range of temperature (Azam *et al.* 2012). Prevalence was significantly higher in winter season and in northern and central areas. This may be due to the more number of samples collected and tested in winter season. *Echinococcus* and *Taenia* are cestodes difficult to differentiate from egg morphology on microscopy, therefore we have reported

these eggs as *Echinococcus/Taenia* eggs in our results. Contact of dogs with wildlife or livestock animals increase the prevalence of *Echinococcus/Taenia*, therefore these parasites are more prevalent in dogs of rural areas (Murnik et al. 2023). Similar to this, higher prevalence has been reported in districts of Dir Lower, Buner, Malakand and Bannu which are rural areas where wildlife and livestock animals are common. Also, *Echinococcus/Taenia* were found the most widespread parasites in this study detected in all the studied districts. This shows that these parasites may pose a potential risk factor especially in rural areas and therefore need special attention.

In the current study, single parasite infection of the same sample was more common than mixed infection with two or more parasites. Higher prevalence of single type parasitism has also been reported from Dir Lower (Khan et al. 2019), India (Sudan et al. 2015), North America (Nagamori et al. 2020), Iraq (Al-Jassim et al. 2017) and Nigeria (Sowemimo and Asaolu 2008). However, the reason for coinfection of two or more parasites is not clearly known as data about the interaction among different parasites is not available (Murnik et al. 2023). The reason for high prevalence of infection in winter than summer season can be that prevalence is usually high in rainy season (Idika et al. 2017). Higher prevalence found in southern regions compared to central and northern regions may be due to less number of samples collected from this area and not because of geographic or environmental factors. Higher prevalence of parasites has also reported in rural areas from United States (Kubas et al. 2022).

Conclusion

Stray dogs harbour many zoonotic parasites in the study area which may pose a potential risk factor for human infection. Molecular studies, both human and canine are recommended to identify the genotypes of parasites circulating in the area and to find out the routes of their transmission to humans which will help to adopt effective control measures.

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Author Contribution

Hussain Shah: Designed the study and conducted the research work, wrote the research paper, Muhammad Zahid: Supervise the whole work, Sher Hayat Khan: Provided laboratory facilities and supervised the lab procedures. Rehman Mehmood Khattak: Helped in data analysis and writing the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

Not applicable.

Ethics Approval

The faecal samples were collected directly from soil without harming any animal; therefore no ethical approval was required.

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