



Prevalence of Potentially Important Intestinal Pathogenic Protozoan Parasitic Infections in Different Occupational Groups of Swat, Pakistan

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ABSTRACT

Intestinal protozoan infections remain a public health problem in low and middle-income countries. The current study was aimed to investigate the prevalence of potentially pathogenic intestinal protozoan parasitic infection in farmers, education concerned and shepherds of Swat, Khyber Pakhtunkhwa, Pakistan. A total of 1041 stool samples were examined from January 2006 to December 2008 using direct smear and concentration methods. One hundred and fifteen (11.04%) participants were found infected with one or more than one intestinal protozoans. Forty one (35.6%) of the participants were infected with single parasite and seventy four (64.3%) with multiple infections. *Entamoeba histolytica* 30.5% (n=77/252), *Giardia lamblia* 15.0% (n=38/252), *Ascaris lumbricoides* 17% (n=43/252), *Trichuris trichura* 11.1% (n=28/252), *Enterobius vermicularis* 9.52 (n=24/252), *Ancylostoma duodenale* 3.96% (n=10/252), *Taenia saginata* 9.52% (n=25/252) and *Hymenolepis nana* 2.77% (n=7/252) were detected in order of their prevalence. The adults were found marginally more parasitized than children (P<0.05). The females were more infected than males with (P<0.05). No significant association was found among the occupational groups and parasitic infection (P<0.05). However, shepherds were found more infected than farmers and education concerned. Due to comparative based approach in different occupational communities the present study is of particular importance and interest. Such studies should continue time to time to know the hazardous of potentially important pathogenic parasitic infections particularly in remote parts of the world.

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Authors' Contribution

WK, NUN and AK conceived and designed the study. WK performed the study, analyzed the data and wrote the manuscript.

Key words

Protozoan infections, Intestinal protozoan diseases, Amoebiasis, Giardiasis, Occupational groups.

INTRODUCTION

Amoebiasis caused by *E. histolytica* is one of the deadliest parasitic infection which kills 40,000 to 100,000 people each year worldwide (Stanley, 2003; Lozano *et al.*, 2010). The prevalence of *G. intestinalis* has been estimated at 2–3% in the industrialized world and 20–30% in developing countries (Escobedo and Cimerman, 2007).

Entamoeba histolytica and *Giardia lamblia*: the parasites of diarrhoea are the most common intestinal protozoan parasites which infect about 60 million and 200 million people worldwide, respectively (Murray *et al.*, 2002). It is estimated that about 3.5 billion people are infected with digestive tract parasites and at least 450 million became ill after infection (WHO, 2001). Whereas much

effort was being made towards a better comprehension of helminth epidemiology, relatively few studies were done on intestinal protozoan parasites (Lammie *et al.*, 2006).

E. histolytica/dispar is cosmopolitan in distribution, but more common in tropical countries or the areas with poor sanitary conditions. About 10% of the world's population is infected with *E. histolytica/dispar*, and in many different countries the prevalence may reach up to 50%. It is also estimated that about 100,000 deaths and 50 million cases of amoebiasis occur per year in the world and humans are the only host of *E. histolytica/dispar* passing virulent cysts that are transmitted chiefly by ingestion of contaminated foods or water or through direct contact (Bethony *et al.*, 2006). *G. lamblia* is another major intestinal protozoa parasite found especially in temperate and tropical countries and commonly causes diarrheal disease throughout the world. The prevalence rate of giardiasis has been found to be 2 to 7% in developed and 20 to 30% in developing countries (Jerlstrom-Hultqvist *et al.*, 2010). The variation in prevalence depends on factors

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like geographical position; the setting of the society as urban or rural, the age group composition and the socio-economic conditions of the study subject (Sebastian *et al.*, 2007). *G. lamblia* mostly infects children than adults particularly those who are malnourished (Mehraj *et al.*, 2008). It was estimated that about 200 million people are infected each year in Africa, Asia and Latin America. *G. lamblia* exhibits a typical fecal-oral transmission cycle. The infection is acquired through the ingestion of cysts. Factors leading to contamination of food or water with fecal material are positively correlated with transmission (Partovi *et al.*, 2007).

Reports from different parts of Pakistan showed the difference in the prevalence rate of amoebiasis and giardiasis as: 3.5 and 13.8% (Farooqi, 1964); 11.6 and 12.0% (Farooqi, 1965); 13.5 and 5.4% (Haleem *et al.*, 1965); 0.8 and 1.8% (Ansari and Naru, 1968); 11.9 and 41.9% (Pal and Malik, 1979); 14.5 and 22.7% (Siddiqi and Bano, 1979); 60.5 and 26.9% (Bilqees *et al.*, 1982); 5.31 and 32.9% (Pal and Rana, 1983a); 5.64 and 31.7% (Pal and Rana, 1983b); 12.6 and 22.8% (Nawaz and Nawaz, 1983); 0.6 and 5.0% (Baqai *et al.*, 1985); 2.5 and 0.4% (Khan *et al.*, 1988); 36.5 and 43.7% (Baqai and Zuberi, 1986); 4.68 and 7.92% (Khan *et al.*, 1993); 3.2 and 9.0% (Akhtar *et al.*, 1993); 0.80 and 50.8% (Kamran *et al.*, 2005); 15.4 and 39.1% (Shaikh *et al.*, 2003); 5.9 and 1.8% (Chaudhry *et al.*, 2004); 1.8 and 10.9% (Sajjad *et al.*, 2009); 6.43 and 2.07% (Khan *et al.*, 2011); 7.26 and 5.86% (Noor-un-Nisa *et al.*, 2011); 4.36 and 1.69% (Khan *et al.*, 2012); 4.40 and 1.69% (Khan *et al.*, 2015, 2017). In Pakistan high prevalence of intestinal protozoan parasite infection is attributable to factors associated with low socio-economic status, poor personal hygiene, environmental sanitation, low household income, overcrowding living condition and lack of clean water supplies. Owing to the public health importance intestinal protozoan diseases act as a source of morbidity and mortality, only few studies have been made on the prevalence and intensity of intestinal parasitic protozoan in Pakistan. Keeping in view that present paper is of particular importance and interest as these infections have been studied comparatively in different occupational groups of human population in Swat region, Pakistan.

MATERIALS AND METHODS

Study area and population

The study was conducted in Swat region from January 2006 to December 2008. Swat is located in Northwestern parts of Pakistan (34°34' to 35° 55' North and 72° 08' to 72° 50' East), characterized by a temperate climate with pleasant summers (average high temperature 33 °C) and cool winters (average low temperature -2°C). Swat is

located in a high altitude (18000 m above mean sea level: mountainous range and 6000 m plain areas) with an annual recorded rain-fall 242 mm. No gastro-intestinal complaint was reported during the collection.

Socioeconomic status of the inhabitants

Swat valley is the land of flora and fauna of a wide range of diversity. The residential settings are built of wood and mud in rural areas and with blocks and bricks in urban. Most of the population has low socio-economic status. The public uses surface or un-treated tap-water for consumption. Health practices are also very low, in rural areas people use open fields for defecation. During rainy seasons the surface of water runoff and waste water from the agricultural field's ingress in to channels, which is the major source of drinking water. The use of such contaminated water are the main sources of gastrointestinal parasitic infections in the people of Swat and joined other areas.

Stool collection

Stool samples were collected from the Basic Health Units (BHUs), Hospitals and Schools of different site and locations of the district. Samples were collected in sterile plastic containers and carefully labeled. The occupational groups' included farmers, education concerned (staff, students and workers) and Shepherds, children and adults of both the gender. No gastro-intestinal complaint was noted at the time of collection. The collected samples were transported to Medical Zoology Laboratory, Vertebrate Pest Control Institute (VPCI), Southern Agricultural Research Centre (SARC), Karachi, Pakistan Agricultural Research Council (PARC) for final examination.

Stool examination

The stool specimens were examined by naked eyes for the detection of adult or any segmental stage of parasite. The specimens were then subjected for microscopically examination, through wet mount techniques (normal saline and Lugol's iodine solutions). The formol-ether concentration was also used for the confirmation of negative cases to be positive. About 3 g of stool was emulsified in 3 drops of normal saline (0.9%) or Lugol's iodine solution. One drop of the suspension was placed on the center of the slide via wooden applicator and a coverslip was positioned. The slide was examined under the light microscope first under low 10× and then high 40× power objectives. Saline direct smear is used for the detection of intestinal protozoa trophozoites motility. Iodine direct smear was used for the nuclear characterization of *Entamoeba histolytica/dispar* and *Giardia lamblia*. After completion of direct stool examination, one gram

of each sample was emulsified in 10% formalin solution and formol-ether concentration technique was performed as described elsewhere in order to increase the chance of detecting parasites (WHO, 1991).

Statistical analysis

The percentage of infections was calculated from the total number of parasitic infections (Table I). Collected data were analyzed using the graph pad version 5, the statistical software. Prevalence was calculated as percentage value. Statistical association of the parasitic infection prevalence with occupation, gender and ages was analyzed. A statistically significant association between variables is considered to exist if $P < 0.05$.

RESULTS

In total, 1041 patients participated in this study.

Among them 420 (40.3%) were education related, 365 (35%) were farmers and 256 (24.5%) were shepherds. Regarding the gender, 651 (62.5%) were male and 390 (37.5%) were female. The overall prevalence of intestinal parasitic infection among these individuals was determined to be 11.04% (115/1041). It was 5.95% (25/420), 12.0 (44/365) and 18.3% (47/256) among educated community, farmers and shepherd, respectively. The prevalence of infection with intestinal parasites was not significantly different among these three groups ($P = 0.9483$) (Table II). However, females were found to have a higher percentage of infection (12.8%) compared to the male group (9.98%). The association between the gender and parasitic infection was statistically non-significant ($P = 0.5200$) (Table II). Regarding ages above 15 years were slightly more infected (11.8%) than below 15 years of age (10.0%). The association between the ages and parasitic infection was also statistically non-significant ($P = 0.6040$) (Table II).

Table I.- Intestinal protozoan and helminth parasitic infections among different occupational groups in Swat, Khyber Pakhtunkhwa, Pakistan (January 2006 to December 2008).

Parasite	Ages		Sex		Occupation			Over all
	<15	>15	Male	Female	Farmers	Education concerned	Shepherds	
Protozoa								
<i>Entamoeba histolytica/dispar</i>	25 (9.92)	52(20.6)	45(17.8)	32(12.6)	34(13.4)	17(6.74)	26(10.3)	77(30.5)
<i>Giardia lamblia</i>	22(8.73)	16(6.34)	21(8.33)	17(6.74)	10(3.96)	7 (2.77)	21(8.33)	38(15.0)
Nematodes								
<i>Ascaris lumbricoides</i>	16(6.34)	27(10.7)	26(10.3)	17(6.74)	19(7.53)	8(3.17)	16(6.34)	43(17.0)
<i>Trichuris trichura</i>	10(3.96)	18(7.14)	13(5.15)	15(5.95)	11(4.36)	2(0.79)	15(5.95)	28(11.1)
<i>Enterobius vermicularis</i>	9(3.57)	15(5.95)	13(5.15)	11(4.36)	12(4.76)	3(1.19)	9(3.57)	24(9.52)
hookworms	4(1.58)	6(2.38)	7(2.77)	3(1.19)	3(1.19)	0	7(2.77)	10(3.96)
Cestodes								
<i>Taenia saginata</i>	13(5.15)	12(4.76)	10(3.96)	15(5.95)	5(1.98)	4(1.58)	16(6.34)	25(9.92)
<i>Hymenolepis nana</i>	6(2.38)	1(0.39)	3(1.19)	4(1.58)	4(1.58)	1(0.39)	2(0.79)	7(2.77)
Total no. of infection	105(41.6)	147(58.3)	138(54.7)	114(45.2)	98(38.8)	42(16.6)	112(44.4)	252(24.2)
Total no. of samples	466	575	651	390	365	420	256	1041

Table II.- Prevalence of intestinal protozoan parasitic infections according to the occupational groups, genders and ages.

Factor	No. examined	No. infected	Prevalence	Lower to upper 95% CI of mean	P.value
Occupational groups					
Farmers	365	44	12.0	-1835 to 2244	0.9483
Education community	420	24	5.71	-2287 to 2732	
Shepherds	256	47	18.3	-1176 to 1479	
Genders					
Male	651	65	9.98	-3337 to 720.0	0.5200
Female	390	50	12.8	-1961 to 2398	
Ages					
<15 years	466	47	10.0	-2392 to 2907	0.6040
>15 years	575	68	11.8	-2906 to 3548	

Table III.- Proportion of mono-parasitism and poly parasitism of intestinal protozoans, and protozoan with helminths parasitic infections in different occupational groups of Swat, Pakistan (2006 to 2008).

Pattern of infection	No. of species	Farmers						Education department						Shepherds					
		<15			>15			<15			>15			<15			>15		
		M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total
Mono-parasitism	1 species	2	8	7	3	10	Eh	4	4	2	6	8	Eh	4	5	4	5	9	
	GI	3	2	3	2	5	GI	0	4	3	1	4	GI	3	2	3	2	5	
Total mono-parasitism		5	10	10	5	(n=15)		4	8	5	7	(n=12)		7	7	7	7	(n=14)	
Poly-parasitism	2 species	1	5	5	1	6	GI,Al	1	0	1	0	1	Eh,Ev	0	2	0	2	2	
	Eh,Ev	0	3	3	0	3	GI,Tt	1	0	1	0	1	Eh,Al	0	1	0	1	1	
	GI,Ev	1	0	1	0	1	Eh,Al	2	1	3	0	3	GI,Hw	1	0	1	0	1	
	GI,Hn	1	0	0	1	1	Eh,Tt	1	0	0	1	1	Eh,Hw	0	2	2	0	2	
	Eh,Tt	0	1	1	0	1							Eh,Tt	0	1	1	0	1	
Sub-Total		3	9	10	2	(n=12)		5	1	5	1	(n=6)		3	7	6	4	(n=10)	
3 species	Eh, Al,Tt	0	2	1	1	2	Eh,Al,Hn	0	1	1	0	1	GI,Tt,Ts	2	2	1	3	4	
	Eh,Tt,Ev	0	1	1	0	1	Eh,Ts,Hn	0	1	1	0	1	Eh,Al,Ts	2	0	2	0	2	
	Eh, Ev,Hn	2	1	1	2	3	Eh,Al,Ev	0	1	1	0	1	GI,Al,Hn	1	0	1	0	1	
	Eh, Al,Hw	2	1	2	1	3	GI,Ev,Ts	1	0	0	1	1	Eh,Tt,Ts	1	0	1	0	1	
							Eh,Al,Ts	0	1	0	1	1	GI,Al,Ev	0	1	1	0	1	
Sub-Total		4	5	5	4	(n=9)		1	4	3	2	(n=5)		8	6	8	6	(n=14)	
4 species	Eh,Al,Tt,Ev	0	3	2	1	3	Eh,Al,Ev,Ts	0	1	0	1	1	Eh,Al,Tt,Ts	1	3	1	3	4	
	Eh,Al,Tt,Ts	1	0	1	0	1						Eh,Al,Tt,Hw	0	1	1	0	1		
	Eh,Al,Ev,Ts	1	0	0	1	1						Eh,Al,Tt,Ev	1	1	0	2	2		
	GI,Al,Tt,Ts	2	1	1	2	3						GI,Tt,Hw,Ts	1	1	0	2	2		
Sub-total		4	4	4	4	(n=8)		0	1	0	1	(n=1)		3	6	2	7	(n=9)	
Gross total poly- parasitism		11	18	19	10	29		6	6	8	4	12		14	19	16	17	33	
Total individuals infected		16	28	29	15	44		10	14	13	11	24		21	26	23	24	47	

M, Male; F, Female; Eh, *Entamoeba histolytica*; GI, *Giardia lamblia*; Al, *Ascaris lumbricoides*; Tt, *Trichuris trichura*; Ev, *Enterobius vermicularis*; Hw, Hook worms; Ts, *Taenia saginata*; Hn, *Hymenolepis nana*.

Out of the 1041 individuals examined, 11.04% (n=115/1041) were found infected with single or multiple infections either with protozoans or mixed with helminths. Of the infected individuals 35.6% (n=41/115) were found to be infected with single species of intestinal protozoa either *E. histolytica* or *G. lamblia* and the other 64.3% (n=74/115) had mixed infection with both protozoans with helminths (Table III).

Regarding the number of parasites species detected in each sample, 35.6% were infected with a single parasite species with a single protozoan (n = 41/115), two parasite species 24.3% (n = 28/115); three parasite species 24.3% (n=28/115) and four parasite species 15.6% (n= 18/115) were detected. *E. histolytica* was frequently associated with helminth parasites in 45.2% (n=52/115) while *G. lamblia* was reported mixed with helminths in 18.2% of the participants (Table III).

Regarding prevalence of intestinal protozoa infection, *E. histolytica* (30.5%) was the most predominant protozoa identified from stool of the studied participants followed by *Giardia lamblia* (15.0%). Among the helminths *A. lumbricoides* 17.0%, *T. saginata* 9.92%, *T. trichura* 11.1%, *E. vermicularis* 9.52%, hookworm species 3.96%, and *H. nana* 2.77% were detected in order of their prevalence (Table I).

DISCUSSION

Very little is known about the epidemiology of potentially pathogenic intestinal protozoan parasitic infections in Pakistan. The formalin fixed stool samples were obtained from 1041 participants of different occupational groups in a randomized controlled trial in Swat, Pakistan. The study was aimed to determine the comparative prevalence of potentially pathogenic intestinal protozoan parasitic infections, pattern of infection that whether the protozoan parasite is showing mixed with other protozoan or helminth parasites. Current findings confirm that intestinal protozoans are a public health threat in different occupational groups of Swat, Pakistan. Amongst the infected individuals almost half of the individuals were infected with at least one of the two potentially pathogenic intestinal protozoa.

In Pakistan, the prevalence of intestinal parasitic protozoan infections ranges from 0.6% (Baqai *et al.*, 1985) to 60.5% (Bilqees *et al.*, 1982), similarly, the prevalence of *Giardia intestinalis* (*lamblia*) ranges from 0.4% (Khan *et al.*, 1988) to 50.8% (Kamran *et al.*, 2005). These two protozoans remain the most common intestinal parasitic pathogens in the study population. The transmission of these parasites occurs via fecal- oral route, either directly from person-to-person or indirectly by eating or drinking

fecally contaminated food and water.

Present study is comparable with the study conducted by Baqai and Zuberi (1986) but higher in prevalence rate when compared with the studies conducted by Farooqi (1964) in Peshawar, Ansari and Naru (1986) in Lahore, Baqai *et al.* (1985), Khan *et al.* (1988, 2011, 2012, 2015), Akhtar *et al.* (1993), Chaudhry *et al.* (2004), Sajjad *et al.* (2009), Farooqi (1965), Pal and Malik (1979), Siddiqi and Bano (1979), Nawaz and Nawaz (1983), Shaikh *et al.* (2000, 2003) and Noor-un-Nisa *et al.* (2012) from other parts of Pakistan. Interestingly in comparison to studies carried out by Bilqees *et al.* (1982) and Tasawar *et al.* (2006), the present study showed low rate of prevalence.

Less than half of the studied samples were singly infected by the protozoans and the other half were with mixed infections either with protozoans or helminths. Current finding suggests that potentially pathogenic intestinal parasitic infections were mostly co-related with helminth infection.

No statistically significant association was found among the occupational groups, genders and ages, however, age above 15 years in ages showed slightly more prevalent rate than below. Similarly females were found to be more parasitized than males while occupation wise shepherds showed high prevalence rate of infections than all the occupation studied.

Compared with the results of related studies conducted in the country, the present study showed prevalence of infection for *E. histolytica* was 30.5%, this protozoan infection was quite high (60.5%) reported in a survey conducted in Karachi, Pakistan (Bilqees *et al.*, 1982); 36.5% infection in diarrreal patients in Karachi (Baqai and Zuberi, 1986); 50.9% in Mirpur Sindh (Tasawar *et al.*, 2006). High prevalence of this intestinal pathogenic protozoan for particular areas might be the same ecological, cultural and geographical associations. *E. histolytica* has a variable range of distribution in human populations of Pakistan.

The incidence of *G. lamblia* in present investigation was 15.0% which is comparable with Farooqi, (1964), (1965), Siddiqi and Bano (1979) and Nawaz and Nawaz (1983). The study of Maqbool *et al.* (2007) shows the highest prevalence of *Giardia lamblia* in urban and rural settings of Lahore which was 51.7%. Kamran *et al.* (2005) shows the prevalence of *G. lamblia* to be 50.8% followed by Baqai and Zuberi (1986) in diarrreal patients on Karachi. Pal and Malik (1979) in Islamabad school children in which the prevalence is 41.9%. Shaikh *et al.* (2000) and (2003) reported 39.1% and 38.5% prevalence rate when they were studying the mixed population in Larkana and Shikarpur. In Rawalpindi and Islamabad the prevalence was reported to be 32.9 and 31.7% in a survey of mixed population (Pal

and Rana, 1983a, b), while Bilquees *et al.* (1982) recorded this parasite with the prevalence rate of 26.9% conducting a survey in mixed population in Karachi.

In Pakistan studies on the prevalence of human intestinal parasites have mainly been restricted to particular areas and targeted populations. It was desirable therefore to investigate which of the potentially pathogenic intestinal protozoan parasites the occupational group harbors and to obtain approximations of both the prevalence and intensities of such infections.

CONCLUSIONS

This research provides new insight into occupation's parasitic infection status among the farmers, education concerned and shepherds of Swat district Pakistan. Present study results call for the improvement of occupational health and rural communities in Swat, Pakistan. Health education interventions should be imposed to reduce transmission and reinfection in the studied occupational communities. This study will serve as a benchmark for successive post-intervention surveys and analysis.

Statement of conflict of interest

Authors have declared no conflict of interest.

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