Soil-transmitted helminth infections in school children of three districts of Malakand region, Khyber Pakhtunkhwa, Pakistan

Wali Khan[1]*, Jahangir Khan[2], Naeem Khan[1-3], Ruhoollah Iqbal[1-4], Atta Ullah[1], Rukhsana Ghaffar[2], Sardar Azhar Mehmood[5], Shabir Ahmad[5], Sheema Khan[6] and Farman Ullah[1-3]

[1]Department of Zoology, University of Malakand, Chakdara, Khyber Pakhtunkhwa, Pakistan
[2]Department of Pharmacy, University of Malakand, Chakdara, Khyber Pakhtunkhwa, Pakistan
[3]Department of Zoology, Islamia College University, Peshawar, Khyber Pakhtunkhwa, Pakistan
[4]Department of Zoology, Virtual University, Pakistan
[5]Department of Zoology, Hazara University, KPK, Pakistan
[6]Medical B Ward, Khyber Teaching Hospital, Peshawar, Pakistan

Abstract: Information on prevalence of soil-transmitted helminth (STH) infections among school children is scarce in Pakistan. This study was aimed to investigate the prevalence of soil-transmitted helminth in school children of three districts in, Khyber Pakhtunkhwa, Pakistan. A total of 300 stool samples were examined from August 2015 to August 2016 using direct smear (Normal saline and Lugol,s Iodine solution) and the concentration methods. One hundred and eighty seven (62.3%) pupils were found infected with soil-transmitted helminths. One hundred and forty five (77.5%) were infected with single parasite and forty two (22.4%) with multiple infections. *Ascaris lumbricoides* 125 (66.4%), *Trichuris trichura* 50 (26.5%) and *Ancylostoma duedenale* 13 (6.91%) were detected. The children above 8 years in age were more parasitized than below 8 years ($p=0.7832$; $P>0.05$). Males were found more parasitized than females ($p=0.9315$; $P>0.05$). Children in lower Dir district were found more infected followed by Swat and upper Dir ($P< 0.0001$; $p<0.05$). No significant relationship was found among the examined and that of infected children for ages and sex in all the districts. Malakand division is an area with poor hygiene located in temperate zone near the border of Afghanistan and China. The prevalence of reported nematode parasites here compared with the same studies is unexpectedly high. These types of studies should continue time to time to know the hazardous nature of such parasitic infections for the betterment of the human health.

Keywords: School children, intestinal parasites, helminth infections, soil-transmitted infections, Pakistan.

INTRODUCTION

The soil-transmitted helminths comprised *Ascaris lumbricoides* (roundworms), *Trichuris trichiura* (whipworms), and *Necator americanu*s or *Ancylostoma duodenale* (hookworms) are a group of parasitic nematodes which cause infections in the human alimentary canal through contact with eggs or larvae. *A. lumbricoides* and *T. trichiura* are transmitted by eggs that are passed in the faeces of infected people while hookworms through larvae usually found in the faecally contaminated soil. Adult worms live in the intestine where they produce thousands of eggs each day (Bethony *et al*., 2006).

The global burden of STH infection revealed that nearly 70% of the infections occur in Asia (Pullan *et al.,* 2014) including 26.4% of the Asian study population hosted at least one STH species. Three and half billion people are estimated to be infected with intestinal worms including: 1.47 billion with *A.lumbricoides*, 1.3 billion with *A.duodenale/N.americanus* and 1.05 billion with *T.trichura*. More than 4 billion school-age children are expected to be infected with STHs. Children ranges from 5 to 15 years in ages are commonly infected, however incidence of *A.duodenale/N.americanus* infection tends to increase with growing age. Thus, teenage girls and women of childbearing age are mostly infected with *A.duodenale/N.americanus* (Luong, 2003).

A high amount of soil transmitted helminthiasis and related diseases affect school-age children and, therefore control efforts have focused on this group, in some countries like Vietnam and China (Bethony *et al.,* 2002). The global estimated prevalence of anaemia in *A.lumbricoides*, *T.trichura* and hookworms indicated millions of persons may be affected (Murray and Lopez, 1996). The high prevalence of soil-transmitted helminthiasis is closely related to poverty, poor environmental hygiene and impoverished health services. Intestinal helminth infestations are the most common infestations among school age children and they tend to occur in high intensity in this age group (Albonico *et al.,* 1999).

Environmental contamination with soil-transmitted helminths has received little attention from scientists and public health managers all over the world including Pakistan. This study was aimed to investigate the

**Corresponding author:* e-mail: walikhan.pk@gmail.com

prevalence of soil transmitted helminths, pattern of parasitism and coinfections with each other and other helminths or intestinal protozoan parasites, in school children of three districts of Khyber Pakhtunkhwa, Pakistan.

MATERIALS AND METHODS

Study area

The district Swat lies from 34°34° to 35° 55° N and 72° 08° to 72° 50° E, lower Dir lies 34.9161° N and 71.8097° E and upper Dir district coordinates are 35.3356° N and 72.0468° E. Temperature ranges between -2c^{o} in winter and 42.7 C^{O} in summer seasons of the year. The minimum and maximum recorded rainfall of the areas ranges 117 and 242mm during.

Specimens' collection and ethical approval

In a cross-sectional study, 300 stool specimens (100 from each district) were collected randomly from school children in Swat, Lower Dir and Upper Dir districts Pakistan from August 2015 to August 2016. This study was approved by the Ethical Review Committee, University of Malakand. Biodata of pupils such as name, age and sex etc were recorded in a questionnaire that was designed for this study. Each of the students was asked to collect their fresh stool specimens in plastic containers. Each of the containers containing faecal sample referenced with a code number and tag. Faecal specimens collected were directly transferred to the Laboratory of the Parasitology Department of Zoology, University of Malakand, Lower Dir, Pakistan.

Microscopic examination

Each of the stool samples were processed with two different methods: Direct smear method (normal saline solution plus Lugol's Io-dine solution) and formalin-ethyl acetate sedimentation technique. Direct smear method was used in the microscopic investigation of bloody samples or watery fecal samples. In present study both direct smear and concentration methods were used.

STATISTICAL ANALYSIS

The frequencies of age group and sex of children were calculated and parasites found in the fecal samples were reported. The prevalence of soil-transmitted helminths and age group (in years) with 95% CI were also computed. P value if less than 0.05 was considered significant. Soil transmitted nematode eggs were identified by their characteristic features. Infection intensities were classified as light, moderate or heavy based on the EPG calculations according to the WHO criteria (table 4).

RESULTS

One hundred and eighty seven (62.3%) children were found infected with one or more than one soil-transmitted nematode parasites. One hundred and forty five (77.5%) were infected with single parasite and forty two (22.4%) with multiple infections. *A.lumbricoides* 125 (63.1%), *T. trichura* 50 (25.2%) and *A.duedenale* 13 (6.56%) were detected (table 2 and 1). Males children were more infected 49.6% (n=149/300) than females 12.6% (n=38/300) (p=0.9315; P>0.05). Regarding age above 8 years were highly infected 39.3% (n=193/300) than below 8 years 23% (n=69/300) (p=0.7832; P>0.05). Children in lower Dir district were found more infected followed by Swat and upper Dir (P< 0.0001; p<0.05) table 3.

Of the examined children 105 were infected with light, 53 with moderate and 30 with heavy infection intensity (p <0.05) (table 4).

DISCUSSION

Present study results evidenced that soil-transmitted helminth (STH) infections are still constitute a major public health problem among school children in Malakand, Pakistan. No doubt different scientists have investigated human intestinal parasites in Pakistan but Investigation on soil transmitted helminths are lacking in the country except a study conducted by (Khan *et al.*, 2017a) in different occupational groups of Swat, Pakistan and the first nationwide survey on STHs in Pakistan by (WHO, 2017) have been published.

More than 1/3rd of the school children were found infected with single species of soil transmitted helminths and the remaining were with mixed species either protozoans or other helminths infections (table 2). This study shows that soil transmitted helminth parasites were mostly co-related with each other. Of the examined pupils 105 were infected with light, 53 with moderate and 30 with heavy infection intensity (WHO, recommended range, table 4).

Heavy intestinal helminth infections are related with minimized learning ability which could possibly impact school attendance (Jardim-Botelho *et al.* 2008). The presence of high prevalence of soil-transmitted helminths in present investigation highlights the need for thorough investigation of the school children. There was a variable prevalence of the parasitic infections among the districts, ages and sex was noted, however, age below 8 in Swat and Upper Dir districts while in Lower Dir district individuals of more than 8 -years in age were highly infected. Also male students were found to be highly infected than female students (P>0.05).

In present study 66.4% of the students were found infected with *A.lumbricoides*. In comparison with the findings of related studies published in other parts of the country, the prevalence of *A.lumbricoides* was 55.8% in a study conducted on food-handlers in Swat, Pakistan (Khan *et al.*, 2017b). This roundworm infection was compared to a survey conducted in Chitral "a district in

Table 1: Prevalence of soil-transmitted helminths in school children of three districts of Khyber Pakhtunkhwa, Pakistan

Parasites	Districts			Total	%
	Swat	Lower Dir	Upper Dir		
Ascaris lumbricoides (round worm)	58	54	13	125	66.4
Trichuris trichura (whip worm)	17	30	3	50	26.5
Ancylostoma duodenale (hook worm)	3	9	1	13	6.91
Total no. of infection	78 (41.4)	93(49.4)	17(9.04)	188	

Table 2: Pattern of Prevalence of soil-transmitted helminths in school children of three districts of Khyber Pakhtunkhwa, Pakistan

Parasites	Districts			Total	%
Pattern of parasites	Swat	Lower Dir	Upper Dir		
Single parasite	62	10	73	145	77.5
Soil-transmitted nematodes	59	9	68	136	72.7
Protozoans	3	1	5	9	4.81
Multiple	15	7	20	42	22.4
Helminths	14	5	20	39	20.8
Protozoans	0	0	0	0	0
Mixed	1	2	0	3	1.60
Total infected individuals	77	17	93	187	

Table 3: Prevalence of soil-transmitted helminths in school children of three districts of Khyber Pakhtunkhwa, Pakistan

Parameters	Swat			
	Age's n (%)		Genders n (%)	
	>8	<8	Male	Female
Number examined	33	67	85	15
Number infected	27 (27)	50 (50)	67(67)	10(10)
P value (with 95%CI)	0.7736 (0.4873 to 1.707)		0.7030 (0.3572 to 2.003)	
	Lower Dir			
Number examined	42	58	82	18
Number infected	10(10)	7(7)	14(14)	3(3)
P value (with 95%CI)	0.1969 (0.1783 to 1.441)		0.9720 (0.2537 to 3.757)	
	Upper Dir			
Number examined	32	68	73	27
Number infected	32 (32)	61(61)	68(68)	25(25)
P value (with 95%CI)	0.7225 (0.4924 to 1.634)		0.9852 (0.5259 to 1.879)	

the Northern areas of Pakistan" (Stodart, 1999). In male and female students and workers of education department of Swat, Pakistan 40% was reported by (Khan *et al.*, 2015); In School children at University Public School, University of Malakand (Khan *et al.*, 2018) reported 27.7% in prevalence. Although *A.lumbricoides* has a high prevalence on contrary to other soil-transmitted nematodes in human populations of Pakistan.

This high prevalence of *A.lumbricoides* is greatly in northern parts of Pakistan might be due to the similar environmental, traditional and physical conditions. *Ascaris lumbricoides* (the large roundworm) is the first rank STH infecting human population in Pakistan. Three kilograms *A.lumbricoides* were proven from the small intestine in a boy of 12 years old in age (Khan *et al.*, 2016).

The current research indicates incidence of *Trichuris trichura* to be 26.5. The study of (Khan *et al.*, 2017a) reveals 18.1% of the whip worm prevalence rate in different occupational groups of Swat, Pakistan. Prevalence of *T.trichura* was 22.5% in a study conducted in Chitral by (Stoddart, 1999) followed by (Khan *et al.*, 2017b) in food-handlers of Swat, Pakistan in which the prevalence was 14.9%. According to Khan *et al.*, 2015 the prevalence was 19%, they were conducted a study in Swat, Pakistan. *Trichuris trichura* (the whip worm) is the second prevalent STH also in other parts of the country. *Trichuris trichura* is a worldwide nematode of soil-transmitted in origin with a wide range of geographical distribution.

Table 4: Number of school children infected with infection intensity range for soil-transmitted helminth parasites in three districts of Khyber Pakhtunkhwa, Pakistan

Districts	Number of children infected with *Ascaris lumbricoid*es (EPG)			P value
	Light (1 - 4,999)	Moderate (5,000 - 49,999)	Heavy (50,000 and above)	
Swat	32	20	6	0.1655
Upper Dir	27	14	13	
Lower Dir	10	2	1	
Sub-total	69	36	20	
	Number of children infected with *Trichuris trichura* (EPG)			
	Light (1 – 999)	Moderate (1,000 - 9,999)	Heavy (10,000 and above)	
Swat	9	6	2	0.2550
Upper Dir	17	8	5	
Lower Dir	3	0	0	
Sub-total	29	14	7	
	Number of children infected with hookworms (EPG)			
	Light (1 - 1,999)	Moderate (2,000 - 3,999)	Heavy (4,000 and above)	
Swat	2	1	0	0.4761
Upper Dir	4	2	3	
Lower Dir	1	0	0	
Sub-total	7	3	3	
Gross-total	105	53	30	

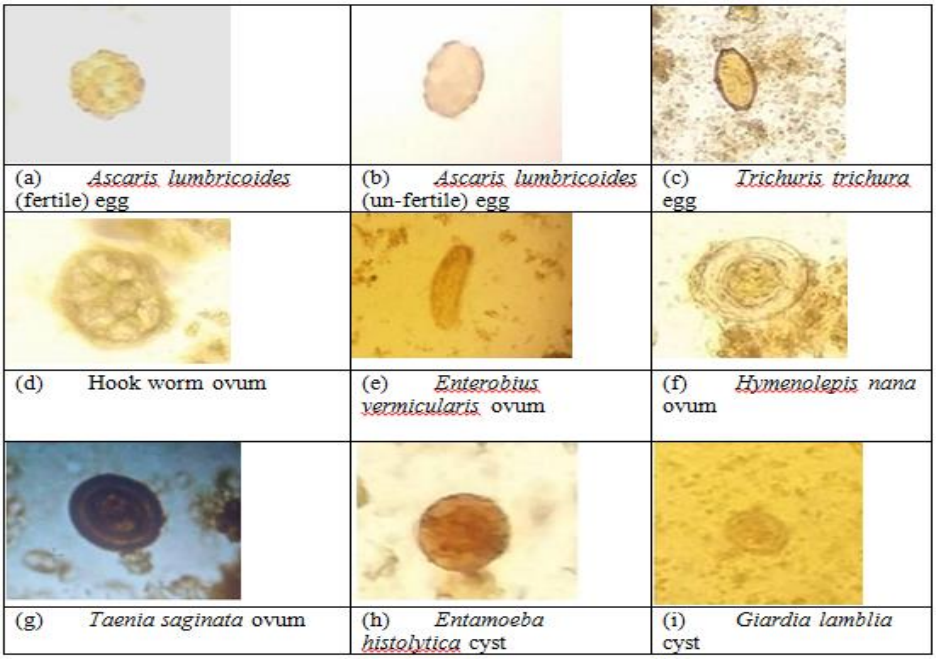

Fig. 1: Eggs of intestinal nematodes with their associated helminth and protozoan parasites: a- *Ascaris lumbricodes* (fertile);b- *A.lumbricoides* (unfertile); c- *Trichuris trichuira* (showing polar plugs); d- *Ancylostoma duodenale* (segmented); e-*Enterobius vermicularis* (plano-conves shell); f- *Hymenolepis nana* (with polar filaments and onchosphere); g- *Taenia saginata* (ovum); h- *Entamoeba histolytica* cyst (quadrinucleated);i-*Giardia lambila* (cysts).

Ancylostoma duodenale was 6.91 in prevalence in current research. This hookworm was reported 5.99% in the study conducted earlier to the present investigation by Khan *et al.,* 2017b. The low prevalence rate was 4.46% in Swat, Pakistan by Khan *et al.,* 2017a); 4.23 and 4.27% prevalence was recorded in Larkana and Shikar Pur (Shaik *et al.,* 200; Shaikh *et al.,* 2003); 3.6% was the lowest prevalence reported in a study conducted by Khan *et al.,* 2015 in Swat, Pakistan. *Ancylostoma duodenale* has variable rate of prevalence in different climatic zones in Pakistan and to be considered as third prevalent STH.

The children >8 years of age were much more affected compared to <8 years suspected children. The children >8 years of age were not aware of personal hygiene

education which played a main role in transmission of parasitic infections. Similar results have been reported by (Chaudhry *et al.,* 2004: Khan *et al.,* 2004) showed that children less than five years were highly infected as compared to more than five years in age. Regarding sex infestation was also high in males than females. According to Chaudhry *et al.,* 2004 similar results from Muzaffarabad whereas Khan *et al.,* 2004 did not find such relationship. The gender is not a risk factor and it does not contribute to prevalence of intestinal parasitic infections. Relationship between the number of examined and that of infected for ages and sex was not significant ($p>0.05$).

CONCLUSIONS

School children in Upper Dir district were found with high parasitic profile than children in Swat district and Lower Dir district. *Ascaris lumbricoides* was highly prevalent soil transmitted helminth followed by *Trichuris trichura* and *Ancylostoma duodenale*. Male students were more parasitized than females. Children above 8 years in Upper Dir district and Swat district while below 8 years in lower Dir district were found more parasitized. The use of human excreta as fertilizer, use of raw vegetables and poor personnel hygiene should be avoided. A campaign about transmission of STHs, unhygienic and illiteracy should be launched in schools and in public sector institutes.

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