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Prevalence And Associated Risk Factors With *Entamoeba histolytica* Infections In School Children

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Abstract

Entamoeba histolytica is medically important pathogenic parasitic protozoan of human in origin which causes amoebiasis that is common in children of devolving nations in tropical and sub-tropical regions of the world. A total of 100 children, comprising 45 boys and 55 girls, were screened to calculate the prevalence and risk factors of *E.histolytica* along with their socio-demographic characteristics among school children at Chkadara, Lower Dir, Pakistan. The stool samples were collected and transported to the Parasitology Laboratory, Zoology Separtment, Malakand University for parasitic investigation. Each of the stool samples were processed through floatation and sedimentation procedures and techniques and examined under the microscope first under low and then high power objectives. The protozoan cysts and the helminth eggs were have been investigated and identified through valid keys. The data was analysed through chi-square test via GraphPad version 5. The p vale was considered statistically significant if less than 0.05 at 95% CI. The results indicates that *Entamoeba histolytica* is a prominent pathogenic protozoan parasite in school children, with a total of 67 cases identified. Boys showed a slightly higher prevalence of this parasite, with 58.8% (n=30/51) cases compared to 58.7% (n=37/63) in boys. *Ascaris lumbricoides*, is another common intestinal parasite, was observed in a total of 35 cases, (30.7%)

with an almost equal distribution among boys (17 cases) and girls (18 cases). Moreover, hookworm species was found in 11 children, with a somewhat higher prevalence in girls 11.1% (7 cases). Giardia spp, although less prevalent, still accounted for 4 cases, equally distributed among boys. *E.histolytica* was highest in prevalence as 58.7 %(67/114) followed by *Ascaris lumbricoides* 30.7 % (n=35/114), then hookworms 9.64 % (n=11/114) while *Giardia* spp was found in least prevalence 0.87 %(n=1/114). Pattern of infection reveal that most of the students were found to be infected with 2 species of parasites including one is *E.histolytica* (37%) followed by single infection 20% with *E.histolytica* while least number of participants were reported to be infected with 3 species of parasites 10%. The overall prevalence was reported as 67%. Age and types of stool were not significantly associated with the prevalence of *E.histolytica* P>0.05, however all other factors sex, diarrheal status, parent education, parent employment status and economic conditions were significantly related to the prevalence of *E.histolytica* infection (P<0.05).

Keywords: Amoebiasis, Ascariasis, hookworm infection, Giardiasis, intestinalpathogenic protozoans

Introduction

Entamoeba histolyica (Eh) is an enteric human pathogenic protozoan parasite (PPP) causes amoebiasis in children living in developing nations of the globe in tropic and sub-tropical regions. Almost 100 million cases per anum of *Eh* resulted in amoebic dysentery, colitis and can develop abscess if left untreated, most commonly the liver. *E.histolytica* was ranked 4th among all the parasitic infection in 2013 which leads 11,300 deaths per year (Abubakar et al., 20151).

Cysts with 4 nuclei is the infective form of this pathogenic protozoan parasite, so it is responsible for carrying the infection from cysts contaminated agencies like water, vegetables and food to the human beings. In most cases the infection is remained asymptomatic.

The encystation is imitated in the colon and the cysts is passed to the external environment along with the passage of stool and contaminated the environmental agencies like food, water, soil and vegetables etc and start the life cycle (Mortimer et al., 2010). An *E.histolytica* infected person can pass 45 million cysts in the stool daily (Mondal et al., 2006). These cysts may entered in to the human host by ingestion of food, water and vegetables and reach to the small intestine. In the small intestine the cysts ruptures and release small anoebulae and recede in to the large intestine for further multiplication.

In large intestine some of them begins the multiplication actively and causes amoebiasis while all others become encysted. So disease is characterized by acute diarrhea often with the presence of blood and mucus, abdominal cramping and fever (Frederick et al., 2005).

Infection with *E.histolytica* was reported during the studies reported earlier in the country with particular emphasis on Malakand region, Pakistan by different workers as (Farooqi, 1964; Haleem et al., 1965; Farooqi, 1965; Ansari & Naru 1968; Pal &Malik 1979; Siddiqi & Bano 1979; Bilqees *et al* 1982; Pal &Rana 1983a; Pal &Rana (1983b); Nawaz&Nawaz 1983; Baqai *et al* 1985; Baqai & Zuberi 1988; Pal & Subhani 1989; Khan *et al* (1993); Akhtar *et al*.,1993; Kamran *et al.*, 2000; Shaik *et al* (2000; Shaikh *et al* (2003); Chaudhry *et al* (2004); Tasawar *et al* (2006); Mehmood et al (2009); Sajjad et al (2009); Nisa et al., 2011; Khan et al., 2015; Khan et al., 2017; Khan et al., 2018; Khan et al., 2019; Arshad et al., 2019; Khan et al., 2022; Khan et al., 2022; Haq et al., 2022; Subhan et al., 2022.

Entamoeba histolytica, is a parasitic amoeba, has long been recognized as a significant public health challenge due to its role in causing amoebiasis, a gastrointestinal disease with potentially severe consequences in the human population. This research study is focused on exploration of prevalence and associated risk factors among school children of Chakdara, Lower Dir, Pakistan.

METHODS AND MATERIALS

STUDY AREA

Different schools at Chakdara, Lower Dir Pakistan were visited from 20 August 2022 to 14 September 2023 to collect the stool samples. Chakdara is located to the north of Malakand, alongside of River Swat. The maximum temperature is reported as 38° C and minimum is 26.5° respectively in the locality (fig 1 and 2).

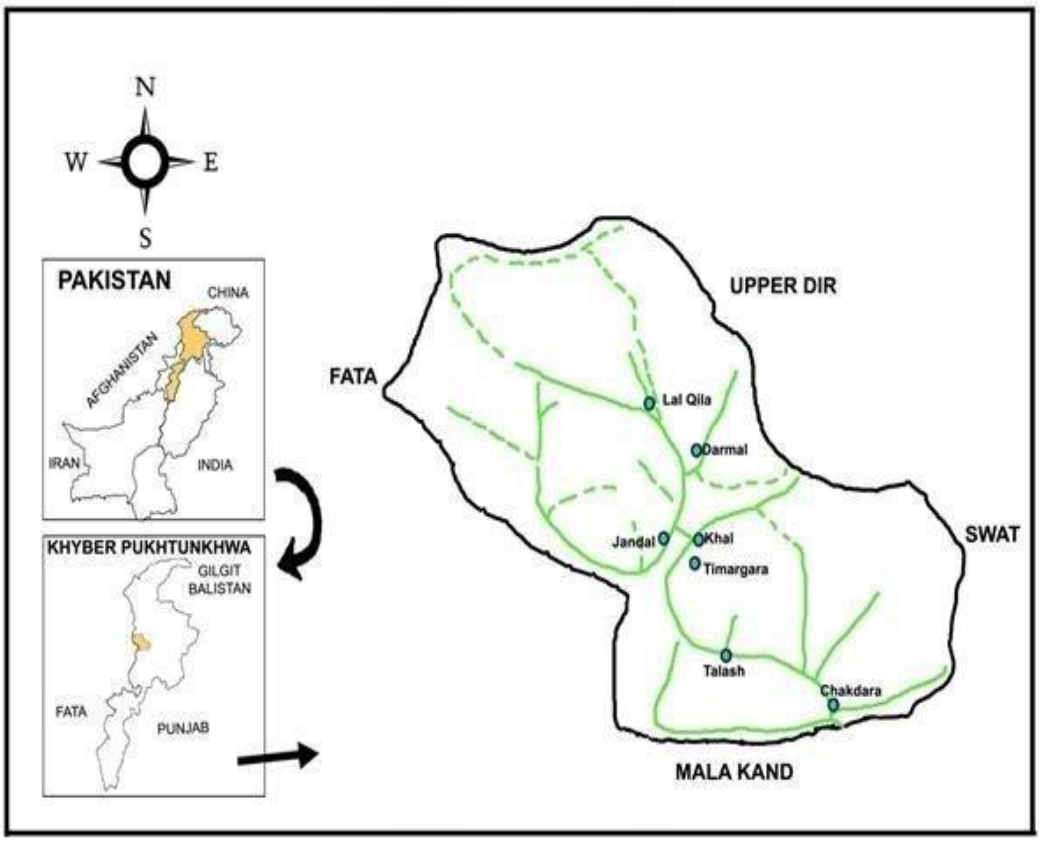

Figure 1. Location of the Study area

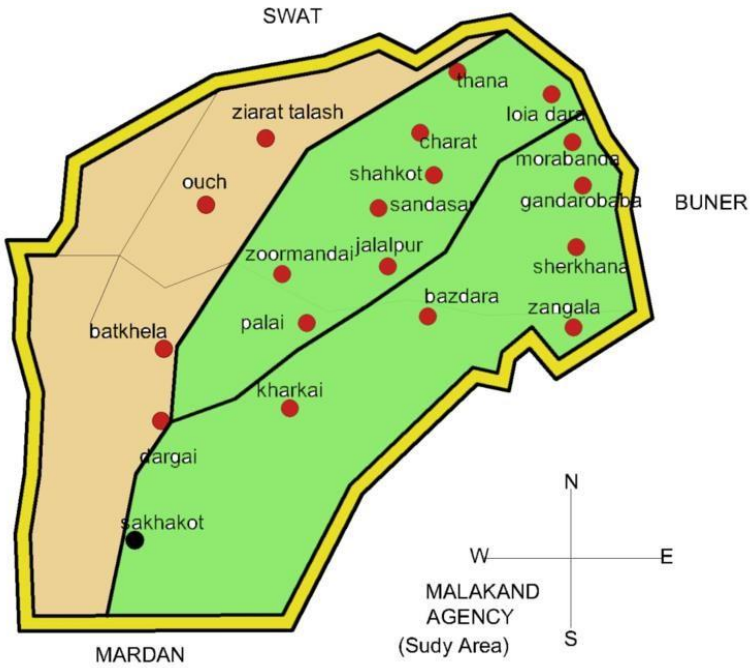

Figure 2: Map of the study area (Chakdara)

Data Collection

Prior to initiation of the study, meeting with the principal of the concerned schools were conducted for the purpose of discussing the important of the study and to take permission to carry it out. Study information was shared with the parents during the parent's teacher meeting days before the exercise to be done including stool sample collection, information on anthropometric measurements were also have been obtained. The data of each respondent was recorded in written on the developed questionnaire. During the collection of data 150 labelled plastic containers with lids were distributed among school children but a total of only 100 consented individuals of age 09-15 years participated in the study from school of Chakdara, Lower Dir, Pakistan. Both male and female students were included.

Laboratory-Based Analysis

Each of the specimens was transported to the Parasitology laboratory, Zoology Department of Malakand University Lower Dir, Pakistan for laboratory investigation. All the samples were seen first under naked eyes for detection of any adult parasite of segments of tapeworms or larval stages of the nematode parasites.

Laboratory Examination

Each of the samples were submitted for the detection of paraite species, and undergoes the process of floatation and sedimentation. The slides were prepared through normal saline solution and lugols iodine procedure and techniques. Each of the prepared samples were screened under the microscope for detection of the parasitic infection and confirm through matching with the standard keys.

Data analysis

Data analysis was carried out through chi-square test on GraphPad version 5. P value was considered statistically significant if less than 0.05 at 95% confidence interval.

Results

A total of 100 children, comprising 45 boys and 55 girls, were surveyed to assess the extent of parasitic infections within this socio-demographic characteristics. The results indicate that *Entamoeba histolytica* is a prominent parasitic infection in school children, with a total of 67 cases identified. Notably, boys exhibited a slightly higher prevalence of this parasite, with 58.8% (n=30/51) cases compared to 58.7% (n=37/63) in boys. *Ascaris lumbricoides*, is another common intestinal parasite, was observed in a total of 35 cases, (30.7%) with an almost equal distribution among boys (17 cases) and girls (18 cases). Additionally, Hookworm was found in 11 children, with a somewhat higher prevalence in girls 11.1% (7 cases). Fasciola, although less prevalent, still accounted for 4 cases, equally distributed among boys. *E.histolytica* was highest in prevalence as 58.7 %(67/114) followed by *Ascaris lumbricoides* 30.7 %(n=35/114), then hookworms 9.64 %(n=11/114) while *Giardia* spp was found in least prevalence 0.87 %(n=1/114) table 1.

Table 1: Over all prevalence of *Entamoeba histolytica* infection among school children in Chakdara, Lower Dir, Pakistan

Characteristics	Boys N=45	Girls N=55	Both sex N=100
Parasite species	Number (%)	Number (%)	Number (%)
Entamoeba histolytica	30 (58.8)	37 (58.7)	67 (58.7)
Ascaris lumbricoides	17(33.3)	18 (28.5)	35 (30.7)
Hookworms	4 (7.84)	7 (11.1)	11 (9.64)
Giardia spp	0	1 (1.58)	1(0.87)
Total number of infection	51	63	114

Pattern of infection reveal that most of the students were found to be infected with 2 species of parasites including one is *E.histolytica* (37%) followed by single infection 20% with *E.histolytica* while least number of participants were reported to be infected with 3 species of parasites 10% (table 2). The overall prevalence was reported as 67%.

Table 2: Pattern of *Entamoeba histolytica* infection in association with other parasites among school children of Chakdara, Lower Dir, Pakistan.

Infection type	Species number	Associated parasites	Number (%)
Single infection	1 spp (n=20)	*Entamoebia histolytica*	20
Total single infection			20(20)
Double infection	2 spp (n=11)	*E.histolytica+Ascaris lumbricoides*	25
		E.histolytica+hookworms	11
		E.histolytica+Giardia lamblia	1
Total double infection			37(37)
Triple infection	3 spp (n=3)	*E.histolytica+A.lumbricoides+hookworm*	9
		E.histolytica+A.lumbricoides+Trichuris trichura	1
Total triple infection			10(10)
All polyparasitism			10
Total number of individuals infected			67(67)
Total examined			100

Age and types of stool were not significantly associated with the prevalence of *E.histolytica* P>0.05, however all other factors sex, Diarrheal status, parent education, parent employment status and economic conditions were significantly related to the prevalence of *E.histolytica* infection (P<0.05). Table 3.

Table 3: Risk factors analysis for the prevalence of *E.histolytica* among school children of Lower Dir, Pakistan

Variables	**No. examined (%)**	**No. infected (%)**	**No. negative (%)**	**P Value**
Age (Years)				
09-10	35	19(54.2)	16	0.1264
11-12	35	25(71.4)	10	
13-15	30	23(76.6)	07	
Sex				
Male	45	39(86.6)	06	0.0002
Female	55	28(50.9)	27	
Type of stool				
Hard	21	15(71.4)	06	0.6356
Soft	27	20(74.0)	07	
Watery	24	14(58.3)	10	
Loose	28	18(64.2)	10	
Diarrheal status				
Yes	34	25(73.5)	9	0.0371
No	66	42(63.6)	24	
Parents education status				
Can write and read	69	37(53.6)	32	0.0052
Cannot write and read	31	30(96.7)	07	
Parents employments status				
Government servant	50	28(56)	22	0.0193
Non-government servants	50	39(78)	11	
Economic status				
Less than 1000 Pakistani rupees/day	18	9(50)	9	0.0903
More than 1000 Pakistani rupees/day	82	58(70.7)	24	

Review of the published literature in Pakistan revealed that current study showed the higher prevalence 58.7% of *E.histolytica*, hence the highest rate was reported by Arshad et al., 2019 which is 66.1% among the hospital

patients in Karachi, Pakistan. Second to the study of Arshad et al., 2019 a study was conducted by Bilqees et al., 1982 which showed 60.6% of the prevalence of this protozoan parasite. Tasawar et al., 2006 was reported 50.9% of the prevalence in mixed population of Mathelo. The lowest prevalence rate was investigated as 0.8% by Ansari and Naru (1968) in mixed population of Lahore, Pakistan. Similar results were also have been counted by Baqai et al., 1985 in Karachi healthy children (Supplementary table 1).

Supplementary table 1: Review of *Entamoeba histolytica* in Pakistan with special reference to Malakand region

Author (ref)	**Study area**	**Population studied**	**Size**	**Prevalence (%)**
Farooqi 1964	Multan	Well persons	675	3.5
Haleem *et al* (1965)	Karachi	Mixed population	6197	13.5
Farooqi (1965)	Multan	Medical students	224	11.6
Ansari & Naru (1968)	Lahore	Mixed population	1771	0.8
Pal &Malik (1979)	Islam abad	School children	3478	11.9
Siddiqi & Bano (1979)	Peshawar	School children	400	14.5
Bilqees *et al* (1982)	Karachi	Mixed population	3249	60.5
Pal &Rana (1983a)	Rawalpindi	Mixed Population	5360	5.31
Pal &Rana (1983b)	Islam Abad	Mixed Population	3490	5.64
Nawaz&Nawaz(1983)	Peshawar Univ.	Food handlers	166	12.6
Baqai *et al* (1985)	Karachi	Healthy children	160	0.6
Baqai & Zuberi (1988)	Karachi	Diarrhea patients	455	2.50
Pal & Subhani (1989)	Dir district	School children	2027	36.5
Khan *et al* (1993)	H.abad &L. abad	Mixed population	2222	4.68
Akhtar *et al* (1993)	Lahore	Hospital patients	2651	3.2
Kamran *et al* (2000)	Karachi	Hospital patients	124	0.80
Shaik *et al* (2000)	Larkana	M. population	2438	15.8
Shaikh *et al* (2003)	Shikarpur	Mixed population	2540	15.4
Chaudhry *et al* (2004)	Muzaffar abad K	Children	287	5.90
Tasawar *et al* (2006)	Mirpur Mathelo	Mixed Population	3317	50.9
Mehmood et al (2009)	Vehari	U.D. Pakistan	500	20.2
Sajjad et al (2009)	Quetta City	D.C and School	110	1.08
Nisa et al., 2011	Swat, Pakistan	Shepherds	256	7.26
Khan et al., 2015	Swat, Pakistan	Education departments	420	4.36
Khan et al., 2017	Swat, Pakistan	Food handlers	267	14.2
Khan et al., 2018	Swat, Pakistan	Occupation groups	1041	30.5
Khan et al., 2019	Swat, Pakistan	Drug addicts	450	3.33
Arshad et al.,2019	Karachi	Hospital patients	2212	66.1%
Khan et al., 2022	University of Malakand	Food handlers	642	5.64%
Khan et al., 2022	Malakand	School children	184	3.37
Ul-Haq et al., 2022	Lower Dir (rural)	School children	324	7.61
Subhan et al., 2022	Bajaur district	School children	402	15.4
Current study 2023	Lower Dir	School children	100	58.7

DISCUSSION

Findings of the current study underscore the prevalence of Entamoeba histolytica which is 58.7% among school-going children in Chakdara, Lower Dir, Pakistan. The data reveals varying infection rates and emphasizes the importance of tailored public health interventions to address these parasitic infections and improve the overall health and well-being of the local child population.

It is however, the findings of the present research was similar with the findings of Arshad et al., 2019 which is 66.1% among the hospital patients in Karachi, Pakistan. Bilqees et al., 1982 conducted a study on general population of Karachi, Pakistan which showed 60.6% of the prevalence of this protozoan parasite. Tasawar et al., 2006 was reported 50.9% of the prevalence in mixed population of Mathelo, Pakistan.

The lowest prevalence rate was investigated as 0.8% by Ansari and Naru (1968) in mixed population of Lahore, Pakistan. Similar results were also have been counted by Baqai et al., 1985 in Karachi healthy children (Supplementary table 1).
Exploring *Entamoeba histolytica* prevalence of various parasitic infections among school-children highlights the distribution of four distinct parasite species, namely *Entamoeba histolytica, Ascaris Lumbricoides,* Hookworm, and Fasciola spp, and their respective occurrences among both boys and girls.

The findings indicate that *Entamoeba Histolytica* is a notable parasite in this population, with 25 cases detected in total, comprising 10 cases in boys and 15 in girls. Additionally, Ascaris Lumbricoides and Hookworm also exhibit a considerable presence, with 23 and 15 cases, respectively, across both sexes. Fasciola, although less prevalent, still accounts for four cases among the surveyed children.
Such information is instrumental for the research on importance of studying *Entamoeba histolytica* and its interactions within a broader context of co-infections with other parasites. Understanding the prevalence of these parasites is essential for comprehending the health risks and potential synergistic effects that these infections may have on the well-being of the children in the region.

Infection prevalence of *E.histolytica* was reported during the studies reported earlier in the country with particular emphasis on Malakand region, Pakistan. These reports were as 3.5% (Farooqi, 1964); 13.5% (Haleem et al., 1965); 11.6% (Farooqi, 1965); 0.08% (Ansari & Naru (1968); 11.9% (Pal &Malik (1979); 14.5% (Siddiqi & Bano (1979); 60.5% (Bilqees *et al* (1982); 5.31% (Pal &Rana (1983a); 5.64% (Pal &Rana (1983b); 12.6% (Nawaz&Nawaz(1983); 0.06% (Baqai *et al* (1985); 2.50% (Baqai & Zuberi (1988); 36.5% (Pal & Subhani (1989); 4.68% (Khan *et al* (1993); 3.21%(Akhtar *et al* (1993); 0.80% (Kamran *et al* (2000); 15.8% (Shaik *et al* (2000); 15.4% (Shaikh *et al* (2003); 5.90% (Chaudhry *et al* (2004); 50.9% (Tasawar *et al* (2006); 20.2% (Mehmood et al (2009); 1.08% (Sajjad et al (2009); 7.26% (Nisa et al., 2011); 4.36% (Khan et al., 2015); 14.2% (Khan et al., 2017); 30.5% (Khan et al., 2018); 3.33% (Khan et al., 2019); 66.1% (Arshad et al., 2019); 5.64%(Khan et al., 2022); 3.37% (Khan et al., 2022); 7.61% (Haq et al., 2022); 15.4% (Subhan et al., 2022.

CONCLUSIONS

The current study was pinpoint to understand the prevalence and risk factors associated with the prevalence of *Entamoeba histolytica* among school children at Chakadara, Lower Dir, Pakistan. The results indicated that *Entamoeba histolytica* is a prominent pathogenic protozoan parasite while *Ascaris lumbricoides*, is another common intestinal parasite followed by Hookworm and Giardia spp which was less prevalent in both male and females. Regarding pattern of infection 2 species of parasites including one is *E.histolytica* followed by single infection with *E.histolytica* while least number of participants were reported to be infected with 3 species of parasites. Age and types of stool were not significantly associated with the prevalence of *E.histolytica* $P>0.05$, however all other factors sex, Diarrheal status, parent education, parent employment status and economic conditions were significantly related to the prevalence of *E.histolytica* infection ($P<0.05$).

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