Intestinal parasitic infection among middle school boy students in Jeddah, Saudi Arabia

Original Article

Majed H M Wakid[1,2]

King Abdulaziz University, Faculty of Applied Medical Sciences, Department of Medical Laboratory Technology[1], and Special Infectious Agents Unit, King Fahd Medical Research Center[2], Jeddah, Saudi Arabia

ABSTRACT

Background: Intestinal parasitic infection with protozoan and helminthic parasites are among the major health affairs especially in tropical and subtropical regions like Saudi Arabia. Globally, billions including school students are infected and millions suffer from symptoms and complications, while others are asymptomatic.

Objectives: This study was conducted to evaluate infections of middle school boys in Jeddah with intestinal parasites and the risk factors involved.

Subjects and Methods: Stool samples were collected from 265 boys from two middle schools in Jeddah. The samples were examined physically then by different techniques including: direct smears, sedimentation technique, two permanent staining methods and a rapid immuno-chromatographic assay. Information about each student and his family was collected in a questionnaire form related to lifestyle and socio-demographic data.

Results: Forty-six students (17.35%) were infected with intestinal parasites including seven protozoa; mainly *Blastocystis* spp. *Giardia lamblia*, *Entameba histolytica/dispar*, and three helminth parasites including *Hymenolepis nana, Ascaris lumbricoides* and *Trichuris trichiura*.

Conclusion: Several factors were associated with the intestinal parasitic infections, including age, proper washing of hands and cutting fingernails. There is a need for regular health education programs for students and their parents to increase awareness about intestinal parasites.

Keywords: intestinal parasites, Jeddah, Saudi Arabia, school, students.

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Corresponding Author: Majed H M Wakid, **Tel.:** +966-503627311, **E-mail:** mwakid@kau.edu.sa

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INTRODUCTION

The world health organization (WHO) considers intestinal parasitic infections a worldwide major public health problem. This predicament particularly applies to Tropical and poor countries with low environmental hygiene. The infection rate with intestinal parasites reaches some 3.5 billion of which 450 million are symptomatic[1]. Some parasites can cause serious complications such as cancer, anemia, mental and growth retardation. This can lead to poor education achievement among school children[2-4]. In addition, parasitic infection is known to cause antagonistic interaction against other parasites, viruses, bacteria and microbiota[5,6].

Transmission of protozoan intestinal parasites is mainly associated to fecal oral route through consumption of contaminated water and food. In addition, contaminated soil is another important source for infection especially for hookworm, *Strongyloides stercoralis*, *A. lumbricoides* and *T. trichiura*. The level of education, socio-economic status and sanitary status are associated with the high prevalence level[7].

Intestinal parasitic infection among middle school students in Jeddah has not been investigated previously. Our study will investigate data concerning common intestinal parasites and the risk factors among this group of students in Jeddah.

SUBJECTS AND METHODS

Sample design: This cross-sectional study was conducted in Jeddah in Western region of Saudi Arabia. Students studying at middle schools for boys in the South part of Jeddah were included in the present study. The plan targeted 320 students who were registered to participate in the study. Each student was provided with a consent form, labeled wide mouth stool container and instructions for collection. Nationality, age, and other data about each student and his family were obtained in a questionnaire form.

Physical examination of stool samples: Stool color, consistency, associated gross blood or mucous, presence of whole adult worms or tapeworm gravid segments was determined in each sample.

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Microscopic analysis: The stool samples were prepared for examination by the wet direct mount, sedimentation concentration method, and permanent staining using trichrome stain and modified Kinyoun's stain. Samples were examined accordingly under a light microscope using 10x, 40x and 100x objective lenses.

Direct smears: As described previously[8], for each stool sample, 1-2 mg was emulsified in a drop of normal saline and a drop of iodine on a clean glass slide (75X26 mm). A cover glass (22X22 mm) was placed carefully on each preparation and then examined under light microscope using 10x and40x objective lenses.

Formal ether sedimentation technique: About 2-3 gm of each stool sample was emulsified in 10 ml of 10% formal-saline, then filtered through two layers of gauze and centrifuged at 2000 rpm for 5 min. The supernatant was disposed of, then the washing step was repeated. The sediment was re-suspended in 10 ml formal saline (10% v/v), 3 ml of diethyl ether was dispensed and mixed strenuously for 15 sec, then spun at speed of 2000 rpm for 5 min. The top three layers were discarded, the sediment re-suspended in 2 drops of iodine, covered and then examined microscopically under low and high objective lenses[9,10].

Trichrome permanent staining: Para-Pak® Trichrome Stain, (Meridian Bioscience, Germany) of fixed specimens was carried out according to the manufacturer's procedure[11]. Polyvinyl alcohol preserved stool samples were smeared, air-dried then placed in 70% iodine-alcohol for 10 min, followed by two changes of 70% ethanol for 5 min each. After that smears were stained with trichrome stain for 8 min, placed in acidified alcohol for 5-10 sec, two quick dips in 95% ethanol, then in two changes of 95% ethanol for 5 min each, followed by absolute ethanol for 3 min and finally placed in xylene for 3 min. Mounted slides were examined using oil immersion objective lens.

Modified Kinyoun's staining: About 5 mg of stool was smeared on microscope slides, air dried then fixed in methanol. Smears were flooded with carbol fuchsin stain for 10 min, rinsed with tap water, and then decolorized in 1% sulfuric acid for one min. After that, all smears were washed with tap water, counter stained with malachite green for 5 min, washed with tap water and air dried. Stained smears were examined using oil immersion objective lens.

Chromatographic immunoassay for *Cryptosporidium*: Cer-Test rapid card was used and performed according to the instruction of the manufacturer[12]. For each specimen, 150 mg was emulsified in the diluent-tube and then shaken vigorously. After that, 4 drops were dispensed into the circular window of the card. After 10 min, the result was interpreted as positive with the formation of two bands, green for control and red at the test line.

Statistical analysis: Our data were statistically analyzed using SPSS V20 software for windows. *P* value < 0.05 was considered statistically significant.

Ethical considerations: Approval for this study proposal was obtained from the Committee of Research and Ethics at the Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. Permission letter was obtained from Ministry of Education to collect samples from students. We obtained signed consent forms from all participating students or their parents after providing them with the aim and objective of the work. Each infected student was provided with a personal report of our finding for presentation to the authorities in the Health Unit of the Ministry of Education to prescribe treatment.

RESULTS

Demographic description of the students: A total of 265 boy students (83%) at middle school level cooperated and provided stool samples, completed the questionnaire forms and signed the consent forms. The remaining 55 students were excluded. The age of the investigated students ranged from 11-18 years (13.60±1.65), while 51% of them were between 15-16 years old. As shown in table (1), students were of different nationalities: Saudi Arabia, Asian and African countries (Afghanistan, Palestine, Pakistan, Syria, Yemen, Turkey, Chad, Egypt, Nigeria and Somalia).

Table 1. Parasitic infected cases relative to nationality.

Nationality	Total	Positive cases			
	No. (%)	No.	%[a]	%[b]	%[c]
Saudi	64 (24.15)	10	3.77	21.74	15.63
Asian	144 (54.34)	25	9.43	54.35	17.36
African	57 (21.51)	11	4.15	23.91	19.30
Total	265 (100)	46	17.35	100.00	

a relative to the total number of the students.
b relative to the total number of infected students.
c relative to the number of students in the same nationality.

Macroscopic examination of stool samples: Color was brown in most of the samples (77.36%), with no significant relation between color of stool and infection with intestinal parasites (*P*>0.05). No adult worms or gravid segments were detected. The consistency ranged between formed to soft and loose, but no watery or bloody specimens were recorded. There was no significant relation between consistency of the collected stool samples and intestinal parasitic infection (*P*>0.05).

Prevalence of intestinal parasites: A total of 46 students (17.35%) were infected with intestinal parasites (Table 2). Ten different parasites were detected, comprising 7 protozoan and 3 helminth parasites. On the whole 35 students were infected

with single parasites, 8 with double parasites and the remaining three with triple parasites. The detected parasites among the infected cases were *Blastocystis* spp. (15/46, 32.61%); *G. lamblia* (9/46, 19.57%); *E. histolytica/dispar* (9/46, 19.57%); *E. nana* (8/46, 17.39 %); *Chilomastix mesnili* (5/46,10.87 %); *E. coli* (5/46, 10.87 %); *H. nana* (4/46, 8.70%); *Iodamoeba butschlii* (3/46,.6.52%); *A. lumbricoides* (1/46, 2.17%); *T. trichiura* (1/46, 2.17%). None of the students were infected with *Cryptosporidium* spp.

Age groups and distribution of infection: The highest infection rate (35%) was among 14 years old students, and the lowest among 11 and 12 years old students (4.38% for each). There was a significant relation between age of the students and infection with intestinal parasites (P<0.05).

Students' lifestyle: There was a statistically significant association (P<0.05) between handwashing habit (before meals and after toilets) and infection with intestinal parasites among the investigated group (Table 3). A lower rate of infection was recorded (15.64%; 38/243) in those students who washed their hands regularly as compared to those who did not habitually do so (36.36%; 8/22). Similarly, results for eating food from restaurants and fast food outlets, and fingernails trimming were significant (P< 0.05), but association with the source of drinking water was insignificant (P> 0.05).

Table 2. Numbers of parasitic infections relative to nationality.

	Detected parasites	Nationality		
		Saudi	Asian	African
Single (No. = 35)	*Blastocystis* spp. (11)	2	7	2
	G. lamblia (7)	1	4	2
	E. histolytica/dispar (7)	1	3	3
	E. nana (2)	1	1	-
	E. coli (1)	1	-	-
	I. butschlii (1)	1	-	-
	H. nana (4)	1	2	1
	*A. lumbricoide*s (1)	-	-	1
	T. trichiura (1)	-	1	-
Double (No. = 8)	*E. nana* + *C. mesnili* (3)	1	1	1
	Blastocystis spp. + *E. coli* (2)	1	1	-
	Blastocystis spp. + *E. histolytica/dispar* (1)	-	1	-
	E. histolytica/dispar + *G. lamblia* (1)	-	1	-
	Blastocystis spp. + *G. lamblia* (1)	-	1	-
Triple (No. = 3)	*E. coli* + *E. nana* + *I. butschlii* (1)	-	1	-
	E. nana + *C. mesnili* + *I. butschlii* (1)	-	1	-
	E. coli + *E. nana* + *C. mesnili* (1)	-	-	1
	Total infected students (46)	10	25	11

Table 3. Lifestyle factors in relation to parasitic infections.

Factor	Infected	Negative	Total	Statistical analysis
Proper hand washing				
Regular	38	205	243	
Not regular	8	14	22	$X^2 = 6.04$, $P < 0.05$
Fingernails cutting				
Regular	33	192	225	
Not regular	13	27	40	$X^2 = 7.53$, $P < 0.05$
Source of drinking water				
Direct tap water	14	75	89	
Filtered tap water	12	69	81	
Bottled water	20	65	85	$X^2 = 3.52$, $P > 0.05$
Eating outdoor				
Always	22	37	59	
Sometimes	13	90	103	
Rarely	11	92	103	
Never	NA	NA	NA	$X^2 = 21.1$, $P < 0.05$
Residency in Jeddah				
South	18	75	93	
North	3	12	15	
West	10	70	80	
East	15	62	77	$X^2 = 1.89$, $P > 0.05$
Total of each factor	**46**	**219**	**265**	

Residency location, parents' education, and infection: There was no significant association (P> 0.05) between parents' level of education and infection with intestinal parasites among the investigated students, (Table 4). Association of residency location was also insignificant (Table 3). Not all students provided details about the monthly income of their families. Therefore, we were not able to investigate this factor in relation to the infection risk.

Table 4. Parents education level in relation to parasitic infection of their boys.

Education level	Fathers Number	Infected students	Negative students	Mothers Number	Infected students	Negative students
Illiterate	63	10	53	85	11	74
Writer (no degree)	1	0	1	2	1	1
Primary	61	11	50	66	13	53
Middle	0	NA	NA	46	5	41
Secondary	51	4	47	58	12	46
Bachelor	62	13	49	6	3	3
Master	22	5	17	0	NA	NA
PhD	5	3	2	2	1	1
Total (265)	**265**	**46**	**219**	**265**	**46**	**219**
	X^2 = 10.9, P > 0.05			X^2 = 10.6, P > 0.05		

DISCUSSION

Several sets of geographical, demographic, socioeconomic, environmental, behavioral and hygienic factors influence the distribution of entero-parasitosis[13]. To the best of our knowledge, our study is the first in Saudi Arabia to investigate students at the middle school level for prevalence of intestinal parasites. Previous studies involved only primary schools children[14-21].

Macroscopic examination of the stools didn't reveal the presence of any adult worms or segments and none of the samples had abnormal color. Consistency of stool, in relation to symptoms of diarrhea, was around 30%, but with no significant relation to parasitic infection. These results are in agreement with the observations in previous studies dated 2004 and 2019 in Jeddah[14,17].

In the current study, a single infection was detected in 76.09% (35/46) samples including protozoan and helminths parasites: *Blastocysts* spp., *G. lamblia*, *E. histolytica/dispar*, *H. nana*, *A. lumbricoides* and *T. trichiura*. Commensal *E. nana, E. coli,* and *I. butschlii* were also recorded. Meanwhile, among the infected 46 students, double infections with pathogenic and non-pathogenic protozoan parasites were observed in eight students (17.39%). The association included *E. nana* and *C. mesnili; Blastocysts* spp. and *E. coli; Blastocysts* spp. and *E. histolytica/dispar*; *E. histolytica/dispar* and *G. lamblia*; *Blastocysts* spp. and *G. lamblia*. In triple infections three students (6.52%) were infected with commensal protozoa: *E. coli, E. nana* and *I. butschlii; E. nana, C. mesnili* and *I. butschlii; E. coli, E. nana* and *C. mesnili*.

Our results indicate that, similar to a previous observation by a recent study among primary school children in Jeddah[14], the highest infection rate was caused by *Blastocysts* spp., including co-infection cases. In contrast, another previous study in 2004 didn't detect any *Blastocysts* spp.[17]. The co-infection of *Blastocysts* spp. with other intestinal protozoan parasites may be due to resemblance in oral mode of transmission[22]. Although this protozoan parasite infects nearly one billion people all over the world[23], its pathogenicity is still greatly debatable and controversial, as some investigations correlated the parasite to gastrointestinal diseases, while others consider it non-pathogenic[24,25].

Our study demonstrated that the investigated students from Asian countries (including Saudis) represented (78.49%; 208/265), while students from African countries represented (21.51%; 57/265). Although the overall infection rate among the Asian students (including Saudis) in comparison to the total infected cases (76.09%, 35/46), was higher than in the African students (23.91%, 11/46), the infection rate among the investigated African students (19.30%, 11/57) was higher than the Asian students (16.83%, 35/208). Even so, there was no significant association between intestinal parasitic infection and nationality of the students (P> 0.05).

Based on our results among the infected students, two parasites *E. histolytica/dispar* and *G. lamblia* were the second highest detected intestinal parasites (19.75%, 9/46 respectively). *E. histolytica/dispar* is the only pathogenic intestinal protozoan amoeba detected. We know that *E. histolytica* can cause amoebic dysentery or severe extra-intestinal infection in the lung, liver and brain[26,27]. It was estimated that worldwide there are around 50 million infected cases by *E. histolytica/ dispar* per year. Generally, the areas of highest infection rate are related to the inadequate sanitation conditions as well as crowded populations especially in South and Central America, Tropical Asia and Africa[28]. *G. lamblia* is the only pathogenic intestinal protozoan flagellate

observed, while *C. mesnili* is a non-pathogenic flagellate. Annually, some 280 million cases of giardiasis are reported. Among those are asymptomatic cyst passers, cases with acute or chronic diarrhea, failure to thrive in children and malabsorption of fat, vitamins, protein, D-xylose, iron and lactose[29].

In the present study, eight cases (8/46, 17.39%) were infected with *E. nana*, which is another non-pathogenic intestinal protozoan, in addition to *C. mesnili* and *E. coli* (10.87 % respectively) and *I. butschlii* (6.52%). A previous study also from Jeddah, Saudi Arabia, also reported the three non-pathogenic protozoa (*E. nana, E. coli* and *C. mesnili*) with very low prevalence rates[14]. The fundamental mode of infection for pathogenic and non-pathogenic protozoa is through the feco-oral route, possibly leading to co-infection by more than one type, and resulting in diarrhea due to the concomitant pathogenic organisms[30]. Further attention may need to be directed to the importance of reporting non pathogenic protozoans that may be associated with concomitant pathogenic forms. This calls for more accurate laboratory identification and reporting. Furthermore, as described above, the fact that both pathogenic and non-pathogenic protozoa are acquired in the same manner, triggers a message for the patients about the possibility of getting infected by pathogenic parasites if they continue practicing unsanitary conditions in food or contaminated water supplies[31].

To further investigate the possibility of cryptosporidiosis among the study group, two special techniques were conducted. All stool samples reacted negatively with both rapid diagnostic test and modified Kinyoun's stain. Previous studies involving school children in Saudi Arabia did not include *Cryptosporidium* in their investigations. The risk of infection with this coccidian opportunistic parasite is greater in immunosuppressed and immunocompromised patients[32].

Three helminthic infections were detected in our study; one cestode species (*H. nana*) and two nematode species (*A. lumbricoides* and *T. trichiura*). *H. nana* was found in four students; Saudi, Yemeni, Sudanese and Turkish. Meanwhile *A. lumbricoides* was detected among an Egyptian student, and *T. trichiura* infection was only in a Yemeni student. The previous recent study among primary school children in Jeddah detected one case with *H. nana*[14], while the other earlier study reported one case with hookworm[17].

In general, our results indicate that among the infected cases, protozoan infection (70%) was higher than helminth infection (30%). A similar observation was reported by both previous studies among primary schoolchildren in Jeddah[14,17]. We believe this is strongly attributed to the direct feco-oral transmission of intestinal protozoan amoebas and flagellates, in addition to the fact that the environment in Jeddah city is suitable for the survival of the infective stages. This also applies to the cestode *H. nana*, and the nematodes *A. lumbricoides* and *T. trichiura*. On the other hand, there was no record of trematode infection in the studied students. This finding maybe explained by the complicated life cycle of flukes that require one or more intermediate hosts to complete it and produce the infective stage. Suitable conditions for maintaining this cyclic propagation is not available in Jeddah.

Finally, we found that the main three significant risk factors that correlated with intestinal parasitic infections among the investigated students were proper handwashing habits before meals and after toilets, fingernails trimming, and regular dining out rather than eating home cooked meals. Some of these factors are consistent with those obtained in the two previous studies in Jeddah[14,17]. We assume that the first two factors are indoor and related to the level of hygiene and health awareness of the students and their families. Furthermore, as reported previously, the third factor may be related to the outdoor risk of infection from food handlers in Jeddah[33].

Conclusion and recommendation: The present study is the first in Saudi Arabia to include *Cryptosporidium* in an investigation of the prevalence of intestinal parasites, and its exclusion among this sample of students in the middle level education. The prevalence with seven protozoan parasites was nearly 18% including *Blastocysts* spp. and the pathogenic *E. histolytica/dispar* and *G. lamblia*. Three helminth parasites were detected including *H. nana*, *A. lumbricoides* and *T. trichiura*. The recorded prevalence could be correlated to several factors including mainly the age, as middle school students are more mature and eat outside their homes more frequently. In addition, there was an association of intestinal parasitic infection with the unhygienic habits of ignoring hands washing and cutting of fingernails.

There is a necessity for special health education programs oriented for students and their parents to increase the awareness about intestinal parasites to help in effective control. Furthermore, we recommend that the municipality of Jeddah requests food handlers to perform compulsory periodic stool analysis, even the asymptomatic cases. Lastly, regular hygiene and food-handling practice programs are essential.

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Conflict of Interest: The author declares no conflict of interest.

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