Prevalence of Intestinal Protozoan Parasitic Infection and its Risk Factors among Primary School Children in Mukalla City-Hadramout/Yemen

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A B S T R A C T

Intestinal protozoan diseases in Yemen are a significant health problem with high prevalence rates. This study aimed to determine the prevalence of intestinal protozoan parasites among school children, and identifying risk factors associated with infection in Mukalla city-Hadramout/Yemen. In this cross-sectional study, 385 stool samples were collected from 238 males and 147 females of age group children 7-11 years. All samples were examined using direct method to identify microscopically possible diagnostic stages of intestinal protozoan parasites, and data were collected via standard questionnaire. A total of 286 children found to be positive for at least one protozoan parasite infection with an overall estimated prevalence of 74.3%. The distributions of intestinal protozoan parasites among school children were Entamoeba histolytica/dispar 130(33.8%), Giardia lamblia 76(19.7%) and multiple infections of these intestinal protozoan stages 80(20.8%). The prevalence was significantly among the different selected schools (P < 0.05). The rate of infection was highest among the age group 7-8 years 126(32.7%), as well as the infection rates of 180(46.8%) and 106(27.5%) were recorded among males and females respectively. This study indicated that infection of intestinal protozoan parasites was high prevalent among school children and being most common with Entamoeba and Giardia infections. Low personal hygiene, unsanitary condition, economic status and contact with animals were important predictors factors for intestinal protozoan infections.

Keywords
Intestinal protozoa, Entamoeba histolytica/dispar, Giardia lamblia, Prevalence

Introduction

Intestinal parasitic and protozoan infections are amongst the most common infections worldwide. It is estimated that about 3.5 billion people are affected, and cause clinical morbidity in approximately 450 million individuals as a result of these infections (WHO, 2010), the majority of the cases occur among school aged children (Pradhan et al., 2014).

Entamoeba histolytica (E. histolytica) is estimated to inflict severe disease in 48 million individuals around the globe. Severe E. histolytica infections in children often cause dysentery and bloody diarrhea. E. histolytica infects the large intestine, severe
infection result in serious loss of blood and also may cause systemic problem such as liver abscesses due to infection of the liver by the parasite. Amoebiasis can cause nutrient loss and can lower the levels of circulating proteins, this sometimes leads to under nutrition (Stanley, 2003; Feng and Xiao, 2011). *Giardia lamblia* (*G. lamblia*) is the most common protozoal infection of the human small intestine.

Infections with *G. lamblia* damages the intestinal mucosa and results in mal absorption of nutrients, particularly fat, carbohydrates, and vitamins, especially vitamins A. It seems to be commonly seen in children with under nutrition and results in impaired growth and weight loss in children (Eckmann and Gillin, 2001).

The distribution of intestinal protozoan infection depends on many factors, these include socio-demographic variables as well as unpredictable factors such as natural disasters contribute to the problem (Thapar and Sanderson, 2004). In addition to that, some countries mainly have tropics or subtropics climates and relatively humid areas that combined with poverty, malnutrition, personal and community hygiene, high population density, unavailability of potable water and low health status, poor sanitary facilities all provide optimum conditions for the growth, transmission of these parasites and increase the probability of exposure to intestinal parasites (Thapar and Sanderson, 2004; Sayyari *et al.*, 2005; Raza and Sami, 2009). Children ages two to four years old as well as elementary school children ages 5–14 years old are at highest risk for soil-transmitted helminthes infections due to poor personal hygiene, frequent outdoor exposures and high risk behavior. These children are most prone to significant morbidity especially those with chronic untreated infections (Belizario *et al.*, 2011). Yemen is heavily affected by intestinal protozoan infection due to poor personal and environment hygiene, poor water quality and toilet coverage. Indeed, identifying predictors of intestinal protozoan infections is crucial for effective implementation of control strategies in combating these intestinal protozoan infections. Therefore, studies on the prevalence of intestinal protozoan infection and identifying risk factors in the community are important to design appropriate intervention strategies. The aim of this study was to determine the prevalence of intestinal protozoan infection and associated risk factors to improve the general health and suitable solutions to eliminate the intestinal protozoan infections among school children in Mukalla city-Hadramout/Yemen.

**Materials and Methods**

**Study design and setting**

A cross sectional study was carried out in Mukalla city-Hadramout/Yemen. Four primary schools were randomly selected for both boys and girls in this study.

**Sample size and study population**

A total of 385 school children were included in the study, 238(62%) were males and 147 (38%) were females. The study population included school children attending years 7–11 at the selected schools. The age groups distribution categorized into four groups, 7-8 years 172 (45%), 8-9 years 77 (20%), 9-10 years 70 (18%) and 10-11 years 66 (17%).

An informed consent was obtained from all students who participate in the interviews. Students privacy was respected. The permission was obtained from the local educational authority and each school headmaster in the study area.
Inclusion and exclusions criteria

All primary school age children with suspected intestinal protozoan infection were included, whereas primary school age children were excluded when were they are without suspected intestinal protozoan infection.

Data collection

Prior to sample collection, a standardized questionnaire was used to obtained information concerning the socio demographic, behavioral habits, environmental conditions, hygienic awareness. The participants were also asked about health conditions and history of abdominal pain, diarrhea, nausea, vomiting and weight loss.

Samples collection and examination

Stool samples were collected using a sterile, clean, dry, leak-proof, wide-mouthed plastic containers with brief instructions on how to collect the samples. Each sample was labeled properly to correspond with the number of the questionnaire given. Collected stool samples were transported to the laboratory for examination after adding formaldehyde solution. Examination of the samples carried out within two hours of collection. The appearance of faecal specimens was reported macroscopically for colour, consistency, presence of blood and mucus. Microscopic examination of stool samples for the presence of intestinal protozoan cysts or trophozoites was done by direct saline-Logol’siodine wet mount method (Cheesbrough, 2006).

Statistical analysis

The data were analyzed by statistical package for social sciences (SPSS) version 19.0 software. Descriptive statistics was used to measure the frequencies and percentages. Deductive statistics was used to measure the independent-samples, T-test and One-Way ANOVA. Correlation (person coefficient) was used to define a difference between the variables. Statistical significance was defined at P-values less than 0.05.

Results and Discussion

The total number of infected school children with intestinal protozoa were 286 (74.3%). The types of intestinal protozoan parasites detected were E. histolytica/dispar 130 (33.8%), G. lamblia 76(19.7%) and multiple infection of E. histolytica/dispar and G. lamblia 80 (20.8%), the differences statistically was significant among the four selected primary schools (P-value 0.049) as given in table (1).

Furthermore, multiple infections were clearly identified. The results collectively indicated that 206(72%) of the positive cases were infected with one stage of parasite, whereas 80(28%) were infected with twoand three stages of parasites, table (2).

The infection rate of intestinal protozoan parasites in males was 180(46.8%) and in females 106(27.5%). The types of intestinal protozoan detected among males were E. histolytica/dispar 68(17.7%), G. lamblia 49(12.7%) and multiple infection 63(16.4%). For females, E. histolytica/dispar 62(16.1%), G. lamblia 27(7.0%) and multiple infection 17(4.4%). The differences statistically was not significant (P-value= 0.444) as show in table (3).

The infection rate of intestinal protozoan parasites among different age groups is clearly shown in table (4). The distribution of infections was high among the age group 7-8 years 126 (32.7%), followed by 8-9 years 58 (15.1%), 9-10 years 56 (14.6%) and 10-11
years 46(11.9%). The differences statistically not significant ($P$-value= 0.562).

Correlation between behavioral habits, environmental and health conditions variables with the prevalence rates of intestinal protozoan infections showed statistically significant relationship ($P$-value < 0.05) for hand washing with soap before eating, for other variables there was no statistically significant correlations, table (5).

**Table.1** Prevalence rate of intestinal protozoan parasites among four primary schools

| Intestinal protozoan parasite | Infected cases | F-test value | $P$-value |
|------------------------------|----------------|--------------|-----------|
| $E. histolytica/dispar$       | 130            | 2.637        | 0.049     |
| $G. lamblia$                 | 76             | 19.7         |           |
| Multiple infection           | 80             | 20.8         |           |

Statistically significant $P$-value less than 0.05

**Table.2** Multiple infection of intestinal protozoa among school children

| Multiple infection | Infected cases |
|--------------------|----------------|
|                    | No.  | %     |
| One stage          | 206  | 72    |
| Two stages         | 76   | 27    |
| Three stages       | 4    | 1     |
| Total              | 286  | 100   |

**Table.3** Gender distribution of intestinal protozoan parasites

| Intestinal protozoan parasite | Male     | Female    | t-test | $P$-value |
|------------------------------|----------|-----------|--------|-----------|
| $E. histolytica/dispar$       | 68(17.7) | 62(16.1)  | 0.767  | 0.444     |
| $G. lamblia$                 | 49(12.7) | 27(7.0)   |        |           |
| Multiple infection           | 63(16.4) | 17(4.4)   |        |           |

Statistically significant $P$-value less than 0.05

**Table.4** Age groups distribution of intestinal protozoan parasites

| Intestinal protozoan parasite | 7-8 years | 8-9 years | 9-10 years | 10-11 years | F-test | $P$-value |
|------------------------------|-----------|-----------|------------|-------------|--------|-----------|
| $E. histolytica/dispar$       | 51 (13.2) | 21 (5.5)  | 32 (8.3)   | 26 (6.7)    | 0.684  | 0.562     |
| $G. lamblia$                 | 42 (10.9) | 17 (4.4)  | 11 (2.9)   | 6 (1.6)     |        |           |
| Multiple infection           | 33 (8.6)  | 20 (5.2)  | 13 (3.4)   | 14 (3.6)    |        |           |

Statistically significant $P$-value less than 0.05
### Table 5: Correlation between behavioral habits, environmental and health conditions variables with infected cases of intestinal protozoa

| Variable                              | Infected cases | Pearson Correlation | P-value |
|---------------------------------------|----------------|---------------------|---------|
|                                       | No. | %       |                    |         |
| Drinking tap water                    | Yes  | 248  87 | 0.011              | 0.831   |
|                                       | No   | 38   13 |                     |         |
| Food source at home                   | Yes  | 276  97 | 0.037              | 0.468   |
|                                       | No   | 10   3  |                     |         |
| Food source at restaurants            | Yes  | 11   4  | 0.071              | 0.162   |
|                                       | No   | 275  96 |                     |         |
| Food source at fast food              | Yes  | 122  43 | 0.007              | 0.893   |
|                                       | No   | 164  57 |                     |         |
| Hand washing before eating            | Yes  | 243  85 | 0.049              | 0.333   |
|                                       | No   | 43   15 |                     |         |
| Hand washing with soap before eating  | Yes  | 32   11 | 0.103              | 0.043*  |
|                                       | No   | 254  89 |                     |         |
| Hand washing after defecation         | Yes  | 243  85 | 0.014              | 0.789   |
|                                       | No   | 43   15 |                     |         |
| Hand washing with soap after defecation| Yes | 51   18 | 0.027              | 0.601   |
|                                       | No   | 235  82 |                     |         |
| Fruits and vegetables washing         | Yes  | 272  95 | 0.063              | 0.218   |
|                                       | No   | 14   5  |                     |         |
| Provision of sanitation system        | Yes  | 282  99 | 0.028              | 0.582   |
|                                       | No   | 4    1  |                     |         |
| Contact with domestic animals         | Yes  | 216  76 | 0.028              | 0.582   |
|                                       | No   | 70   24 |                     |         |
| Monthly income                        | High | 3    1  | 0.037              | 0.469   |
|                                       | Moderate | 64 | 23              |         |
|                                       | Weak  | 141  49 |                     |         |
|                                       | Very weak | 78 | 27              |         |
| Abdominal pain                        | Yes  | 206  72 | 0.17               | 0.744   |
|                                       | No   | 80   28 |                     |         |
| Diarrhea                              | Yes  | 107  37 | 0.006              | 0.914   |
|                                       | No   | 179  63 |                     |         |
| Nausea and vomiting                   | Yes  | 96   34 | 0.007              | 0.88    |
|                                       | No   | 190  66 |                     |         |
| Weight loss                           | Yes  | 164  57 | 0.007              | 0.893   |
|                                       | No   | 122  43 |                     |         |

* Correlation is significant at 0.05 level

In Yemen, the intestinal protozoan infections are still a public health problem with Giardia and Entamoeba infections and being most common (Alyousefi et al., 2011). In this study, the overall prevalence rate of intestinal protozoan infections among primary school
children was 74.2%, this high prevalence rate agreement with the prevalence of intestinal parasitic infections among school aged children in Jenin (Palestine) 32.0-41.5% (Bdir and Adwan, 2010), Nepal19.8% (Mukhiya et al., 2012), Pakistan 51.5% (Shezana et al., 2012), Saudi Arabia 45.38% (Amer et al., 2016), Nigeria was 86.2% (Gyang et al., 2017), Iran 42.3% (Bahmani et al., 2017), and Ethiopia 21.5% (Gebretsadik et al., 2020).

The commonest intestinal protozoan parasites detected in the present study were *E. histolytica* 45%, *G. lamblia* 27% and multiple infections of more than one stages of these parasites 28%. These findings were similar to findings of other studies conducted in different Yemeni governorates, in Sana’a,*E. histolytica* and *G. lamblia* were the most common of intestinal parasitosis among children (Azazy and Raja’a, 2003), in Ibb, *E. histolytica* 33.7% and *G. lamblia* 23.6% were the intestinal protozoa detected among school children (Alsubaie et al., 2016), and in Al-Mahweet, the distribution of intestinal protozoan infections among primary schools pupils in primary schools were *E. histolytica* cysts 64% and amorphous amoebae 22.5% (Alwabr and Al-Moayed, 2016).

Another studies carried out in different countries showed different percentages the prevalence of intestinal protozoan parasites, in Nigeria 25% and 12.3%were found in *E. histolytica/dispar* and *G. Duodenalis* infections respectively (Gyang et al., 2017). *E. histolytica* and *G. lamblia* were detected 33.1% and 19.7% respectively in Sudan (Ahmed et al., 2010), *E. histolytica/dispar* 16.15% and *G. lamblia* 11.54%were detected in Saudi Arabia (Amer et al., 2016), 19.8% of *G. lamblia* followed by 2.5% *E. histolytica* were detected in Pakistan (Shezana et al., 2012), in Ethiopia *E. histolytica/dispar* 12.1% and *G. lamblia* 12.1% (Sahiledengle et al., 2020), *E. histolytica* and *G. lamblia* were detected as intestinal protozoa in Jenin (Palestine) (Bdir and Adwan, 2010), also, the protozoan of *E. coli* followed by *G. lamblia* and *E. histolytica* were detected in school children in Nepal (Mukhiya et al., 2012). *E. histolytica* infects a significant proportion of many populations in South Africa and Egypt (Stauffer et al., 2006). In Lebanon, *G. duodenalis* 28.5% and other protozoan spp. were detected (Osman et al., 2016), in Ethiopia *E. histolytica* 8.3% was detected among school children (Gebretsadik et al. 2020)and 27.3% of the patients were infected with *G. lamblia* 10.1% and *E. coli* 10% in Iran (Haghighiet al., 2009).

Approximately one in two children 44.8% were found to be infected with at least one intestinal parasite, *Entamoeba histolytica* 25.95% and *Giardia lamblia* 19.6% were most prevalent, and infection with more than one pathogen was noted in a study conducted in Rwanda (Butera et al., 2019). Other study showed that *Giardia lamblia* was the second usually identified protozoan with a diseases rate 10.4% among the analyzed cases in El Behara schoolchildren, Egypt (Radwan et al., 2019). In a study conducted in White Nile State, Sudan, the commonest intestinal parasites among the school’s children were *E. histolytica* 31.2%, and *G. lamblia* 22.9%. The most infected age group was (10-13) with prevalence of 43% (Suliman et al., 2019). Another study revealed that the prevalence of *Giardia lamblia* was the highest 19.95%, followed by *E. histolytica/dispar* 5.9% among primary school children in Ethiopia (Sitotaw et al., 2019).

The multiple intestinal protozoan infection among school children showed of 28% in our study. A study conducted on school aged children showed 39.1% of intestinal protozoa infections was polyparasitism (Gyang et al., 2017). Multiple intestinal parasitic infections were recorded 75.5% among the children
Yemen (Alwabr and Al-Moayed, 2016). A 33.84% were found to be infected with one or more intestinal protozoa in Saudi Arabia (Amer et al., 2016). Other multiple intestinal parasitic infections infection showed in Iran 6.4% (Bahmani et al., 2017).

In this study, the prevalence rate of intestinal protozoan infections according to gender showed that 62.9% for males and 37.1% for females. These results agreement with the results of study of males 46.5% were more infected than females 43.5% in Yemen (Alwabr and Al-Moayed, 2016), other study carried out in Pakistan revealed that males of 60% were the majority of the participants (Shezana et al., 2012). Another studies disagree in the present study results, the prevalence rate of protozoa in boys and girls were 16.9% and 22.0% respectively in school children (Mukhiya et al., 2012), and the prevalence of intestinal parasitic infections in females was significantly higher than in males in Saudi Arabia (Amer et al., 2016). Other study revealed that infection with intestinal parasites were similar in boys and girls in Sana’a/Yemen (Azazy and Raja’a, 2003). In a study carried out among Ethiopian primary school children, the overall infection rate was slightly higher in males 51.85% than in females 45.30% though the difference was not significant (Sitotaw et al., 2019). Another study revealed that females were more infected with intestinal protozoan parasites than males, P-value=0.0001 (Suliman et al., 2019).

The prevalence rate of intestinal protozoan infection of age groups in this study showed high in age group of 7-8 years 44%, followed by 8-9 years and 9-10 years 20%, then 10-11 years 16%. A study done in Yemen revealed that, the intestinal protozoan infection rate was highest among the age group 10-11 years 31.8% followed by 12-13 years 28.4% and infection rates of 23.6% and 16.2% were recorded among 8-9 years olds and 14-15 years old respectively (Alsubaie et al., 2016), other study in Yemen showed a difference in the percentages of infection among the different age groups of the studied primary schools children (Alwabr and Al-Moayed, 2016).

High prevalence rates of intestinal protozoan infections were recorded related to socio-economic characteristics such as water resources for drinking uses, poor hygienic practices, unsanitary condition, contact with animals, economic status and the methods of washing hands. A study in Nigeria, revealed that drinking untreated water was a significant risk factor for these school aged children in acquiring protozoan infections (Gyang et al., 2017).

Among the risk factors assessed, age, hand washing habit before meals, open field defecation habit, habit of eating raw and unwashed vegetables, and finger nail cleanliness were found to be the most important predictors associated with high risk of intestinal parasitic infections (P-value < 0.05) in a study carried out among primary school children in Ethiop (Sitotaw et al., 2019; Sitotaw and Shiferaw, 2020). Other study showed significant correlation between intestinal parasitic infection and sources of drinking water, hand wash after toilet. Whereas, no significant association was observed with father education, mother education and toilet availability in a study conducted in White Nile State, Sudan (Suliman et al., 2019). Children from non-farming families and from households with access to treated drinking were less likely to be at risk of intestinal parasite infections, whereas children from families with improved sources of water were twice as likely to be diagnosed with intestinal parasitoses like E. histolytica and G. lamblia (Butera et al., 2019).
Poor hygienic practices, unsanitary condition and contact with animals were important predictors responsible for high prevalence of intestinal parasites among primary schools in different area in Yemen (Alyousefi et al., 2011; Alwabr and Al-Moayed, 2016). A significant relationship was observed between intestinal protozoa infection with economic status, water resources for drinking uses and the methods of washing vegetables in Iran (Bahmani et al., 2017), other study showed poor hygienic practices was predictor for prevalence intestinal parasites(Sahiledengle et al., 2020). In this study, it was observed that the prevalence rate of parasitic infection associated with frequent symptoms such as abdominal pain, diarrhea, weight loss, nausea and vomiting. Clinical symptoms of nausea, vomiting and abdominal pain were significantly varied (Hawash et al., 2015).

In conclusion the intestinal protozoan infection was common health problem, and the prevalence rate was found to be higher in primary schools. E. histolytica/dispar was more prevalent followed by G. lamblia and multiple infections were showed with two or more stages of parasites. The most commonly infected age group children was 7-8 years and males were infected more than females. High prevalence of intestinal protozoan infection among the student population is influenced and associated by the behaviors practices, poor personal hygiene, health services, and environmental contamination.

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