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Research Note

Different profile of intestinal protozoa and helminthic infections among patients with diarrhoea according to age attending a rural hospital in southern Ethiopia

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Abstract. The aim of this study was to analyze the association of intestinal parasitic diseases with age and gender in patients with diarrhea attending a rural hospital in southern Ethiopia in the period 2007-2012. A total of 32,191 stool examination was performed in patients who presented with diarrhea. The overall prevalence of intestinal parasites in the present study was 26.5%. Predominant parasites detected were *Giardia lamblia* (15.0%), *Entamoeba histolytica/dispar* (5.4%), and *Ascaris lumbricoides* (5.0%). The median of age of diarrheal patients with *Hymenolepis* species, *Schistosoma mansoni* and *G. lamblia* was significantly lower (5 y., 10.5 y., and 18 y., respectively; $p<0.001$). The median age of diarrheal patients with *Taenia* species, *S. stercoralis*, and *E. histolytica/dispar* was significantly higher (24 y., 24 y., and 20 y., respectively; $p<0.01$). In conclusion, *Giardia lamblia* was the most prevalent intestinal parasite and the profile of intestinal parasitic infections is influenced by age.

Diarrheal diseases are the major causes of morbidity expressed as disability-adjusted life year (DALY) and mortality in low- and middle-income countries (Kosek *et al.*, 2003). Globally, it is estimated that two billion people are affected by intestinal parasites, of whom 300 million suffer from associated severe morbidity; however, it is difficult to estimate the actual burden due basically to underreporting (WHO, 2002; Kosek *et al.*, 2003). Moreover, intestinal parasites contribute to malnutrition, protein and iron deficiencies, an increment in health costs, as well as long-term deleterious effects, often forgotten, such as disability and cognitive impairment (WHO, 2002; Kosek *et al.*, 2003). This is true for children but also adults and probably especially fertile women from rural communities who have an increment in their mortality rate and also side effects in their offspring (Larocque *et al.*, 2005; Ngui *et al.*, 2012). These problems occur even in parasitic infestations of mild intensity, and they are particularly evident in concomitant infections (Larocque *et al.*, 2005; Ngui *et al.*, 2012).

Intestinal parasitic infections are one of the major disease causes of public health problems in sub-Saharan Africa including Ethiopia, where helminthic infections are one of the principal causes of morbidity due to the economic situation in the country and poor sanitation (Endeshaw *et al.*, 2004; Huruy *et al.*, 2011). These problems are seen even if the infection intensity is mild, and even worse in concomitant infections (Larocque *et al.*, 2005; Ngui *et al.*, 2012).

The aim of this study was to determine the most prevalent intestinal parasitic diseases in patients with diarrhea and to analyze their association with age and gender at a rural hospital in southern Ethiopia over five years.

The study was conducted at Gambo Rural Hospital (GRH). It is located 245 km from the capital (Addis Ababa) in the Oromia Region, West Arsi Province, Ethiopia. GRH covers 11 municipalities with an estimated population of 100,000 inhabitants. The altitude there is 2200 m.a.s.l. The temperature ranges between 13 and 30 ºC, with monthly rainfall that varies from February to October. Subsistence farming and animal husbandry are the predominant occupations.

This is a retrospective observational study of all stool samples examined for parasites in the period 2007-2012 at GRH. Stool examination was performed in patients presenting with diarrhea (passage of three or more loose stools per 24 hours). All patients whose stool samples were examined at the laboratory were referred from either the outpatient clinic or the hospital wards of our hospital. Stool samples were collected in accordance with WHO guidelines on fecal sample collection. Stool specimens were examined by direct microscopy of the smear in saline and Lugol's iodine for parasite detection (trophozoites, cysts, eggs, and larvae). The study was reviewed and approved by the Institutional Ethical Review Board at GRH.

Demographic and parasitological data were entered into an electronic database designed using Microsoft Excel 2007. Data were cleaned for abnormal figures before being transferred to SPSS (version 15.0) statistical software for analysis. Continuous variables, such as age distribution, were represented as median with interquartile range (IQR). Relationships between proportions of intestinal parasites with sex were analyzed by the chi-square test and with age by the Mann-Whitney U test. Age distribution was tabulated in eleven age groups. Any p-value less than 0.05 was considered statistically significant.

Thirty-two thousand one hundred ninety-one patients with diarrhea were included in this study. Gender was available in 32,066 cases (99.6%), and 52% of the study subjects were female. Patient age was available in 31,333 cases (97.3%). The median age was 18 (IQR: 5-30) years. Twenty-one percent of the patients were children under 5 years. The overall prevalence of intestinal parasites in the present study was 26.5%. The predominant protozoan parasite detected was *Giardia lamblia* (15.0%), followed by *Entamoeba histolytica/dispar* (5.4%). *Ascaris lumbricoides* was the dominant helminthic parasite identified (5.0%), followed by *Hymenolepis* sp. (1.7%), and *Taenia* sp. (1.0%) (Table 1). Multiple infections caused by two or three intestinal parasites were detected in 3.1% and 0.2%, respectively. The predominant mixed infections were *G. lamblia* and E. *histolytica/dispar* co-infections (1.3%), followed by *G. lamblia* and *A. lumbricoides* co-infections (0.5%), *E histolytica/dispar* and *A. lumbricoides* co-infections (0.3%), and *G. lamblia* and *Hymenolepis* co-infections (0.02%).

The median age of diarrheal patients bearing intestinal parasitic infection was significantly less than patients without infection (16 years versus 18 years; $p<0.001$). The median of age of diarrheal patients with *Hymenolepis* species, *Schistosoma mansoni*, and *G. lamblia* was 5 years, 10.5 years, and 18 years, respectively. The median age was significantly less than in other illnesses ($p<0.001$; $p=0.025$ and $p<0.001$, respectively). Moreover, the median age of diarrheal patients with *Taenia* species, *S. stercoralis*, and *E. histolytica/dispar* was 24 years, 24 years, and 20 years, respectively,

and was significantly higher than in other parasitic infections (p<0.001, p=0.003, and p<0.001) (Table 1).

Gender was not a risk factor for harboring intestinal parasitic infections (female prevalence was 26.9% and male was 26.4%). However, the prevalence of *A. lumbricoides* and *E. histolytica/dispar* was significantly higher in females (5.6% and 5.9%) than in males (4.3% and 2.8%) (p<0.001 and p=0.03, respectively). The prevalence of *Strongyloides stercoralis* was higher in male than in female patients (p=0.002) (Table 1).

Table 2 gives a detailed description of intestinal parasites by age group. *Giardia lamblia* was the most common intestinal protozoan in all age groups, and there was a significant decrease in its presence with age (chi-square test for trend = 77, p<0.001). *Ascaris lumbricoides* was the second intestinal parasite under 20 years of age, while over 20 years it was the third intestinal parasite in order of frequency. *Entamoeba histolytica/dispar* was the third intestinal parasite up to 20 years and above. *Hymenolepis* species was a common parasite in children under 5 years and also from the 5 to 9 years group, and it represented 3.4% and 3.1% of the parasites identified, respectively. In higher ages, there was a significant decrease in its percentage with age (chi-square test for trend = 68, p<0.001).

In this cross-sectional study amongst diarrheal patients in a rural located hospital in southern Ethiopia, the overall prevalence of intestinal parasites in stool samples was 26.5%. Our finding is lower in comparison to studies undertaken in other areas of Ethiopia and South Africa (Mengistu *et al.*, 2000; Endeshaw *et al.*, 2004; Huruy *et al.*, 2011). This could be due to differences in hygiene practices of populations, environmental, and host factors or sanitation issues like water supplies or latrine coverage. The methods used for parasite detection (formalin concentration, direct smear, Kato-Katz technique, etc.) may also contribute to the differences observed. That divergence could also be owed to the type of patients recruited (i.e., rural and all age ranges). Other studies either focused on subjects from urban communities, those from the general population, or school-age children.

Giardia lamblia was the predominant protozoan parasite (15%) isolated from stool of the diarrheic subjects. *G. lamblia* is considered one of the leading causative agents of water-borne diarrhea in both children and adults (Dib *et al.*, 2008; Nkrumah & Nguah, 2011). In low-income countries, *G. lamblia* infects children younger than 10 years with prevalence rates of 15-20%, while in some studies it is above 50% (Dib *et al.*, 2008; Worku *et al.*, 2009; Nkrumah & Nguah,

Table 1. Intestinal parasite in diarrheal patients by sex and median age of patients of diarrheal patients with intestinal parasitic infections at Gambo Rural Hospital, Ethiopia

	Total (n=32,191) No. (%)	Female (n=16,668) No. (%)	Male (n=15,398) No. (%)	P-value*	Median (IQR)	P-value**
Giardia lamblia	4,844 (15)	2,466 (14.8)	2,363 (15.3)	0.17	18 (6-30)	<0.001
Entamoeba histolytica/dispar	1,733 (5.4)	944 (5.9)	789 (5.1)	0.035	20 (8-30)	<0.001
Ascaris lumbricoides	1,596 (5.0)	926 (5.6)	662 (4.3)	<0.001	15 (7-30)	0.48
Hymenolepis species	551 (1.7)	271 (1.6)	278 (1.8)	0.23	5 (3-10)	<0.001
Taenia species	322 (1.0)	185 (1.1)	137 (0.9)	0.05	24 (13.5-35)	<0.001
Hookworm infection	214 (0.7)	103 (0.6)	110 (0.7)	0.32	17 (9-26)	0.69
Trichuris trichura	171 (0.5)	102 (0.6)	69 (0.4)	0.05	18 (8-30)	0.91
Schistosoma mansoni	162 (0.5)	75 (0.4)	86 (0.6)	0.19	10.5 (5-26)	0.025
Strongyloides stercoralis	92 (0.3)	33 (0.2)	59 (0.4)	0.003	24 (13-32)	0.003
Multiple infections	1,062 (3.3)	577 (3.5)	484 (3.1)	0.11	30 (16-42)	0.31
Overall prevalence	8,546 (26.5)	4,479 (26.9)	4,037 (26.2)	0.18	16 (6-29)	<0.001

*P-value from the Chi-square test
**P-value from the Mann-Whitney U test
IQR: interquartile range

Table 2. Prevalence of intestinal parasites in stool samples for various age groups of diarrheal patients at Gambo Rural Hospital, Ethiopia

	< 5 yrs. (n=6,776) No. (%)	5-9 yrs. (n=4,601) No. (%)	10-14 yrs. (n=2,438) No. (%)	15-19 yrs. (n=2,438) No. (%)	20-25 yrs. (n=2,592) No. (%)	25-30 yrs. (n=3,403) No. (%)	30-35 yrs. (n=2,118) No. (%)	35-39 yrs. (n=1,836) No. (%)	40-49 yrs. (n=2,272) No. (%)	50-59 yrs. (n=1,193) No. (%)	>59 yrs. (n=2,234) No. (%)	P-value
Giardia lamblia	1,132 (16.7)	810 (17.6)	376 (15.4)	448 (16.5)	372 (14.4)	471 (13.8)	285 (13.5)	230 (12.5)	293 (12.9)	159 (13.3)	266 (11.9)	<0.001
Entamoeba histolytica/dispar	242 (3.6)	262 (5.7)	140 (5.7)	163 (6.0)	158 (6.1)	216 (6.3)	142 (6.7)	111 (6.0)	121 (5.3)	67 (5.6)	111 (5.0)	<0.001
Ascaris lumbricoides	254 (3.7)	331 (7.2)	174 (7.1)	165 (6.1)	99 (3.8)	146 (4.3)	98 (4.6)	80 (4.4)	116 (5.1)	44 (3.7)	89 (4.0)	0.003
Hymenolepis species	232 (3.4)	140 (3.0)	42 (1.7)	28 (1.0)	12 (0.5)	24 (0.7)	17 (0.8)	7 (0.4)	11 (0.5)	6 (0.5)	32 (1.4)	<0.001
Taenia species	15 (0.2)	38 (0.8)	31 (1.3)	33 (1.2)	41 (1.6)	46 (1.4)	30 (1.4)	21 (1.1)	24 (1.1)	19 (1.9)	26 (1.2)	<0.001
Hookworm infection	21 (0.3)	35 (0.8)	42 (1.7)	27 (1.0)	22 (0.8)	18 (0.5)	9 (0.4)	6 (0.3)	13 (0.6)	10 (0.8)	11 (0.5)	0.45
Trichuris trichura	15 (0.2)	39 (0.8)	18 (0.7)	18 (0.7)	7 (0.3)	19 (0.6)	11 (0.5)	10 (0.5)	12 (0.5)	6 (0.5)	16 (0.7)	0.22
Schistosoma mansoni	29 (0.4)	30 (0.7)	12 (0.5)	10 (0.4)	10 (0.4)	14 (0.4)	6 (0.3)	10 (0.5)	5 (0.2)	2 (0.2)	34 (1.5)	0.04
Strongyloides stercoralis	8 (0.1)	10 (0.2)	7 (0.3)	8 (0.3)	13 (0.5)	15 (0.4)	9 (0.4)	6 (0.3)	6 (0.2)	2 (0.2)	8 (0.4)	0.02
Multiple infections	192 (2.8)	204 (4.4)	89 (3.7)	102 (3.8)	82 (3.2)	97 (2.9)	72 (3.4)	52 (2.8)	68 (3.0)	33 (2.8)	70 (3.1)	0.001
Overall prevalence	1,742 (25.7)	1,476 (32.1)	746 (30.6)	791 (29.1)	648 (25.0)	863 (25.4)	529 (25.0)	423 (23.0)	531 (23.4)	278 (23.3)	516 (23.1)	<0.001

P-value from the chi-square test for trends

2011). *Entamoeba histolytica/dispar* was the second protozoan parasite (5.5%) isolated from the stool of diarrheal patients. The rate of *S. mansoni* (0.5%) and hookworm infection (0.7%) was lower compared to other studies performed in South and North Ethiopia (Huruy *et al.*, 2011). The 1.7% of *Hymenolepis* species diagnosed in the study was also in agreement with a study conducted in Ethiopia by Hurry *et al.* (2011).

Gender was not a risk factor for acquiring intestinal parasitic infections. This finding is consistent with previous reports (Mukhopadhyay *et al.*, 2008; Akinbo *et al.*, 2011). However, there was a significant difference in the prevalence of *A. lumbricoides* and *E. histolytica/dispar* in females. There is not a clear explanation for this. The association in our study with the female condition could be influenced by several factors, such as sanitary facilities (the existence of pit latrines), abstaining from housework, drinking water from streams, contact with animals, and not be a true association. However, *E. histolytica* can be transmitted by heterosexual activity as well as male and female homosexual activity. Patients with amebiasis should be counseled about possible sexual transmission. And it is the probable reason for higher infection due to *E.histolytica/dispar* among women (Salit *et al.*, 2009). In our study, males were more frequently affected by *S. mansoni* than females, basically due to their work activities (Black *et al.*, 2010).

The finding that age significantly affected the prevalence of intestinal parasitic infection has been previously reported (Akinbo *et al.*, 2011; Matthys *et al.*, 2012;). However, the observation in this study differs from other studies. In our report, the patients carrying *Hymenolepis* sp., *S. stercoralis*, *G. lamblia*, and *A. lumbricoides* were a young population. *Hymenolepis* species has been identified as the first cause of diarrhea at primary schools in Tajikistan (Matthys *et al.*, 2012). In a survey from a rural population in Egypt, younger age groups were significantly infected by *H. nana* more so than older ones (Bakr *et al.*, 2009). Infections with *Taenia* sp. and *E. histolytica/dispar* are more common in older people. Moreover, patients with diarrhea who have intestinal parasites can be co-infected with gastrointestinal microorganisms, such as the *Shigella* or *Salmonella* species, but unfortunately, we were neither able to identify these agents nor a virus (Huruy *et al.*, 2011).

We can conclude that *G. lamblia* is the most prevalent intestinal parasite in stool samples examined from patients with diarrhea in our area. There are different profiles of intestinal parasites according to sex and age. So measures must be put in place to improve the quality of diagnostic services in the hospital with concentration techniques for stool examination or use modified Ziehl-Neelsen staining of stool smears to identify these protozoa and serial stool samples should be included.

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