

More Than Half Prevalence of Protozoan Parasitic Infections Among Diarrheic Outpatients in Eastern Tigray, Ethiopia, 2019; A Cross-Sectional Study

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Background: Protozoan infections remain a major public health concern in Ethiopia, which causes malnutrition, protein and iron deficiencies, increment of health costs, hospitalization and long-term deleterious effects. In Tigray, particularly, in the study area, there are limited literatures on the prevalence of protozoa and associated factors among diarrheic outpatients. Thus, aimed to determine the prevalence and associated factors of protozoan infections among diarrheic outpatients in Eastern Tigray.

Methods: Health facility-based cross-sectional study was conducted in Eastern Tigray, Ethiopia from April to October 2019 among diarrheic outpatient in health facilities of Adigrat. Four hundred one eighteen study participants were included consecutively. The stool samples were examined via direct wet mount following formal-ether concentration technique. Both interviewer based and self-administrated questionnaires were used to collect demographic data and factors associated with protozoan infections. Data were analyzed and computed using BM SPSS statistics version 22.0. P-value=0.05 at 95% confidence interval declared statistically significant.

Results: Out of 418 diarrheic outpatients, the overall prevalence of protozoan infection was 59.3% (248/418). A higher percentage of intestinal protozoan infection was recorded for females 130 (31.1%). Using well water as a source of drinking, poor hand washing habits before eating, not home latrine, dirty and untrimmed fingernails were the most important risk factors identified. *E. histolytica complex* and *Giardia duodenalis* were the most predominant protozoan infections detected among diarrheic outpatients.

Conclusion: The present study showed that diarrheic outpatients in the study area were heavily infected with protozoan parasitic infection. *E. histolytica complex* and *Giardia duodenalis* were the most predominant protozoans detected among diarrheic outpatients. Using well water as a source of drinking, poor handwashing habit before eating, not having home latrine, dirty and untrimmed fingernails were most important risk factors identified. So, cooperative action and health education on preventive measure are needed to reduce protozoan infections in Tigray, particularly in the study area. The present study showed that diarrheic outpatients in the study area were heavily infected with protozoan parasitic infection.

Keywords: Adigrat, diarrhea, Ethiopia, exposure factors, intestinal protozoa, outpatients

Introduction

Protozoan infections are amongst the leading causes of morbidity and mortality throughout the world with more than 58 million diarrheal cases detected each year.^{1,2} However, it is difficult to estimate the actual burden of protozoan infections due to under reporting.^{3,4} Moreover, intestinal parasites contribute to malnutrition, protein and iron deficiencies, an increment on health costs, as well as long-term deleterious effects.⁴

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Diarrheal diseases transmitted through person to person, waterborne, foodborne, and zoonotic transmissions^{2,5} are the major modes of transmission to humans. *Giardia* and *Entamoeba* are the major causes of acute and persistent diarrhea in humans worldwide with more pervasive in developing countries including Ethiopia.^{1,6,7} Giardiasis is reported to infect about 280 million people every year and contributed to 2.5 million annual deaths. Annually, an estimated 1.2 million giardiasis cases occurred in globe.⁸ On the other than, Amoebiasis is reported to cause an estimated 50 million cases annually with 100,000 deaths. Thus, intestinal protozoan infections are ranked second to malaria as the cause of morbidity and mortality.⁹

Diarrheal diseases are the second leading cause of morbidities and cause 1.8 million deaths annually, despite improved use of oral rehydration solutions, zinc, and vitamin A supplements in developing nations including Ethiopia.^{10,11}

Although there are other causes of diarrhea in Ethiopia, protozoan infections remain principal causes of morbidity due to socio-economic situations and poor sanitation.¹² These problems are seen even if the infection intensity is mild and worse in concomitant infections.¹³ This may increase the prevalence of intestinal parasitic infections and needs attention.¹⁴ Epidemiology of intestinal protozoan infections among diarrheic patients in Ethiopia had been reported. However, there is a need for updating epidemiologic survey of these parasites infection at regular intervals to develop effective prevention and control strategies. Hence, this study is aimed to determine the prevalence and associated factors of protozoan infection in diarrheic outpatients in governmental health facilities of eastern Tigray, Ethiopia and enable decision-makers' to focus on the severity of these parasitic diseases and improve factors believed to be risk for causing and transmitting of these protozoan diseases.

Methods

Study Design, Period and Area

Health facility-based cross-sectional study was conducted in all health facility of Adigrat town, Tigray, Ethiopia from April to October 2019. The town is located around 905 km north of Addis Ababa (capital city of Ethiopia) at a latitude and longitude of 14°16'N 39°27'E, with an elevation of 2457 m (8061 ft) above sea level. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA), the town has a total population of

57,588, people (26,010 are men and 31,578 women). The town has one General Hospital and two health centers. Currently, the Hospital is serving as a teaching hospital, emergency, inpatient and outpatients services for more than one million people who live in eastern zones of Tigray and Afar regional state.

Source Population

All clients visiting Adigrat health facilities for health-care services.

Study Participants

All diarrheic outpatients who visited Adigrat health facilities during the study period.

Exclusion Criteria

Those patients who took medication for intestinal parasites within 1 month prior to the study, patients who were severely ill and unable to give stool sample were excluded from the study.

Sample Size and Sampling Technique

A total of 418 study participants were determined using a single population proportion formula by assuming: 95% level of confidence, 5% margin of error and P (proportion) of 0.453¹⁸ and non-response rate of 10%. There are a total of three health institutions found in Adigrat town, namely; Adigrat hospital, Tekliswuat, and Mayda Agame health centers and the 418 study subjects were proportionally allocated to the health facilities based on the size of patient flow within 1 month prior to our study. Convenience sampling technique was employed to include the study participants.

Method of Data Collection

Questionnaire

Data related demographic characteristics and factors associated with protozoan infections of the study participants were collected using both interviewers based and self-administrated questionnaire by skilled data collectors. The questionnaires were developed from previous different published literatures and pre-tested in Wukro General Hospital.

Stool Examination

Labeled stool cup with leak-proof covers possessing respective sequential numbers were given for the study participants. Fecal specimens were carried out by wet mount and formal-ether concentration techniques.

Quality Control

Questionnaires were pre-tested prior to the actual data collection. The collected data were checked for consistency and completeness on random bases. All the laboratory procedures were conducted as per the standard operating procedures (SOPs).

Statistical Analysis

Data were entered and analyzed using IBM SPSS statistics version 22.0. Then, it summarized using descriptive statistics. Bi-variate and multi-variate regression tests were employed to measure the association between dependent and independent variables. Variables with $p < 0.20$ in the bivariate logistic regression were transferred to multi-variate regression analysis to compute AOR. A p -value less than 0.05 was considered statistical difference.

Operational Definition

Personal Hygiene

Study participants who did not wear foot-ware, proper working clothes, dirty and untrimmed fingernail, those who are not washing their hand before meal and after defecation and general poor looking individuals.

Ethical Consideration

Ethical clearance was taken from Research Ethical Review Committee of Adigrat University, Ethiopia. An official letter was obtained from Tigray Regional Health Bureau to health facilities of Adigrat town. Permission was also obtained from Administrator of each health facility of Adigrat town. Furthermore, after explaining the importance of study, informed written consent were obtained from study participants. Moreover, informed written consent and assent was taken from a parent or legal guardian for participants under the age of 18 years. Additionally, this study was conducted in accordance with the declaration of Helsinki. No name was mentioned during the entire data collection and identification was based on the unique number given for each questionnaire and corresponding specimen. Confidentiality of information (results) was kept between the study participant, data collector/investigator, and authorized physician. Those study participants who have harbored any kind of intestinal parasites were reported to physician to treat.

Results

A total of 418 diarrheic outpatients were included in this study. Out of the total respondents, 214 (51.2%) were males. The mean age of the study participants was 30 ± 18 years, ranging 1–88 years. Majority of the study participants were in the age group of 15–24 years

(26.3%), 1–8 education level (33%), urban dweller (67%) and unemployed (60.3%) (Table 1).

Prevalence of Intestinal Parasitic Infections

The overall prevalence of intestinal protozoa infection was 59.3% (248/418) and higher infection rates were recorded among female participants 31.1% (130/418). *E. histolytica complex*, 39.7% (166/418) was the predominant parasite followed by *Giardia duodenalis*, 11% (46/418), Taenia species and *Enterobius vermicularis*, 0.48% (2/418) each were also detected. The rate of multiple infections accounted for, 8.6% (36/418) with predominant co-existent infection was *E. histolytica complex* and *Giardia duodenalis*, 8.1% (34/418) (Table 2).

Factors Associated with Protozoan Parasitic Infection

Using well water as a source of drinking (AOR = 3.08, 95% CI: 1.29–8.00, $P = 0.021$), not having home latrine (AOR = 1.87, 95% CI: 1.21–2.89, $P = 0.04$), had not washed their hand before meal (AOR= 3.3, 95CI: 1.42–7.90), untrimmed nails (AOR=1.71, 95 CI: 1.11–2.65, $P = 0.016$) and those had dirty nail (AOR =4.28, 95CI: 2.73–

Table 1 Demographic Characteristic of Study Participants in Governmental Health Facilities of Adigrat Town, Tigray, Ethiopia, 2019

Variables	Category	Number	Percentage
Sex	Male	214	51.2
	Female	204	48.8
Age	<5	38	9.1
	5–14	34	8.1
	15–24	110	26.3
	25–34	90	21.5
	35–44	56	13.4
	≥45	90	21.5
Occupations	Civil servant	66	15.8
	Self employed	60	14.4
	Farmer	40	9.6
	Unemployed	252	60.3
Educational level	Illiterate	86	20.6
	1–8	138	33
	9–12	106	25.6
	College and above	88	21.1
Residence	Urban	280	67
	Rural	138	33

Table 2 Distribution of Parasitic Infection by Sex and Age Among Diarrheic Patients in Governmental Health Facility of Adigrat Town, Tigray, Ethiopia, 2019

Parasite Species	Sex, n (%)		Age (Year), n (%)					
	Male	Female	<5	5–14	15–24	25–34	35–44	≥45
Single infection								
<i>E. histolytica</i> complex	72(17.22)	94(22.49)	16(3.83)	20(4.78)	36(8.61)	44(10.53)	24(5.74)	26(6.22)
<i>G. duodenalis</i>	24(5.74)	22(5.26)	6(1.44)	4(0.96)	6(1.44)	4(0.96)	4(0.96)	22(5.26)
Taenia species	2(0.48)	–	–	–	2(0.49)	–	–	–
<i>E. vermicularis</i>	–	2(0.48)	–	–	2(0.49)	–	–	–
Mixed infection								
<i>E. histolytica</i> complex + <i>G. duodenalis</i>	20(4.78)	14(3.35)	4(0.96)	2(0.49)	8(1.91)	8(1.91)	4(0.96)	8(1.91)
<i>E. histolytica</i> complex + <i>H. nana</i>	2(0.48)	–	–	–	2(0.49)	–	–	–

Abbreviations: *E. histolytica* complex, *Entamoeba histolytica* complex; *G. duodenalis*, *Giardia duodenalis*; *E. vermicularis*, *Enterobius vermicularis*; *H. nana*, *Hymenolepis nana*; –, missing data.

6.73, $P=0.000$) were significant indicators of protozoan infections in the present study (Table 3).

Although not statistically associated, the prevalence of the protozoan infections was higher among those who had

habits of eating raw vegetables and fruits, participants with poor personal hygiene, those who had not washed their hands after toilet and absence of regular waste disposal system.

Table 3 Factors Associated with the Occurrence of Protozoa Among Diarrheic Patients in Governmental Health Facilities of Adigrat Town, Tigray, Ethiopia 2019

Variables		Parasite Status, n (%)		COR (95CI)	P-value	AOR (95CI)	P-value
		Positive	Negative				
Source of drinking water	Pipe water	168(57.9)	122(42.1)	2.38(0.97–5.85)	0.059	3.08(1.19–8.00)	0.021
	Well water	8(36.4)	14(63.6)				
	River water	22(64.7)	12(35.3)				
	Hand pipe	54(75)	18(25)				
Presence of Latrine	Yes	154(53.4)	124(46.6)	1.88(1.22–2.88)	0.004	1.87(1.21–2.89)	0.04
	No	98(70)	42(30)				
Habit of hand washing after toilet	Yes	174(57.2)	130(42.8)	1.62(1.03–2.55)	0.04	0.94(0.56–1.58)	0.823
	No	78(68.4)	36(31.6)				
Habit of handwashing before food	Yes	238(94.4)	14(5.6)	2.06(0.99–4.28)	0.050	3.3(1.42–7.90)	0.006
	No	148(89.2)	18(10.8)				
Habit of nail trimming	Yes	112(47.5)	124(52.5)	3.69(2.40–5.67)	0.00	1.713(1.11–2.65)	0.016
	No	140(76.9)	42(23.1)				
Presence of dirty nail	Yes	179(75.2)	59(24.8)	4.44(2.93–6.76)	0.000	4.28(2.73–6.73)	0.000
	No	73(40.5)	107(59.5)				
Eating raw vegetables	Yes	134(59.8)	90(40.2)	1.043(0.704–1.545)	0.834		
	No	118(60.8)	76(39.2)				
Personal hygiene	Yes	164(54.3)	138(45.7)	0.804(0.49–1.31)	0.38		
	No	88(75.9)	28(24.1)				
Presence waste disposal	Yes	140(55.6)	112(44.4)	1.66(0.93–2.956)	0.086	1.17(0.612–2.24)	0.63
	No	112(67.4)	54(32.6)				

Note: 1 (referent).

Abbreviations: COR, crude odds ratio; CI, confidence interval; AOR, adjusted odds ratio.

Discussion

Parasitic infections particularly, protozoans infections are a significant causes of diarrheal diseases at individual and public health levels in lower-income countries due to lack of safe water supply, poverty, ignorance of health promotion practices and impoverished health services.^{15–17} In this study, the overall prevalence of intestinal protozoan infection was 59.3%. Our finding was higher than the study conducted in Ethiopia 45.3%,¹⁸ 35.5%,¹⁹ 41.1%,²⁰ 20.4%,²¹ 10.6%,²² 44.6%,²³ Iran, 30.1%,²⁴ 37.5%,²⁵ Ghana 42.9%,²⁶ Nigeria 19.3%.²⁷ However, it was comparable with study conducted in South Africa 68%.²⁸ In the other hand, our study was lower compare from study conducted in Tripoli, Lebanon 85%²⁹ and Nigeria 97%.³⁰ The variation might be due to difference in personal hygiene, population density, unavailability of potable water, socioeconomic status, poor environmental sanitation, malnutrition, health status, immunity and laboratory techniques used by different researchers.^{31–33}

E. histolytica complex was the predominant parasitic species detected in this study with a prevalence rate of 39.7%. This was higher than studies conducted from Ethiopia; 5.6%,³⁴ 10.3%,³⁵ 2.2%²² and lower than study conducted in other parts of Ethiopia 57.5%,²³ 50.8%.¹⁹ This variation might be due to poor access to safe drinking water, personal hygiene, environmental sanitation, and waste disposal system as indicated in this and other study.¹⁸ Similarly, in this study, the prevalence *E. histolytica complex* is higher than studies conducted Saudi Arabia 4.7%,³⁶ Malaysia 0.4%,³⁷ Italy 4.1%,³⁸ Myanmar 6.2%,³⁹ and Iran 0.25%.²⁴ In addition to the above variations the higher prevalence might be due to the over-diagnosis of *E. dispar* and *E. moshkovskii* as *E. histolytica*

G. duodenalis was detected among 11% of the study participants which is comparable with studies conducted in Ethiopia 11.2%,¹⁸ 8.4%,²² Iran 11%,²⁴ and lower than studies conducted in Ethiopia 42.4%,²³ 32.7%.¹⁹ The higher prevalence of *G. duodenalis* in other part of Ethiopia this might be due to lack of access to safe drinking water, inadequacy of medical care and lower degree of sanitation.²³ Likewise, the prevalence of *G. duodenalis* was lower than study conducted in Tripoli, Lebanon 28.5%²⁹ and higher than studies conducted in India 5%⁴⁰ and china 6.8%.⁴¹ The lower finding of *G. duodenalis* than *E. histolytica complex* in the present study might be due to the single study that can underestimate the prevalence of *G. duodenalis*.

Some studies have shown that males are at greater risk of developing protozoan infections^{18,24} and other studies indicated females were affected than males,^{42,43} while other studies have showed comparable prevalence.^{19,23} But, the present study indicated that occurrence of protozoan infections (*E. histolytica complex* and *G. duodenalis*) was higher in females than males. This is comparable with the studies conducted in Pakistan,⁴⁴ Uganda,⁴⁵ and Nepal.⁴⁶ This might be explained due to the reason that females have increased the likelihood of soil contacts during growing vegetables and eating row vegetables than males.⁴⁵

Study revealed that study participants who were using well water as a source of drinking were 3.08 times at higher risk of acquiring intestinal protozoan infections. This is parallel with previous studies.^{47,48} This might be contributed by the presence of resistant cysts of the parasites for routine chemical treatments like wuha agar and chlorine and the well water in the study area is unprotected; hence, it contains high number of pathogens. Similarly, those who had not washed their hands before the meal were 3.3 times at high risk of acquiring protozoan infections than those who were washing their hands before a meal. This is supported by the studies conducted elsewhere that wash their hands before meal is a significant prevention of protozoan infection.^{49–51} this might be due to the direct possibility of acquiring of these protozoan parasitic infections.

On the other hand, study participants who had not home latrine were 1.87 times more likely to harbor protozoan infections than those who had a home latrine. This was in agreement with studies from Ethiopia, Cotdivar and India.^{52–55} This might lead to open field defecation and favors the spread of intestinal parasites via faecal-oral route.⁵⁵ Our study also revealed that the risk of protozoan infections was 1.71 times higher among those who had untrimmed nails and 4.28 times higher among those who had dirty nails. This is consistent with the studies.^{49–51,54–57} This might be due to contaminated hands and fingers can serve as a carrier for the transmission of intestinal parasitic infections.^{50,56,58–60}

Conclusions

Findings of the present study showed that the study area was heavily infected with protozoan infections, implying that they are continued major public health problems. *E. histolytica complex* and *G. duodenalis* were the most predominant protozoans detected among diarrheic outpatients. Using well water as a source of drinking, having

poor handwashing habit before eating, not having home latrine, having dirty and untrimmed fingernails were associated with protozoan infections. Urgent actions are needed to reduce these protozoan infections via combined approaches involving Tigray health bureau, health extension workers, health workers and the community of the study area at large. All these bodies should plan for effective prevention and control of protozoan infections in the study area by giving awareness regarding the mode of prevention and control in the community.

Limitations of the Study

We were unable to differentiate *Entamoeba histolytica* from *Entamoeba dispar* and *Entamoeba moshkovskii* due to lack of molecular techniques. Other possible causes of diarrhea also not identified. Besides, this study was carried out only in symptomatic patients.

Abbreviations

CSA, Central Statistical Agency; SOPs, standard operating procedures, SPSS-Statistical product, and service solutions.

Ethics Approval and Consent to Participate

Ethical clearance was obtained from research and community service of Adigrat University. After discussing the purpose and relevance of the study, written permission was obtained from the administration of each health facility of Adigrat University before the data collection began.

Data Sharing Statement

To generate findings of this study, data were collected and analyzed based on the stated methods and materials. The original data supporting this finding will be available at any time upon reasonable request from the principal investigator.

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Author Contributions

BB designed the study, analyzed data & drafted manuscript; HN, GA, FM, HL, and KT participated in the

design of the study, data analysis and revision of the manuscript; HH, BT, NS, GG participated in data collection. All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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