Parasite Epidemiology and Control 28 (2025) e00408

Contents lists available at ScienceDirect

Parasite Epidemiology and Control

journal homepage: www.elsevier.com/locate/parepi

Intestinal protozoa infections and associated factors among diarrheal under-five children in Borena district, central Ethiopia

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ARTICLE INFO

Keywords:
Diarrhea
Intestinal protozoa
Prevalence
Under-five children

ABSTRACT

In impoverished nations, intestinal protozoan infections (IPIs) are a leading cause of diarrhea in children. However, in the majority of afflicted nations, including Ethiopia, the role played by each intestinal protozoa species in causing diarrhea and the risk factors linked with it are not adequately addressed. This would support focused intervention efforts. The prevalence of IPIs and related variables were evaluated between April and May 2023 among 380 under-five children in Borena district of Amhara region, central Ethiopia, by an institution-based cross-sectional survey. Systematic random sampling was used to select study participants. The study included a structured questionnaire to gather data regarding sociodemographic characteristics and perceived risk factors for IPIs. Using saline and iodine wet mounts as well as Richie's modified formol-ethyl acetate concentration technique, stool samples were collected and analyzed under a microscope. SPSS was used to enter and evaluate the data. At a 95 % confidence level, bivariate and multivariate logistic regressions were used to determine the factors linked to intestinal protozoa infection. Among 380 participants, 136 (35.8 %) were tested positive for one or more intestinal parasite species at least by one of the diagnostic methods. 118 (31.1 %; 95 % CI: 26.6–36.1) children were infected by intestinal protozoans.

The parasites detected were: *G. lamblia* 71 (18.7 %) and *E. histolytica/disar* 54 (14.2 %), *E. vermicularis* 3 (0.8 %), *A. lumbricoides* 2 (0.5 %), *H. nana* 2 (0.5 %) and *T. trichiura* 1 (0.3 %). Seven (1.8 %) participants were infected by both protozoan species. Children whose mothers/guardians did not attend formal education were at higher risk of IPI (adjusted odds ratio (AOR) = 2.801; 95 %CI: 1.666–4.711, $p < 0.001$) than children from literate mothers/guardians. Absence of functional toiltet in the household (AOR = 1.952; 95 %CI: 1.195–3.187, $p = 0.008$), hand washing with water alone, rather than with soap/ash (AOR = 3.052; 95 %CI: 1.203–7.746, $p = 0.019$) and having frequent contact with animals (AOR = 2.103; 95 %CI: 1.238–3.574, $p = 0.006$) were associated with IPIs. These findings revealed that *Giardia lamblia* and *Entamoeba histolytica* are public health problems causing diarrhea among under-five children in the study area, and their transmission is associated with the illiteracy of mothers/guardians, the absence of functional toilets, not using soap/ash during hand washing, and frequent contact with domestic animals. Therefore, when diagnosing, treating, and educating patients about diarrhea, healthcare professionals should take these protozoans into account.

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https://doi.org/10.1016/j.parepi.2025.e00408
Received 23 August 2024; Received in revised form 25 October 2024; Accepted 1 January 2025
Available online 3 January 2025

1. Introduction

Human intestinal protozoan infections are caused by protozoa which primarily reside in the gastro-intestinal tract. Intestinal protozoa infections (IPIs) affect an estimated 3.5 billion individuals worldwide, posing a serious threat to public health, particularly in poorer nations (Abu-Madi et al., 2017). Every year, 1.7 billion episodes of diarrhea are brought on by parasitic protozoan epidemics (Artemis et al., 2017). The population groups most susceptible to infection and serious morbidity include children under-five years of age (Harhay et al., 2010; Knopp et al., 2010).

Giardia, Cryptosporidium, pathogenic *Entamoeba*, *Blastocystis*, and *Cyclospora* species are some of the most common protozoa (McHardy et al., 2014). Notably, the intestinal protozoan parasites that cause acute diarrheal disorders most frequently include *Giardia lamblia* (*G. lamblia*), *Entamoeba histolytica* (*E. histolytica*), and *Cryptosporidium parvum* (*C. parvum*), especially in under-five children and immunocompromised people (WGO, 2012). One of the most common IPIs, giardiasis, affects over 280 million people and is responsible for 2.5 million fatalities; most of these cases affect under five children in low- and middle-income nations (Kalyoussef and Goldman, 2010). Every year, amoebiasis causes over 50 million instances of amoebic dysentery worldwide and accounts for over 100,000 fatalities (Choudhuri and Rangan, 2012). As a result, IPIs are listed as the second most common cause of death among under-five children (WHO, 2017).

The excrement of infected hosts sheds intestinal protozoa, which can live in water sources and damp areas as resistant cysts or oocysts for future infection (Arnone and Perdek, 2007; Barcelos et al., 2018). Therefore, the feco-oral route, which involves consuming food or drink tainted with infectious oocysts or cysts, is the way by which transmission happens. Direct contact with animals or people who are affected can also spread the infection (Ryan et al., 2018; Squire and Ryan, 2017). As a result, there are risks for infection associated with a variety of host and environmental factors, such as family size, parent literacy rates, poor hygiene, lack of access to clean drinking water, and inadequate sanitation (Anwar et al., 2015; Mumtaz et al., 2009).

Children under the age of five in rural areas are more vulnerable to IPIs. This susceptibility is linked to childhood habits like picking at their fingernails, not washing their hands before eating, and using toilet improperly. The transmission and incidence of infestations rise when these behaviors are combined with low socioeconomic level and low educational attainment of parents, particularly women (Okyay et al., 2004).

Intestinal protozoans can cause significant diseases, despite the fact that they are frequently associated with asymptomatic infections (Barcelos et al., 2018). When pathogenic intestinal protozoa colonize the human gut, they cause diarrhea and other intestinal symptoms. For example, diarrhea, dysentery, and infrequently extra-intestinal consequences like an amoebic liver abscess can be experienced by those infected with *E. histolytica* (Adnan Bashir Bhatti et al., 2014; Tanyuksel and Petri Jr., 2003). Furthermore, these parasite infections have detrimental effects on one's physical, emotional, and social well-being (Siddiqui et al., 2002).

Due to issues including poor hand washing facilities, a lack of toilet facilities in rural homes, and restricted access to improved sources of drinking water, impoverished children's health in Ethiopia is still severely impacted by IPIs (EDHS, 2017). Recent studies conducted in Ethiopia have shown that the prevalence of IPIs in diarrheal under-five children ranges from 10.85 % to 25.5 % (Eyasu et al., 2022; Gadisa and Jote, 2019; Mohammed et al., 2016; Wadilo and Solomon, 2016). Different studies are available in the Amhara region and in Ethiopia at large but it is important to update the epidemiology of IPIS. Moreover, the available data do not adequately represent isolated rural areas like Borena district. In the district, 10,087 under-five children received treatment for diarrhea in 2022, according to reports from the Borena district health office. Nonetheless, the majority of the therapies were assumed, and the common causes of diarrhea have not yet been addressed. Therefore, local epidemiological data regarding the diarrhea's causal agents will be essential.

2. Materials and methods

2.1. Study design, area and period

Between April and May 2023, an institution-based cross-sectional study was carried out on under-five children who had diarrhea and were enrolled in three rural health clinics located in the Borena district in central Ethiopia. Borena district is located in north-central highlands of Ethiopia at 10^0 34′N and 10^0 53′N and 38^0 28′E and 38^0 54′E. Mekane Selam serves as the administrative center.

It is located 469 km from Ethiopia's capital, Addis Ababa, and 289 km from the capital of the Amhara National Regional State, Bahir Dar. The district has an elevation of 500 to 4000 m above sea level, a mean annual temperature between 14 °C and 19 °C, and an average rainfall between 889 mm and 1500 mm.

2.2. Sample size determination and sampling technique

The sample size was determined using a single population proportion formula and a previous intestinal protozoan prevalence of 34 % from Bahir Dar city in northwest Ethiopia (Abera et al., 2020).

$$\text{Sample size}(n) = \frac{\left(z_{\alpha/2}\right)^2 \times p(1-p)}{d^2} = \frac{(1.96)^2 \times 0.34(1-0.34)}{(0.05)^2} = 345$$

where, n = the minimum sample size required, Z = standard score corresponds to 1.96 at 95 % confidence interval, d = is margin of sampling error tolerated (5 % marginal error was used), p = prevalence of intestinal protozoa. After adding 10 % (35) for nonrespondents, the final sample size was 380. At the time of data collection, there were six rural health centers in the district, out of these, three health centers - Dega Dibi, Tewa and Dilfrie, − were selected by lottery method. Based on the patient flow of under-five diarrheal children from each health center's previous year's report, the sample size was distributed proportionately to each chosen health center. Based on the 2022 health center data, 972, 864 and 811 under-five diarrheal children were expected to visit Dega Dibi, Tewa and Dilfrie health centers, respectively during the data collection period. Hence, based on the case flow, the sample size was proportionally allocated to each health center and 140, 124 and 116 participants were allocated to Dega Dibi, Tewa and Dilfrie health centers, respectively. Then sampling interval (k) was calculated for each health center by dividing the expected number of cases during the data collection period by the allocated sample size. The first participant was selected by lottery method among the first k cases, and then every k^{th} child was included in the study. The under-five outpatient department was the point of contact for children with diarrhea. Children less than five years old who were attending out-and-in patient clinics with diarrhea and were able to provide stool samples during the study period, and their parents/guardians were willing and able to participate in the study, were included in the study. Children who took treatment for intestinal parasite infection and/or antibiotic therapy within one month preceding the data collection were excluded from the study. For this study, diarrhea is defined as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the child).

2.3. *Data collection*

2.3.1. *Socio-demographic data*

A structured questionnaire prepared in English and then translated into Amharic (the local language) was used to gather data on sociodemographic characteristics, water, sanitation, and hygiene status. Trained nurses conducted in-person interviews with mothers/guardians of children to administer questionnaires.

2.3.2. *Fecal sample collection and examination*

The child's mother/guardian was given a graduated stool cup to collect approximately 5 ml of stool samples from the child. The stool cup was marked at 5 ml volume level using a permanent marker and children/parents were instructed to collect samples up to the marked level. The mother/guardian received instructions on how to collect, handle, and transport samples. An identification number and the collecting date were written on the labels of each container. Within 30 min of defecating, wet mount smears with direct saline and iodine were made and analyzed from each sample. Two wet mount preparations were done beneath 22 × 22 mm coverslips and on a clean glass slide. In order to detect motile trophozoites, the specimen for one preparation (located on the left side of the slide) was emulsified with normal saline (0.85 % *w/v* NaCl). In order to show the structure of cysts, the material in the other preparation (on the right side of the slide) was emulsified using Lugol's iodine. Using a 10× objective, the whole region beneath the coverslip was methodically inspected. The 40× objective was used to confirm the presence of parasites and correctly identify species (Agmas et al., 2021).

The remaining stool sample was subjected to the Richie's Modified Formalin-Ethyl Acetate Sedimentation Technique for analysis. The Ritchie's tube was filled with 2.5 ml of 10 % formalin and 1 ml of ethyl ether, respectively. The tube was then screwed shut after 0.5 g of the fecal sample was introduced. Following a minute, the cover was taken off, and the filtration concentration unit was added. It was then centrifuged for three minutes at 1000 x*g*. The 10× and 40× objectives were used to investigate the sediment. Positive stool samples were reported based on the results of one or more methods (Agmas et al., 2021).

2.4. *Data quality assurance*

Data collectors received training in order to maintain the quality of the data. The collection, transportation, and processing of the specimens were all done in strict accordance with standard operating procedures. Every reagent - normal saline, formalin, iodine, and ethyl acetate - had its expiration date verified on a regular basis. Two lab professionals inspected each smear in blind, and disagreements were resolved through conversation.

2.5. *Data analysis*

Version 25 of the Statistical Package for the Social Sciences (SPSS) program was used to enter and analyze data. In order to characterize the study participants and infection prevalence, descriptive statistics such as percentages were computed. To evaluate relationships between dependent and independent categorical variables, bivariate logistic regression was employed. After doing a bivariate analysis, variables with a *p-value* < 0.2 (Bursac et al., 2007) were subjected to multivariate logistic regression analysis to eliminate the potential influence of confounders. A *p-value* < 0.05 at a 95 % confidence level was deemed to indicate a statistically significant association between the variables.

2.6. *Ethical consideration*

Ethical approval was obtained from the Institutional Review Board (IRB) of the College of Medicine and Health Sciences, Bahir Dar University (protocol number 743/2023). Support letters were obtained from the Amhara Regional Health Bureau, South Wollo Health Department, and Borena District Health Office. Letters of permission were obtained from the administrator of each selected health center. Additionally, after explaining the importance, purpose, and procedure of the study by the data collectors, written informed consent was obtained from the study participants' parents/guardians. Participants who were tested positive for any parasitic infections were linked to the respective health centers for treatment.

3. Result

3.1. Sociodemographic characteristics of the study participants

Of the 380 participants in the study, 187 (49.2 %) were male and 193 (50.8 %) were female. The age range of the study participants was 2–59 months, with a mean (± standard deviation (SD)) and median age of 32.2 ± 16.5 and 36.0 months, respectively. The majority of the children's mothers/guardians (70.0 %) were farmers (Table 1).

3.2. Prevalence of intestinal protozoa infection

One hundred twenty-six (33.2 %; 95 % CI: 28.7–38.2) participants were positive for one or more intestinal parasite species at least by one of the diagnostic methods. The parasites detected were: *G. lamblia* 71 (18.7 %), *E. histolytica/disar* 54 (14.2 %), *E. vermicularis* 3 (0.8 %), *A. lumbricoides* 2 (0.5 %), *H. nana* 2 (0.5 %) and *T. trichiura* 1 (0.3 %). Two intestinal protozoan species, *G. lamblia* and *E. histolytica/disar,* were detected, with 118 (31.1 %; 95 % CI: 26.6–36.1) participants being infected at least by one species. Seven (1.8 %) children were co-infected by both protozoans (Table 2). Only protozoan cysts, only protozoan trophozoites and both cysts and trophozoites were detected in 55 (14.5 %), 40 (10.5 %) and 23 (6.1 %) participants, respectively.

3.3. Factors associated with intestinal protozoan infection

Binary logistic regression results show that children whose mothers/guardians did not attend formal education were at higher risk of IPI (adjusted odds ratio (AOR) = 2.801; 95 %CI: 1.666–4.711, $p < 0.001$) than children from literate mothers/guardians. Toilet availability in the household was also associated with protozoan infections that the odds of infection among children who have no toilte was 1.952 (95 %CI: 1.195–3.187, $p = 0.008$) compared to children who have functional toilet in their home. Similarly, participants who washed their hands with water alone, rather than with soap/ash, (AOR = 3.052; 95 %CI: 1.203–7.746, $p = 0.019$) and those who frequently contacted animals (AOR = 2.103; 95 %CI: 1.238–3.574, $p = 0.006$) were at higher risk of infection than their counterparts (Table 3).

4. Discussion

The overall prevalence of IPIs in the present study (31.1 %) among diarrheal under-five children was in line with previous reports of 28.0 % in Ethiopia (Lewetegn et al., 2019), 28.3 % in Kenya (Ondara et al., 2019) and 31.9 % in Pakistan (Haider et al., 2018). However, it was higher than previous findings in Ethiopia (Eyasu et al., 2022; Mekonnen and Ekubagewargies, 2019; Mohammed et al., 2016; Wadilo and Solomon, 2016; Zemene and Shiferaw, 2018). The finding was also higher than studies from other countries, where prevalences of 21.4 %, 14.3 %, 10.7 % and 16.9 % were reported in Mozambique (Bauhofer et al., 2021), India (Banerjee et al., 2020), Nepal (Ansari et al., 2012) and Saudi Arabia (Al-Megrin, 2015), respectively. The variation might be due to the poor hygienic and sanitary conditions of children and their caretakers in the present study area. 43.9 % of the participants had no functional toilet and 59.7 % had untrimmed fingernails in the present study. Moreover, 51.6 % did not have access to pipe/protected water for drinking (Table 3). Poor living conditions, improper disposal of faeces, and overcrowding favor for the transmission of intestinal protozoa. These factors were reported by other authors as well, along with a climatic difference, temperature, and humidity (Chelkeba et al., 2020; Haider et al., 2018). Differences in study population, study design and diagnostic methods might also be responsible for these variations. For instance, a study from India (Banerjee et al., 2020) was community-based, but our study was institution-based

Table 1
Socio-demographic characteristics and IPIs among under-five children attending rural health centers in Borena district, Amhara, central Ethiopia, 2023 (*N* = 380).

Variable	Catagory	Examined N (%)	Infected by intestinal protozoa N (%)	X^2	*p-value*
Age group (in months)	< 24	114 (30.0)	38 (33.3)	0.396	0.529
	≥24	266 (70.0)	80 (30.1)		
Gender	Male	187 (49.2)	59 (31.6)	0.043	0.836
	Female	193 (50.8)	59 (30.6)		
Family size	≤5	195 (51.3)	48 (24.6)	7.752	0.005
	>5	185 (48.7)	70 (37.8)		
Mother's/guardian Educational status	No formal education	169 (44.5)	76 (44.8)	27.537	<0.001
	Litrate	211 (55.5)	42 (19.9)		
Mother's/guardian Occupation	Farmer	262 (70.0)	83 (31.7)	1.898	0.594
	Housewife	45 (11.8)	15 (33.3)		
	Merchant	55 (14.5)	17 (30.9)		
	Government	18 (4.7)	3 (16.7)		

Table 2
Prevalence of IPIs among under-five children attending rural health centers in Borena district, Amhara, central Ethiopia, 2023 (N = 380).

Parasite species detected	Frequency	Percent (%)
Giardia lamblia	71	18.7
Entamoeba histolytica/dispar	54	14.2
Enterobius vermicularis	3	0.8
Ascaris lumbriciodes	2	0.5
Hymenolepis nana	2	0.5
Trichuris trichiura	1	0.3
Overall	**136**	**35.8**
Co-infections		
Giardia lamblia + *Entamoeba histolytica/dispar*	7	1.8

Table 3
Logistic regression factors associated with intestinal protozoan infections among study participants.

Variable	Catagory	Number examined (%)	Positive (%)	COR (95 %CI)	*P-value*	AOR (95 %CI)	*P-value*
Age group (in months)	< 24	114 (30.0)	38 (33.3)	1			
	≥24	266 (70.0)	80 (30.1)	0.860 (0.538–1.375)	0.530		
Gender	Male	187 (49.2)	59 (31.6)	1.047 (0.678–1.617)	0.836		
	Female	193 (50.8)	59 (30.6)	1			
Mother's/guardian Educational status	No formal education	169 (44.5)	76 (44.8)	3.288 (2.088–5.177)	<0.001	2.801 (1.666–4.711)	**<0.001**
	Litrate	211 (55.5)	42 (19.9)	1			
Mother's/guardian Occupation	Farmer	262 (70.0)	83 (31.7)	2.318 (0.653–8.228)	0.193	0.291 (0.065–1.301)	0.106
	Housewife	45 (11.8)	15 (33.3)	2.500 (0.625–9.996)	0.195	0.505 (0.102–2.504)	0.403
	Merchant	55 (14.5)	17 (30.9)	2.237 (0.571–8.760)	0.248	0.697 (0.151–3.218)	0.643
	Government	18 (4.7)	3 (16.7)	1			
Family size	≤5	195 (51.3)	48 (24.6)	1			
	>5	185 (48.7)	70 (37.8)	1.864 (1.199–2.897)	0.006	1.603() 0.985–2.608	0.057
Toilet availability	Yes	213 (56.1)	49 (23.0)	1			
	No	167 (43.9)	69 (41.3)	2.357 (1.512–3.672)	<0.001	1.952 (1.195–3.187)	**0.008**
Habit of hand washing after toilet	Always	338 (88.9)	97 (28.7)	1			
	Sometimes	42 (11.1)	21 (50.0.0)	2.485 (1.298–4.755)	0.006	1.846 (0.904–3.771)	0.093
Wash hands with soap/ash	Yes	57 (15.0)	7 (12.3)	1			
	No	323 (85.0)	111 (34.4)	3.740 (1.641–8.522)	0.002	3.052 (1.203–7.746)	**0.019**
Wash fruits/vegetables before use	Yes	229 (60.3)	66 (28.8)	1			
	No	151 (39.7)	52 (34.4)	1.297 (0.835–2.016)	0.247		
Fingernail status	Trimmed	153(40.3)	48 (31.4)	1			
	Untrimmed	227 (59.7)	70 (30.8)	0.975 (0.626–1.518)	0.912		
Water source	Pipe/protected water	184 (48.4)	43 (23.4)	1			
	Surface water	196 (51.6)	75 (38.3)	2.032 (1.300–3.177)	0.002	1.556 (0.946–2.559)	0.082
Availability of animals	Yes	299 (78.7)	97 (32.4)	1.372 (0.789–2.385)	0.262		
	No	81 (21.3)	21 (25.9)	1			
Contact with animals	Yes	245 (64.5)	90 (36.7)	2.219 (1.359–3.624)	0.001	2.103 (1.238–3.574)	**0.006**
	No	135 (35.5)	28 (20.7)	1			
Contact with animals' dung	Yes	114 (30.0)	32 (28.1)	0.817 (0.504–1.323)	0.411		
	No	266 (70.0)	86 (32.3)	1			

recruiting children who were sick and had a high probability of infection. Similarly, a study from Mozambique (Bauhofer et al., 2021) used ELISA technique which targeted only cysts, ignoring trophozoites, which are more common in diarrheal stool.

On the other hand, the prevalence of intestinal protozoa in the present study was lower than previous findings in Ethiopia (53.3 %) (Aiemjoy et al., 2017), Egypt (55 %) (Elmonir et al., 2021), and Port Blair, Andaman and Nicoba Islands (57.8 %) (Thamizhmani et al., 2017). The use of different diagnostic methods might be one factor in such differences. A study from Egypt (Elmonir et al., 2021) used modified Ziehl-Neelson staining for oocysts, in addition to direct wet mount and formol-ether concentration.

Children whose mothers/guardians didn't attend formal education were at higher risk of intestinal protozoan infection ($p < 0.001$). This could be due to less awareness among illitrate parents about the transmission methods of protozoa, so that they may not practice infection prevention activities. Children who had no functional toilet in their home were also at higher risk of infection ($p = 0.008$) which was in line with previous studies in Ethiopia (Mohammed et al., 2016; Tsegaye et al., 2020). This might lead to open field defecation and favor the spread of intestinal parasites via feco-oral route (Patel et al., 2013). This is more aggravated by a lack of protected water supply, as more than half (51.6 %) of the participants in the present study use unprotected water for drinking. This is supported by previous studies in Ethiopia (Hailegebriel, 2017; Berhe et al., 2020) and Iran (Bahmani et al., 2017), where people who drink unprotected water were at higher risk of protozoan infection than those who drink pipe water. This is also supported by the fact that, in developing countries, intestinal parasites are mainly transmitted due to fecal contamination of water and food, environmental, and sociocultural factors (Kiani et al., 2016; Bahmani et al., 2017; Orish et al., 2021).

Infective cysts that contaminate children's hands during play or defecation might not be effectively inactivated or removed through hand washing using only water. As a result, children who washed their hands with water were at higher risk of infection than those who used detergents, in addition to water ($p = 0.019$). Some species/strains of protozoa, like *G. lamblia* are zoonotic that availability and frequent contact with animals or their dung might be attributive to human infection. In line with this, children with frequent contact with domestic animals were at higher risk of infection ($p = 0.006$).

The present study had some limitations: we didn't detect intestinal coccidians using the recommended modified Ziehl-Neelson staining due to logistic problems. We were unable to differentiate between *E. histolytica* and *E. dispar* due to the lack of molecular assay techniques.

5. Conclusions

Intestinal protozoa are public health problems causing diarrhea in under-five children in the study area. Illiterate mothers/guardians of children, absence of functional toilet, not using soap/ash during hand washing and frequent contact with domestic animals predispose for intestinal protozoan infection. Therefore, when diagnosing and treating children who come with diarrhea, medical professionals in the study area should take protozoans into account. Additionally, intestinal protozoa must to be covered in the health education package. It is advised that more research be done to determine how intestinal coccidians contribute to diarrhea in under-five children in the study area.

CRediT authorship contribution statement

Alebie Mesfin: Writing – original draft, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Woynshet Gelaye:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation. **Getaneh Alemu:** Writing – original draft, Validation, Supervision, Software, Project administration, Methodology, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Bahir Dar University College of Medicine and Health Sciences for ethical approval of the study; Amhara Regional Health Bureau for giving support letter; study participants for volunteer participation in the study.

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