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The Transmission Pattern of Amoebiasis in Bale Zone, South East Ethiopia

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ABSTRACT

Amoebiasis is a primarily zoonotic disease, mainly transferred through the fecal-oral route and waterborne. Amoebiasis is still a big challenge for human and animal health and is a major cause of diarrhea in developing countries, including Ethiopia. Therefore, the study was conducted to assess the epidemiology of the disease in humans, dogs, and the occurrence of the parasite in water bodies. A prospective cross-sectional study was conducted in selected districts of the Bale zone in southeastern Ethiopia. Pet owners were selected randomly. Socio-demographic data were collected using a questionnaire and fecal samples were used to perform microscopic examination. A total of 383 fecal samples of humans, 383 fecal samples of dogs, and 58 water samples were studied from December 2019 to July 2020. Of 383 humans, 179 were males and 186 were females, while 94 individuals were grouped as children younger than 8 years, 164 were grouped as youth within the age range of 8-18 years, and 125 were grouped as adults who were older than 18 years. Of 383 local breeds, dogs were grouped as 87 puppies younger than one year, 192 young dogs with the age range of 1-2 years, and 104 adult dogs who were older than 2 years. Fecal samples were taken from 173 male and 210 female dogs. The water samples were taken randomly from the water sources (river, lake, pond, or water tank) at different sites where dogs and humans can easily contact water to use for different purposes. Of the total samples, 70 humans (18.3%), 63 dogs (16.5%), and 16 water samples (27.6%) were contaminated with the parasite. The major risk factors for the transmissions of parasites were contaminated drinking water, large family size, open-air defecation, and improper handwashing. The present study revealed that the human reservoir was a major risk factor for the spread and transmission of amoebiasis in dogs. The high prevalence of the disease might be due to open-air defecation, unhygienic health practices, domestic animals inside the houses, and using local water bodies as a drinking source.

Keywords: Amoeba, Dog, Human, Transmission, Water

INTRODUCTION

Amoebiasis has been introduced to the scientific community since 300 BC. *Entamoeba histolytica* (*E. histolytica*) was identified in human fecal samples in 1875. The genus *Entamoeba* comprises several species, such as *E. histolytica*, *E. dispar*, *E. moshkovskii*, *E. polecki*, *E. nutalli*, *E. chattoni*, *E. coli*, and *E. Hartmanni* (Ekanayake et al., 2006), which infects a wide range of mammals, including humans, non-human primates (dogs, cats, swine, rats, rabbits), and reptiles (such as snakes and lizards). Recent studies revealed the presence of *E. polecki* in swine, goats, and humans (Abioye et al., 2019). Among reptiles, snakes and lizards are infected by *E. invadens* which causes amoebiasis in these animals (Chia et al., 2009). Amoebiasis is a major cause of death in developing countries with an estimated rate of 50 million cases per year and 100000 deaths per year (Xim´enez et al., 2010).

The main hosts of *E. histolytica* are humans and primates. Infected dogs and cats can carry this pathogen. Dogs cannot transmit this pathogen, they only pass non-infective fragile trophozoite. However, dogs and other animals can be infected by humans (Ashar et al., 2014). The resistant cysts are passed through the excreta of chronic carriers and asymptomatic individuals. Seropositive Human immunodeficiency virus (HIV) case is a risk factor for invasive extra-intestinal amoebiasis (Hung et al., 2008). The main reservoir and source of infection are humans (Verkerke et al., 2012).

Amoeba produces cysts during unfavorable conditions and spreads to hosts due to unhygienic conditions. Infective cysts reach the hosts through unprotected water and contaminated food (Prakash and Bhimji, 2022). The life cycle of amoebiasis starts with the ingestion of infective cysts. The cysts germinate out and become feeding trophozoites, and then the trophozoites multiply asexually (Tanyuksel and Petri, 2003). The drinking water contaminated with fecal matter, human carriers (such as food handlers and livestock workers), and poor hygienic conditions all become major causes of the infection spread (Ashar et al., 2014). Microscopic examination of fecal samples needs three specimens taken on a

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separate day to increase the sensitivity of the test (Fotedar et al., 2007). The molecular diagnosis is the best method of identification; however, it cannot be used as a routine technique to identify the parasite due to the high cost and lack of standardization (Ngui et al., 2012).

The epidemiological studies of amoebiasis in domestic animals, reptiles, and other animals are rare. In Nigeria, the prevalence rate of amoebiasis in drill monkeys and chimpanzees was reported as 40% and 66%, respectively (Akpan et al., 2010). In a study conducted in the United Kingdom at Twycross zoo, the prevalence of amoebiasis was 81.7% in primates among which Old World Colobinae primates showed the highest prevalence of *Entamoeba* infection (Regan et al., 2014). Studies from Pakistan indicated that the prevalence of amoebiasis was 6% for local dogs, 2% for exotic, and 1% for crossbreeds (Ashar et al., 2014). Appropriate health practices (food hygienic practice, controlling open field defecation, hand washing, maintaining good environmental sanitation, especially in controlling the quality of drinking water, health education to the general public and food trade on the observance of good personal, environmental, and food hygiene) are the best methods to prevent this disease (Dickson et al., 2017). There is a gap in studies addressing the prevalence of amoebiasis in humans and dogs interface to measure the effect of the human amoebiasis on dogs amoebiasis as well as the effect of environmental health on the prevalence of amoebiasis in the study area. Therefore, the current study was designed to measure the prevalence and associated risk factors of amoebiasis in humans and dogs, and the occurrence of *Entamoeba* species in water bodies.

METHODS AND MATERIALS

Study area and duration

The present study was carried out in Delomena and its surrounding districts (Haranabuluk and Medawalabu) of the Oromiya regional state, Southeast Ethiopia about 430 km away from Addis Ababa, Ethiopia, from December 2019 to July 2020. The area was selected due to the proximity of Delomana Hospital, Ethiopia, to sample examination and ease of access to transportation. The altitude of the selected districts is between 850 m and 2800 m above sea level. The area gains a bimodal rainfall rained from September to November and March to June. An average annual temperature of 20-25°C and rainfall of 200 mm were recorded in 2020 (BZANRO, 2020).

Study population and sources

The study population consisted of dogs with their owners. The dog owners were included in the study using a simple random sampling technique. A total of 383 dog owners with their dogs (383 human and 383 dogs) were included in the study. Out of 383 humans (179 males and 186 females), 94 were grouped as children under the age of 8 years old, 164 individuals with the age range of 8-18 years were grouped as young, and 125 were grouped as adults who aged over 18 years old. Regarding the dogs, they were grouped as 87 puppies younger than one year, 192 young dogs with the age range of 1-2 years, and 104 adult dogs who were older than 2 years. All dogs included in the current study were local breeds, among which there were 173 males and 210 females. The owner's sociodemographic data were collected. Dogs under different management systems were sampled. The dog's demographic characteristics, management practices, and history of deworming and vaccination were also recorded. The questionnaires were distributed among the dog owners. The plastic container of 1-liter volume was used for water sampling from different water sources (river, lake, wells, springs, and piped water supply). Using this technique, 58 water samples were collected.

Study design

A cross-sectional study was conducted based on a questionnaire survey (Thrusfield, 2005) and microscopic examination of stool samples by direct fecal smear and floatation method. The presence of *Entamoeba* cysts and trophozoites in fecal and water samples were recorded as positive for *Entamoeba* species and further investigation of the historical background of dog owners with their dogs and the conditions of water bodies were carried out to identify the risk factors for the disease through questioner surveys.

Sampling methods and sample size

A total of 24 kebeles were selected by simple random sampling from the study area. A random sampling method was used to select the pet owners from the selected kebele. The desired sample size was calculated using a standard method based on the previous finding with a 5% expected prevalence of amoebiasis in Delomana town by Begna et al. (2014) results with 5% absolute precision. So, the calculated sample size included 383 dog owners, 383 dogs, and 58 water samples.

Data collection and analysis

Data were collected through direct smears and floatation method of fecal samples (microscopical examination) and a questionnaire survey. Each participant was asked to provide the fecal sample three times on different days to avoid

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false-negative results. All samples were placed in the air. Watertight samples and the vials were labeled with specific codes. Then, all stool samples kept in dry universal bottles were sent immediately to the Delomena Hospital Laboratory, Mena town, Ethiopia, for further studies. Ritchie's fecal concentration technique (Ritchie, 1948) and polyvinyl alcohol fixation of stools were carried out to obtain maximum sensitivity (Jensen et al., 2000). The fresh stool specimen was preserved with polyvinyl alcohol, and kept cool (4°C). Microscopic examination of fresh stool samples was carried out according to Soulsby (2006). A saline method and Lugol's iodine method were used to evaluate trophozoite and cysts. Motile trophozoites were observed and identified. All fecal samples were examined for *E. histolytica* analysis as described by Adam et al. (1979) and Soulsby (2006). A positive sample would contain motile amoebic trophozoites measuring 10-60 µm in size. The number of trophozoites and cysts was counted as described by Soulsby (2006). Stool specimens were stained with Lugol's iodine for the identification of cysts. The ocular micrometer was used to measure the size of the trophozoite or cysts. The *Entamoeba* cysts were identified based on the size and number of nuclei, as well as the presence and shape of the glycogen mass of chromatid bars. Maximum care was taken to select pathogenic *E. histolytica*. However, the result was confirmed as *Entamoeba* species due to the lack of confirmation by molecular diagnosis.

A questionnaire was prepared based on the standard questionnaire of the World Health Organization (WHO) and demographic information (sociodemographic factors, issues related to amoebiasis, hygiene, food handling system, drinking water sources, dog management practices, knowledge of parasitic diseases, and use of anthelmintics, the purpose of keeping dogs and the breeds of dogs) were collected.

Methods for detecting *Entamoeba* cyst in water

A total of 58 samples of high-turbidity water (100 mL) were collected from different sites of water bodies, including sludge, surface water, and groundwater. The water samples were taken randomly from the water sources at five different locations. Four different specimens, namely wet soil, mud, turbid water, and clean water were collected from each sample location. Each sample was then subjected to centrifugation and floatation techniques (Xiao et al., 2004). Slides were prepared, stained, and subjected to microscopy (XSZ-210, China).

Statistical analysis

Data were analyzed by the SPSS statistical software, version 23. The Chi-Square test and multivariable logistic regression were performed. Odds ratios were recorded. The Chi-Square test was used to evaluate the association between hypothesized risk factors and amoebiasis infection. Further analysis of the association was made by the multivariable logistic regression, and the odds ratio (OR), computed as the exponent of the respective regression coefficients, was used to quantify the effect of risk factors on the likelihood of amoebiasis. The confidence level was held at 95% and $p \leq 0.05$ was used to check the significance level in the analysis of generated data by logistic regression.

RESULTS

The overall prevalence of *Entamoeba* species in humans, dogs, and the water bodies in study areas is presented in Table 1. Of the samples, 70 (18.3%) humans, 63 (16.5%) dogs, and 16 (27.6%) water samples were positive for *Entamoeba* species.

Table 1. The overall prevalence of *Entamoeba* species in humans, dogs, and the water bodies in Delomena, Haranabuluk, and Medawalabu districts from December 2019 to July 2020

Study Districts	Humans		Dogs		Water bodies	
	Tested samples (N)	Positive (prevalence percentage)	Tested samples (N)	Positive (prevalence percentage)	Tested samples (N)	Positive (prevalence percentage)
Medawalebu	105	26 (24.8)	105	25 (23.8)	12	5 (41.7)
Haranabuluk	125	24 (19.2)	125	20 (16)	20	5 (25)
Delomena	153	20 (13.1)	153	18 (11.8)	26	6 (23.1)
Total	383	70 (18.3)	383	63 (16.5)	58	16 (27.6)

N: Number

Risk factors and prevalence of *Entamoeba* species in humans

The prevalence of amoebiasis was determined based on family size, living conditions, district, sources of drinking water, age, and educational status of the study population. Of those risk factors, family size, age, district, standards of

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living condition, sources of drinking water, and educational status were statistically significant ($p \leq 0.05$, Tables 2 and 3). The prevalence of human amoebiasis was higher in participants with the largest family size, defecation in the field, poorest living conditions, age range of 8-18 years, being users of the lake as water sources for household consumption, and illiteracy ($p \leq 0.05$), compared to other groups. However, there was no significant difference between male and female participants as shown in Table 3 ($p > 0.05$).

Statistical analyses of risk factors (demographics of participants) with amoebiasis through logistic regression analysis are illustrated in Tables 2 and 3. The contamination of drinking water (occurrences of *Entamoeba* species in water bodies) was statistically associated with the prevalence of human amoebiasis ($p \leq 0.05$). The prevalence of amoebiasis was higher in individuals who used contaminated drinking water (OR= 67.05, CI= 31.303- 143.618, $p \leq 0.05$), compared to those who used clean water for household consumption (absence of *Entamoeba* species in water bodies).

In terms of defecation, the open-air defecation group showed a higher infection rate of 26.4% (OR= 0.367, CI = 0.195- 0.689, $p \leq 0.05$), compared to the users of a toilet with a 6.4% infection rate of *Entamoeba* species. Moreover, a univariate analysis of the socio-demographic factors indicated that the prevalence rate of amoebiasis was associated with the living condition and the family size of participants.

The users of lake, river, well and spring water for household consumption were at higher risk, compared to the users of the piped water supplied by the municipalities. A significant association was observed between the sources of drinking water (lakes, rivers, and spring uses for drinking water, OR = 0.762, CI = 0.633-0.917, $p \leq 0.05$) and the prevalence of amoebiasis in participants. It was observed that the prevalence rate decreased from 29.8% to 4.2%, with the lower education level of the participants (OR=0.569, CI = 0.409-0.790; $p \leq 0.05$).

A total of 105 (27.41%) participants were from Medawolebu district, Ethiopia. Infection was higher in respondents from the Medawolebu district, Ethiopia (OR= 1.477, CI = 1.072-2.036, $p \leq 0.05$), compared to those from the Delomena and Heranabuluk districts of Ethiopia. The prevalence rate confirmed an age dependency association, with a significantly highest prevalence of amoebiasis in respondents of the age group between 8-18 years old (OR = 1.588, CI = 1.015-2.485, $p \leq 0.05$), compared to other age groups.

There was no significant association between the prevalence of *Entamoeba* species infection and the sex of the participants, however, males (19.8%) had a slightly higher prevalence rate, compared to females (16.7%).

Table 2. The potential risk factors associated with *Entamoeba* species in human Delomena, Haranabuluk, and Medawalabu districts from December 2019 to July 2020

Variable		Tested samples (N)	Number of positive (%)	X^2 (Chi-square)	Logistic regression	
					OR (95% CI)	P-value
Presence of *Entamoeba* species in waterbody						
	Occurrence	314	54 (78.3)	202.743	67.050 (31.303-143.618)	0.001
	Absence	69	16 (5.1)		57.968 (25.652-130.994)	
Defecation						
	Open-air	193	51 (26.4)	20.463	0.367 (0.195-0.689)	0.001
	Sometimes use toilet	65	11 (16.9)		0.352 (0.174-0.710)	
	Use toilet	125	8 (6.4)		0.337 (0.172-0.659)	
Family size						
	<6	307	32 (10.4)	63.883	6.513 (2.787-15.220)	0.001
	>6	76	38 (50)			
Income						
	High income	117	11 (9.4)	8.884	0.252 (0.111-0.568)	0.001
	Low income	266	59 (22.2)		0.257 (0.108-0.610)	
Handwashing						
	Proper handwashing	168	18 (10.7%)	11.459	0.46 (0.23- 0.9)	0.0016
	Improper handwashing	215	52 (24.2%)			

N: Number

The prevalence and risk factors of amoebiasis in dogs

The result of the microscopic examination of the fecal samples of the dogs is presented in Table 1. The results indicated that out of 383 fecal samples examined, 63 (16.5%) were positive for *Entamoeba* species. The present study revealed a significant association between amoebiasis in dog owners and amebiasis in dogs ($p \leq 0.05$). A higher prevalence of amoebiasis was observed in dogs whose owners had amoebiasis than in dogs whose owners did not have amoebiasis. The age and sex-wise prevalence of *Entamoeba* species were observed as the pups and females had higher prevalence, compared to adults and males (Table 4).

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Table 3. The potential risk factors (sociodemographic) factor associated with *Entamoeba* species in human Delomena, Haranabuluk, and Medawalabu districts from December 2019 to July 2020

Variable	Tested samples (N)	Positive samples (%)	X^2 (Chi-square)	Logistic regression	
				OR (95% CI)	P-value
Sources of water					
River	199	40 (20.1)			
Lake	40	15 (37.5)		0.762 (0.633- 0.917)	
Well	28	7 (25)	23.496		
Spring	14	3 (21)			0.004
Piped-water supply	102	5 (4.9)			
Literacy					
Illiterate	141	42 (29.8)		0.569 (0.409-0.790)	
Read and write	112	17 (15.2)	23.083	0.563 (0.401-0.789)	0.008
Elementary school	63	8 (12.7)		0.536 (0.382-0.751)	
High school	43	2 (4.7)		0.518 (0.366-0.735)	
Professional	24	1 (4.2)		0.536 (0.376-0.765)	
District					
Delomena	153	20 (13.1%)			
Heranabuluk	125	24 (19.2%)	5.803	1.47 7(1.072-2.036)	0.017
Medawolebu	105	26 (24.8%)			
Age					
Child<8	94	9 (9.6)		1.59 (1.01- 2.5)	0.043
Young (>8<18 yr)	164	41 (23.6)	8.08		
Adult (> 18yr)	125	20 (17.4)			
Sex					
Male	179	39 (19.8)	0.628	1.0	0.428
Female	186	31 (16.7)			

N: Number

Table 4. The potential risk factors associated with *Entamoeba* species in dogs of Delomena, Haranabuluk, and Medawalabu districts from December 2019 to July 2020

Variable	Tested samples (N)	Positive samples (%)	X^2 (Chi-square)	Logistic regression	
				OR (95% CI)	P-value
Owners' health status					
Infected by *Entamoeba*	70	18.3	299.01	620 (165.71-2319.73)	0.001
Not infected by *Entamoeba*	313	81.7			
Age					
≤1 yr	87	10 (11.5)		1.993 (1.324-3.00)	
> 1yr ≤2	192	23 (12)	15.9	2.158 (1.417-3.285)	0.001
>3	104	30 (28.8)			
Sources of drinking water					
River	199	37 (18.6)		0.69 (0.57- 0.85)	
Lake	40	12 (30)	17.443	0.72 (0.59- 0.87)	
Well	28	5 (17.9)		0.71 (0.57-0.88)	0.001
Spring	14	4 (28.6)		0.69 (0.54-0.86)	
Piped water supply	102	5 (4.9)			
District					
Delomena	153	18 (11.8)			
Heranabuluk	125	20 (16)		1.673 (1.180-2.371)	0.004
Medawolebu	105	25 (23.8)			
Sex					
Male	348	36 (17.1)	0.163	2.0 (0.48-8.14)	0.340
Female	173	27 (15.6)			

N: Number

Occurrence of *Entamoeba* species in water and its risk factor

A total of 58 samples of high-turbidity water (100 mL) were collected at various points of a water body (lake, river, well, spring, and pipe water), where the water was used for household consumption. Out of the 58 water samples,16 (27.6%) were positive for *Entamoeba* species based on direct microscopical examination (Table 5). During the study, it

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was found that 26.63% of domestic households in study areas were supplied with treated water from piped water supply and there were other remote villages with a sparse population not being covered by the main water supply system. The risk factors, sources of drinking water (lakes, rivers, springs, and wells, versus piped water supply), hygienic (poor hygienic surroundings versus good hygienic surroundings), human activities around water bodies (higher human activities around water bodies versus lower human activities around water bodies), and turbidity (lower turbidity of water bodies versus higher turbidity of water bodies) were significantly associated with the occurrence of *Entamoeba* species in water bodies ($p \leq 0.05$). The study revealed that the occurrences of the parasite in water bodies with poor hygienic surroundings (OR = 39, CI=7.635-199.208, $p \leq 0.05$) were highly comparable to that of water bodies with good hygienic surroundings. The occurrences of the *Entamoeba* species were higher in water bodies with a history of high human activities in and surrounding water bodies (OR = 9.667, CI = 2.347-39.817, $p \leq 0.05$) and lower turbidity (OR = 0.064, CI = 0.007-0.617, $p \leq 0.05$), compared to minimal human activities in and surroundings water bodies and higher turbidities, respectively.

Table 5. The potential risk factors associated with *Entamoeba* species in water bodies of Delomena, Haranabuluk, and Medawalabu districts from December 2019 to July 2020

Variable	Tested samples (N)	Positive samples (%)	X^2(Chi square)	Logistic regression OR (95% CI)	Logistic regression P-value
Surrounding hygiene					
Good	43	4 (9.3)	27.82	39.0 (7.635-199.208)	0.001
Poor	15	12 (80)			
Population and animal activity					
High	26	13 (50%)	11.851	9.667 (2.347-39.817)	0.002
Low	32	3 (9.4%)			
Turbidity					
High	27	3 (9.7)	10.692	0.064 (0.007-0.617)	0.017
Low	31	13 (48.1)			
Protection					
Unprotected	40	15 (37.5)	6.341	0.098 (0.012-0.813)	0.031
Protected	18	1 (5.6)			
Sources of water					
River	25	8 (33.3)	8.358	0.686 (0.472-0.998)	0.049
Lake	2	2 (66.7)			
Well	9	4 (44.4)			
Spring	4	1 (25)			
Piped water supply	18	1 (5.6)			

N: Number

DISCUSSION

In the study area, the high activities of domestic animals and humans surrounded by unprotected water sources caused frequent contamination of surface water, ponds, wells, rivers, and lakes with cysts of *Entamoeba* species. The occurrence of *Entamoeba* species in the water body depended on open-air defecation and high human activity in the surrounding water body. The overall prevalence of amoebiasis in humans was found to be 18.3% which agreed with the findings of previous studies conducted in Omo valley in southern Ethiopia with a prevalence of 16% (Teklehaymanot, 2009). Comparatively, the obtained result of the present study indicated a lower prevalence rate, compared to previous studies conducted in different parts of Ethiopia, such as 27.3% in Gondar (Ayelaw et al., 2011), 33.7% in Diredawa (Dawit, 2006), and in other countries, Kenya which was 74.1% (Robert et al., 2008) and 25.9% in Tajikistan (Matthys et al., 2011). However, the prevalence rate of *Entamoeba* species was higher in the current study, compared to other parts of Ethiopia, including 5% in Delomenatown (Begna et al., 2014), 5.6% in Jimma (Amare et al., 2007), 2.2% in South Wollo (Haji, 2016) and other countries, such as 6.2% in Thai district, Myanmar (Simona et al., 2005),4.1% in Parma Italy (Chukiat et al., 2003), and 0.4% in Kuala Lumpur, Malaysia (Jamaiah and Rohela, 2005). This higher prevalence of *Entamoeba* species might be due to low sanitary conditions and contamination of drinking water.

The results of the present study indicated a significantly higher prevalence of *Entamoeba* species among large-size families, compared to the small families. This finding was similar to a study in Derna city, Libya (Sadaga and Kassem, 2007) which reported higher infection of intestinal parasites in big families. Similarly, exposure to *E. histolytica* was positively associated with overcrowding at home or more household members in the family in the Orang Asli Ethnic Group in Malaysia (Shahrul et al., 2012) and Mexico (Alvarado et al., 2015). However, Gelaw et al. (2013) and Nath et

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al. (2015) reported that infection of *E. histolytica* was independent of family size in Ethiopia and India, respectively. The results of the present study indicated that participants both humans and pets belonging to large size families might be favoring the transmission of *Entamoeba* species. This might be due to overcrowding near homes.

Toilet usage and open-air defecations were statistically significant. This further increased the risk of the high prevalence of *Entamoeba* species. Similar findings were reported by Haji (2016). Indiscriminate stool elimination increased the risk of *Entamoeba* prevalence among communities. A study conducted on antiretroviral-treated HIV/Acquired Immunodeficiency syndrome patients in Ethiopia also reported that unavailability of latrines and lack of handwashing with soap was associated with *E. histolytica*/*dispar* infections (Mahmud et al., 2014). Open field defecation is found to be an important determinant in Nigeria, for *E. histolytica/ E. dispar* infestation which was similar to the findings of the present study (Idowu and Rowland, 2006).

It has been noted that those who did not wash their hands properly were at higher risk of being infected with *Entamoeba* species. The main role of dirty hands in the fecal-oral spread of the disease has been well reported in low-income countries and washing hands before eating or after defecation have been regarded as a secondary barrier. The result agrees with previous studies (Gasem et al., 2001). Poor personal hygiene, poor living conditions, unwashed hands after playing with soil or gardening, and the presence of already infected family members were important predictors for intestinal protozoan infections including *Entamoeba* species (Zhang et al., 2013; Nath et al., 2015; Joyobrato et al., 2018). In Vietnam, the transmission route via contaminated hands is of significant importance through which the infection risk increases by three times if hands are not washed properly (Pham et al., 2011). Previous studies in Italy and Yemen also show the significance of handwashing prior to food (Rinne et al., 2005; Naelahet al., 2011). Hand washing and diarrhea were significantly associated (Knight et al., 1992) as was reported in Myanmar (Han and Hlaing, 1989) and Indonesia (Gasem et al., 2001).

The present finding indicated that the factors responsible for infection by *Entamoeba* species were related to poor living conditions, unhygienic toilet facilities, without habits of washing hands before taking food. The result agrees with other studies from India, Italy, and Yemen (Rinne et al., 2005, Naelah et al., 2011; Joyobrato et al., 2018). The place of defecation and the presence of pets in the houses were found as significant contributing factors. The type of water used for washing utensils was a determinant of disease transmission. The pond water (27%) is a major determinant of parasitic infestation, compared to river water (25.8%) and tube wells 14.9%, (Sintu et al., 2011). The literacy of the parents was a crucial contributing factor in *Entamoeba* transmission. This was confirmed by the recent studies conducted in Ethiopia and other countries (Wordemann et al., 2006; Ayalew et al., 2011; Begna et al., 2014).

A significant difference in the prevalence of amoebiasis was observed among the three age groups. The highest prevalence rate of amoebiasis was seen among the young group (8-18 years), and the lowest infection rate was seen among children. Similar age distribution of infection had been observed in Dire Dawa, Ethiopia (36.1%), where children within the age range of 6-14 years had the highest infection rate, compared to other age groups (Dawit, 2006). In Kenya, individuals within the age range of 10-14 years recorded a higher prevalence and intensity of *E. histolytica* infection, compared to the older age group, 15 years and over. Garmie (2016) also reported that children of 8-18 years were the most affected group with *E. histolytica* infection. Generally, young children have been reported to be more exposed to *Entamoeba* species infection than adults. The reason might be that children have very active playing habits at home/school and more chances to get in contact with food and drinks that were contaminated with the infective cyst of the parasite.

The prevalence of *Entamoeba* species among different sex was not statistically significant. The present study indicated that 19.8% of males and 16% of females were positive for *Entamoeba* species. This finding was in agreement with the previous studies (Ohnishi and Murata, 1997; Sharma et al., 2004; Ozyurt et al., 2007). Other studies conducted in Turkey (Ogzumusand Efe, 2007) have shown a high prevalence of amoebiasis among females. The higher prevalence of *Entamoeba* species in males could be explained on the basis that males are more susceptible than females to infections caused by parasites because males generally exhibit reduced immune responses and increased intensity of infection compared to females (Klein, 2000). These differences were usually attributed to ecological, sociological, and physiological factors.

The prevalence of *Entamoeba* species between humans using pipe water and a hand pump (protected) and lake, river, well, and spring (unprotected water sources) showed a significant difference. In the study area, different activities of humans and livestock surrounding the unprotected water sources led to continuous contamination of surface water, wells, rivers, and the lake with cysts of *Entamoeba* species. A similar observation was recorded by Dawit (2006) in Diredawa. According to Dawit, there is a significant difference in infection of *Entamoeba* species among children using protected (23.3% for amoebiasis) and unprotected (33.3% for amoebiasis) water sources. Besides, a study conducted in the United Kingdom showed that a large number of intestinal parasites, including *E. histolytica/dispar* were detected in drinking water samples from water sources where agricultural and human activities were high. On the other hand, the prevalence of *Entamoeba* species and other intestinal parasites was low in the protected water bodies with no agricultural activities and minimal human activities. The prevalence of amoebiasis was low in areas with access to clean water and

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improved environmental hygiene. In the present study, water sample analysis indicated that the occurrence of *Entamoeba* species cyst in both protected and unprotected water sources was 27.6%. This occurrence of *Entamoeba* species in the water body caused infection in humans and dogs in the study area. The prevalence of *Entamoeba* species in water bodies of the study area depended on the level of protection, which stresses the importance of the water quality and the transmission of *Entamoeba* species. The occurrence of *Entamoeba* species in the water body depended on open-air defecation, and high human activity in and surrounding the water bodies (fetching, swimming, washing, and bathing). These results agree with the report of Ben and Sabbahi (2017) indicating that the occurrence of amoebiasis in waterbodies was significantly higher in uncovered wells (43.3%) than in covered wells (35%), in unclean surroundings (48.9%) than in clean surroundings (20%), in turbid well water (51.9%) than relatively clear well water (30.2%) and wells frequently used by grazing animals (53.1%).

The present study revealed an overall prevalence of *Entamoeba* species in pets was 16.5% in Delomena, Haranabuluk, and Medaworeda districts, Ethiopia. The study reported a lower level of infection with a prevalence of enteric protozoan infections in dogs at 50.9% in Hawassa, Ethiopia (Mekibib and Sheferaw, 2018). These findings were relatively similar to the reports from various areas as 12.11% in Spain by Segovia et al. (2010), 13.4% in Bangladesh by Mahmud et al. (2014), and relatively higher than those reported by Alam et al. (2015) in dogs (7%) and Gillespie et al. (2017) in Australia (1%), and Justin et al. (2020) in Cameroon (1.5%). The differences in the prevalence of enteric protozoa could be due to variation in geographic location, owner awareness, and the number of stray dogs in an area. The prevalence of enteric protozoan parasites in dogs was significantly higher in younger dogs. A higher prevalence of *Entamoeba* species in puppies was reported by other studies (Adejinmi et al., 2001, Sager et al., 2006, Lorenzini et al., 2007). This might be due to either immune incompetence or a low level of passive immunity in puppies received from their dam. The prevalence of amoebiasis was higher in the dogs whose owners had a history of *Entamoeba* species infection, compared to the dogs whose owners were free from the amoebiasis. This appeared to be due to the cross-infection of *Entamoeba* species from the owner to the pet. Most of these dogs became chronic carriers of *Entamoeba* species. The dogs that were kept by low-income groups of people receive less medical care. The prevalence of *Entamoeba* species in the dog was related to the income of the owner. The prevalence of the dog amoebiasis was low, in which the pet owners had a high income (2%), compared to those with low income.

During a cross-sectional study of dog owners in the study area, the analyses of fecal samples of dogs and their owners revealed that both harbor the *Entamoeba* species. Both the samples from the drinking water source and the stool samples of dog owners showed the presence of cysts or trophozoites. This result indicated that the source of infection was contaminated drinking water, poor management practice, and level of awareness of dog owners about dog parasites and associated risk factors, in addition to the lack of veterinary attention. These exacerbated the risk of transmission of canine parasitic zoonoses to the human community or vice versa.

In the present study, it was surprising to see that amoebiasis was transmitted between humans and dogs. Additionally, the prevalence of amoebiasis in humans and dogs had a great contribution to the contamination of water bodies by cysts of *Entamoeba* species. *Entamoeba* was identified or diagnosed in both pets and pet owners from the same households with similar sources of drinking water. During a cross-sectional survey of pet owners in the study area, 63 individuals and their dogs were positive for *Entamoeba* species. This result agrees with the findings of Endrias et al. (2010). It is now accepted that *E. dispar* infection is much more common than *E. histolytica* worldwide (Ramos et al., 2005). Human infections with *E. moshkovskii* have also been reported in Tanzania, Bangladesh, India, Iran, Australia, and Turkey, and in general, they are not associated with the disease (Solaymani et al., 2006; Tanyuksel et al., 2007; Fotedar et al., 2008).

CONCLUSION

In the present study, a higher prevalence and occurrence of *Entamoeba* species have been found among humans, dogs, and water bodies with the average prevalence rates of 18.3%, 16.5%, and 27.6%, respectively. Amoebiasis was identified or diagnosed in both dogs and dog owners from the same households with similar sources of drinking water. This indicated that the transmissions and spread of the amoebiasis occurred among humans, dogs, and water bodies. The increasing prevalence of *Entamoeba* species in the present study was associated with the socioeconomic burden of the community. Improved personal and environmental sanitation is the most effective sustainable option for controlling and preventing amoebiasis in humans and dogs. People often depend on open-air defecation systems might lead to infection in humans, dogs, and contamination of water bodies by *Entamoeba* species. The microscopic examination for intestinal parasites and comparison with symptom profiles of the patient remains the crucial routine diagnostic tool. However, further studies using molecular approaches are needed to distinguish the morphologically identical pathogenic and non-pathogenic species in the studied area.

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Authors' contribution

Sufian Abdo Jilo generated the idea, proposal, and paper write-up and completed the paper. Mukarim Abdurahman Kadir, Johar Aliye Hussein, and Sureshkumar P. Nair have taken part in the paper write-up, data analysis, and edition of the manuscript. All authors read and approved the final version of the manuscript and conceived the study.

Competing interests

The authors have not declared any conflict of interest.

Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

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