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The Transmission Pattern of *Entamoeba Spp.* In and Around Dello Mena District, South East Ethiopia

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Abstract

Amoebiasis is primarily a disease of humans and animals and its transmission is mainly faecal-oral route and waterborne. Amoebiasis is still a big challenge for humans, animals and is a major cause of diarrhea in least-developed tropical and subtropical regions including Ethiopia and its prevalence data in animal and environmental occurrence is rare. Recent information on these diseases is essential to design suitable control interventions in a specific setting. This study was conducted to assess the magnitude of Entamoeba species infestation in humans, dogs and its occurrence in water.

A prospective cross-sectional study was conducted among the population of DelloMana District. Pet owners were selected randomly and sociodemographic data were collected using a standard questionnaire. Microscopic examinations of stool samples were done. Parasites were identified using morphometric techniques using the known pathogenic spp. positive control slides. A total of 383 faecal samples of humans, 383 their dogs, and 59 water samples were studied from December 2019 to July 2020.

Out of 383 humans, 70 (18.2%), 383 dogs 63 (16.4%) and 59 water samples 16 (27.11%) were contaminated with *Entamoeba spp.* The major risk factors for the spread and transmissions of parasites were contaminated drinking water (prevalence: 78.3%, p-value ≥ 0 and OR (95% CI), 67.050 (31.303, 143.618), family size (prevalence: 50%, p-value ≥0 and OR (95% CI), 6.513 (2.787, 15.220), open air defecation (prevalence: 26.4% p value ≥0 and OR (95% CI), 0.367 (0.195, 0.689) and improper hand washing (prevalence 76.5% p-value=0.002 and OR (95% CI): 2.5 (1.36-4.4). Of the 59 water samples from different sites of Dellomena, Haranabuluk and Medawolebu 16 (27.11%), samples were positive for Entamoebiasis based on direct microscopical examination. This study revealed that human reservoirs are a major risk factor for the spread and transmission of Amoebiasis in the dog (prevalence: 18.2%, p-value: ≥ 0.000 and OR (95%CI), 620.000 (165.709, 2319.733).

This study revealed that the overall prevalence of amoebiasiswas high among humans and dogs. The high prevalence of the disease might be because of open air defecation, unhygienic health practices, keeping domestic animals inside the houses or using local water bodies as a drinking source.

Keywords: Amoebiasis; Human; Dog; Water

Introduction

Amoebiasis is known to the scientific community since 300 BC. *Entamoeba histolytica* was identified from human samples in 1875 by FredorLosh. This Cosmopolitan genus is an intestinal protistof diverse host as humans, Nonhuman Primates (NHPs), and other animals such as dogs and cats. 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*, Amoebiasis contribute a major cause of death in developing countries with an estimated 50 million cases per year and 100,000 deaths [1].

Amoeba produces cysts during unfavorable conditions and spread to the hosts because of unhygienic conditions. Infective cysts reach the hosts through unprotected water and contaminated food. The life cycle of Amebiasis starts with the ingestion of infective cysts. The cysts germinated out and become feeding trophozoites. The trophozoites multiply asexually. The drinking water contaminated with faecal matters, human carriers like food handlers, live stick workers and poor hygienic conditions all become major causes of the spreading of this infection. The specimens are examined microscopically for the presence of trophozoites and cysts for three subsequent days can give good results. The molecular diagnosis is the best method of identification and due to the high cost and lack of standardization; it cannot be used as a routine technique to identify the parasite. Good health practices are the best method to prevent this disease [2].

There are several species of Entamoeba and are common in humans, nonhuman primates and other different mammals but the only known pathogen is *Entamoeba histolytica.* Humans are the natural host for this species and the usual source of infection for domestic animals. Mammals become infected by ingesting food or water contaminated with faeces containing infective cysts. *E. invadens* of reptiles is not communicable to mammals and are morphologically similar to *E. histolytica.* The *Entamoeba dispar* usually infects humans but it is a nonpathogenic species. But some medical reports indicated the pathogenicity of this species in humans and may cause symptomatic colitis. *Entamoeba histolytica* is an anaerobic parasite predominantly infecting humans and other primates causing amoebiasis. This protest parasite includes several species like *E. histolytica, E. dispar, E. moshkovskii, E. polecki, E. nutalli, E. chattoni, E. coli, E. hartmanni, E. ecuadoriensis* and *E. Bangladeshi*. Nonhuman primates harbor several *Entamoeba spp.* of varied importance to human and domestic animal health. The *Entamoeba* species like *E. histolytica, Entamoeba dispar, Entamoeba moshkovskii, Entamoeba polecki, Entamoeba coli* and *Entamoeba hartmanni* can reside in the human interstitial lumen of these *E. dispar* and *E. moshkovskii* are from patients with GastroIntestinal (GI) symptoms, but the pathogenicity of these species remains to be confirmed [3].

Entamoeba can tide over the unfavorable conditions in soil and water for about one month, and up to 45 minutes under fingernails. The incubation period of this parasite is about 14 to 28 days but it may vary up to months or years. The onset of incubation period *Entamoeba* invades colonic mucosa and cause ulcerative lesions and bloody diarrhea often called amoebic dysentery [4].

Humans act as a reservoir *Entamoeba* and cause disease to domestic animals. The studies on domestic animals, reptiles and other animals are rare. Team of experts, reported that the prevalence of amoebiasis in drill monkeys and chimpanzees from Nigeria is 40% and 66% respectively. A study in the UK at Twycross zoo indicated that the prevalence of amoebiasis is 81.7% in primates. Old World Colobinae primates showed the highest prevalence of *Entamoeba* infection. Studies from Pakistan reported that the prevalence of amoebiasis in local dogs is 6%, exotic 2% and [5] cross breed 1%. The detection of cysts or haematophagous trophozoites in stool is the common method used in the routine diagnostic method of the pathogenic form. But by using this method species identification is difficult and sensitivity and specificity are less than 60%. But if formalin ether sedimentation and trichome techniques are performed together a better sensitivity can be achieved. Advanced techniques like polymerase chain assay can be used for the accurate detection of species. According team of experts around 500 million people with *Entamoeba* infection are colonized by experts reported that the prevalence of *E. hystolytica* varies based on the regions, for example, Brazil 11% and that Mexico 8%. Some studies revealed that the prevalence of amoebic liver abscesses is high in endemic areas of HIV infection [6].

The high prevalence of Entamoeba spp. in developing countries might be due to low socioeconomic and unsanitary conditions. The areas of endemic infection were usually seen in developing countries because of ignorance, poverty, overcrowding, inadequate and contaminated water supplies. These conditions favour direct faecal-oral transmission of cysts among the population. It is now known that even in areas where invasive amebiasis is common *E. dispar* is by far the most prevalent species reported that Jimma town that an occurrence of amoebiasis was 3.1% [7].

Entamoeba (family *Entamoebidae*) is a genus of intestinal protistsand it comprises several species, including *E. histolytica, E. dispar, E. moshkovskii, E. polecki, E. nutalli, E. chattoni, E. coli* and *E. hartmanni*. *Entamoeba* genus includes both pathogenic and nonpathogenic species. *Entamoeba* infects a wide range of hosts dogs, cats, swine, and rats and rabbits. *E. dispar* was considered as a separate species recently and it is seen in humans. Recent studies revealed the presence of *E. polecki* in swine, goats and also in humans. *Entamoeba invadens* were isolated from reptiles, such as snakes and lizards and it can cause amoebiasis in these animals. The main hosts of *E. histolytica* are humans and primates. Infected dogs and catscan carrythis pathogen. Dogs cannot transmit this pathogen. Dogs pass only non-infective fragile trophozoite. But dogs and other animals get an infection from humans. The resistant cysts are passed through the excreta of chronic carriers and asymptomatic persons. Seropositive HIV case is a risk factor for invasive extra intestinal amoebiasis. The main reservoir and source of infection are humans [8].

Materials and methods

Study area and duration

The present study was conducted in selected districts of Bale Zone namely Dello-Mena, HaranaBuluk, and Medawalabu Woredas of the Oromiya Regional State, Southeast of Ethiopia about 430 kms away from Addis Ababa. The altitude of the study area ranges from 850 to 2800 m.a.s.l, where the lowland area predominates with a narrow strip of high land area in the Northern part of Dello-Mena, Heranabuluk and Medawolebu district. The area experiences a bimodal rainfall occurring from September to November and March to June. An average annual temperature of 20-25°C and rainfall of 200 mm are recorded in Dello-Mena. The vegetation of the area changes with altitude ranging from scattered trees and bushes in the low land to dense woody forest area in the high land. Dello-Mena district is endowed with several rivers, nine perennial rivers flow across the district namely: Welmel, Yadot, Erba-1, Erba-2, Deyu, Denda, Doya, Bidimo, Mandisa, Gobele, and Shawae. The rivers and other deep wells, ephemeral ponds, seasonal streams are sources of water for livestock and people. Among Dello-Mena district and its surrounding woredas communities, most of them are pastoralists and others have an agricultural vocation and a mixed farming system with crop-livestock production. The study was conducted from December 2019 to July 2020 [9].

Study population and sources

The study population consists of dogs and their owners. The owner's sociodemographic data were collected. Dogs under different management systems were sampled. The dog's demographic characteristics, management practices, history of deworming and vaccination were recorded. The questionnaire survey was taken from the dog owner. The water samples were taken from the drinking water sources such as a rivers, lakes, wells and springs in the study area [10].

Study design

A cross-sectional study based on a questionnaire survey and microscopic examinations of stool samples were done [11].

Sampling methods and sample size

The kebele was selected randomly from each woreda by using the computer. A random sampling method was used to select the pet owners from the selected kebele. The desired sample size was calculated using standard methods [12-18].

Data collection and analysis

Data were collected through parasitological examination and questionnaire survey. Three faecal samples from each human and dog were collected on three consecutive days to avoid false negative results. All samples were placed in air and watertight sample vials, labeled accordingly. Then all stool samples received in dry universal bottles were sent immediately to the Delomena hospital laboratory, for further studies. Ritchie's faecal concentrationtechnique and polivinyl alcohol-fixation of [19-24] stools were carried out to get maximum sensitivity. Fresh stool specimen was preserved with a fixative such as polyvinyl alcohol or kept cool (4°C). Microscopic [25-36] examination of fresh stool samples was carried out. A saline method and Lugol's iodine method were used to evaluate trophozoite and

cysts. Occasionally motile trophozoites were seen even after 4 hours at this temperature, although the trophozoites generally disintegrate rapidly in unfixed stool specimens. All faecal samples were examined for *Entamoeba histolytica* analysis as described by team of experts. A positive sample would contain motile amoebic trophozoites measuring 10-60 µm in size. The number of trophozoites and cysts were counted, as described by Soulsby, 2006. Stool specimens were stained with Lugol'siodine for the identification of cyst. Iodine stains make the nucleus perfectly visible. The ocular micrometer is used to measure the size of the trophozoite or cysts. The *Entamoeba* cysts were identified based on the size, number of nuclei and presence and shape of the glycogen mass of chrometoidal bars. Maximum care was taken to select pathogenic *E. histolytica.* But the result is given as *Entamoeba spp.* because of the lack of confirmation by molecular diagnosis [37-45].

A questionnaire was prepared based on the standard questionnaire of WHO and informations like sociodemographic factors, issues related to amoebiasis, hygiene, food handling system, drinking water sources, dog management practices, knowledge on parasitic diseases and use of anthelmintics, the purpose of keeping dogs and the breeds of dogs were collected in Delo Mena Town, Ethiopia. The sample size is calculated based on Dirk, 2002 and Thrusfield, 2005 [45-50].

Methods for detecting *Entamoeba cyst* in water

Water samples were collected from different sites such as from sludge, surface water and groundwater of [51] drinking water that used for drinking for themselves and their animal such as dogs. The samples were taken randomly from the water sources at five different locations. Four [52] different specimens namely wet soil, mud, turbid water and clean water was collected from each sample location. Each sample was subjected to centrifugation and floatation techniques. Slides were prepared, stained and subjected to microscopy [53-60].

Statistical analysis

Data were analyzed by the SPSS statistical software. The Chi-Square test and multivariable logistic regression were performed. Odd ratios were recorded. The confidence level was held at 95% and p value ≤ 0.05 was used to check the significance level [61-63].

Results and Discussion

The studied population includes three Districts Delomena, Haranabuluk and Medawalebu of Bale Zone, southeast Ethiopia. This cross-sectional study comprises a total of 383 humans, 383 dogs and 59 water samples collected from the studied districts. A random sampling technique was adopted. Among the sample 70 (18.6%) from human, 63(16.4%) from dogs and 16 (27.11%) from water [64-68] sample were positive for *Entamoeba spp.* The [69-75] statistical analysis of the data revealed that there was significant association ($p<0.05$) between the prevalence of amoebiasis in dogs and the owner of the dog. The risk factors such as contamination of drinking water by *Entamoeba sps,* family size, socioeconomic, educational background of the owner, open air defecation, improper hand washing and age with that of dog and owner also indicates high significance (p value ≤ 0.05). Also, the result indicates that there is no significant association between the location and sex of owners [76-78]. Table 2 indicates a high prevalence of *Entamoeba* infection in male owner population. The studied areas showed a high prevalence of amoebiasis (Table 1).

Study	Human		Dogs	Water		
Districts	No. tested	No. positive (% prevalence)	No. tested	No. positive (% prevalence)	No. Tested	No. positive (% prevalence)
Delomena	153	20 (13.1%)	153	18 (11.8%)	26	6 (23.1%)
Haranabuluk	125	24 (19.2%)	125	20 (16%)	20	5 (25.0%)
Medawalebu	105	26 (24.8%)	105	25 (23.8%)	12	5 (41.7%)
Total	383	70 (18.3%)	383	63 (16.4%)	58	16 (27.8%)

Table 1: Overall prevalence of *Entamoeba spp*. In human, dog and water body of the studied districts.

Risk factors and prevalence of *entamoeba spp.* In human

The prevalence of Amoebiasis was determined based on family size, living condition, woreda, sources of drinking water, age, educational status of the study population of these risk factors family size, age and woreda were statistically significant and p value<0.05 (Tables 3 and 4). A statistical analysis showed that there was a highly significant variation in family size, defecation, living condition, between woreda, sources of drinking water and educational status($p<0.05$) with a high prevalence of Entamoeba spp. in humans was reported in the big families with more family members, open air defecation, age between 8-18 age group, lake as the source of the drinking water and literacy, sex, lower age group, and living standard respectively. But no statistical significance was seen among gender ($p>0.05$) as shown below by Tables 2 and 3.

Logistic regression analysis of [79] demographics and the prevalence of E. histolytica infection was presented in Tables 2 and 3. The prevalence showed an age dependency association, with significantly higher infection rates among respondents aged between 8 to 18 years (OR=.131 (0.077, 0.184)95% CI=(0.077, 0.184) P= ≥

0.001) and in the other age group. 105 (27.415%) of the participants were from Medawolebu. Infection was higher among respondents from the Medawolebu (OR=1.477; 95% CI=1.072, 2.036; P=0.017) than those from Delomena and Heranabuluk. There was no significant association between the prevalence of *E. histolytica* infection and sex, but males (19.8%) had a slightly higher prevalence rate compared to females (16.7%). Among [79] other socio-demographic factors, as in various sources of drinking water, the participants who depend on the lake, river, and spring or well for drinking water (OR=0.762; 95% CI=(.633, 0.917); P=0·004), the river, well and the spring water users were at higher risk compared to the pipe water (supplied by the municipalities) users. In context to the defecation, open air defecation groups showed an infection rate of approximately 26.4% (OR=0.367; 95% CI=((0.195, 0.689); P<0.001) compared to the users of toilet [80].

Variable	No. tested	No. positive (%)	X^2 (p-value)	Logistic regression	
				OR (95% CI)	P-value
District					
Delomena	153	20 (13.1%)	5.803 (0.055)	1.477 (1.072,2.036)	0.017
Heranabuluk	125	24 (19.2%)			
Medawolebu	105	26 (24.8%)			
Sex					
Male	179	39 (19.8)	0.628 (0.428)	1	
Female	186	31 (16.7)			
Sources of drinking					
river	199	40 (20.1)	23.496 (0.00)	0.762 (.633, 0.917)	0.004
lake	40	15 (37.5)			
well	28	7 (25)			
spring	14	3 (21)			
piped water supply-	102	5 (4.9)			
Presence of *Entamoeba spps* in waterbody					
Occurrence					
Absence	314	54 (78.3%)	202.743 (0.000)	67.050 (31.303, 143.618)	<0.001
	69	16 (5.1%)		57.968 (25.652, 130.994)	<0.001
Defecation					
Open air	193	51 (26.4%)	20.463 (0.000)	0.367 (0.195, 0.689)	<0.001
Sometimes use toilet	65	11 (16.9%)		0.361 (0.194, 0.485)	0.002
Use toilet	125	8 (6.4%)		0.352 (0.174, 0.710)	0.004
				0.337 (0.172, 0.659)	

Table 2: *Chi-square* and logistic regression of potential risk factors associated with *Entamoeba spp.* in human.

Further univariate analysis of the socio-demographic factors showed that the infection was independent of living conditions and family size. It was observed that the prevalence rate decreased from 58.6% to 12%, with higher education level of the participants (OR=0.53695% CI=0.376, 0.765; P=0.008) [81].

Variable	No. tested	No. positive (%)	X2 (p-value)	Logistic regression	
				OR (95% CI)	P-value
Family size					

<6	307	32 (10.4%)	63.883(0.000)	6.513(2.787, 15.220)	<0.001
>6	76	38 (50%)			
Income					
high income	117	11 (9.4)	8.884 (0.003)	0.252 (0.111,0.568)	≤ 0.001
low income	266	59 (22.2)		0.257 (0.108,0.610)	0.002
Handwashing					
proper hand washing	168	18 (10.7%)	11.459 (0.001)		
improper hand washing	215	52 (24.2%)			
Literacy					
illiterate	141	42 (29.8)	23.083 (0.000)	0.569 (0.409,0.790)	≤ 0.001
read and write	112	17 (15.2)		0.563 (0.401,0.789)	≤ 0.001
elementary school	63	8 (12.7)		0.536 (0.382,0.751)	<0.001
high school	43	2 (4.7)		0.518 (0.366,0.735)	
professional	24	1 (4.2)		0.536 (0.376,0.765)	0.008

Table 3: *Chi-square* and logistic regression of potential risk factors (sociodemographic) factor associated with *Entamoeba spp.* in human.

The prevalence and risk factors of amoebiasis in dogs

The results of the microscopic examination of the fecal samples of the dogs are presented in Table 2. The results showed that out of 383 fecal samples examined, 63 (16.3%) were found to be positive for *Entamoeba histolytica* (the causative agent of the amoebiasis) [82].

This study revealed that there was a significant association between the infected owners and their dogs ($p<0.01$). *Entamoebiasis* were identified or diagnosed in both dogs and dog owners from the same households with similar sources of drinking water. During cross sectional survey of dog owners in study area, sixty-seven owners responded and coproscopy analysis revealed that all dogs harbor parasite. Both the drinking water source and the user showed the presence of cysts or trophozoites. This result encompasses the source of infection as contaminated drinking water, poor management practice and level of awareness of dog owners about dog parasites and associated risk, in addition to lack of veterinary attention. These exacerbate the risk of transmission of canine parasitic zoonoses to the human community or vice versa. The age and sex wise prevalence of this disease was observed as, the pups and female had higher prevalence compared to adults and male, as shown in Table 4.

Variable	No. tested	No. positive (%)	X^2 (p-value)	Logistic regression	
				OR (95% CI)	P-value
District					
Delomena	153	18 (11.8%)		1.673 (1.180, 2.371)	0.004
Heranabuluk	125	20 (16%)			
Medawolebu	105	25 (23.8%)			
Age					
(≤ 1 yr.)	87	10 (11.5)	15.975 (0.00)	1	-
(> 1 yr ≤ 2)	192	23 (12.)		1.993 (1.324, 3.00)	≤ 0.001
>3	104	30 (28.8)		2.158 (1.417,3.285)	
Sex					
Male	348	36 (17.1)	0.163(0.687)	1	-
Female	173	27 (15.6)		2.0 (0.48, 8.14)	0.34
Sources of drinking					

river	199	35 (17.6)	23.594 (0.000)		
lake	40	14 (35)			
well	28	7 (25)			
spring	14	3 (21.4)			
piped	102	4 (3.9)			
Owners health status					
Infected by *Entamoeba*	70	0.183	299.013a (.000)	620.000 (165.709, 2319.733)	<0.000
Not infected by *Entamoeba*	313	0.817			

Table 4: *Chi-square* and logistic regression of potential risk factors associated with *Entamoeba spp.* in dogs of study districts.

Occurrence of Entamoebasps in water and its risk factor

Fifty nine, 100 mL grab samples of high-turbidity water were collected at various points of a water body (lake, river, well, spring, and pipe water) that participants use for household consumption of the 59 water samples from different sites of Dellomena, Haranabuluk and Medawolebu 16 (27.11%), samples were positive for *Entamoebiasis* based on direct microscopical examination.

While about 26.63% of domestic households in study areas were supplied with treated water from WSD, there are other remote villages with sparse population not being covered by the main water supply system. These villagers rely on the raw water systems from river, lake, spring, and well water for domestic consumption. River, well, and spring (unprotected water sources showed) significant variations in the study area. In the study area, the high activities of domestic animals and humans in the unprotected water sources might lead to repeated contamination of surface water, well, rivers, lake with cysts of *Entamoeba sps* [83]. The occurrence of *Entamoeba sps* in the water body depends on open defecation, high human activity of surrounding water body. Another risk factor such as the hygienic nature of the surroundings of the water body, human activities such as washing clothes and bathing, around water body and turbidity of water were statistically significant $p<0.05$ for the occurrence of *Entamoeba* in the water body. Higher occurrence Entamoeba sps in a water body that has poor hygienic surroundings 12 (80.0%) than good hygienic surroundings (9.3%), higher human activity around water (50.0%) than lower human activity around water (9.4%) and lower turbidity (48.1%) than higher turbity (9.7%)of the water body, Table 5.

Variable	No. tested	No. positive (%)	X^2 (p-value)	Logistic regression	P-value
				OR (95% CI)	
District					
Delo Mena	26	6 (23.1%)	1.523 (0.467)		
HeranaBuluk	20	5 (25.0%)			
MedaWolebu	12	5 (41.7%)			
Sources of water					
River	25	8 (33.3%)	8.358 (.079)	1	-
lake	2	2 (66.7%)		.686 (.472, .998)	0.049
well	9	4 (44.4%)			
spring	4	1 (25.0%)			
piped water supply	18	1 (5.6%)			
Protection					
Unprotected	40	15 (37.5%)	6.341 (.012)	.098 (.012, .813)	0.031
Protected	18	1 (5.6%)			

Surrounding hygiene					
good	43	4 (9.3%)	27.825 (.000)	39.000 (7.635,199.208)	-
poor	15	12 (80.0%)			<0.001
Turbidity					
high	27	3 (9.7%)	10.692 (.001)	1	-
low	31	13 (48.1%)		.064 (.007,.617)	0.017
Population and animal activity					
high		13 (50.0%)	11.851a (.001)	9.667 (2.347, 39.817)	0.002
low		3 (9.4%)			

Table 5: *Chi-square* and logistic regression of potential risk factors associated with *Entamoebiasis* in Water body of study districts.

The overall prevalence of Amoebiasis in humans was found to be 18.3% which agrees with findings of semi-pastoralist tribes in lower Omo Valley, Southwestern Ethiopiaby Teklehaymanot (2009) (16%) and lower than previous studies conducted in different parts of Ethiopia.

A significant difference in the prevalence of amoebiasis was observed among the three age groups. The highest prevalence rate of amoebiasis was seen at 8-18 age groups, and the lowest infection rate was seen at less than eight age groups. Similar age distribution of infection had been observed in studies in Ethiopia Dire Dawa (36.1%) that children 6-14 years had highest infection than others by *Entamoebiasis* in Kenya age group (10-14 years) recorded a higher prevalence and intensity of *E. histolytica* infection compared to older age group.

The prevalence of amebiasis between humans using pipe water and a hand pump (protected) andlake, River, well, and spring (unprotected water sources showed) significant variations in the study area. In the study area, the high activities of domestic animals and humans in the unprotected water sources might lead to repeated contamination of surface water, wells, rivers, the lake with cysts of *Entamoeba sps.* A similar observation was recorded by expert. In Diredawa suggested that there was a significant difference (P<0.05) in infection amebiasis between children using protected (23.3% for amebiasis) and unprotected (33.3% for amebiasis) water sources. Besides, a study conducted in British 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, in protected water with no agricultural activities and minimal human activities, the prevalence of amoebiasis and other intestinal parasites were reduced. The low level of *Entamoebiasis* among participant using water sources in Delomena town highlights the importance of water development projects in the parts of the country in reducing parasite burden. This was of course, in addition to either drinking good quality water or keeping personal hygiene and environmental sanitation. In agreement with the present findings, explained the fact that in the United States, inadequately protected groundwater sources cause twice as many waterborne parasites outbreak than did surface water.

In this study water sample laboratory diagnosis and analysis in the study area indicated the presence of cysts of *Entamoeba* in both protected and unprotected water sources by percent of 28.7%. The occurrence of *Entamoeba sps* in the water body is 28.7% that causes infection of humans, dogs, and cats in the study area. The prevalence amoebiasis in water body in the study area depended on its protection from contamination by pathogen that the highest occurrence was in the lake, river, well, spring and pipe water, and water hand pump respectively. The present finding strengthens the water quality of the area which showed lower prevalence rate in those participants and dogs using water from water pipe (protected sources) at Delomena town and higher prevalence rate in those participants and pets using the lake, river, well and spring (unprotected water sources) (heranabuluk and medawolebu) respectively irrespective of other contaminant factors. The occurrence of *Entamoeba sps* in the water body depends on open defecation, high human activity surrounding the water body. These results agree with the report of Ben and Sabbahi that the occurrence of amoebiasis in water bodies was related to four relevant parameters such as effects of the well casing, nature of surrounding hygiene, clarity of water and effect of grazing animals around the wells and was significantly more important in uncovered wells (43.3%) than in covered wells (35.0%) in unclean surroundings (48.9%) than in the clean surroundings (20.0%), in turbid well water (51.9%) than in relatively clear well water (30.2%) and wells frequent by grazing animals (53.1%).

The result indicated that the factors responsible for infection by *Entamoebiasis* can be pointed to poor living conditions, unhygienic toilet facility, not washing hands before taking food due to which infection rates increased. The result agrees with that similar risk factors have been described for the infection in population from India, Italy and Yemen. Place of defecation, pets in the house were found as an important statistically associated factor. The source of water used for washing utensils was important statistically with pond water (27.0%) being a major determinant of parasitic infestation, compared to river water (25.8%) and tube well (14.9%).

Though it was not significantly associated with *Entamoebiasis* in this study, low educational status of parents was known to be among the significant factors in recent studies conducted in Ethiopia and other countries. This can be explained as the parents of children at a high level of educational status can provide better sanitation condition for their children than those parents with low educational level. The results of this study showed that a significantly higher prevalence of *E. histolytica* was reported among participants (P=0.010) of large households compared to the small size of families. This finding was similar to the study in Derna city, Libya who reported that infection of

intestinal parasites was higher in large family size and noted that those who had a large family size have significantly (P<0.05) higher risk of being infected with *E. histolytica* in sebya Libya. Similarly, exposure of *E. histolytica* was positively associated with overcrowding at home or more household members in the family in Orang Asli Ethnic Group in Malaysia and in general population in Mexico. However, experts reported that infection of *E. histolytica* was independent to the size of family or household members in Ethiopia and India, respectively. The results of present study indicate that participant (human and pets) belonging to large size families might be favoring the transmission of *E. histolytica* among participants (human and pets) due to crowding in the vicinity of homes in study area.

Latrine coverage or use was good (87.8%), but still a considerable number (39.2%) of study participants eliminate their stool in the field. Furthermore, statistically significant association was observed between infection prevalence and stool elimination in field among the study participants. This further increases the risk of significantly high parasite prevalence of the infection by the *Entamoebiasis*. This has a direct relation with possession of the toilet. Similar findings were also reported expert, indiscriminate stool elimination increases the risk of significantly high prevalence among communities. A study conducted on antiretroviral-treated HIV/AIDS patients in Ethiopia also reported that unavailability of latrines and lack of hand washing with soap was associated with *E. histolytica/dispar* infections. And also Open field defecation was found to be an important determinant in Nigeria, for *E histolytica/E. dispar* infestation which is comparable to our finding.

It has been noted that those who do not wash their hands properly were at 2.98 to 6.21 higher risk of being infected with *E. histolytica/E. dispar/E. moshkovskii*. The major role of contaminated hands in the fecal-oral transmission of the disease has been well documented in developing countries and washing hands before eating or after defecation have been considered as a secondary barrier. The result agrees with previous studies also indicated low personal hygiene, poor background, not washing hands after playing with soil or gardening and presence of already infected family members were important predictors for intestinal protozoan infections including E. *histolytica* and in Vietnam, the transmission routes *via* contaminated hands play a major role with a more than three folds risk increase if hands are not washed properly. Also, previous study in Italy and Yemen supported that individuals who do not practice proper hand washing before eating are at a two-fold higher risk of getting *E. histolytica/E. dispar* infection. Besides, not washing hands has been reported to be significantly associated with diarrhea in Malaysia, Myanmar, and Indonesia.

This study revealed an overall prevalence of amoebiasis in pets was 16.3% in Delomena Haranabuluk and medawelabu woreda Ethiopia. This was a relatively low level of infection; concerning in Hawassa Ethiopia that enteric protozoa in dogs were 50.9% and to the report of Adejinmi and Osayomi. This finding is relatively similar to the reports from various areas who reported 12.11%, 13.4%, 21.7% and 13.26% from Spain, Galapagos Islands of Ecuador, Bangladesh and Iran, respectively and relatively higher than reported of prevalence was 55 (7%) in dog and Gillespie et al. 2017 is 1% and also 1.5% in Cameroon. Such differences in the prevalence of enteric protozoa could be due to variation in geographic location, owners' awareness, and the number of stray dogs' population in an area. The prevalence of enteric protozoa parasites of dogs was significantly higher in younger dogs. This finding is in agreement with team of experts who reported a higher prevalence of protozoa parasites in younger dogs. The prevalence of this disease observed in puppies was high, which was following findings by other workers. This might be either due to immune incompetence or low level of passive immunity in puppies received from their dam or high stocking density as observed in our study.

The prevalence of amoebiasis to be highest in the dogs that the owner has also positive for amoebiasis compared to dogs owners has not infected by Amoebiasis. This appeared to be due to Amoebiasis in the pet was contracted from owner or human. Due to they were frequently in contact with human and bird excreta and other infected waste materials and get the infection. Also, most of these dogs were either infected or carriers of intestinal protozoan parasites, which they share playing fields, with each other's, directly or indirectly. Also, dogs that were kept by low-income groups of people receive less often medical help. The prevalence of amoebiasis in the dog was related to the income of the owner. The high-income group owner's dog shown a low prevalence (2%) This result agrees with previous studies in Hosana.

In the present study, it was surprising to see that the transmissibility in the human-pet-environment interface was detected. *Entamoebiasis* were identified or diagnosed in both pets and pet owners from the same households with similar sources of drinking water. During cross-sectional survey of pet owners in the study area, sixty-seven responded owner's coproscopy analysis revealed positive for entamoebaand their dogs results also shows positive ie they were also harboring a parasite. Their drinking water sources were examined for entamoeba and found that water bodies also diagnosed the same result observed in stool and feces. This might be due to the sharing of the same house, children played with the pet, poor management practice and level of awareness of dog and cat owners about dog parasites and associated risk, in addition to lack of veterinary attention, could exacerbate the risk of transmission of canine parasitic zoonoses to the human community. This result agrees with team of expert. Finally, we found that there was no significant association between *E. histolytica/E. dispar/E. moshkovskii* infection and gastrointestinal symptoms including diarrhea. It was well documented that 90% of *E. histolytica/E. dispar/E. moshkovskii* infected individuals were asymptomatic. Our results agree with the study of expert. The possibility of harboring the nonpathogenic species, *E. dispar or E. moshkovskii* cannot be ruled out. Moreover, it was now accepted that *E. dispar* infection is much more common than *E. histolytica* worldwide Human infections with *E. moshkovskii* had also been reported in Tanzania, Bangladesh, India, Iran, Australia, and Turkey, and in general, they were not associated with the disease. However, further studies using molecular approaches are needed to distinguish the morphologically identical species of pathogenic and non-pathogenic species among different at the study site.

Conclusion

In the present study, a higher prevalence of amebiasis has been found among human pet and water with an average prevalence rate of 18.3%, 16.7%, and 27.11%, respectively. Increasing prevalence in this study was associated with factors such as scarcity of water for consumption, indiscriminate defecation by the inhabitants and their animals, absence of toilet, keeping of livestock waste products in their backyards, living in overcrowded situations with many children per household coupled with poor sanitation, poor personal hygiene, and high illiteracy. In this study significant difference was observed in the prevalence of endameba among pets and its owner using protected and

unprotected water sources in study area. This mean that water development project does have a role in reducing illness as a result of waterborne parasites; rather this water development should be conducted side by side with other disciplines particularly with the health sector. Also, untreated protected drinking water sources were not free of the waterborne parasitic pathogens. It was estimated that the prevalence of *Entamoeba sps* does not vary between sexes. Sex related prevalence of amoebiasis in human and pet were not observed in this study, which implies male and female have equal chances of infection since they are engaged equally in all activities. In light of the high proportion of *Entamoebia sps* prevalence in Human, dog, and occurrence of *Entamoeba sps* in water bodies in the present study. We suggest that prevention, treatment, and control of parasitic infections amongst human, animal, and water.

In these parts of Ethiopia are necessary before an outbreak occurs. So the decision-makers should mobilize the community to improve health situations through provision of adequate and safe water supply health education in their local languages related to personal hygiene such as hand washing after using the toilet and before handling food. Hygiene depends on the quantity of water that people have. In many areas, hand washing after defecation or before preparing food seems like a luxury when the water has to be fetched from a water point far away. However, washing hands with soap can reduce the risk of diarrheal diseases by 42 to 47%, and the promotion of hand washing might save a million lives per year. Therefore, Hand washing promotion should be one of the intervention methods. Environmental sanitation cost-effective water purification mechanisms such as boiling and chlorination. Provisions such as listed above will help in enhancing the health and well-being of the community particularly that of children. Besides, the best way to prevent fecal-orally transmitted diseases is the sanitary disposal of human feces in latrines or other improved sanitation facilities. Improved sanitation is also the only long-term sustainable option for controlling Amoebiasis. Improved sanitation has important additional benefits, especially for women. In many part of the country, the only time when women or girls can defecate is at night because they have no toilet. The walk to the defecation field, often in the dark, may lead to the highrisk of sexual harassment and assault. Since the rate of amoebiasis was higher among the studied subjects, molecular techniques such as ELISA and PCR should be conducted to differentiate invasive (*E. histolytica*) with the non-invasive one (*E. dispar*).

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