SciMedCentral

Journal of Veterinary Medicine and Research

Review Article

Epidemiology of Cryptosporidiosis in Dairy Calves and Humans in Ambo and Toke Kutaye Districts of West Shewa Zone

Waktole Terfa[1]* and Dejene Getushe[2]

[1]*Department of Veterinary Science, School of Veterinary Medicine, Ethiopia*

[2]*Veterinary Service at the District of Liban Jawi,West Shewa Zone, Ethiopia*

***Corresponding author**

Waktole Terfa, Department of Veterinary Science, Mamo Mezemir Campus, School of Veterinary Medicine, University of Ambo, Ethiopia, Tel: 251923003177

Submitted: 21 August 2023

Accepted: 30 September 2023

Published: 30 September 2023

ISSN: 2379-948X

Keywords

- Cattle
- Cryptosporidium
- Human
- Risk factors
- West Shewa

Abstract

Cryptosporidium is an enteric protozoan organism that causes gastrointestinal disorders in humans and different animals, mainly in calves. The cross-sectional study was conducted during October 2019 to May 2020, to estimate the prevaelence of Cryptosporidium infection in humans and calves and identify risk factors of Cryptosporidium infection in West Shewa. Faecal samples collected from 275 calves and 149 humans were examined by Modified Ziehl Neelsen techniques to detect the presence of the parasite oocysts. Data on risk factors of the infection were collected using a pre-tested questionnaire. The overall prevalence of Cryptosporidium in cattle and humans was 17.1% (95% CI: 12.61–22.51) and 11.4% (95% CI: 3.59–8.47), respectively. In cattle, Cryptosporidium infection was significantly associated with intense management system (OR=4.55, 95% CI: 1.05–4.49), absence of calving pen (OR=4.94, 95% CI: 4.79–13.55),poor body condition score (OR=6.40, 95% CI: 3.16–9.23), drinking well/ river water (OR=7.09, 95% CI: 4.54–11.08) , group penning of calves (OR=8.54, 95% CI: 4.81–15.17), medium/unclean pen (OR=3.75, 95% CI: 4.98–13.94), unclean hind quarters/flanks (OR=4.78, 95% CI:4.58 13.75), less than two months age (OR=5.04,95% CI: 2.86 8.90) and presence of other disease (OR=3.04, 95% CI: 1.56 5.93). In humans, the infection showed significant association with presence of animal at home (OR=6.38, 95% CI: 1.03 62.30), high level contact with calves and their faeces (OR=5.40, 95% CI: 3.01 6.98), under five years age groups (OR=1.50, 95% CI: 1.01–2.21) and drinking well/ river water (OR=6.75, 95% CI: 2.95–5.93). This study clearly figures out that Cryptosporidium infection is prevalent in the study area. Therefore, community education is recommended in order to adopt integral approach involving good hygienic practice, such as preventing environmental contamination and proper disposal of contaminated material.

INTRODUCTION

Cryptosporidiosis is one of the infectious diseases caused by protozoan parasites under the genus Cryptosporidium, in the phylum Apicomplexa. It isubiquitous intracellular, extra-cytoplasmic coccidian parasites which infecting, develop and multiply in the epithelial cells of the gastro-intestinal tract of a wide range of vertebrate hostsincluding mammals, birds, reptiles and fish resulting in gastroenteritis manifested as diarrhea of varying severities [1]. The infection is acquired orally, usually by routes of direct contact with infected hosts or ingestion of contaminated water or food. In humans, the highest impact is on immune- compromised individuals such as AIDS patients [2]. Cryptosporidiosis is especially common in developing countries, creating additional challenges for the poorly supported public health infrastructure. Zoonotic *Cryptosporidium parvum* is known to occur widely in direct contact with infected animals, ingestion of contaminated food and drinking water and has caused waterborne outbreaks of gastroenteritis. Farm animals and human sewage discharges are generally considered the major sources of surface water contamination with *C. parvum.* Since Cryptosporidium infection is common in wildlife, it is conceivable that wildlife can also be a source of Cryptosporidium oocysts in water [3].

Several Cryptosporidium species are commonly found in humans and their distribution differs depending on socioeconomic development and the intensity of animal farming. At present, 26 *Cryptosporidium* species and over 61 genotypes have been recognized; eight valid species were reported to infect humans among which *C. hominis* and *C. parvum* are the most important ones [4-7]. Alsoseven species (i.e. C. andersoni, C. bovis, C. felis, C. hominis, C. parvum, C. ryanae and C. suis) and two genotypes of Cryptosporidium (i.e. “pig genotype II”and a new “C. suis-like genotype”) have been recorded in cattle [8]. Cryptosporidiosis can be transmitted from human to human (anthroponotictransmission) or from animal to human (Zoonotic transmission) [9]. Ruminants often have been implicated as a major source of human cryptosporidiosis [10].

The public health significance of the disease had been reported

by a number of researchers around the world [6,11,12]. In healthy individuals, the infection is usually self-limiting and resolves within 2–3 weeks of profuse, watery, non-bloody diarrhoea, weight loss, abdominal pain, anorexia, fatigue and cramps and often lethal diarrhea in immunocompromised individuals [13,14]. Cryptosporidium hominis and *Cryptosporidium parvum* in general are responsible for the majority of human Cryptosporidium infections [15]. Studies have indicated that cattle are commonly infected with four major Cryptosporidium species, namely: C. parvum, C. bovis, C. andersoni and C. ryanae [15-18].

The distribution of Cryptosporidium species in dairy cattle is age-related. Thus, the zoonotic species (C. parvum) is mainly found in pre-weand calves. C.bovis and C. ryanae usually infect weaned calves with C. bovis being more prevalent than C. ryanae. On the other hand, C. andersoni is commonly seen in yearlings and adult cattle. Additionally, Cryptosporidium hominis and Cryptosporidiumserpentis were also found in dairy cattle in some provinces of eastern China [19]. Life cycle of Cryptosporidium is monoxenous that causes diarrhea in immunocompromised individuals and neonates that believed as resulted from parasite invasion and epithelial destruction with the result of mild to moderate villus atrophy and microvillii shortening and destruction. Age, immune status, concurrent infections, management and hygienic condition are the potential risk factors [20].

Diagnosis of cryptosporidiosis is traditionally based on the detection of fecal oocysts by fecal flotation but immunofluorescent assay visualization of oocysts is currently being used as diagnostic techniques in most clinical laboratories while Molecular technique like PCR required for species identification. Regarding its treatment, there is not guarantee for an effective treatment in both human and veterinary medicine. However, Nitazoxanide and Halofuginone are approved drugs for pro- and metaphylaxis treatment respectively [21]. In Ethiopia, studies conducted on HIV/AIDS patients showed prevalence of cryptosporidiosisranging from 12.1% to 43.9% [22-24]. Although studies on dairy farms and drinking water sources are scarce in the country, few studies conducted so far showed occurrence of Cryptosporidium oocysts in samples from both sources (river and well water source) signifying their importance to human infections. Forinstance, a study conducted on twenty two drinking water sources in Addis Ababa and some nearby towns pointed out 100% positivity for Cryptosporidium oocysts [25]. Studies carried out on dairy farms in central and southern part of the country reported the prevalence median value30.1% [26-30,7]. Control of cryptosporidiosis has to rely on reducing the prevalence of the parasite and on breaking the transmission pathways of Cryptosporidium species causing disease in animals, transmitting them to humans (zoonotic) or those perpetuating infection in humans only (anthroponotic). However, there is limited information in Ethiopia on the status of Cryptosporidium infection and cryptosporidiosis in calves and humans.

STATEMENT OF THE PROBLEM

In Ethiopia it has been reported Cryptosporidium infection is highly prevalent; there was scarcity of well documented information regarding public health importance of this zoonotic parasite in the current study area.

OBJECTIVES

General objective

- To estimate the prevalence of Cryptosporidium infection in dairy calves and human sand identify risk factors of the disease in the study area.

Specific objectives

- To estimate the prevalence of Cryptosporidium infection in humans in and around Ambo and Gudar town
- To estimate the prevalence of Cryptosporidium infection in calves in and around Ambo and Gudar town
- To investigate the risk factors of Cryptosporidium infection in humans in and around Ambo and Gudar towns.
- o investigate the risk factors of Cryptosporidium infection in calves in and around Ambo and Gudar towns.

MATERIALS AND METHODS

Study area

This study was conducted in Ambo and Toke Kutaye districts of West Shoa Zone of Oromia Regional State (Figure 1) from October2019 to May, 2020G.C. The districts were purposively chosen based on livestock number and crop production potential and accessibility.

Ambo district is found in West Shewa zone of Oromia Regional State at a distance of 114 kms west of Addis Ababa. The district is located between longitudes of 37° 32′ and 38° 3′ E and latitude of 8° 47′ to 9° 20′ N. The total livestock population size is about 158,973 cattle, 68,988 sheep, 31,533 goats, 30,517 pack animals (donkeys, horses and mules) and 92,030 poultry (CSA, 2015) [31]. Toke Kutaye district is found along the highway from Addis Ababa to Nekemete at a distance of 126km from Addis Abebaor 12km West of Ambo town. It is located between 08° 59′ 01.1′ N latitude and of 37° 46′ 27.6′ E longitude. Toke Kutaye districthas 185,596 heads of cattle, 47,349 sheep, 34,782 goats, 84,530 chickens, 10,850 horses, 2,371 mules and 1,398 donkeys (Toke kutaye livestock Resource Development Agency office, 2019) [32].

Study population

The study population for this study includes alldairy calves born in the study dairy farms during the study period, and all human subjects working as animal attendants in dairy farms of the study area.

Figure 1 5Map of the study area. Source: CSA, (2013)

Study animals and sampling technique

The systematic random sampling method was used throughout the study to select farm size and age group strata and finally 275 calves as sample animals.

Study design and sample size determination

Across sectional study design was employed for this study. The sample size for the study was determined using the formula by [33]; at a precision level of 5%, confidence interval of 95%, estimated prevalence16 % in cattle [7] and 8% in humans [30].

d2 n = 1.962 Pexp (1- Pexp)

Where: n = required sample size

Pexp = expected prevalence

d =desired absolute precision

Accordingly, the calculated sample size for estimating prevalence in simple random sampling for the studies on cattle and humans were 205 and 113, respectively. Random sampling method was engaged to select the sample population. In order to adjust the sample size required for the present Stratified random sampling method and to make a prevalence estimate more precise, the sample size was inflated approximately by one over three times (205+70 and113+36) than in simple random sampling and set to 275and 149, respectively. Hence, a total of 275calvesand149humansweresampledfor this study.

Sample collection and preparation

Approximately 1-2 gram of faecal specimens was collected directly from the rectum of calves using sterile gloves, and the same amount of stool samples. Collected faecal and stool specimen'swerekept in a separately sterile stool cups in a cold box.

Inclusion and Exclusion criteria

Inclusion criteria: All dairy calves born in the study dairy farms during the study period and and all human subjects working as animal attendants and diarrheic patients in hospitals and health centers during data collection of the study area.

Exclusion criteria: Calves were not present during data collection and humans (non diarrheic patients) and human swere not present during data collection sex cluded.

Questionnaire data: At the time of sample collection from dairy farm owners, farm attendants, and Peasant association (PA) members, sampling date, faecal consistency (normal/diarrhoea), presence/absence of close contact with other domestic animals, and the calf age, sex, breed body condition score dairy farm location (urban, peri-urban, or rural), type of pen floor (concrete, kraal/stone, wooden), level of floor hygiene (clean, medium, dirty), source of drinking water (tap, well, river, spring) presence of infectious diseases and disposal of farm waste water were collected thoroughly and data from human participants include socio-demographic data (age and sex), contact with animals, cleaning habits after animal contact, source of drinking water (tap, well, river, spring) and water treatment practices, presence of diarrhea, were recorded for each animal and humans on a recording sheet. After collection, the samples were then transported to Ambo University parasitology laboratory on the same day of collection for further process and if the specimens cannot be examined at the time 10% formalin were added as a preservative solution.

Variables of the study

Dependent variables

- Cryptosporidium infection

Independent variables: Water and sanitation related variable **(**source of drinking water, pen type, pen clean liniens, and cleanliness of hind quarter), socio demographic variable (study area, age, sex, and study group). General management related variable (podduction system, presence of calving pen, body condition scor).

Laboratory analysis: The laboratory analysis of the samples was performed using Modified Ziehl Neelsen (mZN) staining**.** Briefly, thin slide smears of faecal/stool samples were made by spreading a small amount of faeces over the surface of a clean slide on an area of approximately 2cm x 1cm, then, the slides were placed on dryer with smeared surface upwards and air-dried for about 10 minutes. The dried smear was fixed with absolute methanol for 3–5 minutes. Carbol-Fuchsin solution was added to the slide covering the whole smear for 15–20 minutes. The slide was washed gently with tap water using a dropper. After this, 4–6 drops of decolorizer acid alcohol was added to the smear and the slide was washed off with clean water again. Then counter-stained with 0.33% malachite green solution for 2 minutes, and washed with water. The back side of the slide was rubbed, cleaned and put in the draining rack for 5 minutes to air dry the smear. The smear was examined microscopically, using the 40x and 100x (oil immersion lens) objectives and scanned thoroughly for parasite identification. Oocysts of Cryptosporidium species stained by this method show a variety of stain reactions from pale pink to deep red. Oocysts measure 4–6µm, and the sporozoites within the oocysts have an outer rim of deep stained material with a pale centre. This differentiates oocysts from some yeast that may stainred but have a homogeneous smooth appearance. A sample is considered positive for Cryptosporidium spp. if an oocyst of correct morphology: optical properties, internal structure, size and shape is detected as described by [34]. When Cryptosporidium oocysts were identified microscopically the positive results were recorded. The intensity of infection was estimated semi quantitatively according to the average number of oocysts in 14 randomly selected fields observed at 1000x magnification, following the criteria used by [35]: 0 (0 oocyst); I (1 oocyst); II (2–5oocysts); III (6 –10 oocysts); IV (>10 oocysts).

Quality control: Before starting the actual work, quality of reagents and instruments were checked by experienced laboratory technologist. The specimens were also checked for serial number, quality and procedures of collection. Each stool sample was examined by two laboratory technicians. The laboratory technicians were not informed about the health and other status of the study participants to eliminate observer bias. In cases where the results were discordant, a third senior technician was used, and his report was considered the final result. All data were entered timely to the database and checked for its accuracy before proceeding to analysis.

Statistical analysis

The data obtained from the humans, calve owners and laboratory results (Modified Ziehl Nelsons staining technique) were recorded on Microsoft excel work sheet. Then analysis was made by STATA version 14 statistical software. Descriptive statistics were utilized to summarize the raw data. The percentage of Cryptosporidium infection was calculated by dividing the number of infected calves and humans by the total number of calves and humans examined, multiplied by 100. Univariate logistic regression method was used to determine the association between potential risk factors and occurrence of Cryptosporidium infection. Variables with significance at $P < 0.05$ were selected for further multivariate logistic regression. The adjusted odds ratio (OR), was used to quantify the effect of risk factors on the likelihood of Cryptosporidium infection. Confidence level was held at 95% and $P < 0.05$ was set for significance level.

Ethical considerations

Ethical clearance for the study on animals was obtained from Ambo University College of Agriculture Veterinary science. The aim of the study was explained and permissions were obtained from farm owners before collection of samples and data. Ethical clearance for the study on human subjects was obtained from Ambo University, College of Medicine and Health Sciences, Ethical Review Committee. Informed verbal consent was obtained from the study participants at the time of sample collection after they have been informed that their specimen and records are examined by authorized person, personal information is treated strictly confidential and that they are free to withdraw the consent at any time.

RESULTS

Prevalence in Calves

Out of 275 faecal samples examined, Cryptosporidium infection was detected in 47 calves with an overall prevalence of 17.1 % (95% CI: 12.61–22.51). The farm prevalence was 26% with a median value of 27.5%. The prevalence of 38.7% obtained in calves under two months age groups was significantly higher than the 3.3% prevalence in calves between four to six months of age groups (p<=0.001).

The prevalence obtained in calves managed under the intensive production system, 20.8%, was significantly higher than the 10.8% prevalence in calve managed under the extensive production system (p<= 0.036). Prevalence among calves with diarrhea35.4% was significantly higher than the11.4%

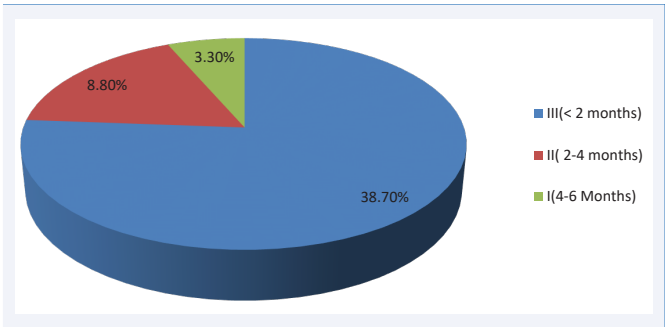

Figure 2 MZN-stained oocyst of Cryptosporidium infection 100xmag.

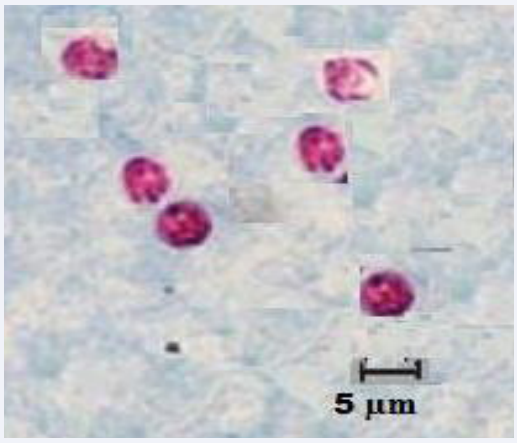

Figure 3 Age wise prevalence of cryptosporidium infection in calves

prevalence in non-diarrheaic (p<=0.001). Also, the prevalence of cryptosporidiosis infection in poor body conditioned calves 23.8 was significantly higher than the 9.9% calves with good body condition (p=0.002). Prevalence of the infection was similar across the sex (p< = 0.329), farm site (p<=0.507) and between the districts (p< = 0.507).

Intensity of infection in calves: Twenty-two semi-quantitatively examined samples 10 showed an average of > 10 (45%) oocysts, 3 showed 6-10 (14%) oocysts, 4 showed 2-5 (18%) oocysts and 5 samples showed an average of 1(23%) oocyst. All of the highest intensity of infections was in calves less than 2 months of age while 4-6 months age calves showed the least intensity of infection.

Risk factors in calves: By using univariate logistic regression analysis, nine risk factors were identified that affect the Prevalence of Cryptosporidium infection in calves [Table 1]. Risk factors assessed during this study were farms using well/river water sources, group penning, unclean pen, absence of calving pens and unclean tail, hindquarter and flank of animals, occurrence of other diseases, age, poor body codiition, intensive production system were significantly associated with increased prevalence of Cryptosporidium. Farms using well/river water sources were 7.1 times (OR=7.09, 95% CI: 4.5 - 11.08, p<=0.006) more likely to acquire Cryptosporidium infection compared to farms using tap water sources. The infection occurred 8.5times more likely (OR=8.54, 95% CI: 4.81-15.17, p <=0.010) in farms practicing group housing of calves compared to farms practicing individual pens. Farms at which Pasteurellosis and foot and mouth disease had been documented were 3tims (OR=3.04, 95% CI: 1.56 5.93, p<=0.001) more likely to acquire Cryptosporidium compared to farms without any record of these diseases. Farms practicing unclean calve pen and un clean hind quarter and tail were 3.75 and 4.78 times more likely (OR= 3.75, 4.78, 95% CI: 4.98-13.94, 4.58-13.75, p < = 0.024, p< = 0.043) affected by the parasite when compared to farms practicing clean calve pen and hind quarters respectively, intensive farming, absence of calving pen, age and poor body condition showed significant association with increased infection rate [Table 1]. In contrast to the above

Table 1: Univariable analysis of poentialrisk factors of calves Cryptosporidiumin West Shewaand its environs,

Risk factors	No.animals	No.+vanimals	Prevalence	OR (95% CI)	P value %
Source of drinking water					
Pipe	178	22	12.361		
Well/river	97	25	25.8	7.09(4.54-11.08)	0.006
Pen typ					
individual pen	124	13	10.481		
group pen	151	34	22.51	8.54(4.81-15.17)	0.010
Pen cleanliness					
clean	59	46.81			
medium/unclean	216	43	19.9	4.98 (3.75-13.94)	0.024
Cleanliness of hind quarters					
Clean	40	25	1		
Medium/unclean	235	45	19.1	4.78(4.58-13.75)	0.043
Presence of other disease					
no	149	15	10	1	
yes	126	32	25.4	3.04(1.56-5.93)	0.001
Production system					
Extensive	102	11	10.81		
Intensive	173	36	20.8	4.49(1.05 -4.55)	0.036
Presence of calving pen					
yes	145	16	11	1	
no	130	31	23.8	4.94(4.79 -13.55)	0.006
Body condition					
good	132	13	9.9	1	
poor	143	34	23.9	6.40(3.16-9.23)	0.003
Study site					
Rural	140	26	18.6	1	
Urban	135	21	15.6	5.43(3.40-8.64)	0.507
Breed					
Indigenous	91	14	15.41		
Holstein-cross	184	33	17.9	1.20(0.61-2.37)	0.597
Sex					
Male	146	28	19.21		
Female	129	19	14.7	1.37(0.73-2.59)	0.329
Disposal of farm waste					
to a fild	175	27	15.41		
to a well	100	20	20	5.48(3.63-8.26)	0.334
Age					
4-6 months	91	3	3.3	1	
2-4 months	91	8	8.8	1.14(2.41-4.26)	0.013
<2 months	93	36	38.7	5.04(2.86-8.90)	0.001
Over all prevalence	275	47		17.1(12.61–22.51)	

OR = odds Ratio CI =Confidence IntervalNo. +v= number of positive animals

findings the study results showed absence of statistically significant association between Cryptosporidium infection and method of colostrum feeding (hand feeding/dam suckling), farm location(urban/rural), presence of bedding, disposal of farm waste water (to a field/well/river), type of barn floor (concrete/soil/stone), weaning age (<6 month/ >6 month). Experience of attendants (≤5 years vs.>5 years), farm age (1–5 years, 6–10 years, 11–30 years), breed (local zebu vs. crossbreed (Holstein Friesian x zebu), access to water (free access/limited) and sex.

Prevalence in humans

The overall prevalence of Cryptosporidium in human participants was 11.4 % (95% CI: 3.59-8.47) [Table 3]. The prevalence in patients with diarrhea, 12.6 was not different (p<= 0.434) from that of dairy farm community, 7.9%. There was statatisticllay no difference in the Prevalence of the infection between participants in Ambo (10.4 %,) and (Toke Kutaye, 12.5%), (p = 0.106), there is an association with age and hose < 5

Table 2: Multivariable logistic analysis of risk factors that were significantusing univariable analysis as shown in Tables 4

Risk factors	OR	Sez	|z|	P >	95% confidence interval	
Calves<2 month	4.873	0.25	14.71	0.000	1.172	2.164
River water	6.592	0.786	3.53	0.001	1.446	4.263
Absence ofcalving pen	5.526	0.649	2.13	0.034	1.266	5.251
Presence of other diseas	3.194	0.432	2.52	0.012	1.242	5.007
Group pen	7.162	0.534	2.31	0.022	1.228	4.047
Poor body scor	6.753	0.370	3.35	0.001	1.269	5.102

Table 3: Univariable analysis of poentialrisk factors of human Cryptosporidiumin West Shewa and its environs,

Risk factorscategories	No. humans	No.+ve	Prevalence	OR95% CI	P value
Sampledstool%					
Presence of animals at home	No	45	1	2.21	
	Yes	104	6	5.77	6.38(1.03-6.30) 0.047
Level contact with animals &their faeces	No	37	2	5.41	
Medium	67	6	8.96	2.16 (1.66-4.71)0.024	
High	45	9	20	5.40(3.01-6.98)0.001	
Age in year					
> 65	26	1	3.8 1		
46-65	42	5	11.9	1.36(1.59-2.04)0.045	
26-45	34	3	8.8	1.32(1.84-2.15)0.047	
6-25	23	1	4.3	1.29(1.07-2.02)0.049	
< 5	24	7	29	1.50(1.01-2.21)0.044	
Source of drinking water	Pipe	80	4	5	1
	Well/river	69	13	18.8	6.75(2.95-5.93) 0.013
Sex	Female	80	9	11.31	
	Male	69	8	11.6	1.48 (2.94-5.78)0.947
Over all prevalence		149	17	1	1.4 (3.59-8.47)

years age are more at risk (p<= 0.044) (Figure 4). The prevalence of Cryptosporidium in female and male subjects 11.3% and 11.6%, respectively, was not significantly different (p < = 0.947),

Intensity of infection in humans: Fifteen samples were examined semi quantitatively, of which, 4 showed an average of > 10 (27) oocysts, 2 showed 6 10 (13) oocysts, 3 showed 2-5 (20) oocysts and 6 showed an average of 1(7) oocyst.The highest intensity was observed in children < 5 years age group.

Risk factors in humans: Risk factors considered tobe associating with human Cryptosporidium and assessing in this study includes: presence of animals at home, level of contact with animals and their faeces, age group, source of drinking water. Among these factors, statistically significant associations were encountered with presence of animals at home (p< = 0.047), level of contact with animals and their faeces (p <= 0.001), age group (p< = 0.044) and source of drinking water (p = 0.013) [Table 3]. Whereas, no associations (p> = 0.05) were encountered with the rest of the assessed factors. Individuals having animals at home were about 8 times more likely to be infected as compared to those lacking animals at home (OR =6.38, 95% CI: 1.03-62.30). People possessing cattle, having high contact with animals and their faeces were about 5.40 times more likely infected compared to people without animals and people having no contact with animals and their faeces. Among the age groups < 5 year were

Table 4: Multivariable logistic analysis of risk factors that were significantusing univariable analysis as shown in Tables 6

Risk factors	OR	SE	ZP >	|z|	95% confidenceInterval	
High contactwith animals	2.368	0.927	2.73	0.018	1.648	4.092
River water	4.821	0.543	2.95	0.015	2.37	4.641
Childs < 5 year	1.936	0.351	2.43	0.038	1.093	3.263

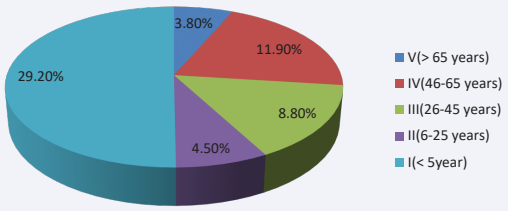

Figure 4 Age wise prevalence of Cryptosporidium infection in humans in the study area.

1.50 tims (OR=1.50, 95% CI: 1.01–2.21) more likely to acquire Cryptosporidium compared to age groups 6-25, 26-45, 46-65 and >65 years. The prevalence of Cryptosporidium infections in study participants using well or stream water for drinking, 18.8%, was statistically significantly higher (p = 0.047) than the prevalence in people using pipe water as drinking water source.

DISCUSSION

The overall prevalence of Cryptosporidium infection in calves in the present study was found to be 17.1 %. This is comparabel with the report of [26,7] who noted 17.6% and 15.8 infection rates in dairy calves in Central Ethiopia. However lower than the 18.6% and 27.8% prevalence report of [36,28] and higher than the 2.3%, 7.8%, and 13.6% prevalence reports by [37,30,29], respectively. Studies conducted in other parts of the world also stated varied prevalence: comparable values of 16.3%, reported in India [38], higher prevalence ranging from 27.2% to 57.9% had been reported in Pakistan and Chile, [39-40], respectively and lower values of 14% were reported [41]. The difference in the overall prevalence of Cryptosporidium among different studies could be due to variations in ecology, study design, season, management system, age, herd size and laboratory techniques employed [42]. Animals reared under intensive management system were more affected by Cryptosporidium (20.8%) than those under the extensive system (10.8%) which could be due differences in confinement, higher stocking rate and crowding in the intensive dairy farms favoring more contamination of barns, high contact of animals and rapid dissemination of oocysts compared to extensive farms. In the semi-intensive or intensive management system of rearing animals are confined to a restricted area, thus continuously contaminated the surroundings**.** This result is in agreement with the findings of [42,36] that reported prevalence of 42.8% and 21.4% for animals reared under intensive system and 6.3% and 11.2% for animals under extensive system. Comparable lower prevalence had been reported in extensive farms compared to intensive farms [43].

Cryptosporidium was significantly associated with absence

of calving facilities and practice of dam suckling; higher chance of infection might have resulted due to exposure of neonates to their dams or other group of the herd in farms where calving facilities are absent, or if newborns stayed with their dams in maternity pens in case of farms with calving facilities. This result is in agreement with findings of [44] that reported higher exposure and prevalence of the disease in newborns that stayed with their dams in maternity pens. A significant association was also observed between the prevalence of Cryptosporidium oocysts and faecal consistency of calves; where diarrheic animals had shed the oocysts more frequently than those calves with the normal faecal matter. This is in accordance with [45] who reported a strong association between Cryptosporidium oocyst shedding and calf diarrhoea. Thus, it seems that Cryptosporidium is the enteropathogen which strongly associated with diarrhoea. This might be due to the fact that the pathogen causes villous atrophy and crypt hyperplasia, which results in a decrease in the absorptive surface area of the intestine; thus glucose, water and sodium absorption are hindered and results in diarrhoea [46]. Moreover, the parasite could have a capability in reducing disaccharides activity resulting in the reduced breakdown of sugars resulting in bacterial overgrowth, the formation of volatile fatty acids, and changes in osmotic pressure; these changes, then cause the characteristic severe and watery diarrhoea.

Increased risk of Cryptosporidium was seen in farms using river/stream water sources; this could be due to exposure of these water sources to faeces of human, domestic and wild animals which have been contaminated with oocysts of Cryptosporidium. River water is heavily contaminated with oocyst of Cryptosporidium in proportion to the number of cattle in the adjacent area and livestock waste was more pollutant of river water compared to sewages [47]. Results of this study showed that animals having unclean hindquarters and/or housed in unclean pens showed higher infection rates than animals with clean hindquarters and/or housed in clean pens. This could be due to the fact that wet and soiled pen floors create favorable environment for the persistence of oocysts and spread of the infection among the herd. Our results are in accord with the findings of [26] that reported 5.2 times odds of infection in calves housed in poorly cleaned farms compared to calves in well-cleaned farms [18], illustrated significant association between daily cleaning of pens and reduction in the risk of Cryptosporidium infection.

The association of age with the infection was in agreement with the study by [42,48,49]. This supports the present finding in which higher prevalence recorded in calves less than < 2 months of age than 2-4 and4- 6 months. This could be owing to the fact that the immature immune system of young calves [50], also reported calves less than 4 months of age are more at risk for Cryptosporidium infection. This is also supported by [51] who described that resistance to infection could be developed with age due to immune development through time. There was significant difference in Cryptosporidium infection within body condition score with higher prevalence in calves with poor body condition scores than good body condition scores. This result is in agreement with the finding [52] that reported prevalence of 30.4%, 20.2% and 8% for calves' body condition score, poor, medium and good respectively. This can be related to immunity of poor body conditioned calves as immune status of the animal is decreased. Additionally, some synergic infection of enteric pathogens can result in poor body condition, immuno-compromisation and increase new born calves the susuptablity to cryptosporidium infection [53]. The present study illustrates that infections were significantly higher in farms with previous record of Foot and Mouth Disease (FMD) or Pasteurellosis compared to farms without these diseases. It is likely to get higher prevalence in such farms given that Cryptosporidium is an opportunistic parasite mostly affecting immunocompromized animals [54] and these diseases are highly infectious and known to cause severe illness with immune suppression effect.

The overall prevalence of Cryptosporidium infection in humans was 11.4%, this was comparable to previous studies reported from Ethiopia (11.7%) [55] and of 12% in Indonesia, [60]. The prevalence obtained in the farm community, 7.9%, was comparable within the median vlueof 7.7% prevalence reports in apparently normal children [30, 37,56] but lower than the prevalence report of 14.8% in diarrheic children [57]. The prevalence of Cryptosporidium obtained in the diarrheic patients 12.6% was comparable to 11.7% of HIV seropositive study group [55], but was lower than earlier reports of 20.1% [58] and 17.7% [59] in HIV seropositive persons at different parts of the country. It is also lower than the prevalence25.6% in Iran [61] in HIV patients with chronic diarrhoea and in diarrheic and non-diarrheic humans. The Cryptosporidium infection has been reported in people from age 3 days to 95 years however, statistical analysis show that the young are more vulnerable to Cryptosporidium infection. The current findings suggest that children in the age group of less than 5 years were more exposed and infected (29.2%) with Cryptosporidium infection. Different environmental factors and low standard of personal cleanliness may have attributed to this higher infection of children. Similarly, some investigations from Malaysia and Pakistan show that most of infected individuals were children having less than four and five years of age [62]. This statement is an agreement with other described study from Nepal, Bhutan reporting infected cases from children below 3 years of age [63]. A similar study from Peshawar (Pakistan) also reported the infection in children less than 2 years of age [64]. Most of the positive cases were found that they were previously suffering from gastrointestinal symptoms and diarrhea. Cryptosporidium was more common in persons with mucus stool, 23%, compared to persons without mucus stool. This could be due to ingested oocysts release sporozoites, which subsequently attach to and invade the intestinal epithelial cells (IECs). The parasite has a particular predilection for the jejunum and terminal ileum and binds on the apical surface of the intestinal epithelium [4].

The prevalence of Cryptosporidium was higher in people using well or stream water than people using pipe water, which could be due to more exposure and contamination of well and stream water with faeces of animals and humans compared to pipe water. This result is in agreement with findings of [64]

that reported 77.8%of the total Cryptosporidium infections in children using well water. A study from district Buner Pakistan showing that most of the participants were from villages and rural areas where birds, cats and dogs are commonly wandering freely, which may be a route for subsequent zoonotic spreading of oocysts, contaminating the soil and water with their feces. Cows and other domestic animals are also seen drinking and bathing in the surface water (rivers, streams and canals) along with children. A study from three Districts of KP Pakistan showed that Cryptosporidium infection was prevalent (19.5%) in surface water as compared to other water-borne parasites [65]. In lowland UK, the Cryptosporidium infection was pre-dominant in livestock and deer samples, suggesting a significant risk to surface water quality and public health [66]. In 2002, a high prevalence (66%) of Cryptosporidium infection in surface waters on a coastal farm in England was reported, where Cryptosporidium spp oocysts were being spread by at least one livestock or wild animal inhabitants [67]. Similarly, other studies from North West Wales and Scotland evidenced that wildlife contributes to the oocyst counts in surface waters [68,69]. A study from China reported that the drinking of surface water is the main cause of the Cryptosporidium exposure route and infection [70]. A study from Pakistan reported that the human feces were often found near surface water and houses and in some towns the sewage and toilets waste water were freely flowing to the surface water sources which is a concern for possible water-borne transmission of Cryptosporidium infection. Significant association of Cryptosporidium infection was seen with owning animals and having high level of contact with their faeces, and this association was particularly evident in persons having contact with cattle. In this study, no association was detected between infections of humans and having contact with pet animals and their faeces. This finding is in agreement with earlier studies that reported close contact with cattle and their faeces as the major risk factor of Cryptosporidium infections in humans [71-73,30,37]. In a study in Pakistan majority of the infected children had history of contact with animals and the authors suggested that animals could be reservoirs of human infection [64]. While a study in Thailand reported that 30%-40% of the infections in dogs and cats were attributed to C. parvum, and suggested the potential role of zoonotic transmission [71]. Moreover, among farm community participants of the study, high prevalence (85%) of Cryptosporidium was obtained in persons working in dairy farms with high prevalence of the infection.

CONCLUSION AND RECOMMENDATIONS

This study indicated that the prevalence of Cryptosporidium infection in calves and humans was 17.1%, and 11.4%, respectively.Hygienic status, drinking water source, management, age, contact with infected animals, presence of other disease were the identified potential risk factors in calves and contact with infected animals, drinking water source, hygienic status and age were the identified potential risk factors in humans, which had significant association with the occurrence of Cryptosporidium infection. River and stream water as well as contaminated pipe water are the major routes of transmission for Cryptosporidium in both cattle and humans in the study area. Therefore, based on the above conclusion the following recommendations are forwarded:

- It is recommended to avoid faecal contamination of nearby water and soil through proper management of calves and farm waste-water disposal.
- Awareness creation should be practiced in the community about public health of cryptosporidiosis and about the proper care to be involved.
- Drinking water contaminated by sewage is the major vehicle for Cryptosporidium oocyst. Hence, public health and municipal water authorities should regularly check safety of the water supply from Cryptosporidium oocyst and provide the community with sufficient information for control.
- In humans, one of the control options is avoiding ingestion of oocysts via water (drinking/swimming pool) or contaminated food, however, regular hand washing and escaping from contact with faeces of animals or humans is an important hygienic measure.
- Dairy farm barns, pens and indoor animals should always be clean and hygienic, farm gates should be provided with disinfectant solution to decontaminate humans, animals and vehicles entering in to the farm.
- In addition, molecular studies to be conducted- to identify species and genotypes.

ACKNOWLEDGEMENTS

We are very grateful to all dairy farm owners who found at the study area for permitting to work on their farms. We are grateful to all respondents (dairy farm owners and attendants, peasant association members and diarrheic patient participants) in the study area for their genuine cooperation.

We acknowledge all staffs of Guder and Toke Erensa Health Centers, Ambo University Referral Hospital staffs and livestock and Fishery Development Bureaus staffs of the study area for their whole hearted help during sample collection laboratory works and for their helpful collaboration throughout the study.

We also thank Ambo University College of Agriculture and Veterinary Sciences and Ambo University College of medical Sciences department Heads, Laboratory workers for their genuine help in sample preservation, laboratory material and equipments and for permitting to work in their Labs.

REFERENCES

1. Smith HV, SM Caccio, A Tait, J Mc.Lauchlin, RC Thompson. Tools for investigating the environmental transmission of Cryptosporidium and Giardia infection on Humans. Trends Parasitol. 2006; 22: 160-167.
2. Chalmers RM, Davies A. Minireview: clinical cryptosporidiosis. Exp Parasitol. 2010; 124: 138-146.
3. Robinson G, Chalmers RM, Stapleton C, Palmer SR, Watkins J, Francis

C, et al. A whole water catchment approach to investigating the origin and distribution of Cryptosporidium species. J Appl Microbiol. 2011; 111: 717-730.

4. Bouzid M, Hunter PR, Chalmers RM, Tyler KM. Cryptosporidium pathogenicity and virulence. Clin Microbiol Rev. 2013; 26: 115-134.
5. Lebbad M, Beser J, Insulander M, Karlsson L, Mattsson JG, Svenungsson B, et al. Unusual cryptosporidiosis cases in Swedish patients: extended molecular characterization of Cryptosporidium viatorum and Cryptosporidium viatorum and Cryptosporidium chipmunk genotype I. Parasitology. 2013; 140: 1735-1740.
6. Adamu H, Petros B, Zhang G, Kassa H, Amer S, Ye J, et al. Distribution and clinical manifestations of Cryptosporidium species and subtypes in HIV/AIDS patients in Ethiopia. PLoS Negl Trop Dis. 2014; 8: 2831.
7. Wegayehu T, Karim R, Anberber M, Adamu H, Erko B, Zhang L, et al. Prevalence and Genetic Characterization of Cryptosporidium Species in Dairy Calves in Central Ethiopia. Plos One. 2016; 11: e0154647.
8. Hunter PR, S Hughes, S Woodhouse, Q Syed, NQ Verlander, RM Chalmers, et al. Sporadic cryptosporidiosis case-control study with genotyping: Emerg Infect Dis. 2004; 10: 1241-1249.
9. Madi MA, JM Behnke, A Ismail, N Al-Olaqi, K Al-Zaher R El-Ibrahim, et al. Comparison of intestinal parasitic infection in newly arrived and resident workers in Qatar. Parasite Vectors. 2011; 4: 211.
10. Xiao L, Fayer R. Molecular characterization of species and genotypes of Cryptosporidium and Giardia and assessment of Zoonotic transmission. Int J Parasitol. 2008; 38: 1239-1255.
11. Zaidah AR., Chan YY, Asma HS, Abdullah S, Nurhaslindawati AR, Salleh M, et al. Detection of Cryptosporidium parvum in HIV infected patients in Malaysia using a molecular approach. South East Asian J Trp Med Public Health. 2008; 39: 511-516.
12. Szonyi B, Wade SE, Mohammed HO. Temporal and spatial dynamics of Cryptosporidium parvum infection on dairy farms in the New York City Watershed: a cluster analysis based on crude and Bayesian risk estimates. Int J Health Geogr. 2010; 9: 31.
13. Warren C, Guerrant R. Clinical Disease and Pathology. In: Fayer R, Xiao L, editors. Cryptosporidium and Cryptosporidiosis. (2008): 2nd ed. Boca Raton, FL: CDC. Press. 2008; 235–254.
14. Gabr, Nabil S. Azza K. Ahmad, Usama S. Belal Ekhlas H, et al. Prevalence of Cryptosporidium species in human faecal specimens in Minia Governorate. Egypt. MJMR. 2018; 29: 225-229.
15. Xiao L. Molecular epidemiology of cryptosporidiosis: an update. Exp Parasitol. 2010; 124: 80-89.
16. Fayer R, Santin M, Trout JM. Cryptosporidium ryanae n. sp. (Apicomplexa: Cryptosporidiidae) in cattle (Bos Taurus). Vet Parasitol. 2008; 156: 191-198.
17. Brook EJ, Anthony C, French NP, Christley RM. Molecular epidemiology of Cryptosporidium subtypes in cattle in England. Vet J. 2009; 179: 378-382.
18. Zhang CY, Lin MM, Gong YC. Calf cryptosporidiosis: pathogen isolation and identification and prevention measures. J Anim Husb Vet Med. 2013; 34: 17-18.
19. Chen F, Huang K. Prevalence and molecular characterization of Cryptosporidium spp. in dairy cattle from farms in China. J Vet Sci. 2012; 13: 15-22.
20. Thomson, S. Efficacy of halofuginone lactate in the prevention of cryptosporidiosis in dairy calves. Vet Rec. 2016; 168: 509.
21. Shahiduzzaman M, Daugschies A. Therapy and prevention of cryptosporidiosis in animals. Vet Parasitol. 2012; 188: 203-214.
22. Zelalem TM., Gemeda A, Andargachew M. Opportunistic and Other Intestinal Parasitic Infections in AIDS Patients, HIV Seropositive Healthy Carriers and HIVSeronegative in Individuals in Southwest Ethiopia. East Afr J Public Health. 2008; 5: 169-173.
23. Adamu H, Petros B. Intestinal protozoan infections among HIV positive persons with and without Antiretroviral Treatment (ART) in selected ART centers in Adama, Afar and Dire-Dawa, Ethiopia. Ethiop J Health Dev. 2010; 23: 133-140.
24. Getaneh A, Medhin, Shimelis T. Cryptosporidium and Strongyloidesstercoralis Infections among people with and without HIV infection and efficiency of diagnostic methods for Strongyloides in Yirgalem Hospital, southern Ethiopia. BMC Res Notes. 2010; 3: 90.
25. Fikrie N, Hailu A, Belete H. Determination and enumeration of Cryptosporidium oocysts and Giardia cysts in Legedadi (Addis Ababa municipal drinking water system) Ethiop. J Health Dev. 2008; 22: 68-70.
26. Abebe R, Wossene A, Kumsa B. An epidemiological study of Cryptosporidium infection in dairy calves on selected dairy farms of central Ethiopia. Rev Med vet-toulouse. 2008; 2:107-111.
27. Adamu H. The prevalence of intestinal parasites and molecular characterization of Cryptosporidium species in Ethiopia, PhD dissertations submitted school of graduate studies AAU.pp.65. 2010.
28. Alemayehu R, Oda G, Fufa A, Rahmeto A, Desta B, Bekele M, et al. Cryptosporidium in Calves, Lambs and Kids at Haramaya, eastern Ethiopia. Ethiop Vet J. 2013; 17: 81-94.
29. Ayana D, Alemu B. Cryptosporidiosis in Calves, Lambs and Goat Kids in Bishoftu, Oromia Regional State, Ethiopia. African J Basic Appl Sci. 2015; 7: 233-239.
30. Wegayehu T, Adamu H, Petros B. Prevalence of Giardia duodenalis and Cryptosporidium species infections among children and cattle in North Shewa Zone, Ethiopia. Bmc Infect Dis. 2013; 13: 419.
31. Central Statistical Agency of Federal Democratic Republic of Ethiopia Livestock and livestock characteristics, agricultural sample survey. Addis Ababa, Ethiopia. Statistical Bulletin. 2:13. 2015.
32. Toke kutaye livestock Resource Development Agency office. 2019.
33. Thrusfield MV. Veterinary Epidemiology, 3rd Edition. Oxford, England: Blackwell Science. 2015; 234-238.
34. Fayer R, Speer CA, Dubey JP. The general biology of Cryptosporidium. In: Fayer, R. (ed.): Cryptosporidium and cryptosporidiosis. Boca Raton: CRS Press. 1997; 1-42.
35. Castro-Hermida JA, González-Losada YA, Mezo-Menéndez M, Ares-Mazás Elvira. A study of cryptosporidiosis in a cohort of neonatal calves. Vet Parasitol. 2002; 106: 11-17.
36. Anberber M, Stomeo F, Mahendra P, Mamo G, Mulatu T, Muthui L, et al. Prevalence, risk factors and molecular characterization of Cryptosporidium infection in cattle in Addis Ababa and its environs, Ethiopia. Vet Parasitol Reg Stud Reports. 2018; 13:79-84.
37. Adamu H, Petros B, Hailu A, Petry F. Molecular characterization of Cryptospori dium isolates from humans in Ethiopia. Acta Trop. 2010; 115: 77-83.
38. Maurya PS, Garg R, Banerjee PS, Kumar S, Rakesh RL, Kundu K, et al. Genotyping of Cryptosporidium species reveals prevalence of zoonotic C. parvum subtype in bovine calves of north India. Indian J Anim Sci. 2013; 83: 1018-1023.
39. Muñoz AP, Mercado PR., Morales TG, Bravo OV, Raffo CE. Cryptosporidium spp.comparative diagnosis and geospatial distribution in diarrheic calves from dairy farms, Valdivia, Chile. Rev Mvz Córdoba. 2014; 19: 3954-3961.

40. Shafiq MAB, Maqbool A, Khan UJ, Lateef M, Ijaz M. Prevalence, water borne transmission and chemotherapy of cryptosporidiosis in small ruminants. Pak J Zool. 2015; 47: 1715-1721.

41. Bawm S, Kyi S, Lay KK, Htun LL, Myaing, Tin T. Prevalence and associated risk factors of Cryptosporidium and Giardia species in cattle within Mandalay Region, Myanmar. J Adv Parasitol. 2014; 1: 49-53.

42. Geurden T, Goma FY, Siwila J, Phiri IGK, Mwanza AM, Gabriel S, et al. Prevalence and genotyping of Cryptosporidium in three cattle husbandry systems in Zambia. Vet parasitol. 2006; 138: 217-222.

43. Ralston BJ, Cockwill C, Guselle N, Van Herk FH, McAllister TA, Olson ME, et al. Prevalence of Giardia and Cryptosporidium andersoni and their effect on performance in feedlot beef calves. Can J Anim Sci. 2003; 83: 153-159.

44. Del Coco VF, Co'rdoba MA, Basualdo JA. Cryptosporidium infection in calves from a rural area of Buenos Aires, Argentina. Vet Parasitol. 2008; 158: 31-35.

45. Lise A Trotz-Williams, S Wayn M, Leslie KE, Duffield T, Nydam DV, Peregrine S, et al. Calf levelrisk factors for neonatal diarrhoea and shedding of Cryptosporidiumparvumin On tario dairy calves. Prev Vet Med. 2007; 82: 12-28.

46. Radostits OM, CC Gay, KW Hinchcliff, PD Constable. Diseases associated with protozoa. 10 editions, In: Veterinary Medicine: A Textbook of Diseases of cattle, horses, sheep, pigs and goats. Saunders Elsevier. 2007; 1483-1540.

47. Yang W, Chen P, Villegas EN, Landy RB, Kanetsky C, Cama V, Dearen T, Schultz CL, Orno KG, Prelewicz GJ, Brown MH, Young KR.

48. Venu R, Latha BR, Bath AS, Sreekumar C, Raj GD, Raman M, et al. Factors influencing on the prevalence of Cryptosporidium infection in South Indian dairy calves. J Parasit Dis. 2013; 37: 168-172.

49. Joute JR, Gill JPS, Singh BB. Prevalence and molecular epidemiology of Cryptosporidium parvum in dairy calves in Punjab (India). J Parasit Dis. 2016; 40; 745-749.

50. Brook E, Hart CA, French N, Christley R. Prevalence and risk factor of Cryptosporidiu spp infection in young calves. Vet Parasitol. 2008; 152: 46-52.

51. Kvac M., Kouba M. and Vitovec J. Age-related and housing dependence of Cryptosporidium infection of calves from dairy and beef herds in South Bohemia, Czech Republic. Vet Parasitol. 2006; 137: 202-209.

52. Ayele A, Seyoum Z, Leta S. Prevalence of Cryptosporidium Infection in Calves Aged less than One Year in Urban and Peri Urban Areas of Gondar Town. Acta Parasitologica Globalis. 2017; 8: 26-32.

53. Lefay D, M Naciri, P Poirier, R Chermette. Prevalence of Cryptosporidium infection in calves in France. Vet Parasitol. 2000; 89: 1-9.

54. Fayer Rand, Santin M. Cryptosporidium xiaoi n. sp. (Apicomplexa: Cryptosporidiidae) in sheep (Ovisaries). Vet Parasitol. 2009; 164: 192-200.

55. Shimelis T, Tassachew Y, Lambiyo T. Cryptosporidium and other intestinal parasitic infections among HIV patients in southern Ethiopia: significance of improved HIV-related care. Parasitol Vectors. 2016; 9: 270-276.

56. Tigabu E, Petros B, Endeshaw T. Prevalence of Giardiasis and Cryptosporidiosis among childen in relation to water sources in selected village of Pawi special District in Benishangul-Gumuz Region, Northwestern Ethiopia. Ethiop J Health Dev. 2011; 24: 205-213.

57. Teshome F, Fufa A, Mekonnen, G. Intestinal Protozoal Parasites in Diarrheal Children and Associated Risk Factors at Yirgalem Hospital, Ethiopia: A Case-Control Study. Int Sch Res Notices. 2014; 357126.

58. Assefa S, Erko B, Medhin G, Assefa Z, Shimelis T. Intestinal parasitic infections in relation to HIV/AIDS status, diarrhoea and CD4 T-cell count. BMC Infect Dis. 2009; 9: 155.

59. Dawit KR, Sissay M, Yitbarek G. prevalence of isospora belli and Caryptospori diumparvum infections among HIV sero-positive patients in Asella hospital, central Ethiopia. Res Rev J Immunol. 2014; 4: 1-4.

60. Kurniawana A, Karyadib T, Dwintasaria SW, Sari IP, Yunihastutib E, Smith HV, et al. Intestinal parasitic infections in HIV/ AIDS patients presenting with diarrhea in Jakarta, Indonesia. Trans R Soc Trop Med Hyg. 2009; 103: 892-898.

61. Mirzaei M. Prevalence of Cryptosporidium sp. infection in diarrheic and nondiarrheic humans in Iran. Korean J Parasitol. 2007; 45: 133-137.

62. Latif B, Rossle NF. Cryptosporidiosis among children with diarrhoea in three Asian countries: a review. Asian Pac J Trop Dis. 2015; 5: 885-888.

63. Bodhidatta L, Wongstitwilairoong B, Khantapura P, Shrestha SK, Wangchuk S, Raj PA, et al. Cryptosporidiosis: Prevalence in Children in Nepal and Bhutan. JV Med Res. 2016; 3: 1059.

64. Mumtaz S, Ahmed J, Ali L. Frequency of Cryptosporidium infection in children under five years of age having diarrhoea int he Northwest of Pakistan Afri. J Biotechnol. 2010; 9: 1230-1235.

65. Ayaz S, Khan S, Khan SN, Bibi F, Shamas S, Akhtar M, et al. Prevalence of zoonotic parasites in drinking water of three districts of Khyber Pakhtunkhwa Province, Pakistan. Pak J Life Sci.2011; 9: 67-69.

66. Bodley-Tickell AT, Kitchen SE, Sturdee AP. Occurrence of Cryptosporidium in agricultural surface waters during an annual farming cycle in low land UK. Water Res. 2002; 36: 1880-1886.

67. Wells B, Shaw H, Hotchkiss E, Gilray J, Ayton R, Green J, et al. Prevalence, species identification and genotyping Cryptosporidium from livestock and deer in a catchment in the Cairngorms with a history of a contaminated public water supply. Parasit Vectors. 2015; 8: 66.

68. Chalmers RM, Giles M. Zoonotic cryptosporidiosis in the UK – challenges for control. J Appl Microbiol. 2010; 109: 1487-1497.

69. Nichols RA, Connelly L, Sullivan CB, Smith HV. Identification of Cryptosporidium species and genotypes in Scottish raw and drinking waters during a one-year monitoring period. Appl Environ Microbiol. 2010; 76: 5977-5986.

70. Xiao S, An W, Chen Z, Zhang D, Yu J, Yang M, et al. Occurrences and genotypes of Cryptosporidium oocysts in river network of southern-eastern China. Parasitol Res. 2012; 110: 1701-1709.

71. Nuchjangreed C, Boonrod K, Ongerth J, Karanis, P. Prevalence and molecular characterization of human and bovine Cryptosporidium isolates in Thailand. Parasitol Res. 2008; 103: 1347-1353.

72. Ng JSY, Eastwood K, Walker B, Durrheim DN, Massey PD, Porigneaux P, et al. Evidence of Cryptosporidium transmision between cattle and humans in northern New South Wales. Exp Parasitol. 2012; 130: 437-441.

73. Ehsan AM, Geurden T, Casaert S, Parvin SM, Islam TM, Ahmed UM, et al. Assessment of zoonotic transmission of Giardia and Cryptosporidium between cattle and humans in rural villages in Bangladesh. PLoS One. 2015; 10: e0118239.