



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

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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 prevalence 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.

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Keywords: Cattle; Cryptosporidium; Human; Risk factors; West Shewa.



Introduction

Cryptosporidiosis is one of the infectious diseases caused by protozoan parasites under the genus *Cryptosporidium*, in the phylum Apicomplexa. It is ubiquitous 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 hosts including 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 socio-economic 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]. Also seven 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 (anthroponotic transmission) 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-weaned 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 *Cryptosporidium serpentis* 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 microvilli shortening and destruction. Age, immune status, concurrent in-

fections, 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 cryptosporidiosis ranging 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. For instance, 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 value 30.1% [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 humans and 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 Gudartown
- To estimate the prevalence of *Cryptosporidium* infection in calves in and around Ambo and Gudartown
- To investigate the risk factors of *Cryptosporidium* infection in humans in and around Ambo and Gudartowns.
- To investigate the risk factors of *Cryptosporidium* infection in calves in and around Ambo and Gudartowns.

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 October 2019 to May, 2020 G.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 Abeba or 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 district has 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].

over three times (205+70 and 113+36) than in simple random sampling and set to 275 and 149, respectively. Hence, a total of 275 calves and 149 humans were sampled for 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 specimens were kept 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 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 humans were not present during data collection excluded.

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 lines, and clean lines of hind quarter), socio demographic variable (study area, age, sex, and study group). General management related variable (paddock system, presence of calving pen, body condition score).

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 a dryer

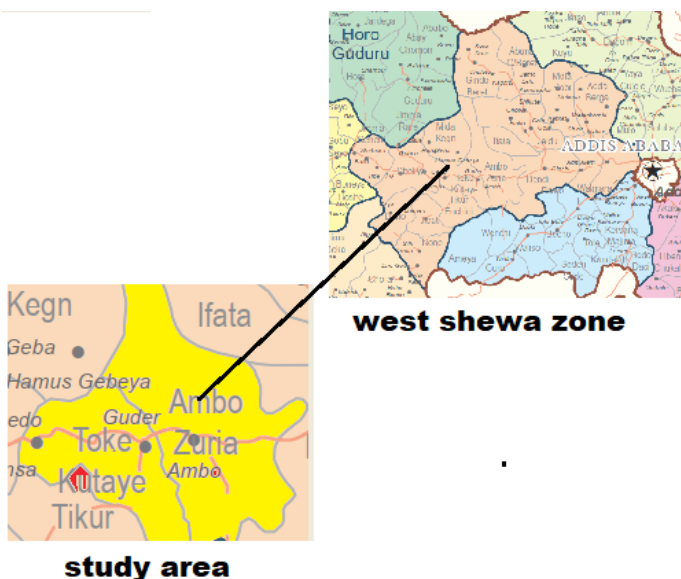


Figure 1: Map of the study area.

Source: CSA, (2013)

Study population

The study population for this study includes all dairy 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.

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 prevalence 16 % in cattle [7] and 8% in humans [30].

$n = \frac{1.96^2 P_{exp} (1 - P_{exp})}{d^2}$	Where: n = required sample size P _{exp} = expected prevalence d = desired absolute precision
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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

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 4-6 μ m, 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 be stained 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 semiquantitatively 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 (2-5 oocysts); 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.

Results

Prevalence in Calves

Out of 275 faecal samples examined, *Cryptosporidium* infec-

tion 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 ($p < 0.001$).

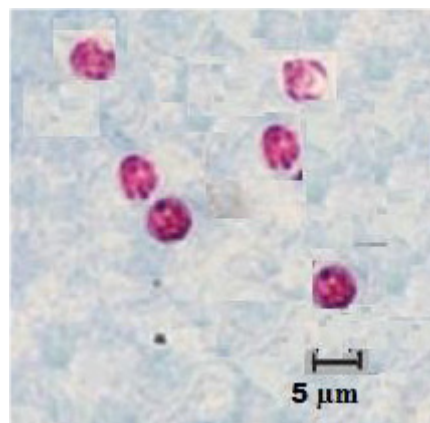


Figure 2: MZN-stained oocyst of *Cryptosporidium* infection 100x mag.

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 diarrhea 35.4% was significantly higher than the 11.4% prevalence in non-diarrheic ($p < 0.001$). 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 ($p < 0.507$).

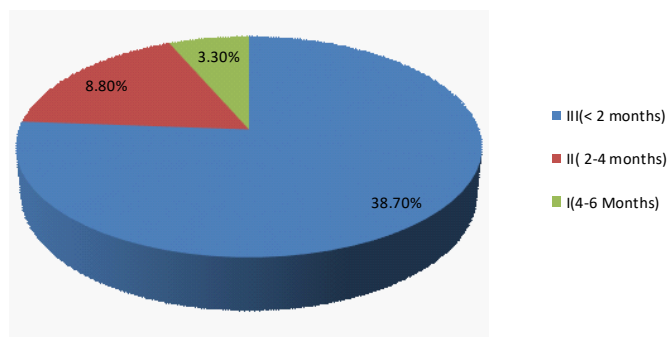


Figure 3: Age wise prevalence of cryptosporidium infection in calves.

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 condition, 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 8.5 times 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 3 times (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 (Table1). In contrast to the above 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.

Table 1: Univariable analysis of potential risk factors of calves-*Cryptosporidium* in West Shewa and its environs.

Risk factors	No. animals	No. +ve animals	Prevalence	OR (95% CI)	P value	%
Source of drinking water						
Pipe	178	22	12.36			
Well/river	97	25	25.8	7.09(4.54-11.08)	0.006	
Pen type						
Individual pen	124	13	10.48			
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			
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			
No	130	31	23.8	4.94(4.79 -13.55)	0.006	
Body condition						
Good	132	13	9.9			
Poor	143	34	23.9	6.40(3.16-9.23)	0.003	
Study site						
Rural	140	26	18.6			
Urban	135	21	15.6	5.43(3.40-8.64)	0.0507	
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 field	175	27	15.41			
To a well	100	20	20	5.48(3.63-8.26)	0.0334	
Age						
4-6 months	91	3	3.3			
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 Interval No. +v: Number of positive animals

Table 2: Multivariable logistic analysis of risk factors that were significant using 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 of calving pen	5.526	0.649	2.13	0.034	1.266 5.251
Presence of other diseases	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

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 statistically 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 those < 5 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),

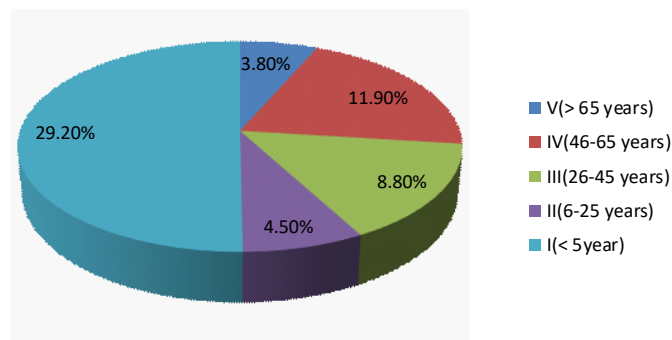


Figure 4: Age wise prevalence of *Cryptosporidium* infection in humans in the study area.

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 to be 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 1.50 times (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

Table 3: Univariable analysis of potential risk factors of human-*Cryptosporidium* in West Shewa and its environs.

Risk factors	categories	No. humans	No.+ve	Prevalence	OR	95% CI	P value
Sampled stool							
Presence of animals at home	Yes	104	6	5.77	6.38	(1.03-6.30)	0.047
Level contact with animals & their faeces	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)	

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

Risk factors	OR	SE	ZP	> z	95% confidence interval
High contact with 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

Discussion

The overall prevalence of *Cryptosporidium* infection in calves in the present study was found to be 17.1%. This is comparable 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 to 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 oocysts 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 and 4-6 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 susceptibility 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 immunocompromised 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 value of 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 prevalence 25.6% 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 faeces. 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.

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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.

Competing interest

The authors declare that they have no competing interests.

Authors contribution

Both authors contributed equally to this work

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