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Prevalence and Assessment of Risk Factors of Cryptosporidiosis in Calves in Selected Districts of Oromia Special Zone, Central Ethiopia

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Abstract: *Cryptosporidium* is an enteric protozoan organism that causes gastrointestinal disturbances in various animals, mainly calves. The parasite also has zoonotic importance in children and immuno compromised patients. The cross-sectional study design and questionnaire survey were conducted from November 2021 to June 2022 to assess the quantitative determination and evaluation of risk factors of cryptosporidium oocyst on calf cryptosporidiosis in central Ethiopia. A total of 279 fecal samples were collected from 40 dairy farms and 53 households by simple random sampling technique. The questionnaire survey was administered to randomly select 100 animal owners and/or workers with a main objective of targeting some basic information about knowledge, attitude and practices towards the disease. The directly collected fecal samples from the rectum of each calf were examined for the existence of *Cryptosporidium* oocysts using the modified Ziehl-Neelsen staining method. Out of 279 examined calves, 42(15.05%) were infected with *Cryptosporidium* oocysts. Risk factors such as study districts and management system were significantly ($p < 0.05$) affected the occurrence of *Cryptosporidium* infection. By using multivariable analysis, the study sites were accepted as possible risk factors for the occurrence of *Cryptosporidium* infection. This study obviously records out that *Cryptosporidium* infection is prevalent in the study area. Therefore, extension services on awareness creation for the community are recommended to adopt prevention and control approaches.

Key words: *Cryptosporidium* · Calves · Prevalence · Risk Factors · Central Ethiopia

INTRODUCTION

Cryptosporidium is one of the most common causes of gastrointestinal disease in a variety of vertebrate hosts, including humans. The disease is distributed worldwide and can infect a variety of hosts [1, 2]. Reports have shown that the prevalence of bovine cryptosporidiosis ranges from 6.25 to 39.65% in different parts of the world [3-5]. Similarly, in humans it ranges from 0.1% to 73.3% [6-8].

In Ethiopia, livestock farming is the main income of the widespread human population, giving farmers traction, income, food sources, means of investment and an important source of foreign exchange [9]. The country has a huge livestock population in Africa with an estimated cattle population of 70 million. Of these, about 97.8% are local breeds and the rest are hybrid and exotic breeds. The performance of cattle depends largely on their reproductive performance and the presence of calves. Calf morbidity and mortality are persistent problems for dairy farmers and newborn calf mortality in the first month of life is more than 80% [10].

The numerous types of gastrointestinal parasites in calve is a worldwide problem [8, 11]. Out of gastrointestinal infections, such as *Escherichia coli*, salmonellosis, rotavirus, cryptosporidiosis, coccidiosisis and giardia are the most predominant causes of diarrhea in calves [12]. Clinical sign of diarrhea is the most significant sign of disease in young calves and accounts for approximately 75% of the mortality of dairy calves within the first 3 weeks of age [13]. *Cryptosporidium* species are protozoa that interrupt livestock production [14]. These parasites belong to the phylum Apicomplexa, class Sporozoasida, Genus *cryptosporidium* and are regularly implicated in outbreaks of diarrhea worldwide [15]. There are over 30 species and more than 70 genotypes have been recognized, with new genotypes continually being identified by molecular means [16, 17].

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Cryptosporidium species found in cattle are usually associated with four main species. These are *C. parvum, C. andersoni, C. ryanae* and *C. bovis*. However, other species including *C. suis, C. hominis, C. xiaoi, C. ubiquitum, Meleagridis, C. muris* and *C. felis*, have also been identified in cattle [18].

The *cryptosporidium* infection may be chronic and life-threatening in immunosuppressed animals. Diarrhea is usually voluminous and watery, sometimes mucous but rarely bloody. Abdominal pain, nausea, vomiting, anorexia, weight loss, fever and fatigue may be also present [19]. Calves begin shedding *cryptosporidium* as early as 2 days of age, but peak shedding occurs at 14 days [20]. From an economic point of assessment, the body weight gain in infected calves is 55% lower than in healthy animals, which compromises livestock production [21]. The risk factors that affect the prevalence of cryptosporidiosis in calves include; age, hygiene, colostrum feeding, season, fecal consistence, breed, body condition score, feed source, drinking water source, contact with other infected animals, overcrowded living style, feed and water sources, diarrhoea, climate, sex, age, type of pen floor, management system, immunological competence and concomitant rearing of cattle with other animal species are the most relevant [7, 8]. Transmission of cryptosporidiosis, both within and between host species including humans, is by the fecal-oral spread of the environmentally resistant oocysts, which are fully sporulated and infective when excreted in feces [22].

Methods accessible for the diagnosis of *Cryptosporidium* species include microscopic, serological and molecular techniques, each with their own characteristics. Molecular detection is used to detect the species/genotype of the parasite [11, 23]. However, microscopic detection is based on the detection of oocysts in fecal samples, of microscopic detection methods, acid-fast staining of fecal smear to visualize *Cryptosporidium* oocytes is a viable and rapid method [24, 25]. The modified Ziehl-Nelson staining technique is the gold standard stain for the detection of *Cryptosporidium* species, conventionally prepared by microscopy after staining fecal smears; with a sensitivity of about 75% for the detection of the round, sporulated oocysts of 4 to 5 m in size [26]. This method is widely used because it is inexpensive, simple and has a high positive predictive value for *Cryptosporidium* [27]. As a specific therapy or vaccine to combat this parasite is not yet available, infection prevention depends on avoiding contact with the parasite and maintaining immune competence [28].

In Ethiopia, only few studies conducted on dairy cattle in central and southern part of the country report prevalence rates ranging from 7% to 27.8% [29-33]. However studies looking at the long term effects of *cryptosporidium* infection in calves have not been carried out, even though it impairs the growth rates of the calves [34]. Reduction in the long term growth of calves affected by *Cryptosporidium* may prove costly for farmers due to loss of income from lower carcass weights, management of enteritis, reduced feed conversion and production efficiency, treatment cost and losses due to animal death [35]. Despite its significant economic losses, the study of distribution and associate risk factors of *Cryptosporidium* in the study area under consideration is so scanty. Therefore, this study was conducted to investigate the prevalence and risk factors of *Cryptosporidium* oocytes in calves in four districts of Oromia special zone, central Ethiopia.

MATERIALS AND METHODS

Description of the Study Area: The study was conducted from November 2021 to June 2022 in four selected districts of Oromia special zone, central Ethiopia. Oromia special zone is located between latitudes of 80 46’ 34’’ and 90 23’ 00’’ N and longitude of 380 32’ 27’’ and 390 14’43’’ E and situated at an altitudinal range of 1950 to 2600 m.a.s.l. The mean annual temperature of the zone ranges between 20 and 25°C in the lowlands and 10 to 15°C in the central highlands. The mean annual rainfall of the special zone varies from 700mm to 1400mm in lowlands and highlands respectively. The human population of the area is estimated to be around 117.88 million and Over thirty thousand peoples directly depend on incomes earned from the dairy subsector, which is estimated to be about 5200 dairy farms (large, medium and small scale) with 58, 500 cattle (almost 50 percent cross breed [36]. There are three Livestock production system of dairy herds in the study area; the highland small holder milk production, Urban and peri-urban milk production, Intensive Dairy Farming [36, 37].

From the study areas, Sululta is one of the districts in special zone surrounding Addis Ababa in Oromia regional state. The district is bordered by Wuchale and Yaya-Gulale *woreda* in the north, Addis Ababa city administration and Walmara *woreda* in the south, Jida and Bereh *woreda* in the east and Mulo *woreda* in the west direction. The area is geographically situated between 9.07-9.52°N and 38.53-38.98° E, while the altitude is ranging from 2851-3700 meters above sea level. Livestock

husbandry and crop production are the predominant economic activities and the major sources of livelihood in the area [38].

Barak district, a second study area is also a district in oromia region special zone and located at 7°57'44''N latitude and 39°7' 42''E longitude. The district is bordered on the south by Akaki and east Shewa zone, on the southwest by the city of Addis Ababa, on the west by Sululta, on the north by North Shewa zone and on the east by Amhara regional state. The total population of the district is 80, 808, of whom 41, 023 were men and 39, 785 were women [39].

Sabata Hawas district is located at 25km southwest of Addis Ababa, at latitude and longitude of 8° 55'N, 38° 39'E/ 8.917°N 38.650°E respectively and an elevation of 2369meters above sea level. The district is bordered with Akaki district in the east, Kersa and Tole districts in the south, Walmara district in the north and Ilu and Ejere districts in the west. The livestock population of the district is 7540 cattle, 2670 sheep, 1200 goat, 1620 horses, 765 mules, 3030 donkeys, 12380 poultry [40]. The altitude of the district ranges between 1800 and 3385 meter above sea level with mean rainfall range from 860 to 1200mm. The annual mean maximum and minimum temperature of the area are 25.4°C and 13.9°C, respectively. The district is divided in to two agro ecological zones called highland 12% and midland 88% [40].

Burayu is located in the oromia special zone surrounding Addis Ababa. Astronomically, the town extends roughly from 9° 02' to 9° 02'30" N latitudes and 38° 03'30" to 38° 41'30" E longitudes. The town is located about 15 kilometers from the center of Addis Ababa, the capital of Ethiopia. Burayu is bordered by Sebeta town in the south, Walmara district in the west, Sululta town in the north and Addis Ababa city in the east.

Study Population: Study animals were calves up to 12 months of age, those kept under small dairy farms and house holder. Local and hybrid calves of both sexes (male and female) were included in the present study. As there was no recorded file to identify the exact age of the calves, the age of each calf were estimated based on owner's response. Hence, the study units were categorized in to three age groups; <2months, 2-6 months and >6 months according to [41] whereas, the body condition scoring of animal was recorded as poor, medium and good [31].

Study Design: A cross-sectional study design was conducted from November 2021 to June 2022 to assess *cryptosporidium* infection in dairy calves; prevalence and potential risk factors of the parasite in the study area.

Sample Size Determination and Sampling Methods

Sample Size Determination: The required sample size was determined by the formula which has been given by Thrusfield and Christley [42]:

$$N = \frac{1.96^2 * P\exp(1 - P\exp)}{d^2}$$

Were,

N = required Sample size

P_{exp} = Expected Prevalence

d^2 = Degree of Precision/desired absolute precision at 95% confidence interval

Therefore, using an expected prevalence of 18.6% [33] at 95% confidence interval and 5% precision value, a sample size of 232 calves was calculated. However, the sampled study units were increased to 279 by adjusting for clustering effect using intra-cluster correlation (rho)

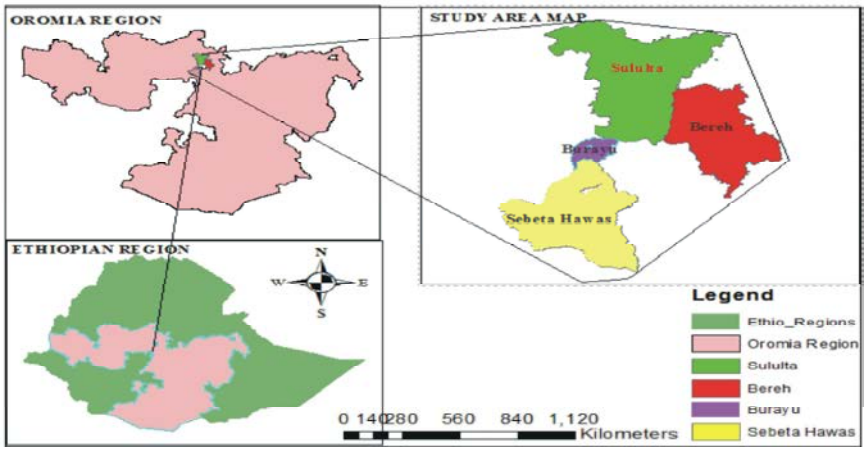

Fig. 1: Map of the study area

of 0.105 for GIT parasite [42]. An average of 3 calves (m) from small scale farms or household were sampled using random sampling techniques. Hence, the design effect (DE) formula of $n' = n(1 + \rho(m - 1)$ was used for increment of the sampled study unit. Where, n' is the adjusted sample size; n=the sample size for simple random sample; ρ is intra-cluster correlation coefficient and m was the average calve sampled per farm/household. Generally, from the four study districts a total of 93 clusters (40 dairy farms and 53 households) were involved in the current study.

For the questionnaire survey sample size was determined based on Arsham formula [43]:

$$N = \frac{0.25}{SE^2}$$

N = sample size
SE = standard error

Therefore by assuming the standard error of 5% at a precision level of 0.05 and the confidence interval of 95%, 100 households were selected for questionnaire survey data.

Sampling Methods: For this particular study, the districts were selected by purposive sampling method based on the abundance of dairy cattle populations. From each districts PAs and farms/households were selected by cluster sampling methods and hence PAs were considered as primary sampling units (PSU). Farms/households were considered as secondary sampling units (SSU). The study units here, calves from each cluster of farms/households were selected by simple random sampling with average of three individuals (n=3) from each.

Accordingly, four districts (Sebeta Hawas, Burayu, Barak and Sululta) with two PAs from each district (Sebata 01, Sebata 02, Gafersa Guje, Nono Gafersa, Beke 02, Girar, Chanco Buba and Guto Ilamu) were selected respectively. For the questionnaire survey households were selected by random sampling technique from the study area.

Study Techniques

Sample Collection and Transportation: Fresh fecal samples were collected directly from the rectum of each calf using sterile gloves and kept in a separate labeled bottle, preserved with potassium di-chromate preservative and then placed in a cold box (Figure 8). At the time of sample collection, the name of the owner, sampling date, age, sex, breed and body condition score and duration of colostrum feeding, fecal consistency (formed/diarrhoea), presence/absence of close contact with other domestic animals, feed and drinking water source and hygiene status were recorded for each animal on a recording sheet. After collection, the samples were then transported within 24 h to the laboratory of Animal Health Institution (AHI) and stored at +4°C until the laboratory activity conducted (maximum of 48 h).

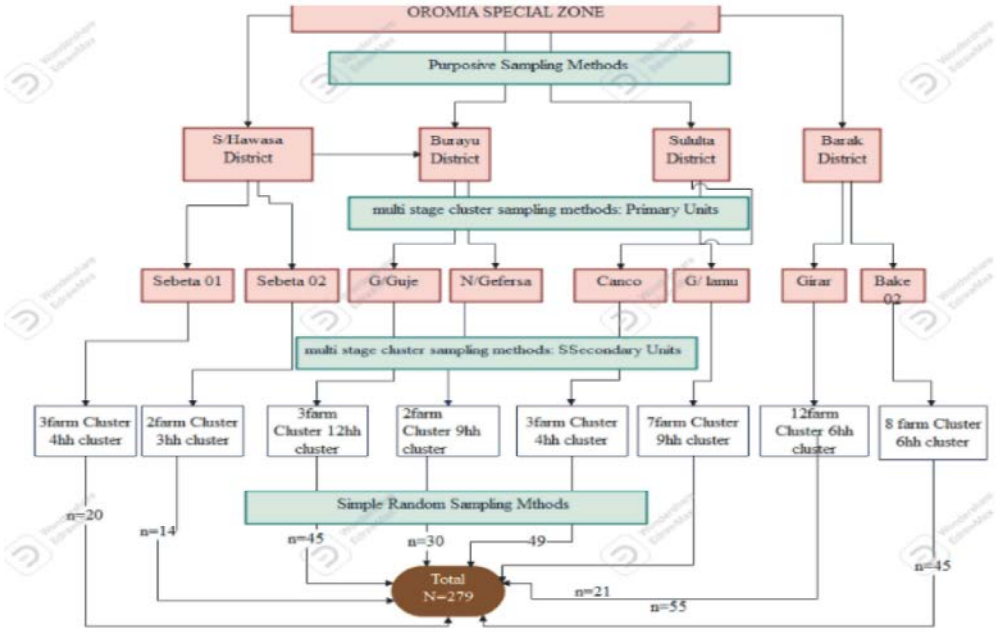

Fig. 2: Schematic Presentation of Sampling Methods

The Modified Acid Fast Ziehl-neelsen Stain: This staining technique was a gold standard for the detection of *Cryptosporidium* oocyst. A thin smear was prepared from the collected fecal specimens and dried in air. Then the prepared thin smear was fixed in methanol for 3 minutes and then stained with undiluted carbol-fuchsine solution for 15 minutes. Successively, the slide was rinsed in tap water and placed in an acid-alcohol solution (3% hydrochloric acid in 70% ethanol) for 10 seconds to remove the stain, whereas acid-fast structures were resist to the acid-alcohol's distaining action. The slide was placed for 30 seconds in a methylene blue that provides contrast between background material and acid-fast structure. Again the slide was rinsed in tap water, then air-dried and examined using x40 eyepieces and an oil-immersion objective of x100 magnification. *Cryptosporidium* oocysts were appear as pink stained, round to oval structures of about 4 to 6 µm in diameter, containing distinct internal structures [44].

The modified acid-fast staining was a time-consuming procedure (about 30 to 45 minutes) and good staining and visual skills were necessary. A common problem was distinguishing *Cryptosporidium* oocysts from other elements, such as molds and yeast, these "pseudo-Cryptosporidia" can be ruled out based on their dimensions [45]. Although the modified Ziehl-Neelsen staining remains the Gold Standard for the detection of *Cryptosporidium* species it was claimed to lack sensitivity and specificity [45]. It has about 70% sensitivity compared with immunofluorescent antibody stains [46]. However, lack of specificity could be resolved by lowering the sensitivity of the test. For instance, a sample could be considered positive if five oocysts or more were observed, causing samples with low-level shedding of oocysts to be interpreted as negative. This extra loss of sensitivity in turn could be resolved by using repeated stool sample examinations on consecutive days [47].

The modified Ziehl-Neelsen staining was a low cost technique and provides a permanent stain that makes it possible to send doubtful or scanty positive slides to a reference laboratory for confirmation [48]. The result was interpreted as: A scoring system for positive samples were used, based on the number of oocysts observed under the ×40 objective. However, microscopic examination cannot be considered as a quantitative determination as oocyst numbers vary considerably during the course of infection. So that:

+ = 1 per field of view (less positive)
++ = 2 to 10 oocysts per field of view (moderate Positive)
+++ = 11 or more oocysts per field of view (strong positive)

Data Management and Analysis: Data collected from the field (both questionnaire and coprological data) were recorded, coded and stored in separate spreadsheet of Microsoft Excel 2007 and analyzed using STATA version 13.0 for MA Windows (STATACorp. LP, Texas USA). Prevalence was calculated by dividing the number of positive animals against the total number of animals tested. The Chi-Square test was used to evaluate the relationship between risk factors and *Cryptosporidium* infection. Univariate logistic regression method was used to determine the association between covariates and occurrence of *Cryptosporidium* infection. Further analysis of the association was made by the multivariable logistic regression, the adjusted odds ratio (OR), computed as the exponent of the respective regression coefficients, was used to quantify the effect of risk factors on the likelihood of *Cryptosporidium* infection. Confidence level was held at 95% and $P < 0.05$ was set for significance level.

RESULTS

Overall Prevalence: *Cryptosporidium* infection was identified in 42 calves from 279 fecal samples examined with modified Ziehl-Neelson staining techniques with an overall prevalence of 15.05%. In the present study, *Cryptosporidium* infection was observed in all sampled districts. A higher prevalence of *Cryptosporidium* infection was observed in Sululta 17 (24.28%) and Barak 21 (21%), while a lower prevalence 2 (5.88%) and 2 (2.66%) were detected in Sabata Hawas and Burayu districts respectively (Table 1). Of the positive samples, more than half (59.52%) were moderately positive (2-10 oocysts per field), however 26.19% and 14.28% of them were less positive (1 oocyst per field) and strong positive (more than 10 oocysts per field) respectively.

The prevalence of *Cryptosporidium* infection in eight Peasant associations, as described in the following table, showed that a higher prevalence was in Bake 02 (28.88%) and Guto Ilamu (28.57%) of Barak and Sululta districts, respectively. Followed by Chanco Buba (22.44%) from Sululta district, Girar (14.54%) from Barak district, sebeta 01(10%) from Sabata Hawas district.

Table 1: Prevalence of calf cryptosporidiosis in study areas

Variables	Categories	No of samples	Positive	(%)	χ^2	p-value
District	Sululta	70	17	24.28	18.6663	<0.001
	Burayu	75	2	2.66		
	S/Hawas	34	2	5.88		
	Barak	100	21	21		
Total		279	42	15.05		
Peasant Association	Sabata o1	20	2	10	23.74	0.001
	Sabata 02	14	-	-		
	Bake 02	45	13	28.88		
	Girar	55	8	14.54		
	Gafersa Guje	45	1	2.22		
	Nono Gafersa	30	1	3.33		
	Cahanco Buba	49	11	22.44		
	Guto Ilamu	21	6	28.57		
Total		279	42	15.05		

Table 2: Relationship of reputed host related risk factors with the outcome variable using Pearson chi-square analysis

Potential risk factors	No of sample	No of positive	Percentage	χ^2	P-value
Sex				0.8456	0.358
Female	177	24	13.55		
Male	102	18	17.64		
Age				0.6018	0.740
<=2month	71	11	15.49		
2-6month	80	10	12.5		
>=6month	128	21	16.40		
Breed				0.6858	0.408
Local	60	7	11.66		
Hybrid	219	35	15.98		
BCS				0.5990	0.741
Poor	73	13	17.80		
Medium	165	23	13.93		
Good	41	6	14.63		

However, in the current study, lower prevalence of the parasite infection was obtained from Nono Gafersa (3.33%) and Gafersa Guje (22%) from Burayu districts and no prevalence from Sabata Hawas district of Sebeta 02 kebele.

Risk Factors

Host Related Risk Factors: The prevalence of infection was higher in calves > 6 months (16.40%) than in calves < 2 months (15.49%) and 2-6 (12.5) months old, but the difference between the ages of the calves was not statistically significant (X^2 = 0.6018, P = 0.74). The prevalence verified between the sex of the calves and the study showed that the highest prevalence was recorded in male 18 (17.64%) than in female 24 (13.55%), but the difference is not significant (P>0. 05). Among the calf breeds, a higher distribution of *Cryptosporidium* infection was found in the hybrid calves 35 (27.13%) than in the local breed 7 (11.66%) and no statistically significant difference between the two breeds was observed (p = 0.408). *Cryptosporidium* infection was identified with a prevalence of 13 (17.80%), 23 (13.93%) and 6 (14.63%) in poor, moderate and good body condition calves, respectively (Table 2).

Non Host Related Risk Factors: Calves without diarrhea (with formed fecal consistency) were more frequently infected 23 (15.43%) than calves with diarrhea 19 (14.61%). For hygiene status, a higher prevalence was found in poorly hygiene calves 22 (16.05%) than in good hygiene calves 20 (14.08%) and is not significant (Table 3). Regarding feed sources, *Cryptosporidium* infection was 21 (15.90%), 18 (13.63%) and 3 (20%) in calves fed Pasteur, milk and Pasteur and milk alone, respectively and this shows the higher prevalence was found in calves feeding milk alone. Farms which were prone to use river water sources experience higher *Cryptosporidium* infection compared to farms using pipe water sources (Table 3). Regarding the type of pen floor and contact with other domestic animals, the higher prevalence of

Table 3: Relationship of reputed non-host-related risk factors to outcome variable using Pearson's chi-square analysis

Risk factors	No of samples	No of positive	Percentage	χ^2	P-value
Fecal consistency				0.0366	0.848
Formed	149	23	15.43		
Diarrheic	130	19	14.61		
Hygiene status				0.2125	0.645
Good	142	20	14.08		
Poor	137	22	16.05		
Feed source				0.5699	0.752
Pasture	132	21	15.90		
Milk and Pasteur	132	18	13.63		
Milk	15	3	20		
Water source				0.0505	0.822
Pipe	124	18	14.51		
River	155	24	15.48		
Type of pen floor				0.0055	0.941
Concrete	227	34	14.97		
Soil	52	8	15.38		
Contact with other domestic animals animal			0.0126	0.911	
No	124	19	15.32		
Yes	155	23	14.83		
Management system			5.7547	0.016	
Extensive	40	1	2.5		
Intensive	239	41	17.15		

Cryptosporidium was observed in calves kept in soil pen floor housing and those without contamination with other domestic animals, but the difference was not significant. Finally, the occurrence of cryptosporidiosis was recorded in intensively managed calves 41 (17.15%) than in extensively managed calves 1 (2.5%) (Table 3).

Association of Explanatory Factors with Response Variables Using Logistic Regression Analysis: By using univariate logistic regression analysis, two risk factors affecting the prevalence of *Cryptosporidium* infection in calves were identified. The prevalence of *Cryptosporidium* infection was observed significantly ($p > 0.05$) in the districts of the study area, with the prevalence of *Cryptosporidium* in Sululta, Barak and Sebeta Hawas districts being 12, 10 and 2 times more likely to contract cryptosporidiosis than Calves in Burayu District (OR = 11.71, 95% CI = 2.593-52.851), (OR = 9.70, 95% CI = 2.198-42.832) and (OR = 2.28, 95% CI = 0.308-0.308- 16.916). Similarly, the management system was found to affect the incidence of *Cryptosporidium* infection in the study area ($p<0.05$). Accordingly, calves on intensive farms were 8-fold more likely to develop cryptosporidiosis than calves on an extensive management system (OR = 8.07, 95% CI = 1.07-60.46). Other risk factors such as; Farmer associations, sex, age, breed, body condition score, fecal consistency, hygiene status, food sources, drinking water source, pen floor type and contact with suspect animals were found to be statistically insignificant for *Cryptosporidium* infection rate ($p>0.05$) (Table 4).

Multivariable analyzes were performed for these significant risk factors in the Univariate analysis and the test result showed that study districts and management systems were statistically significant ($P<0.05$) using univariate analysis. However, in the final multivariate logistic regression, only study districts were still observed as a significant factor for *Cryptosporidium* infection in calves in the study area. Consequently, Sululta was 11 times, Barak 9 times and S/Hawas district 2 times more likely to contract cryptosporidiosis than calves in Burayu districts.

Questionnaire Survey

Respondents Socio- Demography: Out of 100 farmers interviewed in the questionnaire survey, all districts were equally interviewed (25%). Of the respondents interviewed, 28% were male and 72% of the participants were female. Regarding the educational status of respondents, most (37%) were illiterate and the other 25%, 19% and 11% respectively had elementary school, high school and literacy status, while only 8% of respondents had certificate. In terms of age category, the majority of respondents were in the 25 to 50 age groups and other respondents 18% and 17% were in the 15 to 24 and > 50 age groups, respectively (Table 5).

Table 4: Association of accepted explanatory variables with response variables using logistic regression analysis

Variable	No of sample	No positive (%)	COR(95% CI)	P-Value	AOR(95% CI)	P-Value
District						
Burayu	75	2(2.66%)	*		*	-
S/Hawas	34	2(5.88%)	2.28(0.31-16.92)	0.420	2.18 (0.28-17.08)	0.457
Barak	100	21(21%)	9.70(2.20-42.83	0.003	9.41(2.06-42.97)	0.004
Sululta	70	17(24.28%)	11.71(2.59-52.85)	0.001	11.20(2.31-54.28)	0.003
Peasant Association						
Sabata o1	20	2(10%)	*	-	-	-
Sabata 02	14	-	0.000(0.000)	0.999	-	-
Beke 02	45	13(28.88%)	3.66(0.74-18.05)	0.112	-	-
Girar	55	8(14.54%)	1.53(0.30-7.91)	0.611	-	-
G/Guje	45	1(2.22%	0.21(0.02-2.40)	0.207	-	-
N/Gafersa	30	1(3.33%)	0.31(0.03-3.67)	0.353	-	-
C/Buba	49	11(22.44%)	2.61(0.52-13.00)	0.243	-	-
G/Ilamu	21	6(28.57%)	3.60(0.63-20.53)	0.149	-	-
Sex						
Female	177	24(13.55)	*	-	-	-
Male	102	18(17.64)	0.73(0.37-1.42)	0.359	-	-
Age						
<=2month	71	11(15.49)	*	-	-	-
2-6month	80	10(12.5)	0.77(0.30-1.96)	0.596	-	-
>=6month	128	21(16.40)	1.07(0.48-2.37)	0.867	-	-
Breed						
Local	60	7(11.66)	*	-	-	-
Hybrid	219	35(15.98)	1.44(0.60-3.42)	0.41	-	-
BCS						
Poor	73	13(17.80)	*	-	-	-
Medium	165	23(13.93)	0.85(0.59-1.44)	0.56	-	-
Good	41	6(14.63)	*	-	-	-
Fecal consistency						
Formed	149	23(15.43)	*	-	-	-
Diarrheic	130	19(14.61)	0.93(0.85-1.81)	0.84	-	-
Hygiene status						
Good	142	20(14.08)	*	-	-	-
Poor	137	22(16.05)	1.16(0.60-2.25)	0.64	-	-
Feed source						
Pasture	132	21(15.90)	*	-	-	-
milk and Pasteur	132	18(13.63)	0.96(0.55-1.68)	0.91	-	-
Milk	15	3(20)	*	-	-	-
Water source						
Pipe	124	18(14.51)	*	-	-	-
River	155	24(15.48)	1.07(0.55-2.09)	0.82	-	-
Type of pen floor						
Concrete	227	34(14.97)	*	-	-	-
Soil	52	8(15.38)	1.03(0.44-2.38)	0.94	-	-
Contact with suspected animal						
No	124	19(15.32)	*	-	-	-
Yes	155	23(14.83)	0.96(0.49-1.86)	0.91	-	-
Management system						
Extensive	40	1(2.5)	*	-	*	-
Intensive	239	41(17.15)	8.07(1.07-60.46)	0.04	1.15(0.07-19.05)	0.924

Table 5: Socio-demographic characteristics of the respondents

Variable	Category	Frequency	Percentage (%)
Districts	Sululta	25	25
	Burayu	25	25
	Barak	25	25
	Sabata Hawas	25	25
Peasant Association	Chanco Buba	12	12
	Guto Ilamu	13	13
	Gafersa Guje	10	10
	Nono Gafersa	15	15
	Beke 01	12	12
	Girar	13	13
	Sabata 01	11	11
	Sabata 02	14	14
Gender	Male	28	28
	Female	72	72
Age	15-24	18	18
	25-50	65	65
	>50	17	17
Educational status	Illiterate	37	37
	Read and write	11	11
	Elementary	25	25
	High school	19	19
	Certificate	8	8

Table 6: Information on farm

Variables	Category	Frequency	Percentage (%)
Have you dairy cattle?	Yes	78	78
	No	22	22
If yes how many animals are there in your farm?	1-5	16	16
	6-10	26	26
	11-15	29	29
	>15	7	7
	No dairy cattle	22	22
How many calves are there in your farm?	1-3	63	63
	4-6	15	15
	No calves	22	22
What breed of calves in your farm?	Local	21	21
	Hybrid	57	57
	No calves	22	22

Information on Farm: The majority of respondents (78%) had dairy cattle and 22% of them had no dairy cattle. 29% of them had 11-15 dairy cattle and 63% of them had 1-3 calves in their farms. Regarding calf breeds, more than half (57%) of them were hybrids and only 21% of them were local breeds (Table 6).

Information on Calf Management: The survey study of calf management practices in the study area showed that most of the calves were housed on concrete pen floors. The source of drinking water consisted of 48% pipes, 32% rivers and 20% ground; however, only 9% of respondents treat river water with chemicals. Regarding the feed additive for the calves; concentrated feed 79% was the main source, Pasteur 15% and Hay 6% were also used in the study are. Many of the farmers interviewed were unaware of the importance of colostrum for the newborn calves and the main signs of the disease were diarrhea and abdominal pain. The large number of respondents, 77%, was fed 3-6 liters of colostrum to the calf within 24 hours after birth and some of them 19% and 4% < 2 liters and > 6 liters respectively. 85% of respondents use hand-feeding methods to feed their calves colostrum and only 15% of them use the suction technique. Finally, according to the respondents, the majority of the calves with diarrhea were dead (Table 7).

Table 7: Information on management of the calves

General information from Total number of 100 respondents	Category	Frequency	Percentage (%)
Type of pen floor	Soil	9	9
	Concrete	91	91
Amount of colostrum feed to the calf within 24 h. of age/lit	<2	19	19
	3-6	77	77
	>6	4	4
Methods of colostrum feeding	Suckling	15	15
	Hand feed feeding	85	85
Calve started feeding in the first week of life	Yes	85	85
	No	15	15
Feed supplement given to the calves	Pasteur	15	15
	Concentrates	79	79
	Hay	6	6
Source of drinking water	Pipe	48	48
	River	32	32
	Ground	20	20
Treating water comes from river	Yes	9	9
	No	91	91
If yes what methods used to treat river water	Chemical	9	9
	Not treated	91	91
Disease seen on calves	Diarrhoea	71	71
	Abdominal pain	23	23
	others	6	6
Calves dead from diarrhoea	Yes	30	30
	No	70	70

Table 8: Knowledge and information of cryptosporidiosis

General information from Total number of 100 respondents	Category	Frequency	Percentage (%)
Knowledge about the disease	Yes	8	8
	No	92	92
Causes of cryptosporidiosis	Yes		-
	No	100	100
Zoonotic importance of cryptosporidiosis	Yes		-
	No	100	100
Is the cryptosporidiosis affect calves	Yes	8	8
	No		-
	Don't know	92	92
Transmission of cryptosporiosis between animals	Yes	8	8
	No		
	Don't know	92	92
Knowledge on sign of cryptosporidiosis on calves	Diarrhoea	8	8
	Abdominal pain		-
	Don't know	92	92

General Knowledge and Information of Cryptosporidiosis: Regarding general information about the disease, only 8% have heard of the disease and know that it affects calves. However, majorities (92%) of respondents have not heard of cryptosporidiosis and are unaware of its transmission between animals. All respondents were unaware of the cause and zoonotic significance of the disease. Clinical signs of the disease are known to 8% of respondents, as the disease causes diarrhea (Table 8).

Knowledge of Communities on Treatment, Control and Prevention of Cryptosporidiosis: Respondents in the present study treat calves showing signs of diarrhea with locally available traditional medicine (9%), purchase and administer veterinary medicine (19%) and take them to a nearby veterinary clinic (72%). The majority of farmers did not use the routine knowledge to prevent and control this parasitic disease. However, 68% clean the calf pen every day. Only 30% of respondents were aware of colostrum feeding; and quarantine methods (34%) and

Table 9: Evaluation of knowledge on treatment, prevention and control of cryptosporidiosis

Evaluation of knowledge Total No of respondents= 100	Category	Frequency	%
How is the diarrheic calf treated	Traditionally available drugs	9	9
	Buying and administering veterinary drug	19	19
	By veterinary drugs	72	72
Clearing of pen floor	Every day	68	68
	Twice a day	18	18%
	Three times a day	14	14%
Facility of calving pen	Yes	12	12%
	No	88	88%
Awareness of colostrum feeding	Yes	30	30%
	No	70	70%
Separating calf from dam within 12 h after birth	Yes	88	88%
	No	12	12%
Preventing the calves contacting with suspected animals	Yes	53	53%
	No	47	47%
Is newborn calves get vaccines against any disease	Yes	1	1%
	No	99	99%
Using quarantine methods	Yes	34	34%
	No	66	66%
Wearing protective clothes while handling diarrheic calves	Yes	8	8%
	No	92	92%
Spraying of farm and vehicles with chemical	Yes	10	10%
	No	90	90%

Table 10: Risk factors associated with cryptosporidiosis in calves based on the chi-square test

			Occurrence of infection			
Risk factors assessment	Category	Positive response	Positive	Negative	χ^2	P-value
Separation of calves from dam within 12 h after birth	Yes	88	28	34	1.176	0.278
	No	12	2	6		
Spraying of vehicles in the farm	Yes	10	3	5	0.106	0.745
	No	90	27	35		
Vaccination of newborn calves	Yes	1	0	1	0.761	0.383
	No	99	30	39		
Cleaning of pen floor	Yes	71	19	30	1.111	0.292
	No	29	11	10		
Methods of colostrum feeding	suckling	15	3	4	0.648	0.421
	Hand feeding	85	26	37		
Facility of calving pen	Yes	12	5	4	0.680	0.410
	No	88	25	36		
Awareness of importance of colostrum feeding	Yes	30	8	11	0.006	0.938
	No	70	22	29		
Presence of diarrhoea on calves	Ye	60	23	16	9.341	0.002
	No	40	7	24		
Use of quarantine methods	Yes	34	10	15	0.130	0.719
	No	66	20	25		
Wearing of protective clothes	Yes	8	0	5	4.038	0.044
	No	92	30	35		
Type of housing used for calves	Separated	53	13	21	0.577	0.448
	Not separated	47	17	19		
Preventing of calves from contacting with other animals	Yes	53	13	21	0.577	0.448
	No	47	17	19		
Type of pen floor	Concrete	91	23	39	7.350	0.007
	Soil	9	1	7		
Calves start feed in the first week of life	Yes	85	25	35	0.243	0.622
	No	15	5	5		

wearing protective clothing (8%). In terms of setting up a calving box, the majority of them (88%) did not have a calving box and 88% of respondents separated the calves from their mothers within 12 hours of birth. The majority of respondents kept their calves with other pets and some of them were protected from other animals (Table 9).

Risk Factors Associated to the Occurrence of Cryptosporidium Infection Regarding the Present Survey: In the current study, various risk factors that influence the occurrence of cryptosporidiosis in calves were evaluated. Chi-square test analysis showed that pen floor type, wearing of protective clothing when handling diarrheal calves and the presence of diarrhea were statistically significant ($P<0.05$). Spraying vehicles on the farm, separating calves from their mothers within a short time after birth, vaccination of newborn calves, cleaning of the pen floor, method of colostrums feeding, setting up the calving pen, awareness of the importance of colostrums feeding, use of quarantine methods, type of calf housing, avoidance of contact of the calves with other domestic animals and start of feeding of the calf in the first week of life were not statistically significant ($P>0.05$) (Table 10).

DISCUSSION

Cryptosporidiosis is one of the important zoonotic diseases causing life-threatening diarrhea in young, immunocompromised and malnourished hosts. It has been reported in animals and humans around the world in more than 106 countries and mainly in developing countries [49, 50, 8]. There are currently various epidemiological reports on the prevalence and associated risk factors of *Cryptosporidium* infection in animals. According to the reports, the prevalence of the infection varied from country to country and from host to host. Conversely, the infection rate is higher in young animals [6].

Ethiopia has different agro-climatic environments favoring the survival of various parasites such as *Cryptosporidium* species affecting domestic animals. Estimating the country-level prevalence with associated risk factors of *Cryptosporidium* infection plays a central role in developing appropriate approaches for the diagnosis, prevention, treatment and control of *Cryptosporidium* infections in Ethiopia. In the present study, calves in the study area were confirmed to be infected with *Cryptosporidium* species, showing that calf Cryptosporidiosis is endemic and locally prevalent. The overall prevalence of *Cryptosporidium* infection in dairy calves in the present study was 15.05% (42/279). This finding was comparable to that of Wegayehu *et al.* [32] who reported prevalence of 15.8% in central Ethiopia, 18.6% by Manyazewal *et al.*[33] in Addis Ababa and surroundings, 18.6% by Ayele *et al.* [51] in Northwest Ethiopia, 17.6% by Abebe *et al.* [52] in central Ethiopia, 13.6% from Dinka and Berhanu [30] in Bishoftu, 13.8% from Adinew and Geremew [53] in and around Nekemte town. However, lower than the prevalence report of other investigators in Ethiopia; 27.8% [29] in central Ethiopia, 24.0% in southeastern Ethiopia and 21.4% [13] in northwestern Ethiopia. Different prevalence rates have also been described in studies from other parts of the world, those higher than the present study 33.5% [54] in Vietnam, 35% [55] in the United States of America, 39.65% [56] in India, 21.65% [57] in Iran, 27.1% [58] in Malaysia, 21.9% [59] in Brazil and 20% [60] in Malaysia.

On the other hand this study was higher when compared to other reports in the country; 10.6% [61] in Debrezeit and its environs, 7.8% [1] in North Shewa. Consistently relative lower prevalence rate of the *Cryptosporidium* in different parts of the world were reported: 3.9% [62] in Turkey, 2.66% [63] in Island of Japan, 1.5% [64] in western Canadian, 1.5% [65] in China, 12% [66] in Norway and 11.7% [67] in India. The variations in prevalence of this disease among different parts of the world and within the countries might be the result of the variation of the stocking rate and husbandry system of livestock production system. Likewise it might be owing to variation in climatic condition, seasonal variation during study and the susceptibility of the target population that related to age difference and breed of study animals, sample size and diagnostic methods employed [68].

The prevalence of *Cryptosporidium in the present study* was significantly affected among study districts ($p<0.05$). The difference in prevalence of the infection within the study area might be owing to agro ecology of the districts, source of drinking water and difference in awareness created by different members of the government such as veterinarian in charges, development agents and others; on feeding methods, giving of clean drinking water, giving of sufficient colostrum, protecting of calves from contacting with other domestic animals, clearing of pen floor frequently and separation of calves from the dam within a short period of time to prevent the prevalence of the infection.

The influence of age on *Cryptosporidium* infection rate confirmed in the present study was a relatively higher prevalence (16.4%) in calves older than 6 months than in

the other age groups. However, the difference was not statistically significant (p>0.05). The higher prevalence in young age calves may be related to their susceptibility to the disease due to their immature immune systems. This finding agreed with the report by Manyazewal *et al.* [41] in central Ethiopia, Adamu *et al.* [69] in Nigeria, Abebe *et al.* [52] in central Ethiopia who reported the insignificant effect (P>0.05) of *Cryptosporidium* infection in calves. Similarly; Geurden *et al.* [68] in Zambia, Halim *et al.* [70] in Malaysia and Winkworth *et al.* [71] in New Zealand also reported the insignificant association between age and *Cryptosporidium* infection in calves. However, this study was contradicted with the report of Adinew and Geremew *et al.* [53] from Ethiopia, Ayele *et al.* [51] Ethiopia, Joute *et al.* [72] in India, Venu *et al.* [4] in India, Lefay *et al.* [73] from France, were reported significant (P<0.05) effect between *Cryptosporidium* infection and different ages of young calves. This justifies that the insignificant effect of *Cryptosporidium* infection between ages of calves in the current study could be due to the management systems applied (it could be because the calves were less exposed to the sources of contamination) and also in the study area the majority of owners had a farm where calves were separated from the Dams within a short period of time after birth limiting transmission of infection. Consequently, the supplementary feed given for calves was concentrated that was not contaminated with feces from other animals.

In the current study the difference in hygienic status and the occurrence of *Cryptosporidium* infection in calves was not significant (p>0.05). This finding was disagreeing with the finding of Ayele *et al.* [51] in Northwest Ethiopia, Abebe *et al.* [52] in central Ethiopia, Geurden *et al.* [68] in Zambia andin Spain stated the significant association between hygienic status of cattle's and *Cryptosporidium* infection. The higher prevalence of *Cryptosporidium* infection was observed in calves kept on soil floor than those kept on concrete floor. This is directly related to the frequency of cleaning, i.e. soil floor was not washed thoroughly when compared with other types of flooring (concrete and gravel). In addition, *Cryptosporidium* oocyst can persist in soil for long periods of times [58, 75].

Regarding the consistency of fecal samples, this study showed *Cryptosporidium* prevalence was insignificantly (P>0.05) higher among non-diarrheic calves, that was supported by reports of Dankwa *et al.* [76] in Ghana, Das *et al.* [77] in India, Rieux *et al.* [78] in western France who indicated the insignificantly higher prevalence of cryptosporidiosis in non-diarrheic cattle. However the current finding was not in line with the report of Ayele *et al.* [51] in Northwest Ethiopia, Manyazewal *et al.*[41] in Addis Ababa and its environs, Geremew *et al.* [53] in and around Nekemte town, Causape *et al.* [79] in Spain, Geurden *et al.* [68] in Zambia, Karanis *et al.* [80] in Japan who indicated the statistical significant (p<0.05) effect of cryptosporidiosis in diarrheic cattle. The insignificant effect of *Cryptosporidium* infection recorded in the present finding based on fecal consistency might be due to management system and absence of secondary infection such as viral, bacterial and parasitic enteric pathogens; such as rotaviruses, corona viruses, *Escherichia coli, Salmonella* species, Giardiasis, Isospora, microsporidia, amebiasis, Crohn disease and inflammatory bowel disease those associated with acute diarrhea.

In the present study, there was no statistical difference (P>0.05) between calves drinking river and pipe water which is in line with the study by Bawm *et al.* [81] in the Mandalay region, Myanmar. However, this observation was not supported by the report of Manyazewal *et al.* [33], Ayele *et al.* [51], Geremew *et al.* [53] and El-Khodery *et al.* [82] who reported the significant occurrence of *Cryptosporidium* infections in calves drinking river water. This could be due to the time of year in which the study was conducted, that implies absence of pollution from rain drainage and runoff from agricultural land.

The prevalence of *Cryptosporidium* species was slightly higher in male calves than in female calves (p>0.05). This was agreed with the previous report by Regassa *et al.* [29] in central Ethiopia, Birhan *et al.* [13] in Gondar, *et al.* [76] in Ghana and Adamu *et al.* [69] in Nigeria who reported an insignificant difference between *Cryptosporidium* infection rates and calf sex. But this was contradicted by the report of Geremew *et al.* [53] and Ayinmode *et al.* [83] who reported a significantly higher prevalence of *Cryptosporidium* in female than in male.

In the present study, the prevalence of *Cryptosporidium* infection was significantly (P<0.05) higher in hybrids than in local breed calves. This report agreed with previous observations by Fikre *et al.* [31] in Ethiopia, Swai *et al.* [84] in Tanzania, Castro-Hermida *et al.* [74] in Spain and Geurden *et al.* [68] in Zambia. However, the findingwas disagreed with the earlier report by Birhan *et al.* [13] and Geremew *et al.* [53] who found a higher prevalence of *Cryptosporidium* in local breed calves. The finding of the current study, which explains the higher prevalence of *Cryptosporidium* infections in

hybrid calves, could be described by management differences between both breeds. One of the differences observed was that the hybrid was kept in farms in large herds than the local breed. Previous studies have reported an association between large herd size and risk of *Cryptosporidium* infection [85]. Large herds can have a higher pathogen load due to increased animal densities, favoring the infection of large numbers of calves contaminating their environment. Likewise in large herds the feed and drinking water might be contaminated by the fecal materials in case of overcrowded that facilitate the transmission of the disease.

Current study indicated the prevalence of *Cryptosporidium infection;* 17.80%in poor, 14.63% in good and 13.93% in medium body condition, which was statistically insignificant (P>0.05). This was agreement with the reported of Birhan *et al.* [13]. However it was disagreeing with Brook *et al.* [47], Geremew *et al.* [53] who report the statistically significant effect of body condition on *Cryptosporidium* infection. In the present study even if the difference was insignificant; higher prevalence of the infection was observed in calves with poor body condition than in medium and good. This difference might be poor calf nutrition, soiled bedding, due to subclinical infection, inappropriate hygienic status, low colostrums feeding, management practices, poor ventilation and droughts, those regarded as risk factors for cryptosporidiosis in poor body condition Clearly [86].

In terms of feed sources, the prevalence of *Cryptosporidium* infection was 15.90%, 13.63%, and 20% in calves fed pasture, milk and pasture and or milk respectively. There was no statistically significant difference between the prevalence of *Cryptosporidium* and the food source. This was supported by the observation by Swai *et al.* [84] and contrary to the report by Brook *et al.* [47], Ayele *et al.* [51] and Geremew *et al.* [53]. The difference could be due to the management system correlating with less contamination of the feed for the calves. In the present study, the prevalence of *Cryptosporidium* infection in calves with and without contact with other domestic animals showed an insignificant effect. This finding contradicted with Ayele *et al.* [51] in India and Maurya *et al.* [87] in Ethiopia who reported the significant effect of direct contact between calves and other domestic animals on *Cryptosporidium* infection.

According to the questionnaire survey, few respondents knew about cryptosporidiosis; however none of them were aware of the source, transmission between animals and humans and its clinical sign. However, diarrhea is the main disease commonly observed in calves, especially in calves under 12 months of age in the study area as indicated by the questionnaire survey. The diarrhea directly affects the young calves, delaying growth, worsening the animal's physical condition and sometimes causing the death of the calves. This could be the cause of *Cryptosporidium* infection, as observed in diarrhea in calves; in which calves with diarrhea had shed oocysts more frequently than calves with normal feces [53, 79, 82] showing a strong association between shedding of *Cryptosporidium* oocysts and calf diarrhea. In addition, the *Cryptosporidium* could have the ability to reduce the activity of disaccharides, leading to reduced breakdown of sugars, leading to bacterial overgrowth, formation of volatile fatty acids and changes in osmotic pressure; then cause the characteristic heavy and watery diarrhea [86].

As the present survey the amount of colostrums given to newborn calves within 24 hours of birth has been associated with the incidence of *Cryptosporidium* infection. Calves fed a lower amount of colostrums were more likely to be infected with *Cryptosporidium* infection than calves fed an adequate amount of colostrums within 24 hours of birth. Bovine colostrums is highly nutritious and contains high levels of antibodies, which are essential for the body's immunological activity. Also, in colostrums there is a high concentration of hormones, growth factors and cell-modulating factors that stimulate the villi growth of the lining of the small intestine [88, 89]. The majority of respondents had no practice of spraying farms, vehicles and other equipment with disinfectants used to reduce the prevalence of *Cryptosporidium* infections. Disinfection of farm with different disinfectants reduces the incidence and transmission of *Cryptosporidium*infection [66] reported that calves kept in disinfected pens were less infected than calves in non-disinfected pens.

The current survey showed that the majority of respondents did not prevent the calves from suspect animals, suggesting that mixing different animal species could help contract and spread *Cryptosporidium* infection. This was consistent with Maurya *et al.* [87] who reported the significant effect of direct contact between calves and other domestic animals on *Cryptosporidium* infection. The risk of infection can be reduced if animals are kept individually or without close contact with different animal species.

The quarantine procedures practiced prior to the introduction of new cattle, preventing calves from contact with suspect animals, separating calves from their mothers within 12 hours of birth and wearing protective clothing

when handling calves with diarrhea were very important to protect the newborn calf from contamination [90]. In general, potential risk factors were observed using multiple (multivariate) logistic regressions, noting that only the study districts were significantly associated with the likelihood of *Cryptosporidium* infection.

CONCLUSION AND RECOMMENDATIONS

The result of the current study showed that the *Cryptosporidium* infection was widespread in calves in the study area with a prevalence of 15.05%. From positive samples; less positive (1 oocyst per field), moderate positive (2-10 oocysts per field) and high positive (over 10 oocysts per field) were observed. Study districts and management systems were the most important possible risk factors for the occurrence of *Cryptosporidium*. Most often, the disease affects poor body condition and hybrid calves. The questionnaire survey in this study showed that the main risk factors contributing to the occurrence of the diseases were the source of food and water, calf husbandry practices and the lack of control practices such as vaccination and quarantine. The lack of community awareness of disease transmission, zoonosis, prevention and management were all possible factors contributing to the spread of the disease and posing a public health threat. In general, the current study provided baseline data regarding the prevalence and key risk factors of cryptosporidiosis in calves in the study area. Based on the above conclusions, the following recommendations were made:

- Community awareness of cryptosporidiosis, clinical signs, its transmission, zoonotic implications and the importance of colostrum feeding of calves in the study area to control the disease should be created.
- Housing practices and management should be kept optimal for calves to reduce infection and transmission of cryptosporidiosis.
- Protective clothing should be worn when handling calves with diarrhea.
- Further investigations should be carried out for species identification and molecular detection of the parasite.

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