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Original Article

Transmission Dynamics of *Cryptosporidium* in Calves and Children from Southwestern Ethiopia

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ABSTRACT

Introduction: *Cryptosporidium* is a protozoan parasite that can affect both humans and animals. The present study aimed to determine the prevalence and risk factors of cryptosporidiosis in bovine calves and children in Jimma, southwestern Ethiopia. This cross-sectional study was conducted from December 2019 to July 2020 to assess the prevalence and risk factors of the infection among calves younger than 1 year and children younger than 5 years.
Materials and methods: Fecal samples were collected from 384 calves and 147 children and examined by the Modified Ziehl-Neelson staining method.
Results: The overall prevalence was 8.1% in calves and 7.5% in children. The multivariable logistic regression analysis showed that the risk of Cryptosporidiosis was significantly higher in younger calves < 3 months followed by river water users and calves kept in dirty pens. Moreover, the analysis of children data indicated that the risk of Cryptosporidiosis was significantly higher in children >1 year, followed by children settled around the rural area, children whose families had a poor habit of handwashing after attending cattle, children whose their family had a high level of contact with calves, and children who were drinking river water.
Conclusion: The present study revealed that the high prevalence of cryptosporidiosis may be due to poor hygienic status, unclean sources of water, attending farms, and contact with calves or their feces. Generally, poor personal and dairy farm hygiene and drinking river water source were the factors contributing to the disease.

1. Introduction

Cryptosporidiosis is a protozoan parasitic disease of humans and animals that commonly causes diarrheal disease. The *Cryptosporidium* belongs to the phylum Apicomplexa and it inhabits in the small intestine of a wide range of vertebrate hosts, such as calves, sheep, fishes, reptiles, and birds[1,2]. It results in an increase in morbidity and mortality of children in developing countries[3-5]. It was indicated that cryptosporidiosis is an emerging neglected zoonotic disease of public health concern[6]. The disease results in about 30-50% of deaths in neonatal calves and is a major cause of diarrhea and deaths in children[1,2]. Cryptosporidiosis leads to watery diarrhea, abdominal cramps, nausea, mild fever, dehydration, and weight loss. It is a self-limiting disease and its recovery occurs within 2–3 weeks. In immune-competent individuals, it is more serious and can cause severe chronic diarrhea, weight loss, and death[2,7]. Due to the lack of appropriate treatment and vaccination, *Cryptosporidium* infection leads to a higher mortality rate in humans and has become an emerging public health issue worldwide[8]. Poor health practices, illiteracy, limitation in drinking water supply, lack of sewage, and its management for drainage of human and animal waste disposals can substantiate the occurrences of this infection in the vulnerable populations of developing countries. The prevalence of Cryptosporidiosis is higher in developing countries 5.9-17%, compared to developed countries 0.1–2%[9].

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Cryptosporidium produces tremendous numbers of resistant oocysts and is present in the environment, such as water bodies and lands. Animals and humans acquire the infection when they consume infested food and drinking water containing oocysts of these protozoa. Oocysts are infective for 2-6 months in the environment and are released with fecal matter during the onset of symptoms. They are shed 5 days after infection with an incubation period of 1 to 14 days. Oocysts are tolerant to several chemicals and disinfectants as well as chlorine as frequently used to treat drinking water, water parks, and swimming pools[10]. Waterborne outbreaks of *Cryptosporidium* oocysts due to the contamination of animal and human sewage discharge with drinking water are common among developing countries[11]. Several studies reported that the degree of pathogenicity and virulence is based on the immune status of the host, *Cryptosporidium* species, and ingested oocyst dose[3,12]. The infection begins with the release of sporozoites from the oocyst. These sporozoites attach and invade intestinal epithelial cells and promote their reproduction and cause impairment in the absorptive and secretory functions of the gut[12]. The modified Ziehl-Neelson technique is the gold standard for the detection of *Cryptosporidium* oocysts in fecal matters[13]. Regular sanitization, reduced number of animal density in the farms, separation of calves from other herds by keeping them in separate cattle sheds, and short calving periods of animals can reduce transmission of Cryptosporidiosis[14]. Appropriate health practices can prevent the outbreak of cryptosporidiosis[15]. Due to the lack of a universal treatment plan, the burden of this disease continues in high frequency among calves younger than 1 year, children younger than 5 years, and patients with HIV seropositive[2,14]. The previous reports from different parts of Ethiopia particularly in eastern, central, and northwest Ethiopia indicated the importance of the disease in cattle and estimated the prevalence range from 2.3% to 27.8% in calves as a result of immature immunity, poor farm management, and hygienic condition[18,19]. Moreover, different studies indicated the significance of the disease in children due to immature immunity, contamination of drinking water, poor personal hygiene, and contact with domestic animals, especially with calves[9,20]. The prevalence of Cryptosporidiosis ranges between 3.3% and 14.8% in underfive years children in different countries[21,23]. Cryptosporidiosis is a zoonotic disease and is prevalent mainly in calves and children in Ethiopia, however, there is a limitation of inclusive information in the country, particularly in the studied area. As the disease is zoonotic, it is highly significant to study the transmission pattern among humans and animals. This type of study has not been conducted in and around Jimma town, southwest Ethiopia. Therefore, to design a cost-effective and appropriate preventive and control strategy, understanding the occurrences and risk factors of cryptosporidiosis in the interface between humans and animals is crucial. The current study was conducted from December 2019 to July 2020 with the objectives of assessing the occurrences and important contributing factors for cryptosporidiosis in calves and children in and around Jimma town, southwest Ethiopia.

2. Materials and Methods

2.1. Ethical approval

Ethical clearance for the study on human subjects was obtained from the Ethical Review Board, Jimma town health Administration (Reference No: W.E.F.M.J-4/580/2012 E.C). Informed written consent was obtained from the study participants at the time of sample collection and they were informed that their specimen and records were examined by authorized persons, personal information is treated strictly confidential and they are free to withdraw the consent at any time. Ethical clearance for the study on animals was obtained from the Ethical Review Board Jimma University College of Agriculture and Veterinary Medicine (Ref. No: R//Gs /293/2012/ E.C). The aim of the study was explained and permissions were obtained from farm owners before the collection of samples and data.

2.2. Study area

The study was carried out from December 2019 to July 2020 in Mancho and Sekachekorsa districts of Jimma zone, Oromia regional state of Ethiopia. Jimma town is the city of Jimma zone and the largest city in southwestern Ethiopia and found 357 Km away from Addis Ababa and lies between 36°50´E longitude and 7°40´N latitude at an average elevation of 1750 m.a.s.l). The area experienced an annual average rainfall of 1000 mm for 8 to 10 months. The mean annual maximum temperature ranges 25-30°C and minimum 7-12°C and it has a sub-humid, warm to hot climate. Mancho district is one of the 22 districts of the Jimma zone. Located at a distance of 387 km from Addis Ababa, it is bordered by Kersa district in the north, Omo Nada district in the east, SNNP regional state in the south, and Dedo district in the west. It has an area of about 1459.1 km^2. The altitude of this district ranges from 880 to 2400 m.a.s.l. The total population is estimated to be 360,745 and 2% of their population were urban residents. Sekachekorsa district is also one of the districts found in the Jimma zone. It is located between 36° 33 ' 53"E and 7°20' to 7° 45"N at an average elevation of 1560 to 3000 m.a.s l and is found at a distance of 375 km from Addis Ababa. It is bounded by Gomma and Manna district in the north, Gera district in the south, Dedo and Jimma Town in the East, and Shabe Sombo district in the west. The climate condition is generally humid. The mean rainfall of the district is between 900 mm to 1400 mm and the maximum and minimum temperature recorded of the area ranges 7°C -11°C and 18°C-28°C, respectively. In general, agriculture is the livelihood for more than 90% of the population in the rural farming community in and around Jimma town. The main agricultural system is mixed crop-livestock production and animals are mainly produced in an extensive system. The total populations of the Jimma zone were 2,486,155. The total population in the zone (88.6%) is the rural population, which directly depends on agricultural activities. Jimma zone has an estimated of 1,718,284 heads of cattle, 466,154 sheep, 194,677 goats, 74,774 horses, 40,555 donkeys, and 30,541 mule populations[26].

2.3. Study design and sample size determination

A cross-sectional study design was employed in this study from December 2019 to July 2020. The sample size for the cross-sectional study was determined using the Thrusfield formula[24] at 95% confidence level and $p < 0.05$ was set for significance level in both animal and human samples. Since there was no previous data on the prevalence of cryptosporidiosis in animals in the study area 50% was the conservative prevalence of cryptosporidiosis to calculate the sample size of calves. The expected prevalence of cryptosporidiosis in humans was set at 3.3% based on the previous studies conducted at different parts of Ethiopia[21-23]. Accordingly, 384 calves and 147 children were considered using the above formula. Both human and animal samples were proportionally divided between selected districts and Jimma town.

2.4. Study population and sampling technique

The population for the cross-sectional study on humans and animals has included calves aged up to one year and under five years of children of the dairy farm community in the study area. The districts namely Mancho and Sekachekorsa were selected purposively from Jimma town's nearby district based on the availability of transportation to deliver the sample as soon collected. From each district and Jimma town, three kebeles (B/Bore, T/Harer, and U/Qoce) were selected randomly. A stratified random sampling method was undertaken at the herd level to classify farms based on their herd size into three, using the previous categorization made by Megarsa et al.[25]. Children of the dairy farm communities (dairy farm owners, attendants, and guards) that were found in the study area were chosen randomly and enrolled in the study using a simple random sampling method. Hence, under five-year children of dairy farm communities in selected dairy farms were the study participant for the present study. A single stool sample and questionnaire data were collected from study participants and analyzed to determine the risk factors of cryptosporidiosis. In case of failure of getting permission, the next sample units were considered for replacement.

2.5. Inclusion and exclusion criteria

The study inclusion criteria in animals were: all types of breed calves < 1 year, owner interest to participate in the study, and ready to provide the required information through questionnaires. Moreover, the inclusion criteria in children were all children < 5 years of age, parents of the children must be dairy farm community and ready to provide the required information. The exclusion criteria were: unwilling to participate in the study, unable to provide the required information, and participants who refused submission of the specimen, specimen delayed for more than six hours after collection. Approximately 2 grams of fecal specimens were collected directly from the rectum of calves using sterile gloves and the same amount of stool samples was collected from the human participants by the individuals themselves. After collection, the samples were kept in sterile stool cups and transported to the Veterinary Parasitology Laboratory, College of Agriculture and Veterinary Medicine of Jimma University using an icebox in the cold chain for further processing on the same day of collection. A qualitative fecal examination technique was used to recover *Cryptosporidium* oocysts from the collected fecal samples. Cryptosporidiosis was diagnosed by Ziehl-Neelsen staining technique after the concentration of oocysts by formalin-ether concentration technique[13] and centrifugal sedimentation then fecal smears were made from the concentrate to differentiate *Cryptosporidium* oocysts from other cells and artifacts. The microscopic investigation was used to check the presence of oocysts in the samples. The oocysts were stained by modified acid-fast stain, therefore, it stained and seemed pink to red color oocysts in contradiction of a blue background. Known positive slides confirmed by molecular techniques were used as positive controls for *Cryptosporidium parvum* and *Cryptosporidium andersoni*[16,17]. These reference species confirmed positive slides were obtained from previous study groups[23]. The size of the oocyst was compared with that of the known pathogenic oocyst using an ocular micrometre[16]. From the concentrated stool samples, slides were prepared. Thin smears of stool samples were stained using the modified acid-fast Ziehl-Neelson staining method. A small number of feces were spread over the surface of a clean slide on an area of approximately 2cm x 1cm, the smears were allowed to air dry and then fixed in absolute methanol for 3 minutes. Then,fet the slides were stained with strong carbol-fuchsin 3% for 15-20 minutes, rinsed thoroughly in tap water, and decolorized using 3% acid alcohol for 15-20 seconds. After washing in water, the slides were counter-stained with 0.1% methylene blue for 30-60 seconds, washed with water, and placed in a slide rack to drain and dry. Then one drop of oil immersion was added to the dried slides and examined under 100X magnification binocular compound microscope with a Halogen bulb (Olympus, Japan) using an oil immersion lens. Oocysts were identified according to standard methods. Oocysts of *Cryptosporidium* species stained by this method show pale pink or deep red. Data on each sample were collected using a properly structured questionnaire aimed at determining the risk factors for occurrences of cryptosporidiosis in humans and calves.

2.6. Statistical Analysis

The processing of data was done by computer software. All data collected were entered into a Microsoft Excel spreadsheet, transferred to software SPSS version 23, and processed for analysis. Descriptive statistics were conducted to summarize the raw data. The results were presented using tables and figures. The Chi-square tests were used to evaluate the association between hypothesized risk factors and the prevalence of cryptosporidiosis in humans and animals. Further analysis of the association was made in two steps. Firstly, the univariable logistic regression analysis was used to evaluate the significance of the effect of different risk factors on the prevalence of cryptosporidiosis. Secondly, all risk factors that had a non-collinear effect p-value ≤ 0.25 in the univariate logistic regression model were subjected to a multivariable logistic regression model to determine the major risk factors for cryptosporidiosis. The adjusted odds ratio (OR) was used to quantify the effect of risk factors on the occurrences of *Cryptosporidium* infection. A 95% confidence interval and 5% were computed, and results were considered significant at $p < 0.05$.

3. Results

3.1. Prevalence and risk factors of cryptosporidiosis in calves

In the present study, the overall prevalence of cryptosporidiosis in calves in the study area was found to be 8.1%. Out of 384 fecal samples examined using the modified acid-fast method, 31 samples were positive for *Cryptosporidium*. The occurrence of the infection was detected in all selected parts of the study area. Out of 179 fecal samples examined from Jimma town, 13(7.3%) were found positive for cryptosporidiosis, while out of 60 fecal samples examined from Mancho district and 145 from Sekachekorsa, 7(11.7%) and 11(7.6%) were found positive, respectively (Figure 1). The prevalence of cryptosporidiosis in calves based on age, sex, herd size, origin, and production system is presented in Table 1. The prevalence of cryptosporidiosis was significantly higher, 23.9% (21/88) in calves aged <3 months when compared to calves 3-6 months 3.9% (6/155) and 6-12 months 2.8% (4/141) with (p = 0.001). Sex-wise prevalence of cryptosporidiosis was 8.5% (18/211) in female calves and 7.5% (13/173) in males, sex was not significantly associated with cryptosporidiosis in the study area (p = 0.716). Also, there was no statistically significant association (p = 0.303) between the prevalence of the infection and herd size, the prevalence was higher in farms containing larger herd size 12.2% (9/74) as compared to medium 8.1% (11/136) and small herd size 6.3% (11/174). The prevalence of cryptosporidiosis was significantly higher in rural farms at 5.9% (9/152), compared to the farm located in per-urban at 8.6% (10/116), and urban at 10.3% (12/116; p = 0.006). Intensive production system had a higher prevalence of 16.9% (15/89) than semi-intensive of 6.4% (11/172) and extensive production system of 4.1% (5/123), with statistically significant association (p = 0.002).

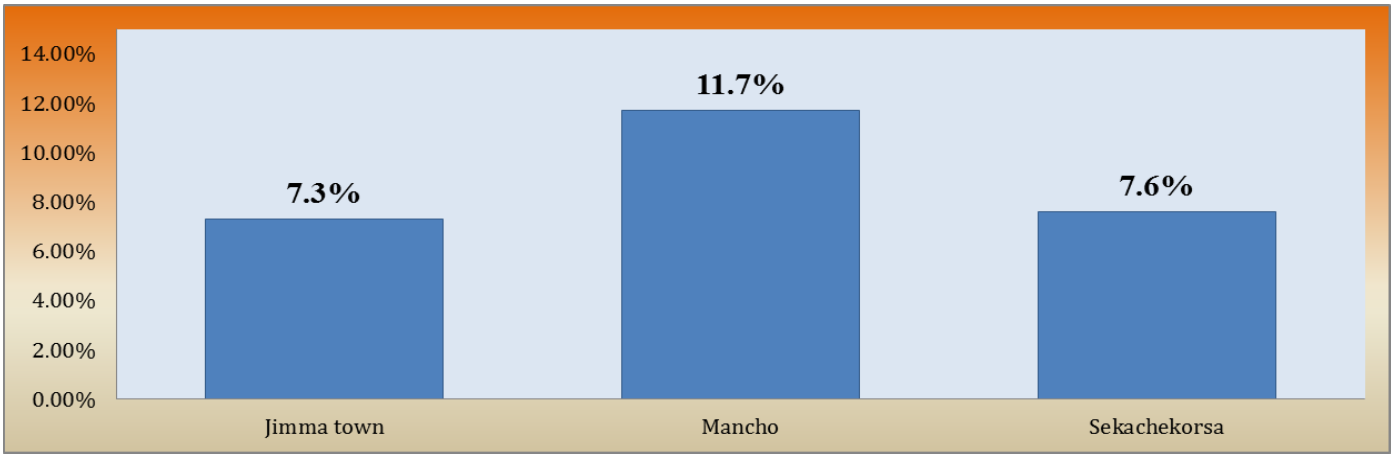

Figure 1. Prevalence of Cryptosporidiosis in claves in and around Jimma town

Table 1. The prevalence of Cryptosporidiosis in calves by age, sex, herd size, origin, and production system in and around Jimma town, Ethiopia

Variables	Number of Examined	Number of Positive	Prevalence	χ^2	P-value
Age					
<3 months	88	21	23.9%	38.46	0.0.001
3-6months	155	6	3.9%		
6-12 months	141	4	2.8%		
Sex					
Female	211	18	8.5%	0.132	0.716
Male	173	13	7.5%		
Herd size					
Small (<10)	174	11	6.3%	2.386	0.303
Medium (10-50)	136	11	8.1%		
Large (>50)	74	9	12.2%		
Origin					
Rural	152	9	5.9%	1.802	0.006
Per-urban	116	10	8.6%		
Urban	116	12	10.3%		
Production system					
Intensive	89	15	16.9%	12.56	0.002
Semi-intensive	172	11	6.4%		
Extensive	123	5	4.1%		
Overall	384	31	8.1%		

P < 0.05 is considered as statistically significant

Table 2. Association of common risk factors with the prevalence of Cryptosporidiosis in calves based on a univariable regression model

Risk Factors	Group	Number of Examination	Prevalence (%)	Crude OR	95% CI	P-value
Age	<3 months	88	23.9	4.29	1.25-14.67	0.022
	3-6months	155	3.9	4.12	1.03-16.53	
	6-12 months	141	2.8			
Sex	Female	211	8.5	0.78	0.3-2.4	0.663
	Male	173	7.5			
Herd size	Small	174	6.3	1.39	0.4-4.82	0.77
	Medium	136	8.1	0.87	0.2-3.53	
	Large	74	12.2			
Origin	Urban	152	5.9	0.59	0.15-2.34	0.219
	Per-urban	116	8.6	0.28	0.1-1.2	
	Rural	116	10.3			
Production system	Intensive	89	16.9	5. 56	1.47-17.7	0.011
	Semi-intensive	172	6.4	5. 09	1.42-21.9	
	Extensive	123	4.1			
Breed type	Exotic	91	14.3	6.14	0.36- 3.6	0.054
	Cross	167	8.4	1.02	1.29- 28.1	
	Local	126	3.2			
Body condition	Poor	81	11.1	2.48	0.56-10.9	0.41
	Medium	189	7.4	3.84	1.01-14.7	
	Good	114	7.0			
Fecal consistency	Diarrheic	132	15.9	5.26	1.75- 15.9	0.003
	Non-diarrheic	252	4.0			
Source of drinking water	River	131	16.0	4.22	1.45-12.3	0.008
	Pipe	253	4.0			
Cleanness of the pen	Unclean	118	16.9	6.37	2.18-18.7	0.001
	Clean	266	4.1			
Method of milk feeding	Suckling	145	15.3	2.45	0.78-7.7	0.124
	Hand feeding	239	3.8			

P ≤ 0.25 is considered as statistically significant

The risk factors of cryptosporidiosis in calves in the study were analyzed in two steps. In the first step, an analysis of the presence of an association between different common risk factors and the prevalence of cryptosporidiosis was done by a univariable regression model (Table 2). In the second step, an analysis of measuring the effect of the significant risk factors on the likelihood of cryptosporidiosis was done by multivariable regression (Table 3). Accordingly, the result of univariable risk factors analyses for cryptosporidiosis in calves showed that age (<3, 3-6 versus 6-12 months), production system (intensive, semi-intensive versus extensive), breed type (exotic, cross versus local), fecal consistency (diarrheic versus non-diarrheic), source of drinking water (river versus pipe), cleanness of pen (dirty versus clean), and method of colostrum feeding (suckling versus hand feeding) were significantly associated with the prevalence of cryptosporidiosis in calves. However, herd size (small, medium versus large), origin (urban, peri-urban versus rural), sex, and body condition (poor, medium versus good) were not significantly associated. All variables with their p ≤ 0.25 were considered as major risk factors and so subjected to the multivariable regression analysis. The result of multivariable logistic regression analysis showed the most crucial risk factors for *Cryptosporidium* infection in calves in the study area. Because of that, calves less than 3 months and 3-6 months were five times and two times more likely to be infected by cryptosporidiosis as

Table 3. The major risk factors of Cryptosporidiosis in calves based on multivariable logistic regression analyze

Risk Factors	Group	Prevalence (%)	Adjusted OR		P-value
			OR	95% CI	
Age	<3 month	23.9	4.68	1.26-17	0.021
	3-6month	3.9	1.63	0.4-6.7	
	6-12 month	2.8			
Production system	Intensive	16.9	4.12	1.12-15.2	0.033
	Semi-intensive	6.4	1.90	0.31-3.9	
	Extensive	4.1			
Breed type	Exotic	14.3	6.83	1.5-31	0.013
	Cross	8.4	6.1	1.44-25.5	
	Local	3.2			
Fecal consistency	Diarrheic	15.9	4.16	1.54-11.16	0.005
	Non-diarrheic	4.0			
Source of drinking water	River	16.0	4.82	1.78-12.9	0.002
	Pipe	4.0			
Cleanness of pen	Unclean	16.9	5.73	2.14-15.4	0.001
	Clean	4.1			
Method of milk feeding	Suckling	15.3	2.88	1.01-8.3	0.04
	Hand feeding	3.8			

P < 0.05 is considered as statistically significant

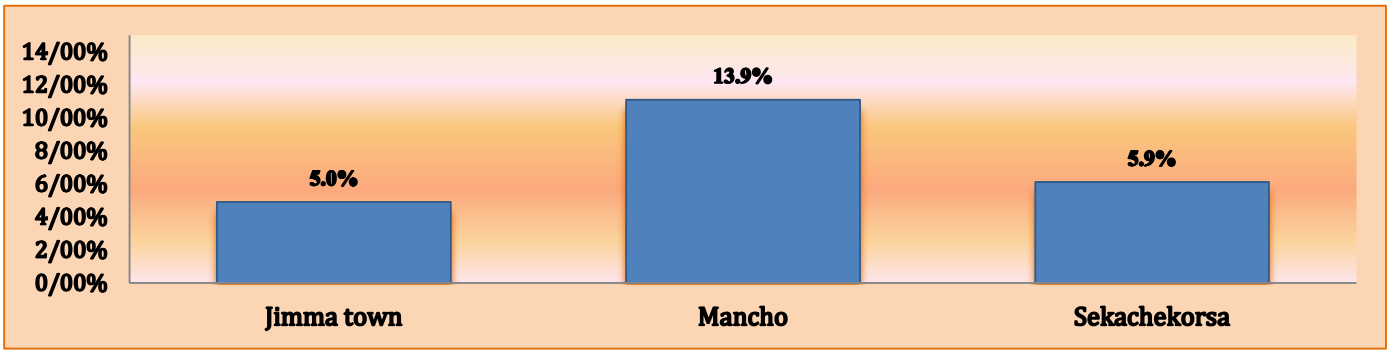

Figure 2. Prevalence of Cryptosporidiosis in children in and around Jimma, Ethiopia

compared to calves between 6-12 months, respectively. Intensive and Semi-intensive production systems were four times and two times more likely to acquire cryptosporidiosis when compared to the extensive production system. Moreover, exotic and crossbreed calves were seven times (OR = 6.83, 95% CI = 1.5-31) and six times (OR = 6.1, 95% CI = 1.44-25.5) more likely to be infected by cryptosporidiosis when compared to local breed calves. Calves with diarrhea were four times more likely to be infected by cryptosporidiosis when compared to non-diarrheic calves. Calves drinking river water were five times more likely to be infected as compared to calves drinking pipe/tap water. In addition, calves kept in dirty pens were six times more likely to be infected by cryptosporidiosis when compared to calves kept in clean pens. Calves suckling was three times (OR = 2.88, 95% CI = 1.01-8.3) more likely to be infected by cryptosporidiosis, compared to hand feeding (Table 3).

3.2. Prevalence and risk factors of cryptosporidiosis in children

The overall prevalence of cryptosporidiosis in children in the study area was found to be (7.5%). Of 147 stool samples collected from children of the dairy farm community, 11 samples were positive for *Cryptosporidium* using a modified Ziehl-Neelson staining technique. The occurrence of the infection was observed in all selected kebeles of the study area, with a prevalence of 5.0% (3/60) in Jimma town, 13.9% (5/36) in Mancho districts, and 5.9% (3/51) in Sekachekorsa (Figure 2).

In the present study, the association between the age group of the children and occurrences of cryptosporidiosis was statistically significant (p= 0.03). Children less than 1 year have a higher prevalence 16.7% (6/36) when compared to children between 1-3 years 5.9% (3/51) and 3-5 years 3.3% (2/60). The prevalence of cryptosporidiosis within the sex of children was 9.7% (7/72), 5.3% (4/75) in females and males, respectively, the association was statistically not significant (p = 0.82). The association between the location of the children and the prevalence of cryptosporidiosis was statistically significant (p= 0.03), a higher prevalence was observed in children settled in a rural area 13.3% (6/45) as compared to peri-urban 5.5% (3/54) and urban 4.2% (2/48, Table 4).

Analogous to calve data, the risk factors of cryptosporidiosis in children were analyzed in two steps. Firstly, the association between different common risk factors and the prevalence of cryptosporidiosis was analyzed by a univariable regression model (Table 5). Secondly, the effect of the significant risk factors on the likelihood of cryptosporidiosis was tested by multivariable regression (Table 6). The result of univariable analyses showed that age (< 1, 1-3 versus 3-5 years), location (urban, peri-urban versus rural), fecal consistency (diarrheic versus non-diarrheic), family occupation (farm attendant, owners versus guards), family level of animal contact (high, medium versus low), the family habit of handwashing after attending cattle (good versus poor) and source of drinking water (river versus pipe), were significantly associated with the prevalence of cryptosporidiosis in calves. However, sex and family education (illiterate versus literate) were not significantly associated. Total variables

Table 4. The prevalence of Cryptosporidiosis in children by demographic characters in and around Jimma town

Variables	L Group	Number of Examined	Number of Positive	Prevalence	x^2 value	P-value
Age	< 1 year	36	6	16.7%	6.07	0.03
	1-3 years	51	3	5.9%		
	3-5 years	60	2	3.3%		
Sex	Female	72	7	9.7%	1.02	0.82
	Male	75	4	5.3%		
Location	Rural	45	6	13.3%	3.27	0.037
	Peri-urban	54	3	5.5%		
	Urban	48	2	4.2%		
Overall		147	11	7.5%		

P < 0.05 is considered as statistically significant

Table 5. The prevalence of cryptosporidiosis in children based on univariable logistic regression analysis

Risk Factors	Group	Number of Examined	Prevalence (%)	Crude OR	95% CI	P-value
Age	< 1 year	36	16.7	0.16	0.17-1.7	0.04
	1-3 years	51	5.9	0.37	0.31-4.54	
	3-5 years	60	3.3			
Sex	Female	75	9.7	2.04	0.236-17	0.31
	Male	72	5.3			
Location	Rural	45	13.3	0.17	0.01-2.9	0.19
	Peri-urban	48	4.2	1.49	0.11-20.7	
	Urban	54	5.6			
Family education	Illiterate	58	6.9	1.29	0.22-7.69	0.87
	Literate	89	7.9			
Family occupation	Attendant	32	18.8	0.17	0.24-1.16	0.03
	Owner	54	3.7	1.42	0.15-13.2	
	Guard	61	4.9			
Family level of animal contact	High	47	12.8	0.15	0.15-1.62	0.016
	Medium	50	6.0	0.05	0.3-0.912	
Family habit of hand washing after attending cattle	Poor	56	12.5	0.19	0.23-1.55	0.07
	Good	91	4.4			
Fecal Consistency	Diarrheic	44	15.9	0.11	0.016-.745	0.01
	Non-diarrheic	103	3.9			
	Low	50	4.0			
Source of drinking water	River	64	10.9	0.18	0.018-1.9	0.031
	Pipe	83	4.8			

$P \leq 0.25$ is considered statistically significant

with their $p \leq 0.25$ were considered as major risk factors and so subjected to the multivariable regression analysis (Table 6).

The result of cryptosporidiosis in children based on multivariable logistic regression analysis is presented in Table 5. Children < 1 year and 1-3 were six times (OR = 5.95, 95% CI = 1.0-57) and three times (OR = 2.67, 95% CI = 0.2-32) more likely to acquire *Cryptosporidium* infection as compared to children between 3-5 years, respectively. Children settled around the rural and peri-urban areas were six times more likely to acquire cryptosporidiosis, compared to children settled in urban areas, respectively. Children with diarrhea were nine times more likely to become positive for cryptosporidiosis when compared to non-diarrheic children. Farmworkers' children and those of farm owners were six times more likely to be infected by cryptosporidiosis when compared to children of dairy farm guards. Children from families with poor handwashing habits after attending the cattle were five times more likely to be infected compared to children from families with good handwashing habits. Children from families with a high level of contact with cattle and medium contact were seven times and five times more likely to be infected compared to children from families with a low level of contact with calves and their manure. Children drinking river water were two times (OR = 2.46, 95% CI = 2-16) more likely to be infected compared to children drinking pipe or tap water. In Ethiopian rural village houses, cattle and poultry were accommodated inside the houses. This is to prevent the attack of wild animals and also thieves. This type of lifestyle contributes to a favorable environment for the spreading of zoonotic diseases.

Table 6. The prevalence of cryptosporidiosis in children based on multivariable logistic regression analysis

Risk Factors	Group	Number of Examined	Prevalence (%)	Adjusted OR	95% CI	P-value
Age	< 1 year	36	16.7	5.95	1.0-57	0.04
	1-3 years	51	5.9	2.67	0.2-32	
	3-5 years	60	3.3			
Location	Rural	45	13.3	5.68	0.34-94.1	0.03
	Peri-urban	48	4.2	0.66	0.04-9.22	
	Urban	54	5.6			
Family occupation	Attendant	32	18.8	5.96	1.0-41.3	0.03
	Owner	54	3.7	0.70	1.0-6.5	
	Guard	61	4.9			
Family level of contact with calve	High	47	12.8	7.43	1.0-67	0.016
	Medium	50	6.0	5.23	1.3-68	
	Low	50	4.0			
The family habit of handwashing after attending cattle	Poor	56	12.5	5.30	2.0-43	0.02
	Good	91	4.4			
Fecal Consistency	Diarrheic	44	15.9	9.24	1.34-63.6	0.01
	Non-diarrheic	103	3.9			
Source of drinking water	River	64	10.9	2.46	2-16	0.031
	Pipe	83	4.8			

$P < 0.05$ is considered as statistically significant

4. Discussion

The present study indicated that the overall prevalence of cryptosporidiosis in calves in and around Jimma town, southwest Ethiopia was found to be 8.1%. The result of this study is comparatively similar with the prevalence reported from North Shewa 7.8% and central parts of Ethiopia 10.6%[27]. The result was higher than the prevalence reported in Ethiopia[28]. However, the present finding was lower than the prevalence reported from central Ethiopia (17.6%), northwest Ethiopia (18.6%), Haramaya (24%), and Asella (27.8%), respectively[18,29-31]. Moreover, the results were comparatively lower than the prevalence reported in different parts of the world, particularly from Australia (23.8%), *Bangladesh (*77%), Tajikistan (63.1%), and New Zealand 86.4%[32-34]. The differences in the prevalence between the countries might be due to differences in the method of laboratory investigation, the season of study, geographical location, husbandry practice, and differences in breed and age of animals. In the current study, significantly a higher prevalence (23.9%) was observed in calves <3 months as compared to calves between 3 to 6 months (3.9%) and 6 to 12 months (2.8%) with (p = 0.001). This is in line with several international studies[35-38]. This higher prevalence in younger calves might be due to the low resistance to the infection. The sex-wise prevalence of cryptosporidiosis was statistically not significant (p = 0.77). This was similar to the previous finding of Ayele et al. from Gondar, Ethiopia[30]. The prevalence based on the herd size was higher at 12.2% in larger herd sizes when compared to medium (8.1%) and small (6.3%). This finding was in agreement with a report by Inpankaew et al., who reported a higher prevalence in large herd size 18.2% than in small herd size 8.3%[39]. This higher prevalence in larger herd sizes might be due to overpopulation of animals with poor husbandry practice and it is relatively difficult to manage the hygienic condition of large herd sizes as compared to medium and small. There was no statistically significant association (p = 0.219) between the prevalence of the infection and farm origin; urban, peri-urban, and rural in the study area. This finding was in line with the study from Gondar, Ethiopia, by Ayele et al. that reported the absence of a statistically significant association between the prevalence of Cryptosporidiosis and farm origin (p = 0.115)[30].

The prevalence of cryptosporidiosis in calves held in an intensive production system was significantly higher (16.9%) when compared to calves held in semi-intensive (6.4%) and extensive production systems (4.1%, p = 0.002). This is consistent with another from Tanzania indicating a higher prevalence (56%) in an intensive production system and 28% in extensive[41]. Similarly, Ketema et al. from Haramaya, Ethiopia reported a significantly higher infection rate of 26.7% in an intensive production system than an extensive 13.7%[35]. This higher prevalence in an intensive production system might be due to flocking a high number of animals in a home with poor hygienic conditions. This probably increases the degree of contact between calves and enhances the oocyst contamination of pen, watering, and feeding material and is consequently stress-free for the transmission of the infection among the calves. In the present study, the prevalence of cryptosporidiosis in exotic breed calves was significantly higher 14.3% when compared to crossbreed and local breed calves 8.4% and 3.2%, respectively. This is in agreement with Alemayehu et al.[29] who reported a higher infection rate in exotic breed calves (28%) than crossbreed (25%) and local breed (27%). This might be due to differences in the host immunity to resist the infection. There was no statistically significant (p = 0.41) association between body condition and occurrences of cryptosporidiosis, but a higher infection rate was observed in poor body condition (11.1%) when compared to medium (7.4%) and good body condition (7%). This is in line with previous findings in Haramaya which indicated a higher 35.5% prevalence in poor than in medium and good body conditions at 11.8% and 6.8%, respectively[35]. This higher prevalence in poor body condition might be related to underfeeding which results in low immune status and increasing exposure rate of calves to the infection. In the current study, a statistically significant association (p = 0.003) was observed between fecal consistency and occurrence of cryptosporidiosis, calves with diarrhea have a higher prevalence (15.9%) as compared to non-diarrheic calves (4%). This is in line with the findings of Causape et al. in Northeastern Spain, and Trotz-Williams et al. in central Canada. They reported a significant association between cryptosporidiosis and calf diarrhea, and also stated that infected calves were five times more diarrheic than non-infected calves[42,43].

In the present investigation, the source of drinking water has been significantly associated (p = 0.008) with the occurrence of cryptosporidiosis*.* This is because the river water is often contaminated with animal fecal matter containing the oocysts of *Cryptosporidium.* This is in line with Manyazewal, who reported a highly significant) association between calves drinking river water and calves kept on farms disposing of liquid wastes to a river, respectively (p = 0.003 and p = 0.001)[23]. Similarly, Yang et al. reported a significant association between cryptosporidiosis and disposing of liquid wastes in a river[44]. The river water is heavily contaminated with oocysts due to the high number of browsing cattle or might be due to sewage contamination. The current study showed, significantly a higher prevalence of disease in calves kept in unclean pens (16.9%) as compared to calves kept in cleaned pens (4.1%). This significant association and higher prevalence in calves kept in unclean pens might result in the transmission of cryptosporidiosis among other calves. Similarly, a report from the central part of Ethiopia revealed that calves housed in poorly cleaned farms were five times more likely to be infected by cryptosporidiosis as compared to calves in well-cleaned farms[18]. The method of colostrum feeding was significantly associated (p = 0.04) with the prevalence of cryptosporidiosis. This significant association could be due to the transmission of *Cryptosporidium* oocysts to neonatal calves through contact with the contaminated udder by feces containing oocysts.

So, this could increase the risk of infection in neonatal calves and higher mortality. This is in line with the report of Kvác et al.[45] who stated, calf to dam contact is the most likely route of transmission, and avoiding this tends to decrease the infection.

In the current study, the overall prevalence of cryptosporidiosis in children in the study area was found to be 7.5%. This can be correlated with the previous report of 6.8% in Nepal and 7.3% in Ethiopia[27,46]. Different prevalence rates were reported from different parts of the world, particularly from Canada (18%), the United States of America (21.2%), Australia (23.8%), and New Zealand (86.4%),[34,40,47]. This difference in the prevalence among countries might be due to the study period or seasonal effects, the difference in the sample size, and the susceptibility of the target population. In the present study, children less than one year had a higher prevalence (16.7%) as compared to children between 1-3 years (5.9%) and 3-5 years (3.3%). This is in line with the finding of Mumtaz et al.[9] from Pakistan who reported that children in the age group of fewer than 2 years were more exposed to cryptosporidiosis. Similarly, the study from Bangladesh and Nepal reported cryptosporidiosis in children below 3 years[32,48]. This might be due to different factors such as low or immature immunity, drinking unclean water, and low level of personal cleanliness. There was no statistically significant association (p = 0.82) between gender and the prevalence of cryptosporidiosis. Similarly, studies from China, Pakistan, and Ethiopia reported that there is no significant association between *Cryptosporidium* infection and gender group[13,49]. Contradictory to earlier studies from Kenya and Malawi[50,51], there was an absence of significant association between the location of children and the prevalence of cryptosporidiosis. However, this study revealed that the location of the children and the prevalence of cryptosporidiosis were significantly associated (p = 0.03). The reason for this significant association might be due to differences in access to safe drinking water, exposure to the animal, and hygiene condition. This study showed a higher prevalence of cryptosporidiosis in children whose parents were illiterate or with low educational levels with poor handwashing habits after handling cattle. This is in line with the studies of Manyazewal[23] from Ethiopia and Khan et al.[54] from Pakistan that stated, literate people, have less infection rate compared to people without formal education or having an elementary level. This could be because literacy is a key factor in improved personal hygiene and the basic ways of disease prevention and control.

A significant association was observed between children whose parents were farm attendants (p = 0.03) and those who had a high level of contact with calves or their manure (p = 0.016). This could be due to farm attendants having close and frequent exposure to animals and their feces. This is in line with the studies conducted by Adamu et al.[21] and Ehsan et al.[26]. The current study specifies the significance of proper washing habits (p = 0.02) with a higher rate of infection. This is in line with the studies of Speich et al.[53]. The prevalence of cryptosporidiosis in diarrheic children was significantly higher (15.9%) when compared to non-diarrheic children (3.9%). This indicates the pathogenicity of the identified *Cryptosporidium* spp. The children participants using river water for drinking purposes were significantly at a higher (10.9%) risk of infection than participants using pipe water (4.8%). This might be due to the contamination of river water by *Cryptosporidium* oocysts. The contaminated water bodies might be a route of spreading cryptosporidiosis. This is in line with a study in England by Wells et al.[52] who reported a high prevalence of cryptosporidiosis in river waters where *Cryptosporidium* oocysts were being spread by at least one livestock or wild animal inhabitants. The lifestyle, accommodating domestic animals inside the house, close contact with the domestic animals, and poor washing habits all play a significant role in the transmission of these zoonotic diseases among rural Ethiopian villages.

5. Conclusion

The present study revealed that cryptosporidiosis is an important health problem of calves and children in and around Jimma town, Southwest Ethiopia with an overall prevalence of 8.1% (31/384) and 7.5%, respectively. In the current study, young age (<3 months), intensive production system, exotic breed type, diarrheic fecal consistency, drinking river water, poor hygienic condition of the pen, and dam suckling were significantly associated with the prevalence of cryptosporidiosis in calves. Likewise, in children of young age (<1 year), rural setting, the presence of diarrhea, family occupation (dairy farm attendance), family level of contact with calves, poor handwashing habits of the family after handling cattle, and using river water were significantly associated with the prevalence of cryptosporidiosis. In general, poor personal and dairy farm hygiene, drinking river water source, drainage of dairy farm liquid waste or manure to a river, and contact with dairy animals were identified as the most important risk factors which had a significant association with the occurrences of cryptosporidiosis in both human and animal in the study area. The lifestyle and accommodating domestic animals inside the houses also play a significant role in the transmission of cryptosporidiosis.

Declarations

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Competing interests

The authors declare that they haven't competing interests.

Authors' contribution

Sadik Zakir Abadura was the principal investigator, who participated in laboratory work, parasite identification data analysis, and result interpretation. Suresh Kumar Pnair participated in the training, supervising laboratory work, parasite identification, proofreading, and preparation of the final version of the manuscript. Wubit Tafese and Abdu Mohammed participated as supervisors, acquisition of sanctions and approving the final version of the manuscript and agreeing to publication.

Availability of data and materials

The data that supports the finding of this study is included in the manuscript.

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