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STUDY ON THE PREVALENCE OF CRYPTOSPORIDIUM IN CALVES SUFFERING FROM DIARRHEA IN NORTHWESTERN SYRIA

H. F. Altaweel[1], F. Al-Daoud[2] and G. Mushtaq[3,*]

[1]Department of Animal Diseases, [2]Department of Microbiology, [3]Center for Scientific Research, Faculty of Medicine, Idlib University, Idlib, Syria
*ORCiD ID: https://orcid.org/0000-0003-4880-4035
*Corresponding Author's Email: dr.goher_mushtaq@idlib-university.com

ABSTRACT

Cryptosporidium is one of the most common causes of diarrhea in calves worldwide, and the risk factors contributing to its control and prevention are extensively studied. This epidemiological study was conducted in an unstable breeding environment. Ninety-six (96) calves suffering from diarrhea were studied from five regions in northwestern Syria. The goal of this study was to assess the prevalence of cryptosporidium in fecal samples and to measure the expected risk and regression for assumed risk factors that may cause diarrhea in calves up to 60 days old. Fecal samples were tested using direct ELISA. The results showed 9.1% (1/11) cases in the western Aleppo countryside, 21.1% (4/19) in the eastern Idlib countryside, 13.6% (3/22) in the Northern Idlib countryside, 2.9% (1/35) in the Jisr Al-Shughur countryside, while no positive case was recorded in the western countryside. This is the first study conducted in the region showing the incidence, regression, and risk rates, and it accentuates the need for a broader study on the effect of certain drugs used in the treatment of diarrhea and its consequences.

Keywords: newborn calves, diarrhea, multiscreen ELISA, Cryptosporidium

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INTRODUCTION

Intestinal and respiratory disorders are the most frequently reported diseases or causes of death in calves (Mõtus *et al*. 2018). Diarrhea in calves is a serious health problem that occurs at the age of less than a month and leads to an incidence rate of up to 20% (Bendali *et al.* 1999b; Waltner *et al.* 1986). The mortality rate as a result of diarrhea can range from 1.5% to 8% (Ammar *et al.* 2014).

Diarrhea is the major cause of economic losses for the dairy and meat industry worldwide (Tajik *et al.* 2012; Bolieau *et al.* 2010). The most common causes of the complex diarrheal syndrome are related to group A rotavirus (BRV), Bovine Coronavirus (BCoV), Enterotoxigenic *E.Coli* (ETEC) (*E.Coli K99*), and Cryptosporidium *Parvum* (Radostits *et al.* 2007; Garcia *et al.* 2000). The most common cause of diarrhea in neonatal calves aged 9 to 21 days is due to infection with BRV, BCoV, ETEC (*E.Coli K99*), Cryptosporidium *parvum* and Salmonella *spp* (Izzo *et al.* 2012; Bartels *et al.* 2010; Mohteshamuddin *et al*. 2020). The most common causative agent of diarrhea in newborn calves is cryptosporidium (*Cryptosporidium spp*). Cryptosporidium is a zoonotic pathogen causing diarrhea in humans, particularly in immunocompromised children and adults. (Nydam *et al.* 2001; Cho and Yoon 2014; Lombardelli *et al.* 2019; Conrady *et al*. 2021).

Cryptosporidia are intestinal protozoans that are parasitic in the intestine of humans and other vertebrates. In cattle, cryptosporidium causes acute or chronic gastrointestinal upset, resulting in mortality, weight loss, and reduced milk production (Brook *et al.* 2008; Smith *et al.* 2006). The incidence of cryptosporidium in calves is specifically correlated with their age and the environmental conditions of the barn (Brook *et al.* 2008). Many factors influence the prevalence of cryptosporidium, including the age of the calves, the general health status of the farm, intake of colostrum, the administrative procedures of the farm, the quality of fodder, the source of drinking water, presence of associated diarrheal cases, and the climate (Ogendo *et al.* 2017).

The cryptosporidium (*C parvum*) in calves over four weeks of age has a low probability of causing infection. Few laboratories put cryptosporidium detection tests on the list of routine tests, and very few veterinarians have paid attention to the importance of this matter (Joachim *et al.*2003).

Several reports indicated the prevalence of cryptosporidiosis from 6.26 to 39.65% in cattle in various parts of the world (Azami *et al.* 2007). There are many diagnostic tests for the detection of diarrheal pathogens, such as Direct Electron Microscopy, Enzyme-linked Immunosorbent Assay (ELISA), Rapid Immunoagglutination Test (RImgT), Acrylamide Gel Electrophoresis (AGE), Polymerase Chain Reaction (PCR), and Immunofluorescence Test (IFT). ELISA is a preferred technique over other laboratory methods for diagnosing diarrhea in calves due to the low cost, high sample throughput and high sensitivity of the test (Mayameei *et al.* 2010). According to Bartels *et al.* 2010 the sensitivity and specificity for *Escherichia coli* were 90% and 98.5% (using ELISA BIO K99), for rotavirus 96% and 100% (using dsRNA electrophoresis on PAGe, for coronavirus 88.9%, and 98.7% (using ELISA BIO K068) and for *C. parvum* test are 94.1% and 95.5% using flotation technique, respectively.

According to Geurden *et al.*2008, the sensitivity and specificity were respectively 68% and 95% for rotavirus ELISA and 33% and 80% for coronavirus ELISA as compared to lateral flow immunochromatography (Izzo *et al.* 2012). However, the sensitivity and specificity were respectively 100% and 91% for *E. coli K99* ELISA when compared to PCR (Izzo *et al.* 2011) and 94% and 96% for cryptosporidium ELISA for other three diagnostic techniques (another EISA assay, immunofluorescence assay, and immunochromatography). Cryptosporidium is a protozoan that causes Coccidiosis. It has a similar life cycle to Isospora and its reproduction alternates between sexual and asexual reproduction. Cryptosporidium is widespread in newborn calves, with prevalence rate ranging from 14% to 80%, depending on the age, general health of the calf, and housing conditions (Haschek *et al*. 2006; Singh *et al*. 2006; Trotz-Williams *et al*. 2005; Joachim *et al*. 2003; Garcia *et al.* 2000; Lefay *et al*. 2000; De la Fuente *et al*. 1999; Lentze *et al*. 1999; Naciri *et al*. 1999; Garber *et al*. 1994). Cryptosporidium in its natural life cycle can develop and multiply in gastrointestinal epithelial cells of infected animals (Shafieyan *et al*. 2014; Chen *et al*. 2003). Generally, cryptosporidium infection is fecal-oral by direct or indirect contact with fully formed oocysts shed from infected animals during the infectious phase (Radostits *et al*. 2007). To date, seven species and two genotypes of cryptosporidium have been identified in cattle (Hunter *et al*. 2004). Oocysts spread and infect calves in the first three days of life and peak in the first two weeks. Infections can occur in adult cows after three months of age, and symptoms appear after infection within 3-7 days and at least after 4-17 days. Previous studies have shown that the morbidity may reach 100% in calves (De Graaf *et al*. 1999; 'O'Handley *et al*. 1999, Fayer *et al*. 1998; Xiao and Herd 1994; Argenzio *et al*. 1990; Harp *et al*. 1990).

There are two possible mechanisms of cryptosporidium diarrhea: The first involves the destruction of the intestinal epithelia and their microvilli as a result of direct toxic effect of sporozoite infection, which impairs absorption from the intestinal lumen. This mechanism is not supported by most researchers (Gookin, *et al*., 2002). The second mechanism involves blocking the sodium chloride absorption by increasing secretion of chloride and bicarbonate ions, which impede absorption of sodium from the intestinal lumen. This results in drawing of water from interstitial tissue of intestine into the lumen. This mechanism depends on the secretion of prostaglandins from the epithelial cells in which the sporophytes are enclosed (foster and smith 2009). Regardless of the mechanism or cause of the occurrence of cellular loss and villous atrophy of epithelial cells, the fluid malabsorption due to the movement of sodium combined with chlorine or some other nutrients in the top of the villi results in diarrhea, which is offset by an increase in the secretion of negative electrolytes (anions) from crypt cells. In addition, the loss of epithelial cells and atrophy of the mature villi and associated transporters leads to a decrease in the total absorbable surface area of the intestine (Gookin *et al*. 2002; Argenzio *et al*. 1994; Argenzio *et al*. 1993; Argenzio *et al*. 1990).

MATERIALS AND METHODS

Ninety-six stool samples were collected from diarrheal calves (Friesian, Simmental and local hybrid species) during the period between September 2021, and June 2022. The selection of samples was done by the stratified cluster sampling method. The number of samples was based on the capabilities provided by the commercial kits for examination and the financial resources of the researcher. Due to apparent changes in fecal structure from semi-natural, creamy texture to watery and fecal color change from green-brown to yellow, sampling was performed directly from the rectum using medical single-use gloves placed in sterile plastic containers with tightly closing. Samples are numbered from 1 to 96 and each number is linked to an epidemiological plate (questionnaire) during sample collection, containing the following information about the collected sample: Number of the sample, farm location, farmer reference, case history, the total number of calves with diarrhea in the farm, health status , the kind of floor of the farm, the kind of roof, age and gender, dystocia, timing and amount of colostrum intake, the consistency and color of diarrhea, and the treatment that was used against the disease. The age of calves was between 1 day to 2 months, divided into the following six categories: (1-9 days old), (10-19 days old), (20-29 days old), (30-39 days old), (40-49 days old), (50-59 days old). Samples have been selected by Multi-stage cluster sampling method from 17 villages (Kafr Karmin, Kafr Nasih, Darat Azza, Sarmada, Binnish, Foah, Kafriya, Zardana, Kelli, Harbnoush, Ma'art Misrin, Mishmishan, Balmis, Ain al-Sooda, Kanest Bani Ezz, Salqin, Meles) which are distributed in five regions (western countryside of

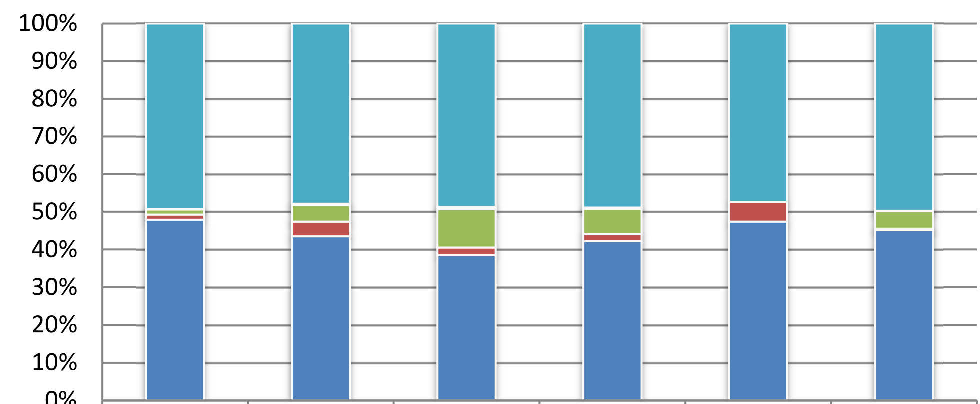
Aleppo, eastern countryside of Idlib, Northern countryside of Idlib, western countryside of Idlib, and Jisr Al-Shughur countryside). Clusters (villages) were selected within the five regions of the study area, and infected calves were chosen randomly from those clusters. The five regions selected for the study have geographical diversity comprising of differences in the environment, altitude above sea level, and the topography (plain or mountainous).

The samples were transferred to the laboratory within a maximum period of eight hours to be stored at -20 degree Celsius in the laboratory until use.

Multiple-link ELISA test was used to detect diarrheal pathogens (EASY-DIGEST Rotavirus - Coronavirus - E. coli F5 - Cryptosporidium Diagnosis test for bovines BIO K 151/2 -Bio-X Diagnostics, Belgique). The Antigen Capturing Enzyme-Linked Immunosorbent Assay (Ag-ELISA), is a rapid assay test to determine the causative agent in samples based on the antibody (Monoclonal Antibody) depending on the target antigen (Lequin 2005). In preparation for the Antigen ELISA test, the antibody is attached to a solid surface such as glass or plastic, or a membrane filter. This antibody will adsorb the target antigen if it is present in the sample. There will be different color reactions to verify that the antibody captures the antigen and visualizes the interaction that occurred between them. The antigen can be quantitatively estimated as optical density (OD) measured by a spectrophotometer which is positively related to the amount of antigen. Sensitivity and specificity of the Easy Digest kit was confirmed by comparing its results with those produced by the Digestive BIO K 348 kit of the same company (Bio-X Diagnostics, Belgique 2021) which is given as follows: Cryptosporidium: Sensitivity: 100%, Specificity: 88%.

RESULTS

The results of the ELISA test showed that out of 96 stool samples, there were 9 cases of cryptosporidium infection (9.4%). The positive cases were distributed as follows: 9.1% (1/11) in the western Aleppo countryside, 21.1% (4/19) in the eastern Idlib countryside, 13.6% (3/22) in the Northern Idlib countryside, 2.9% (1/35) in the Jisr Al-Shughur countryside, while no positive case was recorded in the western Idlib countryside.



	Jisr Al-Shughur	Western Aleppo	Eastern Idlib	Northern Idlib	Western Idlib	Total
Total	35	11	19	22	9	96
Positive%	3%	9%	21.10%	13.60%	0%	9.40%
Positive	1	1	4	3	0	9
Negative%	97%	91%	78.90%	86.40%	100%	90.60%
Negative	34	10	15	19	9	87

Graph 1: Distribution of samples in different regions (negative and positive for Cryptosporidium infection)

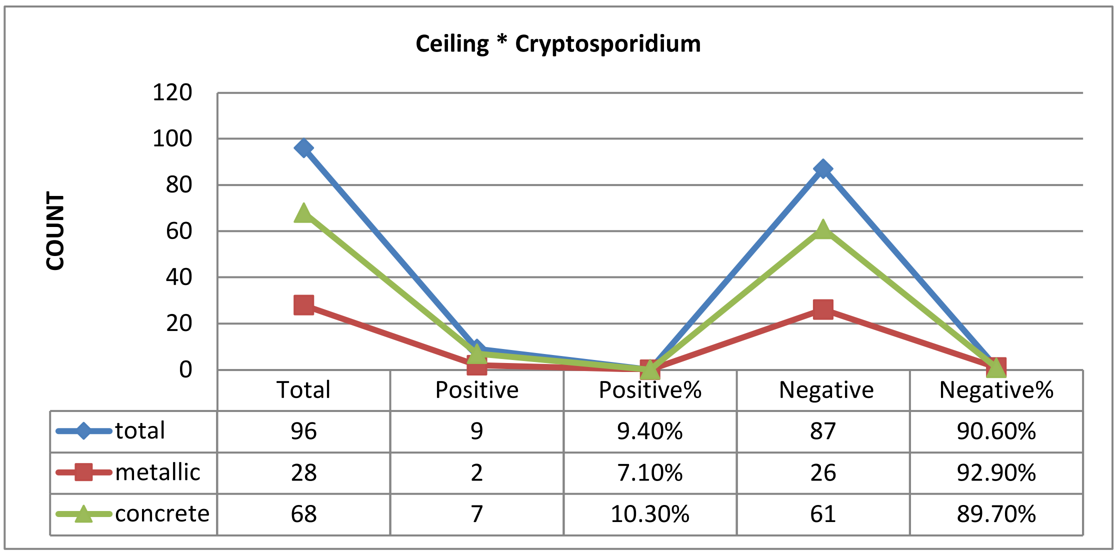
Statistical Analysis: We used the SPSS V23 for statistical analysis, the Nominal and Ordinal logistic regression method to measure the effect, and the Chi-square to test the independence and Odds Ratio (Risk).The prevalence of cryptosporidium in intensive system farms is 25% (5/20) among the calves There is a statistically significant correlation between the type of rearing and the infection with cryptosporidium ($p < 0.05$); Risk Estimate ($R^2 = 7.260$, CI:95% odds: 0.167 (0.040-.695), P = 0.014) (Table 1).

Table (1): Crosstabs, number of cases, the total percent of cases by type of breeding, Chi-square test, Nominal logistic regression test (reference-Negative)

Type of breeding		Negative	Positive	Total	R^2	P value	B	OR	Sig.
Individual	Count	72	4	76	7.26	0.007[a]	-1.792	0.167	0.014[a]
	% of Total	75.0%	4.2%	79.2%					
Intensive	Count	15	5	20					
	% of Total	15.6%	5.2%	20.8%					

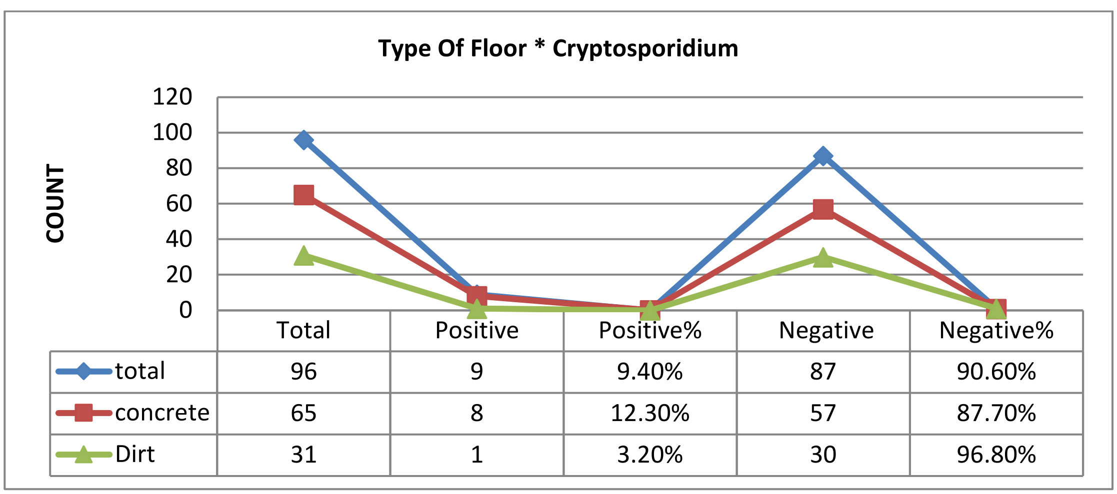
R^2 : chi-square value
P value : for R^2
B : beta value for nominal regression
OR : odds ratio value
Sig : P value for nominal regression
a: $P < 0.05$

There was no correlation ($P > 0.05$) between cryptosporidium infection and kind of the ceiling of the barn (Graph 2), or the floor of the barn (Graph 3), and the health status in the farm (Graph 4) Five out of nine positive cases (55.5%) were recorded in farms with good health status (Graph 4).



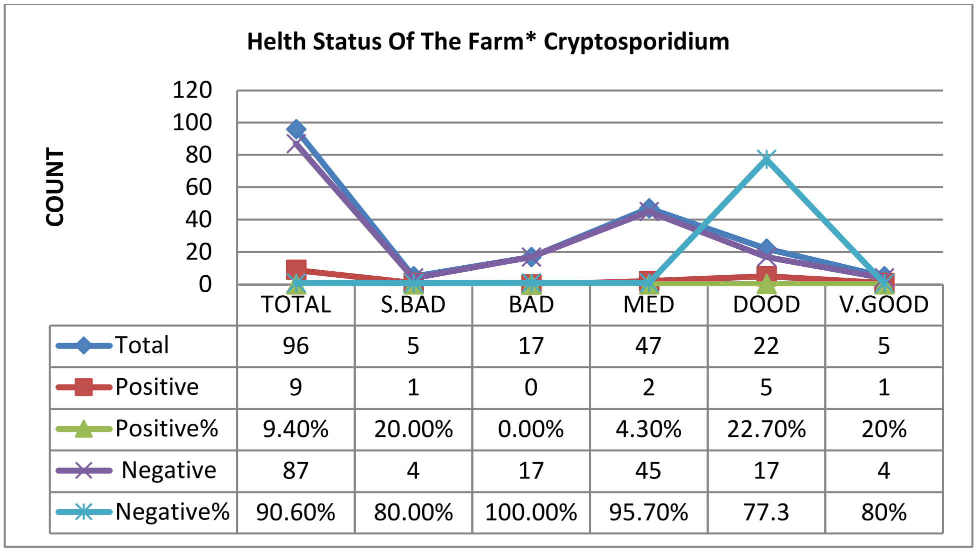
	Total	Positive	Positive%	Negative	Negative%
total	96	9	9.40%	87	90.60%
metallic	28	2	7.10%	26	92.90%
concrete	68	7	10.30%	61	89.70%

Graph (2). Crosstab Type of Ceiling * cryptosporidium



	Total	Positive	Positive%	Negative	Negative%
total	96	9	9.40%	87	90.60%
concrete	65	8	12.30%	57	87.70%
Dirt	31	1	3.20%	30	96.80%

Graph (3). Crosstab Type of floor in the barn * Cryptosporidium



Graph (4). Crosstab health status * cryptosporidium

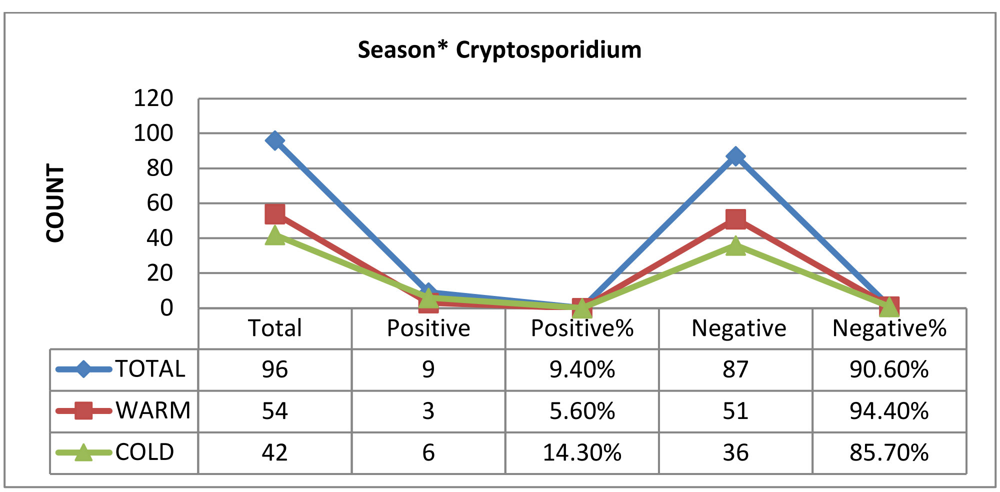
The study also showed the correlation of positive cases in barns where diarrhea is present in more than one calf (P < 0.05) (R^2= 6.874, CI:95% odds: 0.143 (0.028-0.732)) (Table 2).

Table (2): Crosstabs, number of cases, the total percent of cases by Calves with diarrhea in the barn, Chi-square test, Nominal logistic regression test (reference-Negative)

Calves with diarrhea		Negative	Positive	Total	R^2	P value	B	OR	Sig.
Single calf	Count	58	2	60	6.874	0.009[a]	-1.946	0.143	0.028[a]
	% of Total	60.4%	2.1%	62.5%					
common	Count	29	7	36					
	% of Total	30.2%	7.3%	37.5%					

R^2 : chi-square value
P value : for R^2
B : beta value for nominal regression
OR : odds ratio value
Sig : P value for nominal regression
a: P < 0.05

The study showed that the season and prevalence of cryptosporidium are two independent events (P>0.05), where 5.6% (3/54) cryptosporidium-positive calves were detected in the warm months and 14.3% (6/42) in the cold months (Graph 5).



Graph (5): Crosstab Season * Crypto Crosstabulation

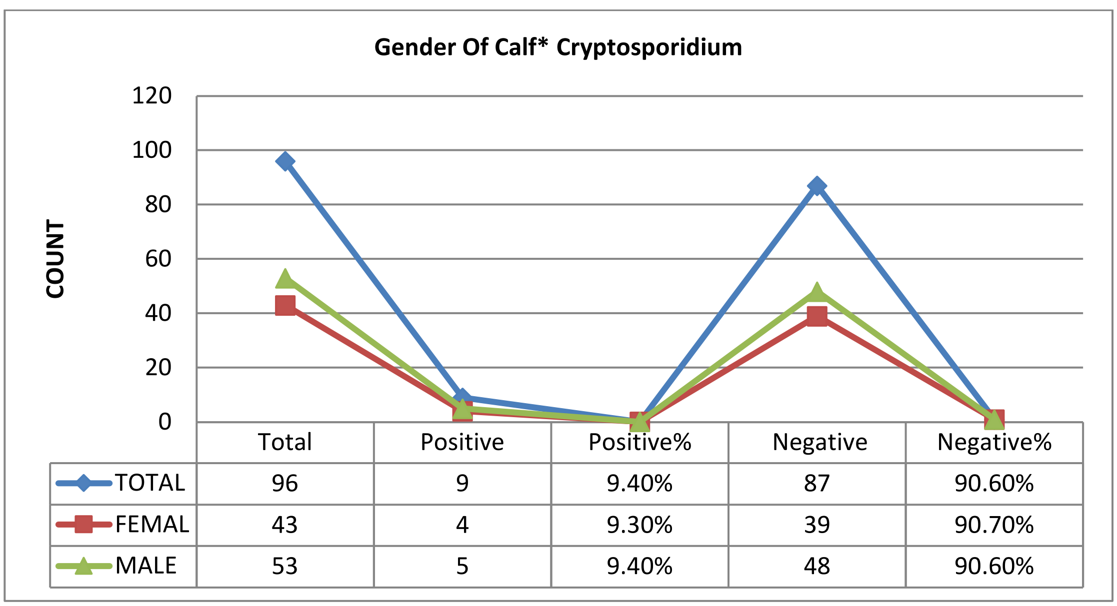
There was a correlation between the category of the age calf and the incidence of infection, as it reached in the first category (1-9 days old) 26.3% (5/19), in the second category (10-19 days old) 23% (3/13) and in the fourth category 24% (30-39 days old), while day categories (20-29 days old), (40-49 days old) and (50-59 days old) did not record any positive case, (R^2: 14.216, df:5, P = 0.014) whereas the correlation of the first age group was the highest compared to the other groups (P<<0.05) (Table 3).

Table (3): Crosstabs, number of cases, the total percent of cases by category of Age, Chi-square test, Ordinal logistic regression test (reference-positive).

Age of calf (Days)		Negative	Positive	Total	R^2	P value	B	OR	Sig.
1- 9	Count	14	5	19			0.425		0.520
	% of Total	14.6%	5.2%	19.8%					
10 - 19	Count	10	3	13			1.227		0.069
	% of Total	10.4%	3.1%	13.5%					
20 - 29	Count	17	0	17			2.037		0.003[a]
	% of Total	17.7%	0.0%	17.7%	14.216	0.014[a]		1.4	
30 - 39	Count	22	1	23			3.141		0.000[a]
	% of Total	22.9%	1.0%	24.0%					
40 - 49	Count	16	0	16					
	% of Total	16.7%	0.0%	16.7%			4.462		0.000[a]
50 - 59	Count	8	0	8					
	% of Total	8.3%	0.0%	8.3%					

R^2 : chi-square value
P value : for R^2
B : beta value for nominal regression
OR : odds ratio value
Sig : P value for nominal regression
a: P < 0.05

The prevalence was 9.3% (4/43) positive among female calves while 9.4% (5/53) among male calves. There was no statistical significance for the association between infection rate and calf gender (P>>0.05) (Graph 6).



Graph (6): Crosstab Gender of calf * Cryptosporidium

There is a correlation between the color of diarrhea and the incidence of infection. This appeared through the incidence of infection that appeared within the two colors recorded in the questionnaire: green-brown and yellow, 2% (1/49) and 17% (8/47), respectively (R^2 = 6.337, CI: 95% odds: 0.102 (0.12- 0.847) (Table 4).

Table (4): Crosstabs, number of cases, the total percent of cases by the color of diarrhea, Chi-square test, Nominal logistic regression test (reference-Negative).

Diarrhea color		Negative	Positive	Total	R^2	P value	B	OR	Sig.
Green	Count	48	1	49	6.337	0.012[a]	-2.287	0.102	0.035[a]
Brownish	% of Total	50.0%	1.0%	51.0%					
Yellow	Count	39	8	47					
	% of Total	40.6%	8.3%	49.0%					

R^2 : chi-square value; P value : for R^2; B : beta value for nominal regression; OR : odds ratio value ; Sig : P value for nominal regression; a: P < 0.05

The study showed a strong correlation (P<<0.05) between the consistency of diarrhea and the rate of infection recorded (R^2=12.768, CI:95% odds:1(0.000-….) sig = 1>> 005. (Table 5).

Table (5): Crosstabs, number of cases, total percentage of cases by consistency of diarrhea, Chi-square test, Nominal logistic regression test (reference-Negative).

Diarrhea consistency		Negative	Positive	Total	R^2	P value	B	OR	Sig.
like-normal	Count	43	0	43	12.76	0.002[a]	.000	1.000	1.000
	% of Total	44.8%	0.0%	44.8%					
Mucous	Count	33	9	42			-18.12	1.342	.
	% of Total	34.4%	9.4%	43.8%					
aqueous	Count	11	0	11					
	% of Total	11.5%	0.0%	11.5%					

R^2 : chi-square value
P value : for R^2
B : beta value for nominal regression
OR : odds ratio value
Sig : P value for nominal regression
a: P < 0.05

Symptoms accompanying diarrhea were recorded when calves were surveyed at the time of sampling, but they did not have any statistical significance linking them with infection.

The study showed that the highest incidence was recorded on the fifth day after diarrhea 33.3% (1/11), on the sixth day 25% (1/4) and on the fourth day 18.2% (2/11). No positive cases of diarrhea were recorded after the seventh day.

There was a correlation between the infection of calves and their dystocia (P = 0.031) (R^2= 0.49, df=1, P = 0.031) (odds: 4.938(1.032-23.617) P = 0.046). The incidence of infection was recorded at 27.3% (3/11) in the calves born with a difficult birth, while it was 7.1% (6/85) in the calves born with natural birth Table 6).

Table (6): Crosstabs, number of cases, total percentage of cases by dystocia, Chi-square test, Nominal logistic regression test (reference-Negative).

Dystocia		Negative	Positive	Total	R^2	P value	B	OR	Sig.
Yes	Count	8	3	11	4.684	0.030[a]	1.597	4.938	0.046[a]
	% of Total	8.3%	3.1%	11.5%					
No	Count	79	6	85					
	% of Total	82.3%	6.3%	88.5%					

R^2 : chi-square value
P value : for R^2
B : beta value for nominal regression
OR : odds ratio value
Sig : P value for nominal regression
a: P < 0.05

The recorded incidence cryptosporidium was 50% (3/6) in the calves whose mothers were found infected when the samples were taken (R^2 = 12.432, CI:95% odds: 14.000 (2.310-84.859) (Table 7).

Table (7): Crosstabs, number of cases, the total percent of cases by mother have diarrhea, Chi-square test, Nominal logistic regression test (reference-Negative).

mother have diarrhea		Negative	Positive	Total	R^2	P value	B	OR	Sig.
Yes	Count	3	3	6	12.432	0.00[a]	2.63	14.00	0.004[a]
	% of Total	3.1%	3.1%	6.3%					
No	Count	84	6	90					
	% of Total	87.5%	6.3%	93.8%					

R^2 : chi-square value
P value : for R^2
B : beta value for nominal regression
OR : odds ratio value
Sig : P value for nominal regression
a: P < 0.05

There were no cryptosporidium-positive cases of calves that had not taken colostrum, as only three cases were recorded of calves that were not taking colostrum at birth but were fed on substitutes. Delay in administering colostrum was recorded in only 7 cases, with only one sample among those found to be infected with cryptosporidium and there was no correlation (P>>0.005) (R^2= 0.320, P = 0.571). There was also no correlation between the amount of colostrum given to the calf and the detection of cryptosporidium infection p>> 0.05. (Table 8).

Table (8): Crosstabs, number of cases, the total percent of cases by given colostrum, Chi-square test, Nominal logistic regression test (reference-Negative).

Colostrum given		Negative	Positive	Total	R^2	P value	B	OR	Sig.
Yes	Count	84	9	93	0.320	0.571	15.192	-	-
	% of Total	87.5%	9.4%	96.9%					
No	Count	3	0	3					
	% of Total	3.1%	0.0%	3.1%					

R^2 : chi-square value
P value : for R^2
B : beta value for nominal regression
OR : odds ratio value
Sig : P value for nominal regression
a: P < 0.05

There was a correlation between the infection detected in the barn and the presence of different ages in it (P<0.05) (R^2 = 4.089) (Table 9).

Table (9): Crosstabs, number of cases, the total percent of cases by Ages in barn, Chi-square test, Nominal logistic regression test (reference-Negative).

Ages in barn		Negative	Positive	Total	R^2	P value	B	OR	Sig.
Yes	Count	59	9	68	4.089	0.043[a]	18.545	113275532	.
	% of Total	61.5%	9.4%	70.8%					
No	Count	28	0	28					
	% of Total	29.2%	0.0%	29.2%					

R^2 : chi-square value
P value : for R^2
B : beta value for nominal regression
OR : odds ratio value
Sig : P value for nominal regression
a: P < 0.05

All calves from which the samples appeared positive for infection were treated with a mixture of antibiotics and analgesics 13% (9/65) (P<0.05) (R^2 = 4.736) as shown in Table 10.

Table (10): Crosstabs, number of cases, the total percent of cases by kind of Treatment, Chi-square test, Nominal logistic regression test (reference-Negative).

Type of treatment		Negative	Positive	Total	R^2	P value	B	OR	Sig.
Antibiotics & analgesics	Count	56	9	65	4.736	0.03[a]	18.59	11934	.
	% of Total	58.3%	9.4%	67.7%					
No treatment	Count	31	0	31					
	% of Total	32.3%	0.0%	32.3%					

R^2 : chi-square value
P value : for R^2
B : beta value for nominal regression
OR : odds ratio value
Sig : P value for nominal regression
a: $P < 0.05$

DISCUSSION

Samples were collected from farms that were placed within the research plan. All calves suffering from a change in feces consistency or color were considered having diarrhea, regardless of their health status and/or other accompanying symptoms. The symptoms were recorded within the questionnaire following Svensson's definition of diarrhea, i.e., a change in feces that is present for two days or more with a soft or watery consistency, which may be associated with other components and may be associated with weight loss and poor general condition of the animal (Svensson *et al.* 2003).

Diarrhea is known by the multiplicity of causes which may be overlapping and are activated as a result of multiple ways of infection. Other factors that may contribute to diarrhea include feeding, environmental factors, and the conditions within the farm. Poor public health measures (biosecurity), lack of colostrum, individual susceptibility to infection, and a combination of these or other factors may lead to the disease and, thus, increase the economic burden on establishments (Alfieri *et al.* 2006).

The questionnaire included supposed risk factors. It included the location of the farm, climatic factors during the season in which the sample was collected, age, gender, colostrum consumption, quantity and time of intake, as well as the medications used when the sample was taken. Studies by many researchers have shown that there is a strong and significant association between infection with *C parvum* and diarrhea in calves (Haschek *et al.* 2006; Singh *et al.* 2006; Trotz *et al.* 2005; Joachim *et al.* 2003; Naciri *et al.*1999). Various reports have indicated a prevalence of cryptosporidium from 6.26 to 39.65% in livestock in different parts of the world (Joute *et al.* 2014, Azami *et al.* 2007). In general, differences exist between studies regarding diarrhea around the world (De la Fuente *et al.* 1999; Garcia *et al.* 2000; Uhde *et al.* 2008). The difference in diarrhea results from different studies may be due to the different diagnostic methods used (Mayameei *et al.* 2010; Ok *et al.* 2009).

ELISA is a preferred method over other laboratory methods for diagnosing diarrhea in calves due to the low cost of the test, high sample throughput, and high sensitivity (Mayameei *et al.* 2010). According to study in the United Arab Emirates, 29 out of 36 fecal samples were positive for cryptosporidium based on the ELISA test (Mohteshamuddin 2020). In the same vein, out of the 28 fecal samples collected from 39 calves, 13 (46.4%) were positive for cryptosporidium in Italy (Loraa *et al.* 2018). Prevalence of cryptosporidium was 18% in calves aged between 1 to 5 days, while the infection rate was 52% among calves aged 9 to 21 days (Al Mawly *et al.* 2015). The prevalence of farm-wide cryptosporidium in New Zealand dairy farms was 52% using ELISA or microscopy. The researchers suggested that the false positive by ELISA was caused by some species whose fragments were not amplified by PCR, other than parvum species, or that there was a deterioration in the samples that were refrigerated for a long time This indicates reliability of the ELISA test (Al Mawly *et al.* 2015). The incidence of 3.92% of the total of 51 calves infected with diarrhea in Turkey (Altug *et al.* 2013) was lower than the percentage that appeared in our study. The number of samples may be different, and geographical environment or other factors might have reduced the incidence.

The incidence of cryptosporidium in southwestern France was 15.6% (Bendali *et al.* 1999a), which is fairly close to our study. Out of the 360 calves examined, 67 (18%) calves carrying cryptosporidium oocysts were identified in feces samples in northwestern Ethiopia (Ayele *et al.* 2018). According to Brandão's study in Brazil, the number of infections (6/9) with *cryptosporidium parvum* which is much higher percentage of infections compared to our study (Brandão *et al.* 2007). The difference between the two studies could be attributed to the geographical variance or due to the presence of another cause such as simultaneous infection. Fonte has shown that simultaneous infection in fecal samples of calves infected with diarrhea, rotavirus and with cryptosporidium were 87%, while it was 11.1% in samples with coronaviruses with cryptosporidium (Fuente *et al.* 1999). Oocysts of cryptosporidium spread and infections occur in calves in the first three days and peak in the first two weeks of life. Infections occur in adult cows after three months of age and symptoms appear after infection within 3-7 days. Some studies indicate an incidence of up to 100% in calves (de Graaf *et al*. 1999; O'Handley *et al.* 1999; Fayer *et al.* 1998; Xiao and Herd 1994; Argenzio *et al.* 1990, Harp *et al.* 1990). Oocytes are not shed during the first three days of a calf's life (Fayer *et al.* 1998). Our study shows a correlation between the age group of the calf and the incidence of infection. The total number of calves on each farm did not exceed 3 calves, except in some farms that were considered intensive breeding farms. There is no correlation between studied variables and the presence of diarrhea-causing factors at the farm level, while Odd's preference for shedding cryptosporidium Oocytes, cofactors, and mixed infections was greater in calves aged 9 to 21 days than calves 1 to 5 days old (Al Mawly *et al.* 2015). The occurrence of severe diarrhea in calves and shedding of oocysts differs from one farm to another which leads to some questions about the confirmation of cryptosporidium as the primary infection (Griffiths *et al.* 1994). Calves that shed oocysts are at greater risk of diarrhea than uninfected calves. This strong correlation suggests an association between the number of oocysts and the occurrence of diarrhea in calves (Trotz *et al.* 2005). According to Trotz and Singh, the secretion of diarrheal agents can be recorded in healthy and sick calves showing diarrhea (Trotz *et al.* 2005; Singh *et al*. 2006). Our study did not find any infection in feces samples that creamy consistency-like-normal texture.

Cryptosporidium is associated with diarrhea in calves up to 14 days of age (Bartels 2010). Through results, we have shown that abnormal mucous-watery texture feces are prevalent in the positive calves, especially at a young age. It may be due to milk content (dams nutrition) or causative factors against which the calf may not have antibodies (the immune system is weak and incomplete). The variable distribution of infection with age and different resistance as age increases is related to the development of immunity over time (Ayele *et al*. 2018). This may be one of the reasons for the unfair management practices of farmers such as lack of care for 'mother cow's vaccinations, unbalanced nutrition during pregnancy, drying periods, giving colostrum, and milk substitutes in large farms). Climate instability (especially cold) may also be considered a factor for intestinal infections. Factors of farm management are influenced by region, general health status, and biosecurity, in addition to environmental conditions and geographical location (climatic factors) (Venu *et al.* 2013; Ok *et al.* 2009). According to the results of the study, there was no correlation between cryptosporidium infection and time sampling, i.e., cold months or warm months, which are two independent events ($P > 0.05$). The prevalence of infections was recorded as 5.6% (3/54) in warm months and 14.3 % (6/42) in cold months. This can be due to factors other than temperature, or it may be due to the environmental privacy of each region.

The highest incidence of diarrhea was recorded in calves during cold months. The age factor is one of the most critical risk factors that increases the incidence of diarrhea during the first month of life (Mansfeld *et al.* 2007; Scott *et al.* 2004; Gutzwiller 2002; Bendali *et al.* 1999a; Frank and Kaneene 1993). The interest in health care in most of the study barns was almost equal in the use of sterilizers and available vaccines. This was especially true for most of individual breeding systems in barns (private breeding), where the farmer has to pay sufficient attention to the administration of the small farm because it is very close to his home. There was a strong correlation between positive cryptosporidium cases and the general health status of calves and farms. Increased prevalence of infected calves may be attributed to polluted and muddy health conditions, which create appropriate conditions and factors to save and store oocysts on a farm or animal itself, fodder, and water sources containing oocysts. It is a source of infection and increases the risk of infection in farms. (Ayele *et al.* 2018; Abebe *et al.* 2008).

The incidence of cryptosporidium in calves is specific and correlated to the barns' age, floor, and environmental condition (Ogendo *et al.* 2017, Brook *et al.* 2008). There was no correlation between the health status of the farm, its floor, and positive cryptosporidium, as most of the floors in the farms sampled were concrete.

According to reports related to cryptosporidium, the incidence of infection was higher in calves in the United Kingdom. This difference may be attributed to the difference in the geographical area, study design, methods of diagnosis, production, and management systems, in addition to the segregation factor during the year in which the study was conducted (Venu *et al.* 2013).

The results of (Gulliksen *et al.* 2009) agree with (Frank and Kaneene 1993) that wintertime poses the most significant risk factor for diarrhea but those results contradict the findings of a Swedish study (Svensson *et al.* 2003, 2006) which are consistent with our current study. Maddox *et al.*2006 found that levels of oocyst shedding of cryptosporidium are higher during wintertime compared to summer which agrees with the study by Hamnes *et al.* 2006. Moreover, cows in Norway that give birth during wintertime produce lower quality colostrum than those that give birth during other seasons of the year (Gulliksen *et al.* 2008). The increase in the size of the herd leads to wider use of advanced techniques and mechanization, which in turn causes a decrease in the time available for the farmer to inspect the herd. It becomes challenging for the farmers to conduct the individual daily examination of the animals. This may explain the high mortality rate in calves in the pre-weaning stage due to diarrhea and dehydration (Lucchelli *et al.* 1992). The prevalence of infection in intensive system farms was 25% (5/20), ($p<0.05$) Risk Estimate (R2:7.260, CI:95% odds:0.310(0.147-0.653)) The rate of infection was higher in the intensive system than in the home individual system. This higher infection rate may be due to overcrowding, which is usually a risk factor for the emergence of pathological infections. Overcrowding of animals in the farm may lead to an increase in the virulence of the causative agent due to the ease of securing a new host thereby increasing the rate of shedding oocysts and spreading them in the herd.

Colostrum is a crucial indicator and a factor contributing to the occurrence of infection (Ayele *et al.* 2018). Colostrum containing low levels of immunoglobulin C (IgG) is a risk factor for increasing the likelihood of diarrhea in calves born during the winter compared with claves born in the rest of the seasons. In addition, no variables involving colostrum intake were affected as a risk factor for cryptosporidium diarrhea (Gulliksen *et al.* 2008). Our study included the subject of colostrum intake, timing, administration, and quantity, in calves (not infected with cryptosporidium) that had not taken colostrum, and only three cases were recorded in calves that were not being feed colostrum at birth but were fed on substitutes milk. Delay giving colostrum was recorded with only 7 cases, only one sample of them was infected with cryptosporidium and we did not see any correlation ($P >> 0.005$). There was also no correlation between the amount of colostrum given to the calf and the detection of cryptosporidium infection. In previous studies of diarrhea due to viral causes, calf gender, and dystocia were identified as risk factors. It has been suggested that the larger size of male calves at birth compared to females causes dystocia and thus results in a deficiency in the absorption of colostrum (Ammar *et al.* 2014; Clement *et al.* 1995). Our study results demonstrate a correlation between the cryptosporidium infection and dystocia ($P = 0.031$) ($R^2 = 0.49$, df:1, F: 8,22, $P = 0.031$) but there was no statistically significant association between dystocia and the gender of the infected calf ($P >> 0.05$).

Bartels found that cryptosporidium shedding increased 3.2 times on farms that routinely used antibiotics for diarrheal calves. The result of the research prompted veterinarians and owners to consider cryptosporidium as one of the possible causes of diarrhea to reduce the use of antibiotics and to include a request for cryptosporidium test in diagnostic protocols when the fecal samples are sent for analysis (Bartels *et al.* 2010).

Our study shows that the samples that were treated with antibiotics had a higher rate of infection than the samples that were not given any drugs, or they were given astringent drugs or the milk was diluted from them as an initial treatment.

Conclusion: The results from our study demonstrate that the incidence of cryptosporidium is significant. It is necessary to include the detection test of cryptosporidium within the protocols for early detection of causes of diarrhea and to reduce the excessive use of antibiotics. Rapid use of antibiotics without thorough diagnosis has more harms than benefits. This practice may lead to animal fatigue and inability to control the cryptosporidium. There exists a need to put strategic plans in intensive breeding to follow up on changes in the color and texture of feces of the newborn in intensive system farms. It is recommended to expand the research on diarrheal causes, especially bacterial causes, using fast, high-reliability, and inexpensive techniques for breeders and comparing their results with the results of traditional bacterial culture.

Statements and Declarations

Conflict of interests: The authors declare no conflict of interest.

Competing interests: The authors declare no competing financial or non-financial interests.

Authors contributions: HFA and FAD contributed to the study conception and design. Material preparation and samples collection were performed by HFA. Processing of the samples and investigation was done by HFA and FAD. Data analysis was performed by HFA and FAD. Original draft preparation (lead) and writing was done by HFA. GM supported in the original draft writing. Draft review and editing were done by GM.

Availability of Data and material: All data generated or analyzed during this study are included in this published article. Raw data are available from the corresponding author upon reasonable request.

Ethics Approval: None of the animals involved in this study were caught or restrained for sample collection, and all samples were obtained in a non-invasive way. Hence, there was no need for ethical permission.

REFERENCES

Abebe, R., A. Wossene, and B. Kumsa (2008). An epidemiological study of Cryptosporidium infection in dairy calves on selected dairy farms of central Ethiopia. Revue Méd. Vét. 159(2): 107–111. https://www.revmedvet.com/2008/RMV159_107_111.pdf

Al Mawly, J., A. Grinberg, D. Prattley, J. Moffat and N. French (2015). Prevalence of endemic enteropathogens of calves in New Zealand dairy farms. N. Z. Vet. J. 63(3): 147-152. doi.org/10.1080/00480169.2014.966168

Alfieri, A.A., M.E. Parazzi, E. Takiuchi, K.C. Medici, and A.F. Alfieri (2006). Frequency of group A rotavirus in diarrhoeic calves in Brazilian cattle herds, 1998-2002. Trop. Anim. Health Prod. 38: 521-526. doi.org/10.1016/S0166-0934(99)00104-4

Altug, N., N. Yuksek, C. Ozkan, I. Keles, Y. Basbugan, Z.T. Agaoglu, A. Kaya and Y. Akgul (2013). Neonatal Buzağı İshallerinin İmmunokromotografik Test Kitleri İle Hızlı Etiyolojik Teşhisi. Van Vet. J. 24 (3): 123-128.

Ammar, S.S.M., K. Mokhtaria, B.B. Tahar, A.A. Amar, B.A. Redha, B. Yuva, and B. Laid (2014). Prevalence of rotavirus (GARV) and coronavirus (BCoV) associated with neonatal diarrhea in calves in western Algeria. Asian Pac. J. Trop. Med. 4: S318-S322. doi.org/10.12980/APJTB.4.2014C778

Argenzio, R.A., J. Lecce and D.W. Powell (1993). Prostanoids inhibit intestinal NaCl absorption in experimental porcine cryptosporidiosis. Gastroenterology. 104 (2): 440–447. doi.org/10.1016/0016-5085(93)90412-6

Argenzio, R.A., J.A. Liacos, M.L. Levy, D.J. Meuten, J.G. Lecce and D.W. Powell (1990). Villous atrophy, crypt hyperplasia, cellular infiltration, and impaired glucose-Na absorption in enteric cryptosporidiosis of pigs. Gastroenterology. 98(5): 1129-1140. doi.org/10.1016/0016-5085(90)90325-U

Argenzio, R.A., J.M. Rhoads, M. Armstrong and G. Gomez (1994). Glutamine stimulates prostaglandin- sensitive Na(1)-H1exchange in experimental porcine cryptosporidiosis. Gastroenterology. 106(6): 1418–1428. doi.org/10.1016/0016-5085(94)90393-X

Ayele, A., Z. Seyoum and S. Leta (2018). Cryptosporidium infection in bovine calves: prevalence and potential risk factors in northwest Ethiopia. BMC Research Notes. 11(1): 1-6. doi.org/10.1186/s13104-018-3219-7

Azami, M. (2007). Prevalence of Cryptosporidium infection in cattle in Isfah, Iran. J. Eukaryot. Microbiol. 54(1): 100–102. doi.org/10.1111/j.1550-7408.2006.00236.x

Bartels, C.J., M. Holzhauer, R. Jorritsma, W.A. Swart, and T.J. Lam (2010). Prevalence, prediction and risk factors of enteropathogens in normal and nonnormal faeces of young Dutch dairy calves. Prev. Vet. Med. 93(2): 162-169. doi.org/10.1016/j.prevetmed.2009.09.020

Barua, R.S. (2019). Clinico-pathology and Molecular characterization of bovine Rotavirus infection in calves in south-eastern part of Bangladesh, Doctoral dissertation, Department of Pathology and Parasitology Faculty of Veterinary Medicine, Chittagong Veterinary and Animal Sciences University, Chattogram-4225, Bangladesh.

Bendali, F., H. Bichet, F. Schelcher and M. Sanaa (1999a). Pattern of diarrhoea in newborn beef calves in south-west France. Vet. Res. 30: 61-74. https://pubmed.ncbi.nlm.nih.gov/10081113/

Bendali, F., M. Sanaa, H. Bichet and F. Schelcher (1999b). Risk factors associated with diarrhoea in newborn calves. Vet. Res. 30: 509-522. https://pubmed.ncbi.nlm.nih.gov/10543385/

Bolieau M.J. and S. Kapil (2010). Bovine coronavirus associated syndromes. Vet. Clin. North Am. Food Anim. Pract. 26: 123–146. doi.org/10.1016/j.cvfa.2009.10.003

Brandão, P.E., L.Y. Villareal, S.L. De Souza, L.J. Richtzenhain and J.A. Jerez (2007). Mixed infections by bovine coronavirus, rotavirus and Cryptosporidium parvum in an outbreak of neonatal diarrhea in beef cattle. Arq. Inst. Biol. (Sao Paulo). 74: 33-34. doi.org/10.1590/1808-1657v74p0332007

Brook, E., C.A. Hart, N. French and R. Christley (2008). Prevalence and risk factor of *Cryptosporidium* infection in young calves. Vet. Parasitol. 152: 46–52. doi.org/10.1016/j.vetpar.2007.12.003

Chen, W., J.A. Harp and A.G. Harmsen (2003). Cryptosporidium parvum infection in gene-targeted B cell-deficient mice. J. Parasitol. 89: 391–393. doi.org/10.1645/0022-3395(2003)089[0391:CPIIGB]2.0.CO;2

Cho, Y.I. and K.J. Yoon (2014). An overview of calf diarrhea - infectious etiology, diagnosis, and intervention. J. Vet. Sci. 15: 1–17. doi.org/10.4142/jvs.2014.15.1.1

Clement, J.C., M.E. King, M.D. Salman, T.E. Wittum, H.H. Casper and K.G. Odde (1995). Use of epidemiologic principles to identificy risk factors associated with the development of diarrhea in calves in five beef herd. J. Am. Vet. Med. Assoc. 207: 1334-1338. https://pubmed.ncbi.nlm.nih.gov/7591931/

De Graaf, D.C., E. Vanopdenbosch, Ortega-Mora, L.M, H. Abbassi and J. E. Peeters (1999). A review of the importance of cryptosporidiosis in farm animals. Int. J. Parasitol. 29(8): 1269–1287. doi.org/10.1016/S0020-7519(99)00076-4

De La Fuente, R., M. Luzon, J. A. Ruiz-Santa-Quiteria, A. Garcia, D. Cid, , J. A. Orden, S. Garcia, R. Sanz and M. Gomez-Bautista (1999). Cryptosporidium and concurrent infections with other major enteropathogens in 1 to 30-day old diarrheic dairy calves in central Spain. Vet. Parasitol. 80: 179-185. doi.org/10.1016/S0304-4017(98)00218-0

Fayer, R., L. Gasbarre, P. Pasquali, A. Canals, , S. Almeria and D. Zarlenga (1998). Cryptosporidium parvum infection in bovine neonates: dynamic clinical, parasitic and immunologic patterns. Int. J. Parasitol. 28(1): 49–56. doi.org/10.1016/S0020-7519(97)00170-7

Fayer, R., M. Santin and L. Xiao. (2005). *Cryptosporidium bovis* n. sp. (Apicomplexa: *Cryptosporidiidae*) in cattle (*Bos taurus*). J. Parasitol. 91: 624–629. doi.org/10.1645/GE-3435

Foster, D.M. and G.W. Smith (2009). Pathophysiology of diarrhea in calves. Vet. Clin. N. Am. Food Anim. Pract. 25: 13–36. doi.org/10.1016/j.cvfa.2008.10.013

Frank, N.A. and J.B. Kaneene (1993). Management risk factors associated with calf diarrhea in Michigan dairy herds. J. Dairy Sci. 76: 1313-1323. doi.org/10.3168/jds.S0022-0302(93)77462-7

Garber, L.P., M.D. Salaman, H. S. Hurd, T. Keefe and J.L. Schlater (1994). Potential risk factors for *Cryptosporidium* infection in dairy calves. J. Am. Vet. Med. Assoc. 205: 86-91. https://pubmed.ncbi.nlm.nih.gov/7928557/

Garcia, A., J.A. Ruiz-Santa-Quiteria, J.A. Orden, D. Cid, R. Sanz, M. Gomez-Bautista, and R. De La Fuente (2000). Rotavirus and concurrent infections with other enteropathogens in neonatal diarrheic calves in Spain. Comp. Immunol. Microbiol. Infect. Dis. 23: 175-183. doi.org/10.1016/S0147-9571(99)00071-5

Geurden, T., E. Claerebout, J. Vercruysse and D. Berkvens (2008). A Bayesian evaluation of four immunological assays for the diagnosis of clinical cryptosporidiosis in calves. Vet. J. 176: 400–402. doi.org/10.1016/j.tvjl.2007.03.010

Gookin, J.L., S.K. Nordone and R.A. Argenzio (2002). Host responses to Cryptosporidium infection. J. Vet. Intern. Med. 16(1): 12–21. doi.org/10.1111/j.1939-1676.2002.tb01602.x

Griffiths, J.K., R. Moore, S. Dooley, G. T. Keusch and S. Tzipori (1994). Cryptosporidium parvum infection of Caco-2 cell monolayers induces an apical monolayer defect, selectively increases transmonolayer permeability, and causes epithelial cell death. Infect. Immun. 62(10): 4506–4514. doi.org/10.1128/iai.62.10.4506-4514.1994

Gulliksen, S.M., E. Jor, K.I. Lie, I.S. Hamnes, T. Loken, J. Akerstedt and O. Osteras (2009). Enteropathogens and risk factors for diarrhea in Norwegian dairy calves. J. Dairy Sci. 92(10): 5057-5066. doi.org/10.3168/jds.2009-2080

Gulliksen, S.M., K.I. Lie, L. Solverod and O. Osteras (2008). Risk factors associated with colostrum quality in Norwegian dairy cows. J. Dairy Sci. 91: 704–712. doi.org/10.3168/jds.2007-0450

Gutzwiller, A (2002). Effect of colostrum intake on diarrhoea incidence in newborn calves. Schweizer Archiv. Für. Tierheilkunde. 144: 59-64. doi.org/10.1024/0036-7281.144.2.59.

Hamnes, I.S., B. Gjerde and L. Robertson (2006). Prevalence of *Giardia* and *Cryptosporidium* in dairy calves in three areas of Norway. Vet. Parasitol. 140: 204–216. doi.org/10.1016/j.vetpar.2006.03.024

Harp, J.A., D.B. Woodmansee and H.W. Moon (1990). Resistance of calves to Cryptosporidium parvum: effects of age and previous exposure. Infect. Immun. 58(7): 2237–2240. doi.org/10.1128/iai.58.7.2237-2240.1990

Haschek, B., D. Klein, V. Benetka, C. Herrera, I. Sommerfeldstur, S. Vilcek, K. Moestl and W. Baumgartner (2006). Detection of bovine torovirus in neonatal calf diarrhoea in Lower Austria and Styria (Austria). J. Vet. Med. B Infect. Dis. Vet. Public Health. 53: 160-165. doi.org/10.1111/j.1439-0450.2006.00936.x

Hunter, P.R., S. Hughes, S. Woodhouse, Q. Syed, N.Q. Verlander, R.M. Chalmers, K. Morgan and K. Osborn (2004). Sporadic cryptosporidiosis case–control study with genotyping. Emerg. Infect. Dis. 10(7): 1241–1249. doi.org/10.3201/eid1007.030582

Izzo, M.M., P.D. Kirkland, V.L. Mohler, N.R. Perkins, A.A. Gunn and J.K. House (2011). Prevalence of major enteric pathogens in Australian dairy calves with diarrhoea. Aust. Vet. J. 89: 167-173. doi.org/10.1111/j.1751-0813.2011.00692.x

Izzo, M.M., P.D. Kirkland, X. Gu, Y. Lele, and A.A. Gunn and J.K. House (2012). Comparison of three diagnostic techniques for detection of rotavirus and coronavirus in calf faeces in Australia. Aust. Vet. J. 90: 122-129. doi.org/10.1111/j.1751-0813.2011.00891.x

Joachim, A., T. Krull, J. Schwarzkopf and A. Daugschies (2003). Prevalence and control of bovine cryptosporidiosis in German dairy herds. Vet. Parasitol. 112, 277-288. doi.org/10.1016/S0304-4017(03)00006-2

Joute, J.R, J.P.S. Gill and B.B. Singh (2014). Prevalence and molecular epidemiology of Cryptosporidium parvum in dairy calves in Punjab (India). J. Parasitic Dis. 40: 745-749. doi.org/10.1007/s12639-014-0571-y

Lefay, D., M. Naciri, P. Poirier and R. Chermette (2000). Prevalence of Cryptosporidium infection in calves in France. Vet. Parasitol. 89: 1-9. doi.org/10.1016/S0304-4017(99)00230-7

Lentze, T., D. Hofer, B. Gottstein, C. Gaillard, and A. Busato (1999). Prevalence and importance of endoparasites in calves raised in Swiss cow-calf farms. Deutsche Tierärztliche Wochenschrift. 106: 275-281. doi.org/10.1016/S0304-4017(99)00230-7

Lequin, M.R (2005). Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA), Clin. Chem. 51(12): 2415-2418. doi.org/10.1373/clinchem.2005.051532

Lombardelli, J.A., M.L. Tomazic, , L. Schnittger and I.K. Tiranti (2019). Prevalence of Cryptosporidium parvum in dairy calves and GP60 subtyping of diarrheic calves in central Argentina. Parasitol. Res. 118(4): 2079-2086. doi.org/10.1007/s00436-019-06366-y

Loraa, I., F. Gottardo, B. Contiero, B.D. Avac, L. Bonfantic, A. Stefanic and A. Barberio (2018). Association between passive immunity and health status of dairy calves under 30 days of age. J. Prev. Vet. Med. 152: 12-15. doi.org/10.1016/j.prevetmed.2018.01.009

Lucchelli A., S.E. Lance, P.B. Bartlett, G.Y. Miller, and L.J. Saif (1992). Prevalence of bovine group A rotavirus shedding among dairy calves in Ohio. Am. J. Vet. Res. 53(2): 169–174. https://pubmed.ncbi.nlm.nih.gov/1315490/

Maddox-Hyttel, C., R.B. Langkjar, H.L. Enemark and H. Vigre, (2006). Cryptosporidium and Giardia in different age groups of Danish cattle and pigs-Occurrence and management associated risk factors. Vet. Parasitol. 141: 48–59. doi.org/10.1016/j.vetpar.2006.04.032

Mansfeld, R., M. Hoedemaker, R. Martin and A. De Kruif (2007). Diarrhea during the first month of life. In: Veterinary herd care for dairy cattle. 2nd edn. Eds A. de Kruif, R. Mansfeld, M. Hoedemaker. Stuttgart, Enke. pp 152-156., Berlin. (Original in German)

Mayameei, A., G. Mohammadi, S. Yavari, E. Afshari and A. Omidi (2010). Evaluation of relationship between Rotavirus and Coronavirus infections with calf diarrhea by capture ELISA. Comp. Clin. Pathol. 19: 553-557. doi.org/10.1007/s00580-009-0920-x

Mohteshamuddin, K., L. Hamdan, A.B. AlKaabi and R. Barigye (2020). Cryptosporidium parvum and other enteric pathogens in scouring neonatal dairy calves from the Al Ain region, United Arab Emirates. Vet. Parasitol. Reg. Stud. Reports. 21: 100435. doi.org/10.1016/j.vprsr.2020.100435

Mõtus, K., A. Viltrop and U. Emanuelson (2018). Reasons and risk factors for beef calf and young stock on-farm mortality in extensive cow-calf herds. Animal. 12(9): 1958-1966. doi.org/10.1017/S1751731117003548

Naciri, M., M.P. Lefay, R. Mancassola, P. Poirier and R. Chermette (1999). Role of *Cryptosporidium parvum* as a pathogen in neonatal diarrhoea complex in suckling and dairy calves in France. Vet. Parasitol. 85: 245-257. doi.org/10.1016/S0304-4017(99)00111-9

Nydam, D.V., S.E. Wade, S.L. Schaaf and H.O. Mohammed (2001). Number of Cryptosporidium parvum oocysts or Giardia spp cysts shed by dairy calves after natural infection. Am. J. Vet. Res. 62: 1612–1615. doi.org/10.2460/ajvr.2001.62.1612

O'Handley, R.M., C. Cockwill, T.A. McAllister, M. Jelinski, D.W. Morck, and M.E. Olson (1999). Duration of naturally acquired giardiosis and cryptosporidiosis in dairy calves and their association with diarrhea. J. Am. Vet. Med. Assoc. 214(3): 391–396. https://pubmed.ncbi.nlm.nih.gov/10023403/

Ogendo, A., M. Obonyo, P. Wasswa, A. Bitek, A. Mbungua and S.M. Thumbi (2017). Cryptosporidium infection in calves and the environment in Asembo, Western Kenya. Pan Afr. Med. J. 28(1): 9. doi.org/10.11604/pamj.supp.2017.28.1.9313

Ok, M., L. Gu., K. Turgut, U. Ok, , I. Sen, I.K. Gündüz, and H. Güzelbekteş (2009). The studies on the aetiology of diarrhoea in neonatal calves and determination of virulence gene markers of Escherichia coli strains by multiplex PCR. Zoonoses Public Health. 56: 94-101. doi.org/10.1111/j.1863-2378.2008.01156.x

Radostits, O.M., C.C. Gay, K.W. Hinchclif and P.D. Constable (2007). Veterinary medicine: a textbook of the disease of cattle, horse, sheep, pig and goats 10th edition. Saunders Elsevier, pp. 1–39. London.

Scott, P.R., G.A. Hall, P.W. Jones and J.H. Morgan (2004). Calf diarrhoea. In Bovine Medicine. Diseases and Husbandry of Cattle. 2nd edn. Ed A. H. Andrews. Oxford, Blackwell Publishing. pp 185-214. London.

Shafieyan, H., A. Alborzi, H. Hamidinejat, M.R. Tabandeh, and M.R.H. Hajikolaei (2014). Prevalence of Cryptosporidium species in ruminants of Lorestan province, Iran. J. Parasit. Dis. 12: 63-94. doi.org/10.1007/s12639-014-0642-0

Singh, B.B., R. Sharma, H. Kumar, H.S. Banga, R.S. Aulakh, J.P.S. Gill and J.K. Sharma (2006). Prevalence of *Cryptosporidium parvum* infection in Punjab (India) and its association with diarrhea in neonatal dairy calves. Vet.

Parasitol. 140: 162-165. doi.org/10.1016/j.vetpar.2006.03.029

Smith, H.V., S.M. Caccio, A. Tait, J. McLauchlin and R.C. Thompson (2006). Tools for investigating the environmental transmission of *Cryptosporidium* and *Giardia* infection in humans. Trends Parasitol. 22: 160–167. doi.org/10.1016/j.pt.2006.02.009

Svensson, C., J. Hultgren, and P.A. Oltenacu. (2006). Morbidity in 3-7-month-old dairy calves in south-western Sweden, and risk factors for diarrhoea and respiratory disease. Prev. Vet. Med. 74: 162–179. doi.org/10.1016/j.prevetmed.2005.11.008

Svensson, C., K. Lundborg, U. Emanuelson and S.O. Olsson (2003). Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. Prev. Vet. Med. 58: 179–197. doi.org/10.1016/S0167-5877(03)00046-1

Tajik, J., S. Nazifi, S.M. Naghib and A.R. Ghasrodasht (2012). Comparison of electrocardiographic parameters and serum electrolytes and microelements between single infection of rotavirus and coronavirus and concurrent infection of Cryptosporidium parvum with rotavirus and coronavirus in diarrheic dairy calves. Comp. Clin. Pathol. 21: 241-244. doi.org/10.1007/s00580-010-1084-4

Trotz-Williams, L.A., B.D. Jarvie, S.W. Martin, K.E. Leslie and A.S. Peregrine (2005). Prevalence of Cryptosporidium parvum infection in southwestern Ontario and its association with diarrhea in neonatal dairy calves. Can. Vet. J. 46: 349-351. https://pubmed.ncbi.nlm.nih.gov/15943123/

Uhde, F.L., T. Kaufmann, H. Sager, S. Albini, R. Zanoni, E. Schelling and M. Meylan, (2008). Prevalence of four enteropathogens in the faeces of young diarrhoeic dairy calves in Switzerland. Vet. Record. 163, 362–366. doi.org/10.1136/vr.163.12.362

Venu, R., B.R. Latha, A.S. Bath, C. Sreekumar, G.D. Raj and M. Raman (2013). Factors influencing on the prevalence of Cryptosporidium infection in South Indian dairy calves. J. Parasit. Dis. 37(2): 168–72. doi.org/10.1007/s12639-012-0153-9

Waltner-Towes, D., S.W. Martin and A.H. Meek (1986). Dairy calf management, morbidity and mortality in Ontario Holstein herds. II. Age and seasonal patterns. Prev. Vet. Med. 4(2): 125-135. doi.org/10.1016/0167-5877(86)90018-8

Xiao, L., and R.P. Herd (1994). Infection pattern of Cryptosporidium and Giardia in calves. Vet. Parasitol. 55(3): 257–262. doi.org/10.1016/0304-4017(93)00645-F.