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Original Research Article

Epidemiology and Impact of Cryptosporidiosis among Calves in the Vina Division, Region of Adamawa Cameroon

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Abstract: Cryptosporidiosis is a cosmopolitan food and waterborne zoonosis responsible for severe diarrhea in young animals, newborns, and immunocompromised individuals. This study was conducted from January to June 2023 in the Vina division to determine the epidemiology of the disease in calves. The search for *Cryptosporidium* spp oocysts in feces was conducted using the modified Ziehl-Neelsen staining technique. Out of 405 fecal samples examined, 270 tested positive, giving an overall prevalence of 66.67%. This prevalence is very significantly higher in calves aged [0-3[months (48.1%) compared to those aged [3-6[months (29.9%) and [6-12] months (29.6%) (P=0.008). It is higher during the rainy season (40%) (P=0.004), in the Nyambaka (48.3%) and Mbe (41.7%) districts (P=0.008), in diarrheic calves (99.13%) (P=0.01), in NEC= [0-2] (38.7%) (P=0.008), and in those weighing less than 40 kg (88.9%) (P=0.001). The established risk factors are the season, the locality, the age, and the diarrheal status. These results show that the infestation of *Cryptosporidium* spp in the Vina department is present and significant, highlighting the urgency to improve the health and hygiene system in farms to prevent human infections.

Keywords: Cryptosporidiosis, Prevalence, Distribution, Impact, Calves, Vina Division.

INTRODUCTION

Cryptosporidiosis is a cosmopolitan parasitosis caused by protozoa of the genus Cryptosporidium, which leads to gastrointestinal damage in mammals, clinically manifested by diarrhea, dehydration, and a deterioration in the general condition of the host that can potentially lead to death (Chapuy 2019). In cattle, although adults are asymptomatic carriers, newborn calves are the most affected (Hatam-Nahavandi *et al.*, 2019). This disease is also a zoonosis because species like C. parvum can be transmitted from animals to humans and cause harm, especially to children and immunocompromised individuals (Caccio and Chalmers 2016). This disease is very dangerous because oocysts can survive for a long time in humid environments and cannot be destroyed by disinfectants or chlorine.

In Cameroon and particularly in the Adamawa region, very little information on the epidemiology of the disease is available, while the farming system, lack of rigorous hygiene, stray animals, and environmental conditions are conducive to infection. Moreover, the parasitosis is little known to farmers and health personnel who might confuse it with helminthiases, microbial infections, indigestions, or diarrhea in animals...

This study aims to contribute to the understanding of the epidemiology of cryptosporidiosis in calves in the Vina department.

More specifically, it will be about:

- Determine the prevalence and distribution of the disease;
- Evaluate the impact of the disease on sick calves;
- Determine the risk factors associated with the disease.

1. MATERIALS AND METHODS

1.1 Study Area

The investigation was carried out in the Vina department between January and December of 2023 (Fig. 1).

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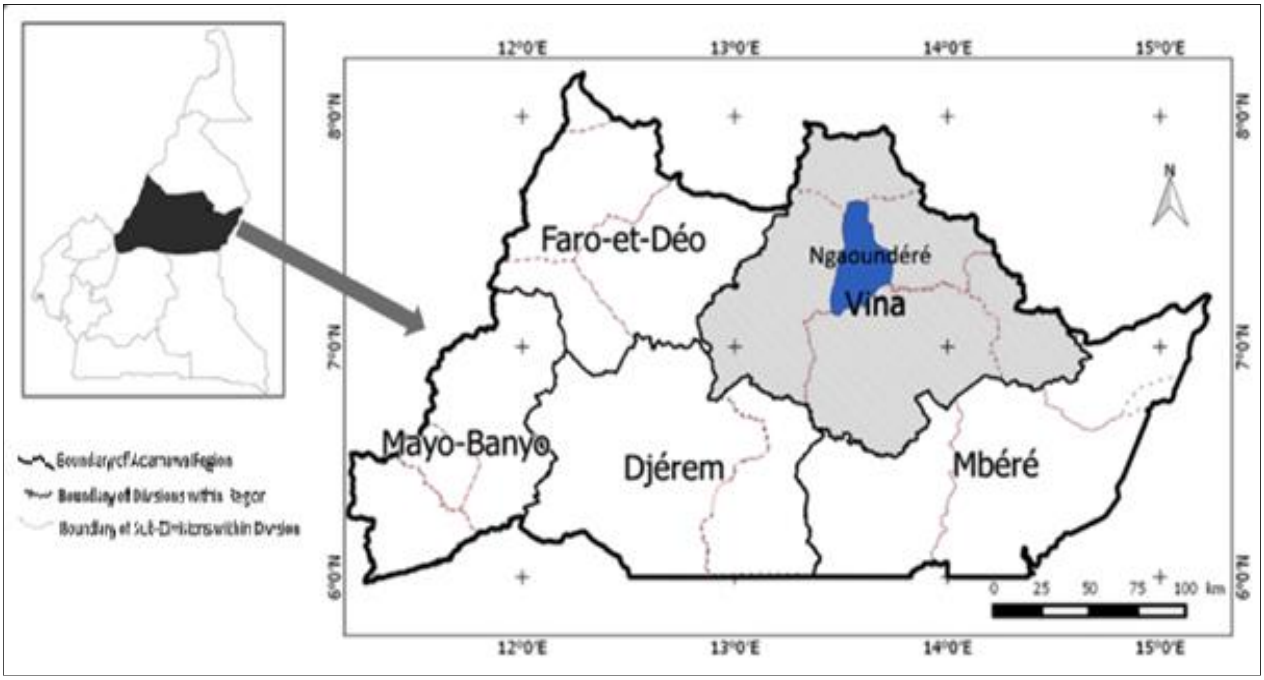

Figure 1: Map displaying Vina Division and study locations

The latter occupies an area of approximately 17,196 km² and is situated in the Adamawa Region. Its climate is rather cool due to its high height, with average temperatures between 22° and 25°C. It has a Sudanese-Sahelian tropical climate, with a long rainy season from April to October and a brief dry season from November to March. Between 900 and 1500 mm of precipitation fall on average each year. Its topography gives it a great place for caverns, wildlife reserves, thermal mineral springs, ranches, and crater lakes (Tchotsoua, 2011).

The Vina Department enjoys great cultural importance mainly due to the presence of Lamidats and ethnic diversity. The main tribes encountered are the Mboum, Dii, Gbaya, Bororos, Foulbé, and Haoussa…. Agricultural activity is dominated by the cultivation of maize, cassava, potatoes, yams, and peanuts (Tchotsoua 2011). Vina is one of the hubs of cattle farming in Cameroon due to its status as the cradle of the Gudali breed from Ngaoundéré, one of the most remarkable beef cattle breeds in Africa (Anaba *et al*., 2019; Tchotsoua, 2011). The livestock farming systems practiced are pastoralism, agropastoralism, and ranching (Tchotsoua, 2008).

1.2 Study Site

The study was conducted in different localities of the seven districts of the Vina Division (Ngaoundere I, Ngaoundere II, Ngaoundere III, Martap, Belel, Mbe, and Nyambaka). The selection of farms and calves was made based on the accessibility and availability of the farmers, the size of the herd, and the availability of the calves.

1.3 Type of Study

The study is cross-sectional with the aim of exhaustively sampling all the calves present in the herds.

1.4 Biological Material and Sample Collection

The biological material consisted of calves under 1 year old, whose reporting was done by collecting information on a form, including details such as breed, sex, age, body condition score...The feces were collected after stimulating the anal opening of the young calves. They were then directly placed in dry labeled vials containing a few drops of formalin (30%). These samples were subsequently transported in a mini cooler equipped with frozen accumulators to the Laboratory of the Special Mission for the Eradication of Tsetse Flies (MSEG) to be stored at 4°C. The blood samples were taken from the jugular vein using a vacutainer tube, and the blood, collected in EDTA (Ethylene Diamine Tri Acetic Acid) tubes, was then transported to the MSEG in a mini cooler equipped with frozen accumulators.

1.5 Sample Size

The minimum sample size was calculated using Thrusfield's (2007) formula, through the formula:

$$n = \frac{Z^2 * P * (1 - P)}{a^2}$$

With: n = sample size; Z = confidence level according to the standard normal distribution (1.96); P = estimated prevalence (50%); a = tolerated margin of error (5%);
The minimum size of our sample is therefore 385 young animals.

1.7 Animal Identification

For the identification of the animals, we collected the locality, farming system, type of housing, breed, sex, age, body condition score, stool quality, and the season. All this information was collected and recorded on a form.

1.8 Oocyst Detection and Research

The modified Ziehl-Neelsen method was used to look for and identify *Cryptosporidium* oocysts. In order to create a thin smear, 0.2g of fecal material that had previously been emulsified using a vortex was spread out on the slide using a loop. These slides were allowed to air dry for a few minutes before being put on the staining rack, inundated with 95% alcohol for five minutes, and then flamed. After five minutes of phenol fuchsin application, the still-hot slides were washed with distilled water. A 95% HCl solution was then used to decolorize them, and they were promptly rinsed. Methylene blue was used for 30 seconds to counterstain. After being drained and given another wash with distilled water, the slides were put in for a fresh.

1.9 Evaluation of the Impact of Cryptosporidiosis

To determine the impact of cryptosporidiosis on the affected animals, parameters such as body condition score, weight, and hematocrit were evaluated. The Body Condition Score (BCS) is a standardized rating of the state of body reserves through visual assessment of muscle and fat coverage at different points on the body on a scale of 0 to 5. The estimation of the animals' weight was carried out by measuring the thoracic perimeter (Pth), considered as the chest circumference, using a tape measure. The barymetric formula is as follows: Live weight = a x Pth^3 where, a = Coefficient and Pth = Thoracic perimeter. Hematocrit is an indicator of the presence and severity of anemia. For its implementation, the blood collected in EDTA tubes by puncture in the jugular vein was placed in capillary tubes. Next, the ends of these capillary tubes are sealed with a paste and centrifuged at 10,000 rpm for 10 minutes in a micro-hematocrit centrifuge. Next, these tubes are read using a hematocrit reader that measures the total height of the blood and that of the red blood cells. The evaluation of the hematocrit level is done according to an assessment grid taking into account the following ratio:

$$Hematocrit(\%) = \frac{Total\ height\ of\ red\ blood\ cells}{Total\ blood\ height} \times \mathbf{100}$$

1.10Statistical Analysis

The statistical analysis was conducted using version 20.0 of the IBM SPSS Statistics software. The prevalence, which represented by:

$$Prevalence = \frac{Number\ of\ samples\ tested\ positive}{Total\ number\ of\ samples} \times 100$$

The confidence interval, which is a range of values likely to include a defined portion of the population, will be estimated at 95% and calculated based on Thrusfield's (2007).

The chi-square test (χ^2) was used to compare the percentages or proportions of the different parameters studied. A logistic regression analysis was used to calculate the odds ratios and to identify the factors promoting the occurrence of bovine cryptosporidiosis, with the significance threshold set at $p<0.05$ in all analyses.

2. RESULTS

1.1. Global Prevalence of Cryptosporidiosis

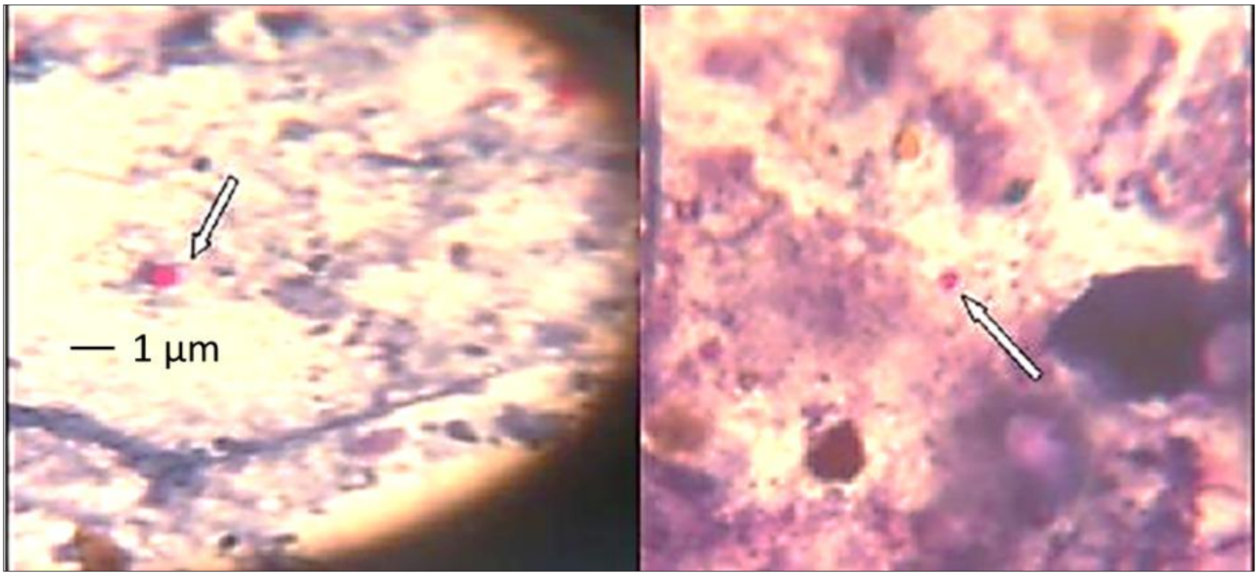

Figure 2: Oocysts of *Cryptosporidium* spp observed under an optical microscope (x100)

Microscopic observation allowed the visualization of *Cryptosporidium* spp oocysts in 270/405 samples, which appear in a round to oval shape and red color on a blue background (Fig. 2). The overall prevalence of cryptosporidiosis in calves of the Vina division is estimated at 66.67%.

2.2.1 Prevalence of Cryptosporidiosis by Age

The results of Table 1 show the distribution of calf infections based on age. It appears that the infection varies very significantly between 29.6% in calves older than 6 months and 48.1% in calves younger than 3 months (χ^2= 9.64; df=2; P=0.008). The Z test shows that the most infected animals are those aged less than 3 months (Z= 9.64; df=2; P=0.008).

Table 1: Shows the distribution of cryptosporidiosis according to the parameters under study

Parameters	NE	NI	P (%)	IC (95%)	χ^2	P Value
Age (month)						
[0-3[	79	41	48,1b	37,1-59,1	9,635	0,008
[3-6[	127	89	29,9a	22,0-37,9		
[6-12]	199	140	29,6a	23,3-36,0		
Race						
Akou	122	71	41,8	33,1-50,6	6,546	0,088
Bokolo	17	11	35,3	12,6-58,0		
Djafoun	37	24	35,1	19,8-50,5		
Goudali	229	164	28,4	22,5-34,2		
Sex						
Female	200	138	31,0	24,6-37,4	0,97	0,325
Male	205	132	35,6	29,1-42,2		
Diarrheal Statuts						
Non-diarrheal	290	156	53,79	25,7-36,4	13.82	0,01
Diarrheal	115	114	99,13	30,2-48,1		
Seasons						
Raining	205	123	40,0	33,3-46,7	8,3	0,004
Dry	200	147	26,5	20,4-32,6		
Weight (Kg)						
[0-39[	235	207	88,09	27,2-39,2	24.91	0,001
[39-43]	24	09	37,50	4,6-37,1		
>43	146	54	36,99	27,8-43,4		
BCS						
[0 ; 1]	185	173	93,51	37,2-49,2	22.5	0,001
[1 ; 3]	159	74	46,54	21,6-40,1		
[3 ; 5]	61	23	37,70	17,8-39,4		

Legend: NA = Number of calves examined; NI = Number of infected calves; CI (95%) = 95% confidence interval; P (%) = Prevalence in percentage;

2.2.2 Prevalence of Cryptosporidiosis by Breed

The results related to the influence of race (Table 1) show that the prevalence of the disease varied between 28.4% in Goudali calves and 41.8% in Akou calves, without presenting a significant difference (χ^2=6.55; df=3; P=0.088).

2.2.3 Prevalence of Cryptosporidiosis by Sex

Regarding the distribution of the infection by sex, it is observed that (Table 1), the prevalence of the disease is higher in males (35.6%) than in females (31%). However, this difference is not significant (χ^2= 0.97; df=1; P=0.325).

2.3. Spatial and Seasonal Distribution of Infection in Calves

2.3.1 Distribution of the Infection Based on Herd Size

The study of the distribution of the infection according to herd size (Fig. 3) shows that herds with a size between [50-100] are the most affected (36.3%), without showing any significant difference (χ^2=2.56; df=2; P=0.293).

2.3.2 Distribution of Cryptosporidiosis by District

Result presented in Figure 4 illustrates the spatial distribution of cryptosporidiosis in seven districts of the Vina department. It appears that the calves of Nyambaka are more affected (48.3%) than those of Mbe (41.7%), Ngaoundere 2 and III (33.3%), Martap (28%), Ngaoundere I1 (26%), and Belel (20%). These results show the existence of a significant difference (χ^2=14.2; df=6; p=0.03).

2.3.3 Distribution of the Infection by Locality

The results of Figure 3 show us that the disease is present in the different studied localities but in different proportions. Thus, we notice that the calves of Labbare are more affected (57.9%) than those of Koukourli (43.9%), Noze (41.7%), Mayanga (37.1%), Maor (35.7%), Mbijoro airport (33.3%), Selbe-darang (33.3%), Mabawa (28.6%), Malang (28%), Marza (26.7%), Maton (22.7%), and Tello (20%). However, these results do not show significant differences (χ^2=16.55; df=11; p=0.122).

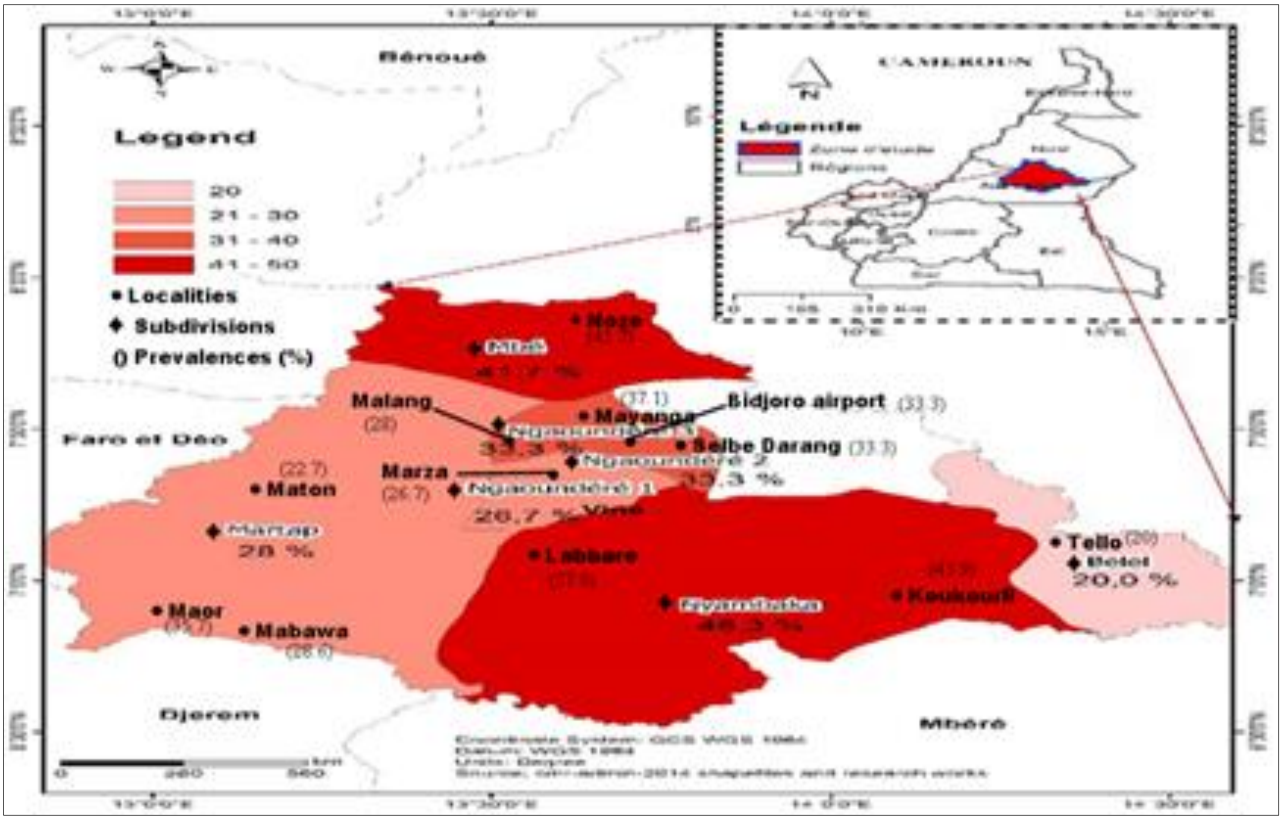

Figure 3: Distribution of cryptosporidiosis in calves by district and locality

2.3.4 Distribution of Cryptosporidiosis According to the Seasons

The results of Table 1 show that during the rainy season, calves were significantly more infected (40%) than in the dry season (26.5%) (χ^2 =8.3; df=1; p=0.004).

2.4 Impact of Cryptosporidiosis on Affected Calves

2.4.1 Impact of Cryptosporidiosis on Weight

The results of Table 1 show the impact of cryptosporidiosis on the weight of calves. It appears that calves weighing less than 39 kg are more infected (88.09%) than those weighing between 39-43 kg (37.5%) and those weighing more than 43 kg (36.99%). These results show very significant differences (χ^2=24.91; df=2; p<0.001).

2.4.2 Impact of the Disease on the BCS

Regarding the prevalence of calf cryptosporidiosis according to Body Condition Score (BCS), we observe that calves with an BCS between [0-1] are very significantly more infested (93.51%) than those with BCS=]1-3] (46.54%) and BCS=]3-5] (37.7%) (χ^2=22.5; df=2; P<0.001).

2.4.2 Impact of the Disease on the Hematocrit Level

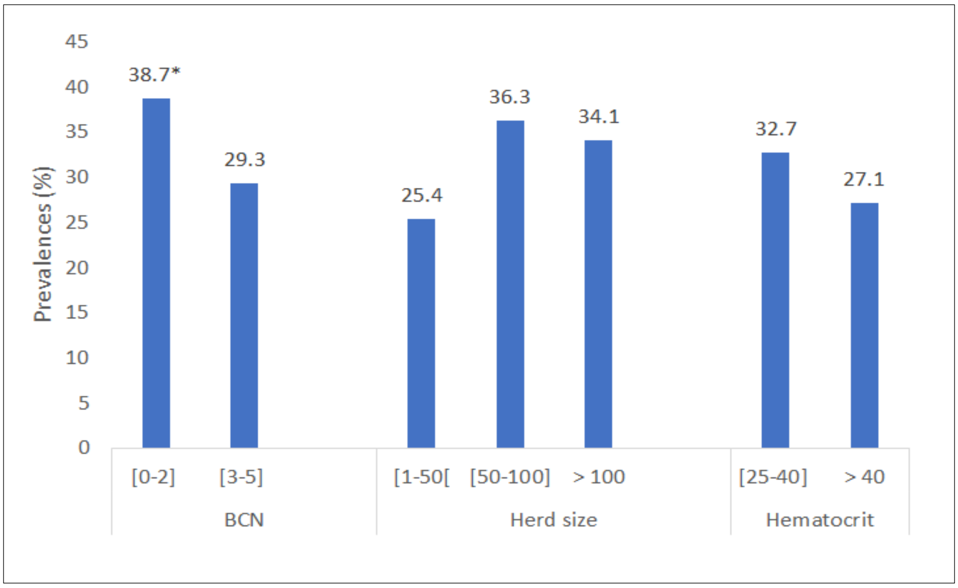

Figure 4: Distribution of the infection according to BCS, herd size, and hematocrit level
*Significant difference at the 5% level

Of the 405 calves studied, 211 (52%) exhibited normal red blood cell counts between [25-40], while 194 (48%) showed counts greater than 40% (Figure 4). However, these results do not show a significant difference (χ^2=0.37; df=1; p=0.541).

2.4.4 Impact of the Disease on the Diarrheal Status of Calves

The results of our investigations (Table 1) show that the infection rates are significantly higher in diarrheic calves (99.13%) than in non-diarrheic ones (53.79%) (χ^2=13.82; df=1; p<0.001).

2.5 Risk Factors

The multinomial logistic regression was performed on the factors that showed significant differences in order to determine the risk factors associated with the disease. It results those calves aged <3 months and [3-6] months are respectively 1.5 and 0.94 times more at risk of being infected by *Cryptosporidium* spp compared to those aged between [6-12] months (OR1=1.5; df=2; p=0.025; OR2=0.94; df=2; p=0.035). Calves with BCS = [0-2] are 1.6 times more at risk of being infected than those with BCS between [3-5] (OR=1.6; df=1; p=0.014). Calves from the Nyambaka district are 4.1 times more likely to be infected by the disease than those from other districts (OR=4.2; df=6; p=0.001). During the rainy season, animals are 2.25 times more likely to be infected than animals in the dry season (OR=2.25; CI=1.40-3.61; p=0.001).

Table 2: Logistic regression of disease risk factors

Variables		OR	IC	Pvalue
Ages(month)	[0-3]	1.5	0.75- 3.02	0.025
	[3-6]	0.94	0.52- 1.70	0.035
	[6-12]			
BCS	[0 ; 1]	4,6	0.89- 3.86	0.001
	[1 ; 3]	2.4	0.72- 2.70	
	[3 ; 5]			
Locality	Ngaoundere 1	1.54	0.61- 3.86	0.361
	Ngaoundere 2	2.13	0.88- 5.14	0.093
	Ngaoundere 3	2.21	0.91- 5.36	0.079
	Nyambaka	4.1	1.72- 9.77	0.001
	Mbe	1.9	0.73- 5.27	0.180
	Martap	1.72	0.67- 4.39	0.260
	Belel			

Variables		OR	IC	Pvalue
Seasons	Raining	2,25	1.40- 3.61	0.001
	Dry			
Diarrheal Statuts	Non-diarrheal	6.2	1.7-8.4	0.001
	Diarrheal			

Legend: OR=Odds ratio; CI (95%)=95% Confidence Interval

3. DISCUSSION

This study of cryptosporidiosis in calves in the Vina department revealed an overall prevalence of 66.67%. This prevalence is higher than those obtained by Ouakli (2018) in Algeria (65%), Sevinc *et al*., (2003) in Turkey (63.92%), Abeywardena *et al*., (2015) in Australia (62%), Aissatou (2022), and Siama *et al*., (2025) in the regions of the Far North (41.5%) and the North (44.55%) of Cameroon. However, this prevalence is lower than those reported by Tumwine *et al*., (2003) in Uganda (100%) and da Silva *et al*., (2019) in Brazil (81.78%). Factors such as the farming method, geographical area, hygiene level of the farms, and the diagnostic technique used could explain the observed differences (Feng *et al*., 2018).

The study of the influence of age showed that young calves under 3 months are more infected (48.1%) than those aged [3-6[months (29.9%) and [6; 12] months (29.6%). These results are similar to those of Aissatou (2022) and Siama *et al*., (2025) who reported higher prevalences in calves under 3 months in the Northern and Extreme-North regions of Cameroon (59.66%; 69.28% respectively). On the other hand, Khelef *et al*., (2007) obtained lower results in calves aged 2 to 3 weeks in the East and Central Algeria (39.6%). These differences show that calves under 3 months old are more susceptible to cryptosporidiosis due to their high sensitivity to cryptosporidial infection caused by the immaturity of their immune system. Indeed, once the barn is contaminated with cryptosporidia oocysts during the calving period, the calves easily become infested either indirectly through the external environment, by suckling their mother's soiled teats, or by contact with other calves. In calves older than 3 months, the decrease in the infection rate would be related to the maturity of their immune system because, although the infection is maintained, the animals do not show the disease (Cho *et al*., 2013).

Animals with BCS= [0-1] are more affected (93.51%) than those with BCS=]1-3] (46.54%) and BCS=]3-5] (37.7%). These results are similar to those obtained by Siama *et al*., (2025) in the Northern Cameroon region. Fikre *et al.,* (2017) and Yeman (2021) in Ethiopia also found higher prevalences in calves of BCS= [0-1] (30.4%; 41.68%). This difference could be explained by the fact that animals with BCS = [0; 1] are the most vulnerable and that cryptosporidiosis, which causes diarrhea, leads to weight loss and consequently to emaciation and a drop in body condition score (Cho *et al.,* 2014).

The infection of calves being higher during the rainy season (40%) than during the dry season (26.5%) was also observed by Tumwine *et al*., (2003) in Uganda (31.2% during the rainy season compared to 22.3% during the dry season), Morse *et al*., (2007) in Malawi (8.1% during the rainy season compared to 3.7% during the dry season). In France, the United Kingdom, and Ireland, the predominance of cases due to *C. parvum* is observed in late summer and autumn (Chalmers *et al*., 2006). This difference could be explained by the resistance capacity of Cryptosporidium spp oocysts and the favorable environmental conditions of the medium (temperature, humidity) (Toukmidine, 2021). Indeed, oocysts can survive up to 18 months in cold water at 4°C and in warm water at 15°C for up to 7 months, but only temperatures above 60°C or below -22°C render them inactive (Innes *et al*., 2020).

The study of the influence of the disease on the hematocrit level revealed normal red blood cell counts (25-40%) in both sick and healthy calves. These results show that cryptosporidiosis has no impact on the decrease of blood levels in the body. This confirms the observations of Toukmidine *et al*., (2021), which report that compared to giardias, cryptosporidia are not hematophagous and cannot directly cause anemia in infected animals.

Following the impact of parasitosis on weight, our study reports that calves weighing less than 39 kg are the most infected. Da Silva *et al*., (2019) in Brazil report that healthy animals have an average weight gain of 2.2 kg more than animals suffering from cryptosporidiosis, in whom a decrease in weight is noted.

The pathology causes diarrhea because 99.13% of the diarrheic calves are infected. These observations are similar to those of Das *et al*., (2018) in India, and Ehsan *et al*., (2019) in Belgium. This is because the disease clinically manifests as diarrhea in patients. The onset of diarrhea would be linked to the action of *C. parvum* oocysts that adhere to and destroy the epithelial cells of the intestinal microvilli, causing a reduction in absorptive activity that leads to diarrhea (Liu *et al*., 2014).

Among the risk factors associated with the disease are age, BCN, season, and locality. These results corroborate with those of Morin (2002), who classifies these risk factors into several categories. However, there are others that were not taken into account in this study, whose importance is not negligible, such as the state of resistance, low general hygiene level, maternity, calf housing, ambiance, calving period... (Garber *et al*., 1999; Eshan *et al*., 2019; Lefay *et al*., 2001). Calves under 3 months are 1.5 times more at risk of being infected by *Cryptosporidium* spp because they are more easily infected by suckling from the udder or by contact with contaminated bedding (Eshan *et al*., 2019; Lefay *et al*., 2000). Calves aged BCS =[0-2] are 1.6 times more at risk of being infected because, according to Morin (2002), malnutrition and/or undernutrition of the calf, concurrent infections, stress, and the health status of the mothers negatively affect the animal's weight and the newborn calf's resistance to disease. The observations that calve with diarrheal stools are 6.2 times more likely to manifest the disease align with those of Ehsan *et al*., (2019) in Belgium, where animals with diarrhea were at higher risk (OR= 6.1). Indeed, factors such as dystocia, sex, twinning, and prematurity can alter the resistance status of the newborn calf and promote the onset and severity of *C. parvum* diarrhea (Mohammed *et al*., 1999; Ola-Fadunsin *et al*., 2022). During the rainy season, animals are 2.25 times more likely to be infected than animals in the dry season because, in the rainy season, climatic conditions favor the dispersion, survival, and sporulation of oocysts and consequently increase the probability of calf infection (King and Monis, 2007; Ikiroma and Pollock, 2020). The calves in the Nyambaka district are 4.1 times more likely to be infected by the disease. These results could be explained by the existence of a particular distribution of cryptosporidiosis depending on the type of farming and environmental conditions (Feng *et al*., 2018).

4. CONCLUSION

From our study, it appears that cryptosporidiosis is indeed present in our study area and affects calves with an overall prevalence of 66.67%. This prevalence is higher in young calves under 3 months old, in diarrheic calves, with BCS ranging from 0 to 2. The disease is distributed in all localities with a high prevalence in the Nyambaka and Mbe districts, and is more significant during the rainy season than in the dry season. Following these results, it seems very important to develop preventive measures against this parasitosis, while awaiting the development of a treatment protocol or an effective vaccine. It should also be noted that prevention must also concern human health given the zoonotic nature of the disease and the very high pathogenicity of the causal agent. In perspective, it would be wise to evaluate the epidemiological factors of the disease by determining the prevalence and the level of oocyst shedding in adult animals.

STATEMENTS AND DECLARATIONS

Funding: No funding was received for conducting this study.

Competing Interests: This study was conducted without any conflict of interest.

Author Contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Augustin SIAMA, ABDOULKADIRI ISSIAKOU, and Alexandre Michel NJAN NLÔGA. The first draft of the manuscript was written by Augustin SIAMA and ABDOULKADIRI ISSIAKOU and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval: No applicable

Consent to Participate: No applicable

Consent to Publish: No applicable

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