

Cryptosporidiosis in Bovine Calf Diarrhea: prevalence and associated risk factors

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Abstract

Cryptosporidium, an enteric protozoan parasite has been documented across the globe for causing morbidity and mortality in bovine calves as well as variety of other animals and human beings. Previous studies documented Cryptosporidium as the second most common cause of neonatal calf diarrhea and fourth leading cause of death among the children below five years of age. Bovine calves are most important reservoir of this parasite. Risk factors are indispensable component of disease epidemics. Non-infectious risk factors are usually overlooked by livestock producers, although they are just as significant as infectious risk factors. The present study was designed with the aim to estimate the prevalence and evaluate the potential risk factors. The overall prevalence of Cryptosporidium observed in current study was 21.02% (148 /704) and was significantly associated with the calf age, colostrum feeding, faecal color and consistency, type of flooring, farm strength and educational status. The variables like sex, season, hygiene, and milking method did not display significant association with the *Cryptosporidium* spp. infection.

Key words: Cryptosporidium, Calf diarrhea, Prevalence and Risk factors

Introduction

Cryptosporidium an intracellular, extracytoplasmic, monoxenous, zoonotic, protozoan parasite has been reported globally to cause heavy morbidity and mortality in bovine calves as well as in animals of other species. The annual global load of *Cryptosporidium* oocysts in animal manure has been projected to be 3.2×10^{23} oocyst. Bovine calves are the largest contributor of it (Vermeulen et al. 2017). Not only animals but immunocompromised human beings are also the main target. Direct life cycle, self-propagation inside gastrointestinal tract, high resistance to most of the disinfectant and faeco-oral route of transmission and huge fecal shedding of oocysts resulted in its biomagnification. Occurrence of recent outbreaks both in animals and human beings have drawn much attention to this parasite (Brar et al. 2017; Liu et al. 2020; Zhang et al. 2022). However, the actual prevalence is grossly under-reported owing to subclinical form of prevailing infections and self-limiting nature of the disease in immunocompetent individuals. (Thomson et al. 2017). Diarrhea is a common symptom associated with *Cryptosporidium* infection. However, in the pathogenesis of diarrhea, besides pathogens, noninfectious risk factors are very crucial (Patel et al., 2016). These noninfectious risk factors may be associated with animal, surrounding environment or management practices (Kalwaghe et al., 2022). A comprehensive study to identify and assess such risk factors is essential requirement to formulate preventive strategies for eradication programme of this parasitic infection from a particular area. Based on these considerations, the present study was formulated with the objective to determine prevalence of *Cryptosporidium* spp. infection in bovine calves and evaluate potential risk factors.

Material and methods

Faecal samples were taken from diarrheic bovine calves (n=704) below six months of age from organized and unorganized farms in and around Ludhiana district of Punjab state, India after getting ethical approval (IAEC/2019/188-221). Ludhiana is located between 30° -33°, 31° -1' north latitudes and 75° -25' and 76° -27' east longitudes, its elevation is from 216 meters (west) to 268 meters (east). According to Köppen climate classification, Ludhiana lies in humid sub-tropical climate with three distinct seasons; summer, winter and monsoon. The climate is hot in summer (27.2°C to 40.6°C) and cold in winter (6.4°C to 19.5°C) with 51% average relative humidity. For the present study, ten hypothesized explanatory risk factors (table 1.0) were evaluated for *Cryptosporidium* spp. infection. These risk factors were further grouped as depicted in the table 01.

Diagnosis of *Cryptosporidium* done by faecal smear examination by mZN (modified Ziehl Neelsen staining), antigen detection by ELISA (Enzyme linked immuno sorbent assay) using Bio K 346/2 monoscreen *Cryptosporidium* ELISA kit (Bio-X Diagnostics, Belgium) and molecular diagnosis by PCR (Polymerase Chain Reaction) after extracting DNA from the faecal samples using the QIAamp FastDNA Stool Kit (Qiagen) according to the producer's protocol with slight modification (five cycles of freeze thaw reaction in initial step). Purity of DNA was measured by measuring the optical density by NanoDrop 2000 (Thermo Scientific). Eluted DNA was subjected to primary and nested set of PCR reactions using COWP (*Cryptosporidial* outer wall protein) gene primers (Primary PCR: forward primer 5'-GTAGATAATGGAAGAGATTGTG-3', reverse primer 5'-GGACTGAAATACAGGCATTATCTTG-3'; nested PCR forward primer 5'-TGTGTTCAATCAGACACAGC-3' reverse primer 5'-TCTGTATATCCTGGTGGGC-3') previously used by Yu et al., (2009). Forty cycles of primary and 35 cycles of nested PCR with annealing temperature 52°C and 60°C were applied respectively. Amplified products were subjected to gel (1.5%) electrophoresis and visualized under gel documentation system.

As there is no gold standard test for the diagnosis of this infection, the samples showed positive results by at least two of the above techniques were identified as true positive. The data of all the diarrheic animals (n=704) were statistically evaluated using SPSS (Statistical Package for the Social Sciences) software version 20.0. Percent prevalence was calculated for each group of risk factor. Chi square test was performed to know the association between *Cryptosporidium* spp. prevalence and possible risk factor. Furthermore, to estimate the strength of association & likelihood, logistic regression analysis was performed by computing odds ratios (OR) for associated risk factors. Fisher's exact test was employed to analyze the relationship between milking practices and cryptosporidiosis, as the assumptions for the Chi-square test were violated by low expected frequencies.

Results

The results obtained for percentage prevalence for each group of risk factor and association between prevalence and possible risk factor is mentioned in table 1, whereas their likelihood is mentioned in table 2. Among the 704 calves with diarrhea that were screened, 148 (21.02%) tested positive for *Cryptosporidium* spp. according to the established diagnostic criteria. Statistical analysis using the Chi square test revealed six risk factors (age group, herd strength, colostrum feeding, flooring, educational status, faecal color consistency) with a significant association ($p < 0.05$) with *Cryptosporidium* spp. infection in calves.

Table 1: Chi square analysis of risk variables for *Cryptosporidium spp.* infection.

Risk Factor	Category of risk factor	Samples examined	Positive results	Prevalence (%)	Chi square (χ^2)	P value
Age of animals	Group I (0-7 day)	221	47	21.3	16.373	0.006
	Group II (8-14 day)	312	82	26.3		
	Group III (15-21 day)	77	11	14.3		
	Group IV (22-30 day)	65	06	9.2		
	Group V (31-60 day)	25	02	8.0		
	Group VI (61-180 day)	04	00	0.0		
Season	Winter	179	31	17.3	3.048	0.384
	Summer	125	25	20.0		
	Monsoon	213	52	24.4		
	Post monsoon	187	40	21.4		
Herd strength	<25	66	04	6.1	10.740	0.005
	26-50	202	41	20.3		
	>50	436	103	23.6		
Sex	Male	369	86	23.3	2.435	0.119
	Female	335	62	18.5		
Colostrum feeding	Before 6 hour	657	132	20.1	5.142	0.023
	After 6 hour	47	16	34.0		
Flooring	Kacha	33	02	6.1	11.146	0.004
	Pakka	550	130	23.6		
	Mix	121	16	13.2		
Educational status	<8 th standard	541	125	23.1	6.104	0.013
	>8 th standard	163	23	14.1		
Hygiene	Clean	32	3	9.4	7.066	0.070
	Dirty (mild)	427	83	19.4		
	Dirty (moderate)	228	56	24.6		
	Dirty (severely)	17	06	35.3		
Milking practices	Machine milking	9	1	11.1	0.539	0.463
	Hand milking	695	147	21.2		
Faecal Color consistency	Porage like yellow	298	53	17.8	8.704	0.033
	Liquid watery	264	58	22.0		
	Liquid blood tinged	22	2	9.10		
	Liquid mucoid	120	35	29.2		

**Fisher exact test

Table 2: Odds ratio (OR) results for various risk factors of *Cryptosporidium spp.* infection.

Sr. No	Risk Factor	Category	OR	95% C.I. for OR	
				Lower	Upper
1.	Age group	30-60 days (V)	1.00		
		0-7 day (I)	3.242	.716	14.675
		8-14 day (II)	4.348	.975	19.386
		15-21 day (III)	2.222	.445	11.106
		22-30 day (IV)	1.168	.214	6.373
		60-180 day (VI)	.000	.000	.
2.	Herd strength	<25	1.00		
		26-50	2.769	.806	9.516
		>50	3.641	1.078	12.294
3.	Colostrum feeding	Before 6 hour	1.00		
		After 6 hour	2.153	1.088	4.262
4.	Flooring	Kacha	1.00		
		Pakka	3.070	.674	13.986
		Mix	1.687	.345	8.253
5.	Educational status	Below 8 th standard	1.00		
		8 th standard & above	1.363	.772	2.406
6.	Faecal Color consistency	Porage like yellow	1.00		
		Liquid watery	1.328	.858	2.058
		Liquid blood tinged	.324	.073	1.447
		Liquid mucoid	1.704	1.010	2.875

Discussion

Our prevalence (21.02%) fell within the global range (0.5–93%) and between Indian reports (2.81–39.65%) (Venu et al. 2012; Singh et al. 2019; Hatam-Nahavandi et al. 2019; Tarekegn et al. 2021). Prevalence estimates are influenced by single sampling (potentially underestimating true rates due to oocyst shedding variation), regional risk factors (calf age, management, hygiene), and diagnostic sensitivity (Ghoshal et al. 2018).

Bovine cryptosporidiosis prevalence consistently declines with age globally (Dahmani et al. 2020; Blanchard 2012; Joute et al. 2016; Kabir et al. 2020), attributed to neonatal immune immaturity and mortality risk. Our study aligns, with peak infection in the second week of life; absence before day three and lower first-week incidence reflect the pre-patent period (Blanchard 2012), while the week-two peak coincides with waning maternal gut antibodies (Caffarena et al. 2021). The rapid post-peak decline indicates acquired immunity and self-limiting infection, confirming cryptosporidiosis predominantly affects calves under 15 days via fecal-oral transmission. Thus, stringent pen hygiene and disinfection are vital to reduce calf disease burden and zoonotic risk.

Higher male prevalence (Hingole et al. 2017) aligns with some reports but contrasts with others showing female association (Díaz et al. 2018; Maurya et al. 2013) or no sex effect (Sharma and Joshi 2020); our finding may reflect lower male calf care. No seasonal influence was observed despite favorable warm/humid season reports (Maurya et al. 2013; Sharma & Joshi 2020; Brainard et al. 2020; Urie et al. 2018), likely due to the direct life cycle, thick oocyst wall, and self-infection.

Liquid mucoid samples had 1.70× higher odds of *Cryptosporidium* (possibly due to goblet cell-rich epithelium; Thomson et al. 2017), while bloody feces had lower odds (OR=0.324) and were positive with co-infections (*Clostridium perfringens*, *Eimeria*), suggesting blood was from other etiologies. Previous reports vary: watery samples showed higher odds (OR 3.59–11.19; Zhang et al. 2022) while Dessi et al. (2020) reported 10.9% in pasty samples.

Medium (20.3%, OR=2.8) and large herds (23.6%, OR=3.6) showed higher prevalence, consistent with most reports (Brainard et al. 2020) though some found highest in small farms (Abdullah et al. 2019); larger herds are worker-managed, likely reducing care. Concrete flooring typically reduces risk (OR 0.3–0.59; Brainard et al. 2020) but not universally (Weber et al. 2016), with discrepancies attributed to cleaning methods and slatted flooring (Brainard et al. 2020). However, higher odds on pakka floors in large farms with uneducated workers and poor hygiene (predominantly urban) explain the higher urban prevalence.

Delayed colostrum feeding (>6 hours) increased infection odds 2.15-fold, aligning with colostrum's protective effect on oocyst shedding and diarrhea (Brunauer et al. 2021), though evidence linking this specifically to antibody levels remains limited (Burton et al. 2011). As infection can occur by week 1, calf vaccination is impractical; vaccinating pregnant dams to confer neonatal immunity might be useful (Thomson et al. 2017). Despite higher infection in dirty animals, hygiene showed no significant association ($p>0.05$), which might be due to self-infection and the direct life cycle. Hand-milking farms had higher prevalence (21.2%) than machine-milking (11.1%), but no association was found (only 9/704 machine samples, 1 positive). No education study was conducted previously, though lower incidence among educated workers is plausible given better hygiene and awareness of zoonotic transmission.

Conclusion

This study confirms that *Cryptosporidium* is locally prevalent in dairy herds in and around Ludhiana. The presence of *Cryptosporidium* oocysts was significantly allied with clinical diarrhea. The risk variables age of calf, color and consistency of faeces, feeding of colostrum, floor type, educational status and farm strength are significantly associated while the parameters sex, season, hygiene and milking practices did not divulge any association with *Cryptosporidial* infection. The study clearly indicates that *Cryptosporidium* is mainly the problem of young calves especially younger than 15 days of larger herds. Non association with hygiene is concerning which demonstrates difficulty to control parasite spread. In conclusion, the current study highlights the need of implementing a practical intervention strategy that focuses on non-infectious risk factors.

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