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Epidemiological factors influencing in prevalence of *cryptosporidium* in buffalo calves of Haryana

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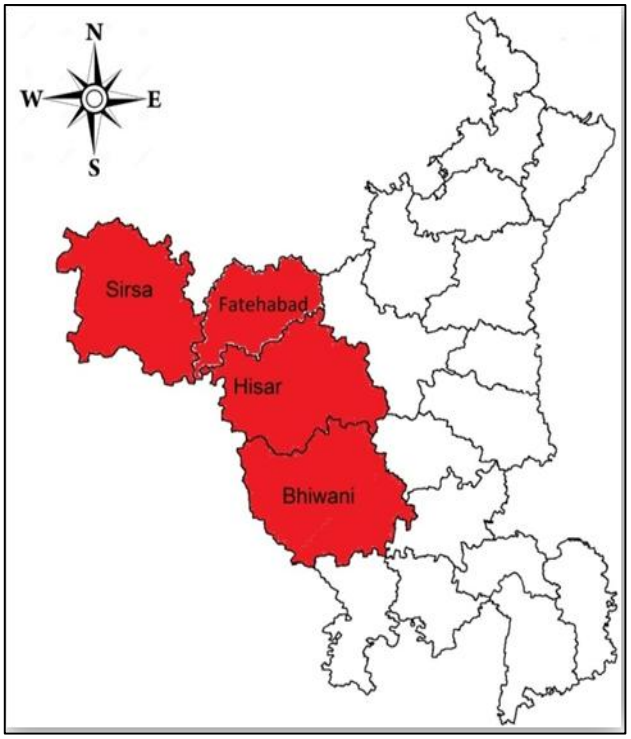
Abstract
The present study report the influence of epidemiological factors like age, sex, season, faecal consistency and rearing system on prevalence of *Cryptosporidium* species in buffalo calves below three month of age from Haryana state. A total of 402 faecal samples from the calves were stained by Modified Ziehl-Neelsen staining (MZN) method. Thirty five samples were found positive with prevalence percent of 8.7%. Age wise comparison showed a significantly high prevalence in calves between the age group of 16-30 days. Prevalence of cryptosporidiosis was higher during rainy (12%) season as compared to autumn (9.3%), winter (7.9%) and summer (6.8%) seasons. Depending on the consistency of dung, the highest prevalence was observed in mucus filled bloody samples. Female calves (10.17%) showed slightly higher prevalence rate than male animals(7.6%). In relation to rearing system, significantly ($p<0.05$) higher prevalence was observed in organized farms (26.7%) as compared to unorganized farms (6.44%). In conclusion, the prevalence of *Cryptosporidium* in dairy calves should be correlated with the factors like age, sex, season, dung consistency and rearing system of the animal to arrive at a reliable epidemiological data on bovine cryptosporidiosis in Haryana.

Keywords: *Cryptosporidium*, Buffalo calves, MZN staining, Prevalence, Haryana

Introduction
Cryptosporidium is an obligate protozoan parasite that commonly infects calves and other mammalian hosts. Cryptosporidiosis posses a significant problem in dairy calves where the prevalence of infection is high, with losses due to increased treatment costs and occasionally causes mortality. Cattle and buffaloes are the most important animal groups which are predominantly recognized to be infected with *Cryptosporidium* [2]. Calf diarrhoea associated with *Cryptosporidium* was for the first time reported by Nooruddin and Sarma (1987) in India and the first confirmed case of *C. parvum* was reported in Uttar Pradesh [5]. Cryptosporidiosis is characterized by acute gastrointestinal disturbances, mucoid or haemorrhagic watery diarrhoea, fever, lethargy, anorexia and loss of condition [14], leading to significant losses in farm animals [24]. Affected calves do not respond to antibiotic therapy and in more severe cases, dehydration and cardiovascular collapse occurs leading to mortality [17]. *Cryptosporidium* is progressively inviting attention as a zoonotic protozoan, largely due to its overriding involvement in worldwide waterborne outbreaks [8]. There is limited data on national prevalence of zoonotic *Cryptosporidium* spp. in dairy calves in India. In the context of the clinical importance, avoiding losses in the production and zoonotic potential of *Cryptosporidium*, more information about its prevalence is required. Therefore, there is an urgent need to conduct research on this aspect. In India, several studies have documented the prevalence of *Cryptosporidium* from different parts of the country based on microscopic detection of oocysts in faecal specimens. The present study was under taken to observe the actual status of cryptosporidiosis in buffalo calves of Haryana with special emphasis on different influencing factors like age, sex, season, management system, etc.

Materials and Methods
Sample Collection
To study the prevalence of cryptosporidiosis, a total of 402 faecal samples of buffalo calves aged less than 3 month were collected from four districts (Bhiwani, Fatehabad, Hisar and Sirsa) in and around Hisar, Haryana.
The faecal samples were collected directly from the rectum in a polythene pouch with a detailed history about their age group, breed and sex and then labeled with particulars of

individual animals on the container. Each sample was studied macroscopically to establish its consistency as liquid, soft or solid, and the presence of mucus or blood was also recorded. The fresh faecal samples were concentrated by formol-ether concentration technique and the sediment
was subjected to Modified Ziehl-Neelsen
staining technique. When immediate processing was not possible, the samples were put in 2.5% potassium dichromate ($K_2Cr_2O_7$) solution or 10% formalin and kept at 4^0C till processed.



Map 1: showing four districts of Haryana (coloured) where from faecal samples of buffalo calves were collected

Examination of Faecal Smears

The hot method of Modified Ziehl-Neelsen staining of faecal smears was used as per the procedure described by [16] with slight modifications.

Thin smears of faecal sediment were made on a clean, grease free glass slide and air dried.Then the smears were fixed transiently over a flame. The smears were then stained with a strong carbol fuchsin solution for 10 minutes. After pouring the stain, the slides were heated until steam appeared but boiling was avoided. Then an additional stain was poured if the slide was dried. After staining, the smears were washed in running tap water for 1-2 minutes. Then the slides were subsequently decolorized in 1% acid methanol for 30 seconds. Again the smears were washed in running tap water for 1-2 min and then, the smears were counterstained with 0.4% malachite green for 1 minute. The smears were finally washed in tap water, air-dried and examined under oil immersion (100X) for detection of *Cryptosporidium* oocysts.

Statistical Analysis

The statistical analysis of results was done using Z-test as described by Snedecor and Cochran (1968) [22]

Results and Discussion

Observation of *Cryptosporidium* oocyst

Cryptosporidium oocyst stained reddish pink on a pale green background (Fig1). The degree and proportion of staining varied with individual oocysts with internal structures taking up the stain to varying degrees. Colour of the background was dependant on exposure time of carbol fuchsin, Malachite green and decolorizer.

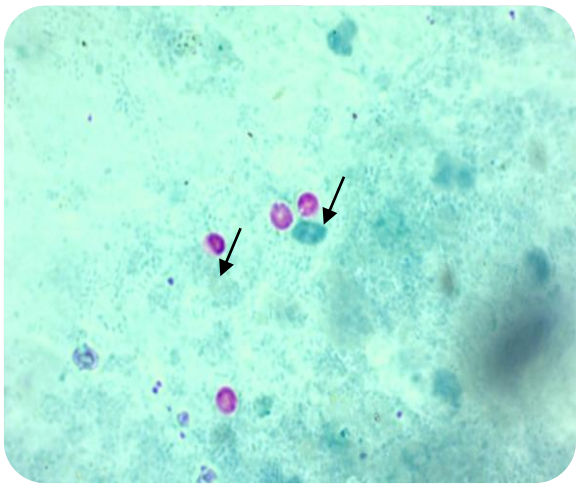


Fig 1: *Cryptosporidium* oocysts in faeces of buffalo calves stained by modified Ziehl-Neelsen method (100X).

Epidemiological factors related to prevalence of cryptosporidiosis

Out of 402faecal samples examined 8.7% (35/402) samples were found to be positive for *Cryptosporidium* oocyst.

Study of age wise prevalence revealed the highest infection rate in calves between 16-30 days (20.5 %) which declined with an increase in age to a minimum of 3.2% in 76-90days age group (Fig 2). Similar age related susceptibility of bovine calves to the *Cryptosporidium* infection has been reported from abroad [1, 6, 13, 20] and in India [3, 7, 9, 11, 18, 19, 21] The higher prevalence of infection in neonates can be attributed to the lower tolerance levels in young neonates due to poor development of acquired immunity. The difference was statistically significant (P≤0.05).

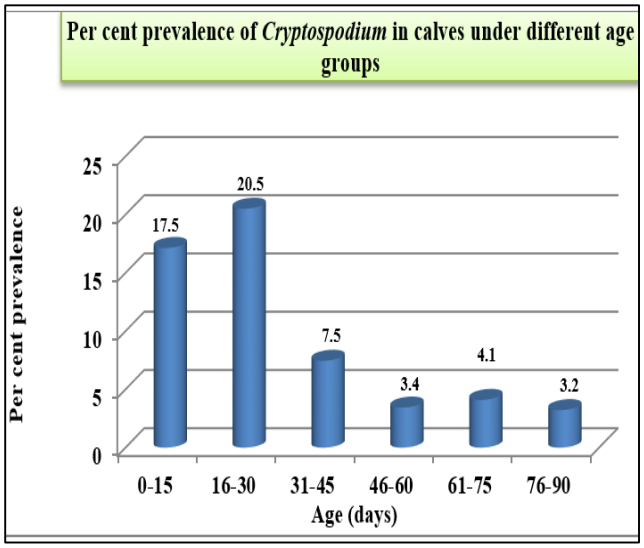


Fig 2: Prevalence of *Cryptosporidium* infection in buffalo calves below three month of age in different age group of Haryana

In terms of seasonal variation, the present study revealed that prevalence of cryptosporidiosis in buffalo calves was highest in rainy (12%) followed by autumn (9.3%), winter (7.9%) and

lowest in summer (6.8%) (Table 1). These findings are similar to those of Roy *et al*. (2006) [19], Paul *et al*. (2008) [18], Maurya *et al*. (2013) [11], Bhat *et al*. (2012) [3] from India and Chai*et al.* (2001) [4] from Korea who also reported highest prevalence during monsoon and lowest in summer. The higher prevalence of cryptosporidiosis during monsoon months can be attributed to the overcrowding of the animals in shelters in the free range farming system. However, Mohanty and Panda (2012) [12] reported highest prevalence in summer and lowest in winter at Odisha. One of the possible reasons might be due to change in the geographical conditions of the area as Odisha is having high humidity as compared to Haryana.

Table 1: Season related prevalence of *Cryptosporidium* in buffalo calves of Haryana.

Seasons	Total examined	Positive	Per cent prevalence
Rainy (June to September)	75	9	12.0
Autumn (October to November)	75	7	9.3
Winter (December to February)	135	11	7.9
Summer (March to May)	117	8	6.8
Total	402	35	8.7

As regards sex wise distribution, female calves showed relatively higher prevalence (10.2%) than male calves (7.6%) but the difference was non-significant (Table2). These findings are similar to the results of Mallinath *et al.* (2009) [10] and Bhat *et al.* (2012) [13]. However, Paul *et al*. (2008) [18] and Maurya *et al*. (2013) [11] found higher prevalence rates in males than in females but the difference was non-significant.

Table 2: Sex related prevalence of *Cryptosporidium* in buffalo calves of Haryana

Sex	Total examined	Positive	Per cent prevalence	Z-test
Male	235	18	7.6	
Female	167	17	10.2	0.887^{NS}
Total	402	35	8.7	

NS Non significant at p< 0.05

Prevalence of cryptosporidiosis was higher in the diarrhoeic calves (20%) as compared to the non-diarrhoeic (0%) thus indicating a relatively higher risk of the disease in diarrhoeic than in normal calves. However, diarrhoea was mostly bloody, mucoid and often foul smelling. Similar were the findings by Paul *et al*. (2008) [18], Mallinath *et al.* (2009) [10], Bhat *et al*. (2012) [3] and Maurya *et al*. (2013) [11].

The prevalence of the *Cryptosporidium* infection was also analyzed in two types of rearing systems. There was a considerable increase in the percentage of infection in organized farm (26.7%) as compared to unorganized farm (6.44%). The statistical analysis also revealed a significant difference between the two farming systems (P≤0.05)(Table 3).However, Venu *et al*. (2012) [23] reported lower prevalence of infection in farm animals as compared to individual animals in bovines of south Indian states but the difference was non-significant. The possible reason might be the difference in location, geographical factors or the management practices of the farm in comparison to our study area.

Table 3: Management related prevalence of *Cryptosporidium* in buffalo calves of Haryana

System	Total examined	Positive	Per cent prevalence	Z-test
Organized	45	12	26.7	
Unorganized	357	23	6.44	4.55*
Total	402	35	8.7	

* Significant at p<0.05

In conclusion, this is the first report on the influence of age, sex, season, dung consistency and rearing system on prevalence of *Cryptosporidium* infection in Haryana. The results obtained in the present study documents a sequential association of *Cryptosporidium* infection in buffalo as they increase in age thereby provides a guide for preventing, managing and tracking sources of *Cryptosporidium*. Clinically, relevant diagnosis of *Cryptosporidium* can be achieved by combining both conventional and molecular techniques with the different influencing factors of the affected animals. In the epidemiological studies of *Cryptosporidium*, factors like age, sex, season, dung consistency and rearing system needs to be included.

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