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Occurrence and Hematological alterations in cryptosporidiosis among newborn Calves

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Abstract
Cryptosporidium spp. is a causative agent of cryptosporidiosis in newborn calves, often resulting in signs such as diarrhea, dehydration, and in severe cases, mortality. *Cryptosporidium parvum*, a predominant species, is known to affect calves. This study was aimed to document the prevalence of cryptosporidiosis in newborn calves. Fecal and blood samples were collected from neonatal calves and adult animals, with modified acid-fast staining used to detect *Cryptosporidium* oocysts in fecal samples. Hematological parameters were analyzed in blood samples, and results were compared to those of healthy control animals. The study revealed a higher occurrence of cryptosporidiosis in diarrheic male calves, highlighting their increased vulnerability. Additionally, prevalence was more common in younger animals, particularly those aged 0-6 months. Notably, the winter season exhibited the highest incidence of cryptosporidiosis. Hematological analysis indicated significant reductions in red blood cell parameters and a concurrent increase in white blood cell count among affected animals.

Keywords: *Cryptosporidium parvum*, modified acid-fast staining, cryptosporidiosis, Anemia, Calves

Introduction
Cryptosporidium spp. causes cryptosporidiosis in newborn calves. Cryptosporidiosis can cause diarrhea, dehydration, and even death in extreme cases (Naciri *et al*., 1999) [4]. *Cryptosporidium parvum* is a leading cause of gastrointestinal disease in newborn and pre-weaned calves under the age of six weeks (Thomson *et al*., 2017) [9]. Eradicating *Cryptosporidium parvum* from the environment poses a significant challenge due to the resilient nature of its oocysts. These oocysts possess an exceptionally durable outer shell, allowing the parasite to persist in the environment for extended durations. Furthermore, they can endure a broad spectrum of temperatures, ranging from -22 °C to 60 °C (Robertson *et al*. 1992) [8]. Additionally, these oocysts exhibit resistance to the impact of numerous farm disinfectants that are commonly employed, (Casemore., 1990) [1]. When newborn calves have received enough colostral antibodies, are kept in warm and dry conditions, receive the necessary supportive care, and are not further infected by other gastrointestinal pathogens, the disease typically resolves on its own (Thomson *et al*., 2017) [9]. The present study was undertaken to record the occurrence of cryptosporidiosis in newborn calves in and around Hassan district Karnataka.

Materials and Methods
Fecal and blood samples were collected from both neonatal calves and adult animals ranging from day 1 to 5 years old. The fecal samples subjected to modified acid-fast staining to detect *Cryptosporidium* oocysts. A small amount of the fecal sample was taken on a clean microscope slide, and the sample was spread evenly across the slide to create a thin smear. Then the slide was passed gently through the flame of a Bunsen burner a few times to fix the sample onto the slide. Afterwards, a few drops of carbol fuchsin were applied to the slide to cover the entire smear. Slide was heated gently for 5–10 minutes using a Bunsen burner until steam started to rise. Later, the slide was rinsed with acid-alcohol decolorizer, and then the slide was rinsed with water to stop the decolorization process. A few drops of methylene blue were added to the smear, and after 1-2 minutes, the slide was rinsed with water to remove excess counterstain (Garcia, *et al*., 1983) [3]. The slide was allowed to air dry and examined under the microscope using oil immersion and identified small, spherical structures pink or red

color cyst after staining (Figure 1). The blood samples were analyzed in Mindray's BC-2300 Series Auto Hematology Analyzers to document any alterations hematological parameters in the affected animals. Additionally, blood samples were taken from animals showing no signs of illness and were utilized as control samples.

Results and Discussion

A total of 372 fecal samples were examined. Out of these, 94 samples were collected from animals displaying symptoms of diarrhea, while 278 samples were obtained from animals without diarrhea. Among the samples, 72 were collected from male animals, with the remaining 300 collected from female animals. Among the entire set of 372 samples, 76 samples tested positive for the presence of *Cryptosporidium* oocysts.

The occurrence of Cryptosporidiosis was notably more common in animals experiencing diarrhea, with 29 out of the 94 diarrheic animals testing positive for the infection (Table 1). In contrast, 47 out of the 278 non-diarrheic animals were found to have Cryptosporidiosis. Prem *et al*. 2013 [6] also observed higher occurrence of Cryptosporidiosis in diarrheic calves than non-diarrheic animals. Diarrheic calves often have compromised immune systems and *Cryptosporidium* is an opportunistic pathogen, this weakened immunity makes diarrheic calves more susceptible to cryptosporidiosis Diarrheic calves may shed more *Cryptosporidium* oocysts in their feces, increasing the likelihood of transmission to other calves through contamination of the environment.

Furthermore, the occurrence of Cryptosporidiosis was more prevalent in younger animals compared to adult animals (Emily, *et al*., 2008) [2]. Specifically, a higher occurrence of the infection was observed in calves aged between 0-6 months, with 43 out of 144 calves testing positive. The 7-12 month age group had 24 positive cases out of 101 animals, and the 1-5 year old animals had 9 positive cases out of 127 animals (Table 1). Neonatal calves, have immature immune systems that are still developing. Their immune defenses are not as robust as those of adult animals, making them more susceptible to Cryptosporidiosis.

The prevalence of Cryptosporidiosis was higher among male calves, with 19 out of 72 males testing positive for the infection, while 57 out of 300 female calves were found to be infected (Table 1). Interestingly, these findings differ from those reported by Prem *et al*. 2013 [6] study, where they noted a higher prevalence of Cryptosporidiosis among female calves. This disparity may be attributed to regional differences and variations in agricultural practices. The increased occurrence of Cryptosporidiosis among male calves in our study is associated with the transition to modern agricultural practices that no longer incorporate male calves into farming operations. Consequently, many farmers opt to cull male calves at an early stage, often without providing them with proper care. This practice results in insufficient colostrum intake for newborn male calves, thereby increasing their vulnerability to *Cryptosporidium* infection.

The occurrence of Cryptosporidiosis varied with the seasons, with the highest incidence observed during the winter season, followed by the summer, rainy, and autumn seasons in that order (Table 2). Reza and Roohollah., 2017 [7] also observed higher occurrence of Cryptosporidiosis in winter season. In the winter season, animals are frequently kept indoors to protect them from severe weather conditions. Additionally, the winter months align with calving seasons, resulting in increased calf births. This combination of factors, including close confinement and a higher number of calves, elevates the risk of *Cryptosporidium* infection during the winter months.

During the hematological examination, significant reductions were observed in several blood parameters compared to the control group animals (Table 3). Specifically, there were notable decreases in PCV to 18.76%, Hb levels to 6.92 g%, RBC count to 8.161 X 10^6/µl, MCV to 34.67 Fl, MCH to 3.3 Pg, and MCHC to 44.37%. Conversely, an increase in WBC count was observed, rising to 11.75 X 10^3/µl. Similar observation were made by Orazali *et al*. 2020 in their study. These findings suggest significant alterations in the blood composition of the animals affected by Cryptosporidiosis as compared to the control group. Cryptosporidiosis can cause damage to the lining of the intestines, leading to malabsorption of nutrients, including iron and vitamin B12, which are essential for red blood cell production. This can result in a decreased number of RBCs and lower levels of hemoglobin (Hb), leading to anemia.

Conclusion

Cryptosporidiosis is more common in diarrhoeic young male calves than non diarrhoeic, female, adult animals. Cryptosporidiosis is commonly encountered in winter season and leads to anemia in affected animals.

Table 1: Based on age and Gender-wise detection of Cryptosporidiosis

	Calves with Diarrhea				Calves without Diarrhea			
	Male		Female		Male		Female	
	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
0-6 M	12	5	24	14	26	7	82	17
7-12 M	9	2	18	6	16	4	58	12
1-5 YR	3	0	28	2	6	1	90	6
Total	24	7	70	22	48	12	230	35

Table 2: Seasonal occurrence of Cryptosporidiosis

Seasons	Total ()
Winter (DEC- FEB)	29
Summer (MARCH-MAY)	20
Rainy (JUNE-AUGUST)	14
Autumn (SEP-NOV)	13

Table 3: Hematological Changes in calves suffering with Cryptosporidiosis

Parameters	PCV %	Hb g%	RBC 10^6/ul	WBC 10^3/ul	MCV Fl	MCH Pg	MCHC %
Affected Calves	18.76	6.92	8.161	11.75	34.67	3.3	44.37
Healthy Calves	23.07	8.472	10.686	8.364	23.207	13.023	33.09

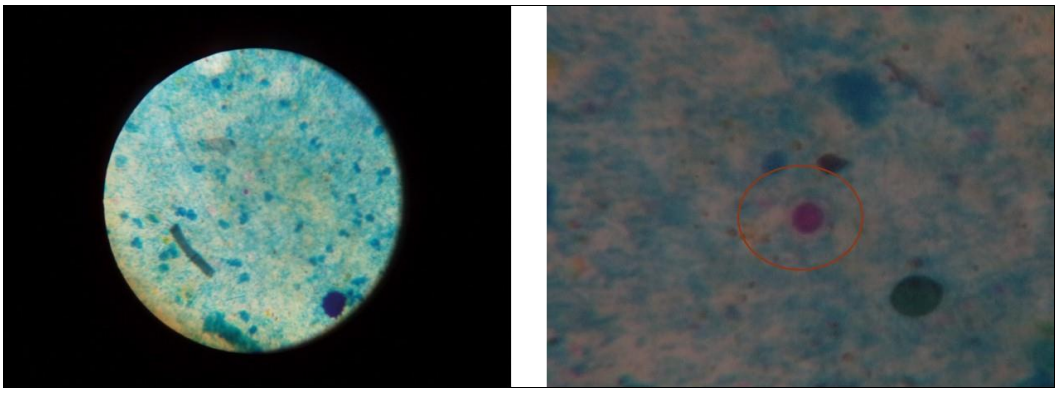

Fig 1: *Cryptosporidium* oocysts

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