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Cryptosporiodiosis in neonatal dairy calves

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ABSTRACT

An overall prevalence of 37.75 % was recorded for bovine cryptosporidiosis on coprological examination of 98 faecal samples collected from neonatal diarrhoeic and non-diarrhoeic cattle calves from an organized dairy farm in Tirupati by modified Ziehl–Neelsen staining. Further, a gradual decline in the percent prevalence was seen with increase in the age of the host from <1 month (52.45 %) to 2–5 months (13.51 %). Further, prevalence of cryptosporidiosis was significantly higher ($p < 0.05$) in the diarrhoeic calves (78.57 %) as compared to the non-diarrhoeic calves (21.42 %). It is clearly indicating a relatively higher risk of the disease in diarrhoeic than normal calves.

Keywords: Cryptosporidium oocysts, Modified Ziehl-Neelsen staining, calves.

INTRODUCTION

Diarrhoea is one of the most important cause of morbidity and mortalility in neonatal peroid of calves [1]. *Cryptosporidium*, is an obligate intracellular zoonotic protozoan parasite that is recognized as a major cause of diarrhoea in man and animal, particularly in immunocompromised hosts. In few cases it causes self limiting watery diarrhoea in immune competent hosts, but has par more devastating effects on immune compromised individuals. In some cases the infection can be life threatening dehydration caused by chronic diarrhoea [2]. *Cryptosporidium parvum* and *Cryptosporidium andersoni* have been identified as cosmopolitan species infecting cattle [3]. Calves with 1-3 weeks of age are highly susceptible but it has also been recorded adult animals above 2 years of age [4]. Cryptosporidiosis in cattle has been reported from different parts of the India in recent years [5,6]. The present communication, report of cryptosporidiosis in neonatal diarrhoeic and non-diarrhoeic calves in an organized dairy in Andhra Pradesh.

MATERIALS AND METHODS

A dairy with 98 cross breed calves in Tirupati had a problem of diarrhoea associated with morbidity. Upon examination of 28 calves with diarrhea showed varying degree of dehydration, shrunken eyeballs, emaciation, matted hair and soiling of perineum. Ninety eight (98) faecal samples were collected into plastic containers directly from the rectum of calves of both diarrhoeic (28) and non- diarrhoeic (70) calves. Faecal samples were diluted with 1:5 normal saline and sieved through strainer for removal of coarser debris. The samples were centrifuged after sieving. A portion of sediment was examined under cover slip floatation by using saturated sugar solution by bright field microscopy (40X). The smears of faeces were made on glass slides and dried in room temperature and stained by modified Ziehl-Neelsen staining method, as described by Henriksen and Pohlenz [7]. Briefly, the air dried smears were fixed in methanol for 5 min and the smears were then flooded with strong Carbol Fuchsin solution (Himedia, Mumbai) for 15 min. The smears were washed in running tap water (5min). Then slides were decolorized in 1% sulphuric acid for 30 sec. The smears were washed in running tap water (5min). Following washing, the slides were counterstained with 0.4% malachite green (S.D. fine Chem. Ltd, Mumbai) for 30 sec. The smears were finally washed in running tap water (5min), air dried and examined microscopically under oil immersion objective of

compound microscope. The length and width of the oocysts were measured by using stage micrometer and ocular micrometer under oil immersion objective of compound microscope. Data pertaining to the prevalence of cryptosporidiosis in different age group of animals were analyzed by Chi-square test using SPSS 17.0 software.

RESULTS AND DISCUSSION

In faecal smears stained by modified Ziehl-Neelsen staining method, oocysts of *Cryptosporidium sp*. appeared as densely stained red bodies clearly distinguishable against a green back ground (Fig-1). The size of the oocysts varied from 2.7-3.2 µm and varying number of dark blue or brownish internal bodies were visible inside them. The sugar floatation technique detected *Cryptosporidium* (Fig-2) when the number of organisms was fairly numerous. The oocysts appear as retractile transparent bodies and yeasts appeared darker in colour. In the present study, 28 diarrhoeic and 70 non-diarrhoeic calves were examined to detect *Cryptosporidium* infection. Out of 28 diarrhoeic samples 22 were positive and Out of 70 non-diarrhoeic samples 15 were positive (Table1 and Table 2). However, infection was more numerous in the diarrhoeic calves. An overall prevalence of cryptosporidiosis in dairy is 37-75 %. Higher prevalence was noticed in calves with below one month age (52.4 %) compare with calves with 2–5 months age (13.5 %). Further, prevalence of cryptosporidiosis was significantly higher ($p < 0.05$) in the diarrhoeic calves (78.57 %) as compared to the non-diarrhoeic (21.42 %) thus indicating a relatively higher risk of the disease in diarrhoeic than normal calves.

Bovine Cryptosporidiosis is reported from all over the world and its prevalence mainly depends up on the management of farms and age of the calves. Prevalence increased when overcrowded, unhygienic housing and improper feeding regimens are practiced [8]. There are a variety of methods, including microscopy, immunological and molecular methods for detection of *Cryptosporidium* oocysts. Microscopic methods include concentration techniques and stainig of faecal smears [1]. Diagnosis of cryptosporidiosis in mammals is generally performed by oocysts detection in faecal samples. There are difficulties in distinguisghing *Cryptosporidium* ooocysts from all other small particles in faecal and environmental specimens such as yeasts, moulds, algae and plant debris by routine faecal examination techniques [9]. Modified Ziehl-Neelsen staining method is useful for oocysts, they appeared as pink to red spherical to ovoid bodies on a blue or purple background, stained smears are permanent and are able to store a long time before examination when the samples are very numbers [10]. The modified Ziehl-Neelsen staining method was considered as a reliable method of *Cryptosporidium* identification because it eliminated the problems of differential diagnosis related to the presence of yeasts [11]. In the present study, prevalence was higher in calves below one month age (52.4 %) than compare with 2-5 months (13 %) aged calves these findings were agreement with the Castro-hermida et al. [12] who recorded higher prevalence in newborn and suckling calves compare with other younger calves. Cryptosporidiosis was also identified in non-diarrhoeic calves (21.4 %) but, it is significantly lower than the diarrhoeic calves (78 %). Bjorkman *et al*. [13] suggested Cryptosporidium parvum may often be identified in non-diarrhoeic calves also.

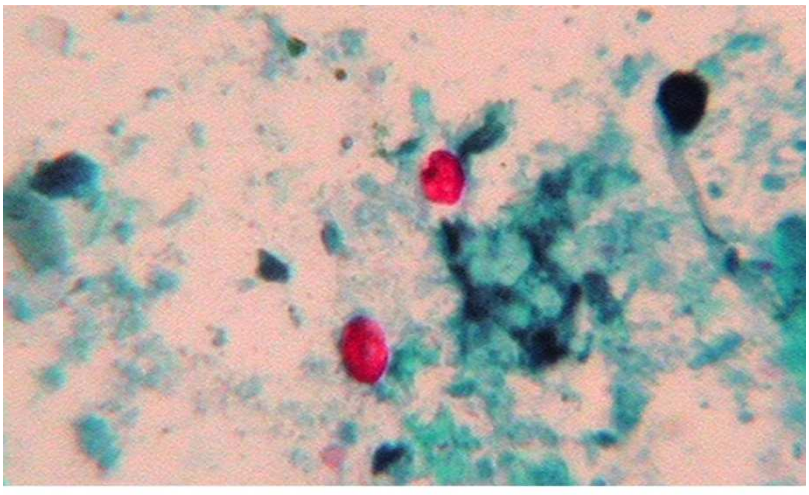

Fig.1: *Cryptosporidium oocyst* in mZN staining method (100X)

Table1and 2: Prevalence of Cryptosporidium oocysts in diarrhoeic and non-diarrhoeic calves

Age of the calves	No of animal examined	No of animal positive	Infection rate (%)
Calves within one month age	61	32	52.45 %
Calves with 2-5 months Age	37	5	13.51 %

Condition of the calves	No of animal examined	No of animal positive	Infection rate (%)
No of Diarrhoeic calves	28	22	78.57 %
No of Non-Diarrhoeic calves	70	15	21.42 %

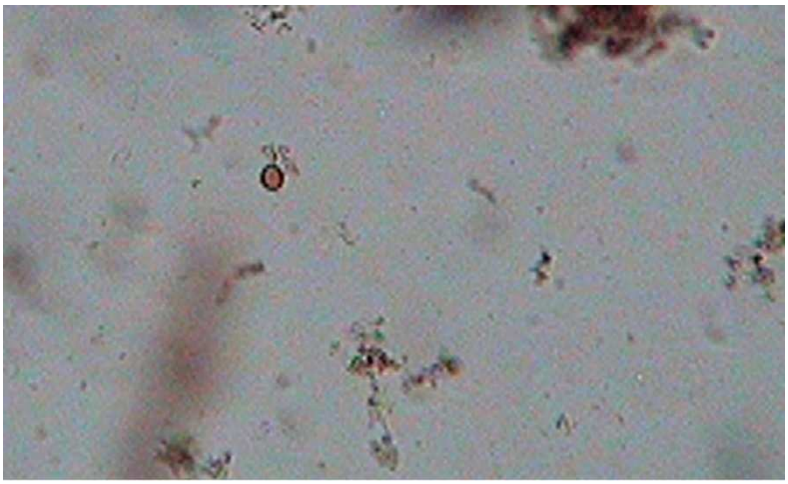

Fig.2: *Cryptosporidium oocyst* in floatation technique (40X)

CONCLUSION

Prevalence of cryptosporidiosis on coprological examination of faecal samples from neonatal diarrhoeic and non-diarrhoeic cattle calves was done by modified Ziehl–Neelsen staining. Prevalence was higher in below one month age calves. High risk was noticed in diarrhoeic calves than compared to the non-diarrhoeic calves. In conclusion overall prevalence of Cryptosporidiosis was recorded as 37.75 % in calves.

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