

CRYPTOSPORIDIOSIS IN BUFFALO CALVES

Archana Bharti*, Madhu Swamy and Amita Dubey

ABSTRACT

Study was conducted to determine the prevalence of naturally occurring *Cryptosporidium* spp. parasite in buffalo calves of Jabalpur region and the pathological effects produced by them contributing to calf mortality. 34.21% (13/38) *Cryptosporidium* oocyst prevalence were found in buffalo calves with Ziehl-Neelsen stain. Maximum 75% (03/04) positive cases were of diarrhoeic calves. More than 05 *Cryptosporidium* oocyst/smear (22 mm x 40 mm) were found in 5 faecal samples including 04 diarrhoeic/enteric and 01 non diarrhoeic animals. Maximum 58.33% (07/12) male and 23.07% (06/26) female were revealed the presence of *Cryptosporidium* spp. The maximum positive cases (06/13) were diagnosed in hot and humid weather. Carcass of buffalo calves found positive for *Cryptosporidium* spp. were dehydrated with soiled perianal region and anal dilatation. Small intestine appeared congested whereas ballooning was observed in the large intestine and mesenteric lymph nodes were uniformly enlarged.

Keyword: *Bubalus bubalis*, buffaloes, buffalo calf, *Cryptosporidium*, diarrhoea, parasite, Ziehl-Neelsen stain

INTRODUCTION

Cryptosporidiosis is the disease caused by the protozoan parasite *Cryptosporidium* spp. now recognised as endemic in buffalo calf worldwide and is one of the most important causes of neonatal enteritis in calves globally. *Cryptosporidium* is known as an opportunistic pathogen which can increase rapidly in numbers if calves are under stress due to environmental factors or other pathogen invasion and frequently cause calf scour as a mixed infection (Naag *et al.*, 2015).

Cryptosporidium parvum has been associated with neonatal calf diarrhoea. Calves become infected with cryptosporidium when they ingest *Cryptosporidium* oocysts (eggs). These oocysts reside in the environment in bedding, pasture, soil and drinking water. They are very infectious, with only ten oocysts required to cause disease in susceptible calves (Tzipori and Ward, 2002). A feature of *Cryptosporidium* spp. is its ability to multiply rapidly in the host leading to a potentially rapid spread of infection through a calf group or farm. An infected calf can spread billions of *Cryptosporidium* oocysts. Neonatal animals infected with *C. parvum* may suffer from profuse watery diarrhoea, inappetence, lethargy, dehydration and in some cases death can

occur (Sahinduran, 2012). Chronically infected or recovered young calves are responsible for enormous economic losses in terms of reduced growth rate, reduced milk yield, rejection of meat and edible offals, depreciation of hides, delayed age of maturity and high production cost due to the use of drugs thereby hampering the development of livestock industry (Maurya *et al.*, 2013). Study was carried out to detect the *Cryptosporidium* spp. in faecal sample of diarrhoeic, non diarrhoeic and dead calves, with enteric lesions of organised dairy farm in Jabalpur.

MATERIALS AND METHODS

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For parasitic examination 20 samples were collected from diarrhoeic and non diarrhoeic buffalo calves for a period of eight months. Diarrhoea was defined as an abnormally loose consistency of faeces and was noted along with the clinical signs present in the animals (anorexia, depression, weakness) and observations including the colour, consistency and smell of faeces were noted. Faecal contents were collected per rectal using sterilized gloves and samples were placed in sterile plastic container which were labelled and sealed properly for identification of animals.

During the study period of eight months total 18 calves were received for post-mortem examination. In all the eighteen calves received for post-mortem examination. The rectal content was collected for examination. Thus the total number of samples collected were 38 including diarrhoeic non diarrhoeic and dead calves.

Acid fast/Ziehl-Neelsen staining was used for identification of *Cryptosporidium* oocyst in faecal samples following the procedure of Chauhan (2006). Thin smear of faecal sample was prepared, air dried and fixed with methanol for 1 minute and also fixed with heat. The smear was stained with strong carbol fuschin for 40 minutes, washed in slow running tap water, destained with 10% H₂SO₄ and washed again with running tap water. Counter staining was done with methylene blue for 5 minutes. The slides was washed air dried and examined under 400X magnification.

RESULTS AND DISCUSSION

Total 38 samples were collected from live and dead calves of which 34.21% (13/38) were found positive for *Cryptosporidium* oocyst (Table 1, Figure 1). More than 05 *Cryptosporidium* oocyst/smear (22 mm x 40 mm) were found in 5 faecal samples including 04 diarrhoeic/enteric and 01 non diarrhoeic animals. Maximum 58.33% (07/12) male and 23.07% (06/26) female were revealed the presence of *Cryptosporidium* spp.

Singh *et al.* (2006) detected 50% and 25.68% *Cryptosporidium* oocysts from 80 diarrhoeic and 74 non-diarrhoeic animals, respectively. Díaz-Lee *et al.* (2011) examined 205 faecal samples, in which oocysts were detected in 115 (56.1%) with auramine (AU) and 102 (49.8%) with ZN. Whereas Rana *et al.* (2011) screened one hundred and forty two faecal samples and reported an overall prevalence of 3.52% for *Cryptosporidium* spp.

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However, clinical finding of diarrhoea was non significantly (NS) associated with *Cryptosporidium* spp. in faeces (Table 2).

The lack of significant association between diarrhoea and presence of *Cryptosporidium* spp. oocyst observed in this study supports a similar observation by Ayinmode and Fagbemi (2010) and Akinkuotu *et al.* (2014). The workers attributed this to subclinical chronic phases of infection with

the parasite which may not have the clinical sign of diarrhoea in the affected calf.

Higher prevalences of *Cryptosporidium* spp. parasites were detected in warm and humid season i.e. 46.15% (06/13) as compared to hot and dry (minimum 25.00%) (Table 3). Rana *et al.* (2011) screened *Cryptosporidium* sp. with the highest infection during peak winters. It was suggested that seasonal variation of *Cryptosporium* spp. affect prevalence with geographical distribution and population size.

All three dead buffalo calves carcass positive for *Cryptosporidium* spp. grossly, appeared dehydrated with soiled perianal region and anal dilatation. Curdled milk was found in the abomasums. Necropsy showed petechiation of the abomasal mucosa and in the intestinal serosa and mesentery (Figure 2). Olson *et al.* (2004) have stated that the *Cryptosporidium* sp. infects the abomasal glands of feedlot and adult cattle. This could be the reason for the prominent abomasal lesions observed.

Small intestine appeared congested whereas ballooning was observed in the large intestine. The mesenteric lymph nodes were uniformly enlarged in all the cases. Similar observations were made by Panciera *et al.* (1971) and Pearson *et al.* (1982). Heine *et al.* (1984) also recorded that the *Cryptosporidium* organisms are most numerous in the lower small intestine causing villous atrophy leading to clinical cases of diarrhoea.

In conclusion, *Cryptosporidium* spp. parasites are highly prevalent in buffalo calves with or without clinical sign of diarrhoea in the study area. It is also recommended that the economic impact of zoologically important cryptosporidiosis on livestock production and molecular epidemiological studies in human population in Jabalpur should be established. In addition, it is necessary to assess the

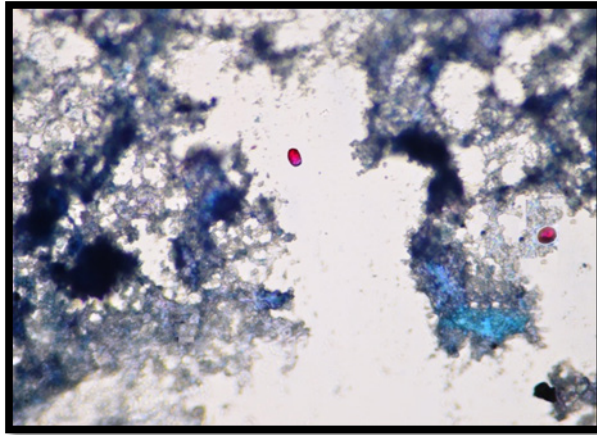


Figure 1. *Cryptosporidium* oocyst in faecal content of 3 months old buffalo calf. ZN X1000.



Figure 2. Viscera of buffalo calf infected with *Cryptosporidium* showing congestion in small intestine, ballooning of large intestine and petechial haemorrhages on abomasal mucosa. (Inset)

Table 1. *Cryptosporidium* oocyst in faecal samples of buffalo calves.

Animal	Category	Sample size	Positive cases	Percent positive
Buffalo calf	Diarrhoeic	04	03	75.00
	Non diarrhoeic	16	07	43.75
	Post-mortem	18	03	16.67
Total		38	13	34.21

Table 2. Association between diarrhoea and *Cryptosporidium*.

Cryptosporidium	Diarrhoeic/Enteric	Non diarrhoeic	Total
Positive	07	06	13
Negative	15	10	25
Total	22	16	38

$\chi^2 = 0.3129$ ^{NS}, Non significant at P<0.05

Table 3. Seasonal prevalence of *Cryptosporidium*.

Month	Calves	Positive	Percent
Hot and dry	12	03	25.00
Warm and humid	13	06	46.15
Cool and dry	13	04	30.76
Total	38	13	34.21

current control strategies to improve production.

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