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Research Paper

# *Cryptosporidium* infection in cattle of sub-tropical region of Assam, India

Veterinary Science

**KEYWORDS :** Cattle, Cryptosporidium sp., Assam

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## ABSTRACT

*The present study was carried out to determine the prevalence of Cryptosporidium infection in cattle of Guwahati, Assam. A total of 1698 faecal samples were collected from cattle and examined by using modified Ziehl-Neelsen (MZN) staining technique, nested PCR (polymerase chain reaction) and PCR-restriction fragment length polymorphism (RPLF). The overall prevalence of Cryptosporidium infections in cattle was 16.43% (28.41% in calves and 10.92% in adult). Cryptosporidium parvum and C. andersoni were recorded form calves and adult cattle, respectively. Infection was prevalent throughout the year with seasonal variations.*

*Summary:*

*A total of 1698 faecal samples were screened for detection of Cryptosporidium infection in different age groups of cattle by using modified Ziehl-Neelsen (MZN) staining technique, nested PCR (polymerase chain reaction) and PCR-restriction fragment length polymorphism (RPLF). Examination of faecal samples revealed an overall prevalence of 16.43% infections in cattle of Guwahati, Assam. Age-wise, 28.41% and 10.92% infections were recorded in calves (< 1 month) and adult, respectively. Season-wise infection was recorded highest during monsoon season (27.88%) followed by pre-monsoon (20.14%), post-monsoon (8.38%) and winter (3.29%) season. Cryptosporidium positive faecal samples were subjected to nested PCR and PCR-RPLF which confirmed presence of two species of Cryptosporidium, viz. C. parvum and C. andersoni.*

## Introduction

*Cryptosporidium*, an ubiquitous intracellular extra-cytoplasmic apicomplexan protozoan parasites known to have multiple hosts such as humans, domestic animals, wild animals, birds, rodents and reptiles. Ernest Edward Tyzzer first described the *Cryptosporidium muris* in the gastric glands of laboratory mice Tyzzer (1907) and in cattle, *Cryptosporidium* infection was first reported in the early 1970's (Panciera *et al.*,1971 and Meuten *et al.*,1974). However, because of the association with other viral or bacterial enteropathogens, the role of *Cryptosporidium* sp. as primary enteropathogens was uncertain until 1980, when Tzipori *et al.* (1980) attributed an outbreak of neonatal diarrhoea due to cryptosporidial infection alone. Bovine cryptosporidiosis is a common disease affecting newborn calves and characterized by acute gastrointestinal disturbances, mucoid or haemorrhagic watery diarrhoea, fever, lethargy, anorexia and loss of condition leading to significant economic losses in farm animals Del Coco *et al.*( 2009) and neonatal morbidity in cattle, resulting in weight loss and delayed growth McDonald (2000). With the attainment of immunological maturity, infection subsides in the older cattle. Affected animals upon recovery become carriers and hence act as source of infection to the susceptible individuals.

## Materials and methods

### Study area and sample collection:

The present study was carried out in Guwahati, Assam lies within the latitude of 26°11′0″ N and longitude 91°44′0″ E. The city is situated on an undulating plain with varying altitudes of 49.5m to 55.5m above mean sea level.

A total of 1698 faecal samples were collected and screened for detection of *Cryptosporidium* infection in different age groups of cattle (calves and adult) for one calendar year from August 2012 to July 2013. Faecal samples were collected directly from the rectum of individual animal and kept in marked plastic pouch/ vials containing 2.5% potassium dichromate solution (1:1 ratio). The study period was divided into four seasons *viz.* Pre-monsoon (March, April, May), Monsoon (June, July, August, September), Post-monsoon (October, November) and Winter (December, January, February).

**Parasitological investigation:** Faecal samples of cattle were examined at first by Sheather's sucrose floatation method for detection and concentration of *Cryptosporidium* oocysts as per the procedure described by Barr (1998). The positive sample was then subjected to modified Ziehl-Neelsen staining technique Henriksen and Pohlenz (1981). The air dried faecal smear was fixed in absolute methanol for 5 mins, air dried and then transiently passed over flame. Staining of faecal smear was done with strong carbol fuchsin solution for 20 mins. After staining, slide was rinsed thoroughly under running tap water. Then it was decolorized with acid alcohol (1%) for 10-15 secs and rinsed thoroughly in tap water. This was followed by counter staining with Malachite green (5%) for 5 mins. Finally, slide was rinsed thoroughly in tap water, air dried and examined microscopically under high power (400X) and oil immersion (1000X) for detection of *Cryptosporidium* oocysts.

Faecal samples which were found positive for Cryptosporidiosis (279) by modified Ziehl-Neelsen staining technique are subsequently processed for confirmation using nested PCR (Xiao *et al.*, 1999) and PCR-RFLP (Feng *et al.*, 2007).

**Statistical analysis:** Data were statistically analyzed by Chi-square tests for significance using SPSS 15 version.

## Results and Discussion

The overall prevalence of cryptosporidiosis in cattle was 16.43% as shown in **Table-1**.The infection was 28.41% and 10.92% in calves (<1 month) and adult cattle, respectively. Chi-Square test revealed highly significant difference ($P<0.01$) in the pattern of prevalence according to age. In Sheather's sucrose floatation, the oocysts appeared as round or oval, refractile bodies with a thin cytoplasmic membrane. However, in modified Ziehl-Neelsen staining, the oocysts appear as spherical to ellipsoidal shaped pink to red stained bodies containing four sporozoites against a pale green background as shown in **Figure-1**.

Present findings were in accordance with the report of Dubey *et al.*(1992) and Jeyabal and Ray (2005) who recorded 17.70% and 35.50% *Cryptosporidium* infection in bovine calves, respectively from Izatnagar, UP by staining faecal smears with modified acid

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fast stain. Decreased infection along with increased age might be ascribed to immunological competence of the host strengthened with increased age and thereby suppressing the infection to a latent stage.

The faecal samples found positive by acid fast staining technique was further confirmed by molecular techniques i.e. nested PCR and PCR-RFLP. Amplification of 18S SSU rRNA gene of *Cryptosporidium* by nested-PCR showed the clear 1325 bp band in the primary PCR and 845 bp band in the secondary PCR in positive samples. RFLP analysis of nested PCR (845 bp) products using *Ssp*I and *Vsp*I revealed presence of *C. parvum* and *C. andersoni* in calves and adult animals, respectively. Out of 279 faecal samples of cattle, 152 faecal samples from calves and 127 from adults were found to be positive for *C. parvum* and *C. andersoni*, respectively. Present findings of RFLP analysis is in accordance with the reports of Radostits *et al.*(2000) for presence of *C. parvum* in cattle by using *Ssp*I and *Vsp*I restriction enzymes. Similar observations were also made by Paul *et al.*(2009) and Feng *et al.*(2007) for *C. andersoni* in cattle with the use of *Ssp*I and *Vsp*I restriction enzymes.

An age related distribution of *Cryptosporidium* sp. has been observed by Xiao and Feng (2008) and they reported that preweaned calves are major source of *C. parvum* while on the other hand, Santin *et al.*(2004) observed *C. andersoni* in calves older than 12 weeks of age. *C. parvum* infects the intestine of young calves, humans and other animals resulting acute enteritis and diarrhoea while *C. andersoni* is associated with reduced milk production due to infection of the abomasums of adult cattle and is neither associated with clinical signs nor known to infect animals other than cattle. It was observed that, the infection was more in diarrhoeic animals (81%) than the non-diarrhoeic (18.99%) ones. The result corroborate the previous findings ranging from 35-50% infection in diarrhoeic neonatal calves with gradual reduction in aged calves has been reported in India (Roy *et al.*,2006 and Singh *et al.*,2006). The shedding of oocysts of *Cryptosporidium* by clinically asymptomatic calves (non-diarrhoeic) indicated a carrier status of cattle to serve as reservoir of infection and transmit the infection to neonatal calves.

Season-wise infection was recorded highest during monsoon season (27.88%) followed by pre-monsoon (20.14%) and post-monsoon (8.38%). Comparatively lower infection rate (3.29%) was recorded during winter season as shown in **Table-2**. Chi-Square test revealed highly significant difference (P<0.01) in the prevalence of infection according to season and sample consistency. In calves, season-wise highest infection was recorded during monsoon (45.27%) followed by pre-monsoon (37.83%), post-monsoon (13.13%) and winter (4.83%). However, in adult maximum infection was recorded during monsoon (18.23%) followed by pre-monsoon (13.62%), post-monsoon (6.03%) and winter (2.65%). High temperature and humidity along with frequent rains in the monsoon season enabled the transmission of oocysts faster. Thus, the warm and humid monsoon season was observed to be the most amicable season for the propagation of the disease than other seasons and these results of the present study are in congruent with Paul *et al.* (2008) who recorded 37.30% prevalence of cryptosporidiosis during monsoon followed by pre-monsoon (25.60%) and post-monsoon (19.60%) seasons.

**Conclusion**

The present study indicates that there is prevalence of *C. parvum* and *C. andersoni* in calves and adult cattle of Guwahati, Assam respectively. *C. parvum* is zoonotic and has public health importance.

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TABLE 1: PREVALENCE OF *Cryptosporidium* IN CATTLE

| Sample Screened | Number Positive | Sample consistency | |
|---|---|---|---|
| | | Diarrhoeic | Non-Diarrhoeic |
| Calves (535) | 152 (28.41) | 124 | 28 |
| Adult (1163) | 127 (10.92) | 102 | 25 |
| Overall | 279 (16.43) | 226 (81.00) | 53 (18.99) |
| $\chi^2$ value, df 2 | 226.30** | | |

**P (<0.01)

**Figures in parentheses indicates percent positivity**

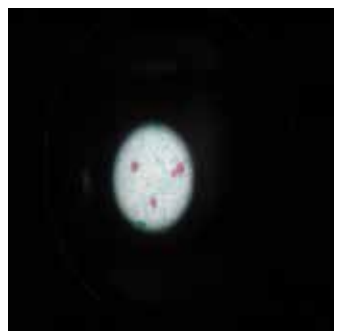

**Figure 1: *Cryptosporidium* oocysts in modified Ziehl-Neelsen stain (1000X)**

**TABLE 2: SEASONAL PREVALENCE OF *Cryptosporidium* IN DIFFERENT AGE GROUPS OF CATTLE**

| Season | Sample Screened | Number Positive | Sample consistency | |
|---|---|---|---|---|
| | | | Diarrhoeic | Non-Diarrhoeic |
| Pre-monsoon | Calves (111) | 42 (37.83) | 33 | 9 |
| | Adult (301) | 41 (13.62) | 27 | 14 |
| | 412 | 83 (20.14) | 60 (72.28) | 23 (27.71) |
| Monsoon | Calves (201) | 91 (45.27) | 74 | 17 |
| | Adult (362) | 66 (18.23) | 57 | 9 |
| | 563 | 157 (27.88) | 131 (83.43) | 26 (16.56) |
| Post-monsoon | Calves (99) | 13 (13.13) | 12 | 1 |
| | Adult (199) | 12 (6.03) | 10 | 2 |
| | 298 | 25 (8.38) | 22 (88.00) | 3 (12.00) |
| Winter | Calves (124) | 6 (4.83) | 5 | 1 |
| | Adult (301) | 8 (2.65) | 8 | - |
| | 425 | 14 (3.29) | 13 (92.85) | 1 (7.14) |
| **$\chi^2$ value, df 6** | 119.05** | | 125.72** | |

****P (<0.01), - (Negative)**
**Figures in parentheses indicates percent positivity**

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