Short Communication
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Prevalence of *Cryptosporidium* Species Isolated from Calves from North 24 Parganas, South 24 Parganas and Nadia Districts of West Bengal

Rahul Bhanja [(1)], Jaita Biswas [(1)], Sayantani Dule [(1)], Soumitra Pandit [(1)], Ruma Jas, Abhijit Nandi [(1)], Rahul Barua[(2)], Shubhamitra Chaudhuri [(3)], Tapan Dutta [(4)], Sanjoy Datta [(5)], Kunal Batabyal [(6)], Surajit Baidya [(1)]*

[(1)] Department of Veterinary Parasitology, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, 37, K. B. Sarani, Kolkata, WB, [(2)] Department of Veterinary Public Health, WBUAFS, Kolkata, [(3)] Department of Veterinary Clinical Complex, WBUAFS, Kolkata, [(4)] Department of Veterinary Microbiology, CVSc & AH, CAU, Aizawl, [(5)] Department of Animal Genetics & Breeding, WBUAFS, Kolkata, [(6)] Department of Veterinary Microbiology, WBUAFS, Kolkata

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Abstract

The present work was carried out to study the morphology of *Cryptosporidium* oocysts recorded in diarrhoeic calves. A total of 148 faecal samples of diarrhoeic calves were screened for *Cryptosporidium* oocysts by modified Ziehl-Neelsen staining technique for morphological studies. Out of 148 samples, 31 (20.9%) samples were found positive for oocyst of *Cryptosporidium* species. The morphometric analysis revealed that the range of longitudinal diameter of the oocysts were 5.05±0.349-5.275±0.248 µm and the transverse diameter of the oocysts were 4.67±0.354-4.775±0.184 µm with a shape index (length/width) of 1.08-1.104.Based on oocyst dimension and shape index, it was assumed that ovoid and sub-spherical oocysts belonged to *Cryptosporidium parvum.*

Keywords: *Cryptosporidium* oocyst, Calves, Modified Ziehl-Neelsen, Morphometry

Cryptosporidium is a zoonotic apicomplexan intracellular obligatory parasite (Rossle and Latif, 2013). The first species of this genus was described by Tyzzer in 1907 and was named *Cryptosporidium muris*; two years later,the author described a similar species in mice and named *Cryptosporidium parvum* (Tyzzer, 1907). *Cryptosporidium parvum* oocysts are smaller than those of *C. muris* and typically infect the small intestine of the host. Cryptosporidiosis is a common protozoan infection in cattle, whereas bovine cryptosporidiosis is also an important source of human *Cryptosporidium* infection. It has enormous zoonotic importance because this parasite is transmitted through contaminated food and water and infects humans and animals (Feng et al., 2007; Helmy and Hafez, 2022). This parasite is ubiquitous in nature and not easily destroyed by ordinary disinfectants. *Cryptosporidium* has the potential for autoinfection, which makes it more fatal. The symptoms like persistent watery diarrhoea, vomition, nausea, abdominal cramps, etc., associated with *Cryptosporidium* spp. are self-limiting, but in immune-compromised individuals, symptoms can become more severe and may persist for months if left untreated. It also causes substantial economic loss in the livestock sector in terms of reduced milk production in dairy cows (Esteban and Anderson, 1995) and decreased weight gain in calves (Anderson, 1992; Innes et al., 2020). Herein, we report the prevalence of *Cryptosporidium* isolates obtained from diarrhoeic calves from three districts of West Bengal, India. For identification, thorough morphological and morphometric studies were also carried out.

Faecal Samples were collected from three districts, namely, South 24 Parganas, North 24 Parganas and Nadia of West Bengal, India. A total of 148 faecal samples were collected from calves under three months of age from organised and unorganised farms. Samples were collected directly from the rectum and stored in a sterile container without preservatives. After collection, faecal matters was stained immediately at the laboratory with modified Ziehl Neelsen's staining method as described by Henriksen and Pohlenz, 1981 with some modifications, for microscopic evaluation and the rest portion was preserved in 2.5% potassium dichromate solution at 4°C for further studies.

For morphometric study, oocysts in the stained faecal preparations were measured under the light microscope by the method described by Pellardy (1965). The micrometer was calibrated under a light microscope and it was found that 1 unit of ocular micrometer equals to 2.5µm.The transverse and longitudinal diameters of 50 oocysts were measured, and the shape index of each oocyst was calculated as follows.

$$\text{Shape index} = \frac{\text{Longitudinal diameter of the oocyst}}{\text{Transverse diameter of the oocyst}}$$

Faecal specimens were analysed for the presence of *Cryptosporidium* and examined by direct microscopy

with modified ZN staining at 400× magnifications. Identification was made on the basis of the oocystic dimensions of *Cryptosporidium* described by Upton and Current (1985), Lindsay et al. (2000) and Kumar et al. (2004).Based on morphology, the smaller, spherical to ovoid purple colour oocysts were found (Figure 1 A and B). Out of 148 samples, 31 (20.9%) samples were found positive for oocyst of *Cryptosporidium* species.

The morphometric studies (Figure 2) revealed that the range of longitudinal diameter of the oocysts was 5.05±0.349-5.275±0.248 µm and the transverse diameters of the oocysts were 4.67±0.354-4.775±0.184 µm with a shape index (length/width) of 1.08±0.0302-1.104±0.035 (Table 1).

Table: 1 Morphological analysis of *Cryptosporidium* oocyst based on micrometry:

Sample no: (n=10)	Longitudinal Diameter (LD): (mean±SD)	Transverse Diameter (TD): (mean±SD)	Shape Index (SI)=LD/TD (mean±SD)
1.	5.05±0.349	4.67±0.354	1.08±0.0302
2.	5.17±0.354	4.7±0.307	1.101±0.031
3.	5.125±0.428	4.77±0.389	1.073±0.027
4.	5.225±0.463	4.8±0.497	1.09±0.042
5.	5.275±0.248	4.775±0.184	1.104±0.035

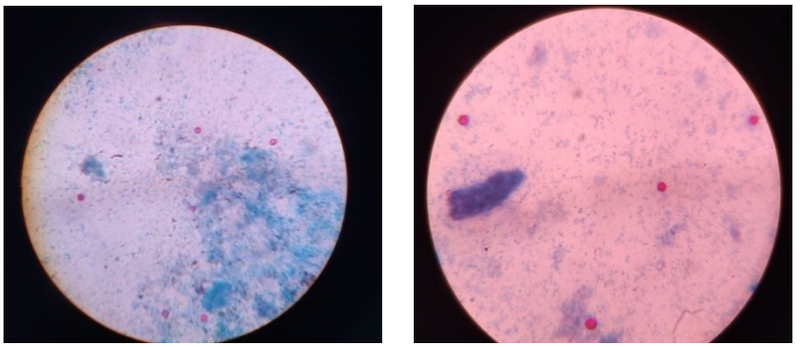

Figure 1: *Cryptosporidium* oocyst in modified Z-N staining under a light microscope. (A) Purple colour spherical oocyst of *Cryptosporidium* under 40X. (B) Purple colour spherical oocyst of *Cryptosporidium* under 100X.

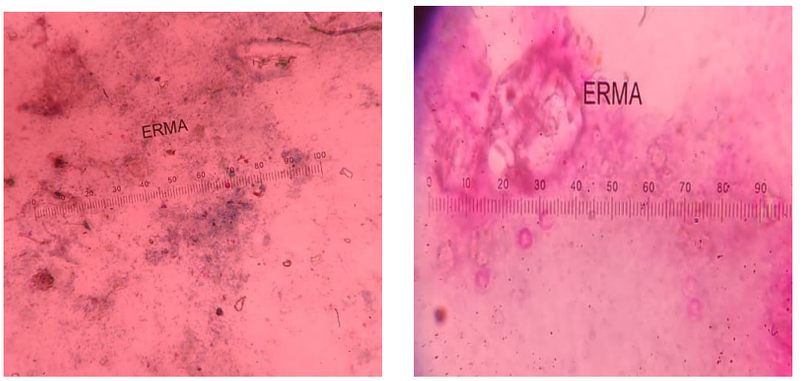

Figure 2: Micrometry of *Cryptosporidium* oocysts

Previously, morphometric studies revealed that the measuring size of *C. parvum* is 5.0 × 4.5 µm [range, (4.5–5.4) × (4.2–5.0) µm] (Tyzzer,1912; Upton and Current, 1985) and the shape indexwas1.16 (1.04- 1.33) (Tilley, 1991).Oocysts of *C. parvum* were also reported to range in size from 4.5 to 5.4 X 4.2 to 5.0 µm, with mean size of 5.0 X 4.5 µm and a shape index of 1.1 (Upton and Current, 1985), from 4.7 to 6.0 X 4.4 to 5.0, with a mean size of 5.0 X 4.7 µm and a shape index of 1.06 (Fayer et al., 2001), and from 5.0 to 5.5µm X 3.7 to 5.0 µm with a mean size of 5.2 X 4.3 µm and a shape index of 1.20 (Fall et al., 2003).

Based on oocyst dimension and shape indices, it was assumed that ovoid and sub-spherical oocysts belonged to *C. parvum*, which was responsible for causing undiagnosed diarrhoea as well as malnutrition, and decreased weight gain in neonatal calves. However, further, confirmation based on molecular characteristics is required to confirm the multi-species nature of *Cryptosporidium.*

Conflict of interest:

Authors declare no conflict of interest for this investigational report.

Data availability:

All raw data and backup photography are preserved at the Department of Veterinary Parasitology, WBUAFS

Ethical statement:

Authors maintained all ethical concern during sample collection and do not require IAEC certificate as it's not experimental.

Author's contribution:

All the authors equally participated in designing, data analysis and interpreting the results, drafting, editing the manuscript and approved the final version of the manuscript.

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***Corresponding author's email ID:** vetsurajit@gmail.com

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