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The Occurrence and Molecular Identification of *Buxtonella sulcata* Linked to Diarrhoea in Cattle from the Western Region of Uttar Pradesh, India

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ABSTRACT

Background: *Buxtonella sulcata* is recognized as an opportunistic parasite that impacts ruminants, especially with a notable prevalence of infections in cattle and buffaloes globally. This organism is a sizable cyst-forming ciliate that shows morphological similarities to *Balantidium coli*, which is present in both humans and pigs. The aim of this research work was to assess the prevalence and confirm the presence of *B. sulcata* infection in cattle through molecular methods in the western region of Uttar Pradesh, India.

Methods: In total, 398 samples were taken from the rectum of both diarrhoeic and non-diarrhoeic animals in seven districts: Meerut, Ghaziabad, Moradabad, Rampur, Gautam Buddha Nagar, Saharanpur and Amroha, from July 2024 to June 2025. The field faecal samples were microscopically examined, revealing the presence of trophozoites and cysts, which were further confirmed by molecular techniques using the 18S rRNA gene.

Result: The overall prevalence detected was 33.41% via microscopy, while the PCR method recorded a higher positive rate of 35.92%, indicating that PCR is a more sensitive technique than microscopy. Among the seven districts, Saharanpur exhibited the highest prevalence through PCR at 43.33%, whereas Ghaziabad had the lowest at 27.27%. Through PCR, the positivity rate in diarrheic animals was significantly greater at 51.85% ($p<0.01$) than in non-diarrheic animals, which stood at 30%. When considering age, young animals (<3 years) showed a significantly higher prevalence through PCR (42.96%) compared to adults (>3 years) at 32.59% ($p=0.047$). Based on sex ($p=0.351$), no significant differences were noted, though males had marginally higher infection rates. This research emphasizes that *B. sulcata* infections are common in cattle within Western Uttar Pradesh and could opportunistically lead to diarrheal issues. For the effective prevention and control of *B. sulcata* infection in cattle, it is suggested to utilize upgraded diagnostic methods alongside refined management practices.

Key words: *Buxtonella sulcata,* Cattle, Microscopy, PCR, Prevalence.

INTRODUCTION

Buxtonella sulcata is a ciliated protozoan parasite that belongs to the family Pycnotrichidae within the genus *Buxtonella* Kalkal *et al.* (2024). It primarily infects the gastrointestinal tract of ruminants, especially cattle and buffaloes. This infection results in diarrhoea, weight loss and decreased productivity in affected animals, which ultimately leads to economic losses in the livestock sector (Patel *et al.*, 2019). An opportunistic protozoan parasite, it inhabits the alimentary canal of ruminants, notably in the “caecum and colon” across the globe (Huang *et al.*, 2014; Kumar *et al.*, 2017). Cattle act as the reservoir for this pathogen. Cattle are the reservoir for this pathogen. Its structure closely resembles that of *Balantidium coli* (*B. coli*), which is found in both humans and pigs. The life cycle of *B. sulcata* includes an intestinal cyst stage and a trophozoite stage. The life cycle consists of two main phases: the trophozoite stage, where the organism actively feeds and reproduces in the host’s large intestine and the cyst stage, which is the environmentally resistant form that is excreted in faeces. Upon reaching the small intestine post-ingestion, the cyst is triggered by the intestinal pH and proteases, subsequently releasing trophozoites that

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invade and settle in the colon wall, causing cytotoxic effects that result in diarrhoea (El-Ashram *et al.*, 2015).

In serious situations, extended diarrhoea can cause imbalances in electrolytes, metabolic acidosis and secondary bacterial infections, potentially deteriorating the animal's health status (Kumar *et al.*, 2020). The distribution of *B. sulcata* is influenced by geographic factors and seasonal shifts, showing higher infection rates during humid and monsoon periods that aid in the parasite's persistence and transmission (Patel *et al.*, 2019). A research study in Gujarat, India, revealed that 23.15% of Gir cattle and 16.18% of Jaffrabadi buffaloes were infected with *B. sulcata*, with a notable rise in cases during the rainy season (Patel *et al.*, 2019). Similarly, in Karnal, Haryana, a study reported 63.7% prevalence rate among buffaloes, with 83.33% of diarrheic animals testing positive for the parasite, highlighting its potential role in enteric diseases (Sharma *et al.*, 2020). *B. sulcata* infection can have a major impact on herd health, particularly in high-density farming systems where the rates of transmission are higher.

The diagnosis of *B. sulcata* infection primarily relies on the microscopic analysis of faecal samples to identify cysts and trophozoites. Common identification techniques include direct smear, sedimentation and flotation methods. Molecular techniques such as polymerase chain reaction (PCR) offer a more sensitive and specific diagnostic approach for confirming infections (Sharma *et al.*, 2021). This investigation intends to analyze the prevalence of *B. sulcata* infection in cattle groups within the Western Uttar Pradesh.

MATERIALS AND METHODS

The ongoing research took place in the Western area of Uttar Pradesh, celebrated for its lush alluvial plains, significant livestock density and subtropical climate. The epidemiological study was conducted from July 2024 to June 2025 in the locations of Meerut, Ghaziabad, Moradabad, Rampur, Gautam Buddha Nagar, Saharanpur and Amroha.

Sample collection

Around 5 grams of faecal samples were aseptically gathered from the rectum of each animal, placed in appropriately labelled plastic containers and transported to the laboratory for microscopy and molecular analysis. In total, 398 faecal samples were collected, comprising 60 from Meerut, 55 from Ghaziabad, 58 from Moradabad, 30 from Rampur, 102 from Gautam Buddha Nagar, 60 from Saharanpur and 33 from Amroha. During the sample collection, the age, diarrhoeic condition and sex of cattle was recorded. In the analysis of 398 samples, 108 were identified as diarrhoeic, compared to 270 that were non-diarrhoeic. In the course of sample collection, the cattle were sorted into two age groups: young (<3 years) and adults (>3 years). A total of 128 samples were gathered from young animals, while 270 from adult animals. Of the samples collected, 124 were male and 274 were female.

Microscopic examination of faecal samples

To screen for *B. sulcata* infection in cattle, the direct faecal wet smears method was employed. A small quantity of faecal material was applied to a clean, grease-free glass slide and then a few drops of tap water were added. A smear was then prepared and examined under the microscope (10X/40X). The identification of enteric protozoan trophozoites and cysts was based on their morphological characteristics as outlined by Soulsby (1982).

DNA extraction

The DNA was isolated by QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany), adhering to the manufacturer's guidelines with slight modifications, from feces. The isolated DNA was preserved at -20°C for subsequent use.

Polymerase chain reaction

Primers designed to amplify a partial segment of the 18S rRNA gene from *B. sulcata* yield a product size of 1047 bp. These primers were produced by BR Biochem Life Sciences Pvt. Ltd, based in New Delhi. The forward and reverse primers utilized are 5′CGCAAATCGCGATTTTGTC GCG-3′ and 5′AAATACATAGTCCCTCTAAGAAGTC3′, respectively, for *B. sulcata* (Pomajbikova *et al.*, 2013).

PCR was performed in an Applied Biosystems (United States). The reaction mixture was prepared a volume of 25 µl, containing 12.5 µl of master mix (BR Biochem), 1 µl each primer at a concentration of 10 pmol, 3 µl of DNA template and 7.5 µl of nuclease-free water. The initial denaturation done at 94°C for 10 minutes, followed by denaturation at 94°C for 1 minute, annealing at 59°C for 1 minute and extension at 72°C for 1 minute, repeated for 35 cycles and concluding with a final extension at 72°C for 5 minutes. To visualize the PCR products, 7 µl of the PCR end products and 9 µl of a 100 bp plus ladder (BR Biochem) were loaded onto a 1.25% agarose gel (Bio-Rad, Japan) and ethidium bromide (0.5 µg/ml from a 10 mg/ml stock solution). The gel was subjected to horizontal gel electrophoresis at 90V for 60 minutes (Bio-Rad, Japan). The resulting resolution pattern was analyzed and an image was captured using the Bio-print (Vilber, France).

Statistical analysis

Using SPSS software, the chi-square test was employed to analyze the data. A p-value below 0.05 suggests a statistically significant correlation between the two categorical variables (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Fig 1 depicts the morphological analysis and essential characteristics of *B. sulcata*, which are mainly seen in a cyst form that typically has a circular diameter between 60 and 68 mµ. Conversely, the trophozoites are generally ovoid and show a size range of about 84 to 127 mµ by 68 to 84 mµ, surrounded by numerous cilia and possess both micro and macronuclei, an anterior cytostome, along with several nutritional food vacuoles in the cytoplasm.

A sum of 398 faecal samples was gathered from seven districts within western Uttar Pradesh, India. Among these samples, 33.41% tested positive for *B. sulcata* through microscopic examination, while 35.92% were confirmed positive using the PCR method (Table 1). The PCR technique exhibited a higher rate of positive results than the microscopic examination, implying that the PCR method is more trustworthy. Earlier research has reported comparable prevalence rates, such as 30.15% in cattle from various areas in El-Minia Province, Egypt (El-Ashram *et al.*, 2015). In contrast, buffaloes in the Hisar district of Haryana, India exhibited a higher prevalence of 54.5% (Kalkal and Sangwan, 2020).

Microscopic analysis revealed that Saharanpur had the highest prevalence at 38.30%, followed closely by Moradabad at 37.90%, Rampur at 36.70%, Gautam Buddha Nagar at 36.27%, Meerut at 28.30%, Amroha at 27.30% and Ghaziabad with the lowest prevalence at 25.50%. Conversely, the PCR technique revealed that the highest prevalence of *B. sulcata* infection was in Saharanpur district at 43.33%, followed by Rampur at 40.00%, Moradabad at 39.65%, Gautam Buddha Nagar at 38.23%, Amroha at 30.30%, Meerut at 30.00% and the lowest in Ghaziabad at 27.27%. There was no variation among the districts in the chi-square test (Table 2). This implies that despite a noticeable numerical variation, the infection is distributed relatively uniformly throughout the districts that were examined. This uniformity may be a result of the largely similar agro-climatic conditions in Western Uttar Pradesh and the comparable agricultural practices in these regions. Bhanot *et al.* (2022) also reported the overall prevalence of *B. sulcata* in Haryana, with the highest occurrence noted in Rohtak (33.8%), followed by Bhiwani (29.4%) and Ambala (15.5%), whereas the lowest was found in Sirsa (6.09%) and Mahendergarh (3.71%).

Based on the analysis of faecal consistency from 398 samples, 108 were identified as diarrhoeic while 290 were

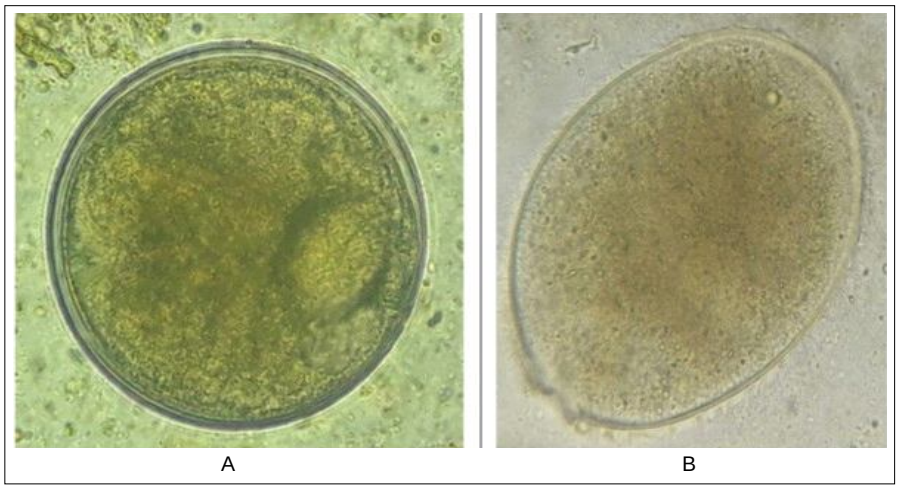


Fig 1: Cyst (A) and Trophozoite (B) of *B. sulcata* extracted from the feces of infected cattle.

Table 1: Overall occurrence of *B. sulcata*.

Methods	Total samples	Non-infected cattle	Infected cattle	Percentage
Microscopy	398	265	133	33.41%
PCR	398	255	143	35.92%

Table 2: District-wise distribution of *B. sulcata*.

Districts	Sample size	Microscopic positive sample	Microscopic %	PCR positive samples	PCR %
Rampur	30	11	36.70	12	40.00
Gautam Buddha Nagar	102	37	36.27	39	38.23
Moradabad	58	22	37.90	23	39.65
Meerut	60	17	28.30	18	30.00
Ghaziabad	55	14	25.50	15	27.27
Saharanpur	60	23	38.30	26	43.33
Amroha	33	9	27.30	10	30.30
Total	398	133	33.40	143	35.92

Chi-square test: χ^2=NS (p=0.57).

classified as non-diarrhoeic. The incidence of *B. sulcata* infection was significantly elevated (p<0.0001) in diarrhoeic cattle relative to their non-diarrhoeic counterparts. Specifically, 50% of the diarrhoeic cases tested positive for *B. sulcata* through microscopic examination, whereas the PCR method revealed a positivity rate of 51.85% among faecal samples. In the non-diarrhoeic group, 27.24% of samples were positive for *B. sulcata* via microscopic examination and 30.00% were positive according to the PCR method (Table 3). This supports the increasing acknowledgment that *B. sulcata*, once regarded merely as a commensal organism, can behave as an opportunistic pathogen in situations of compromised gut health. The organism may either directly induce diarrhoea by damaging the colonic mucosa or may proliferate as a result of dysbiosis triggered by other infections. In buffaloes from the Karnal district, those with diarrhoea exhibited an 83.33% higher association with *B. sulcata* infection compared to those without diarrhoea, who had a 54.74% association, indicating a significant difference (Kalkal and Sangwan, 2020). In addition, Ganai *et al.* (2015) reported that bovines affected by diarrhoea had a markedly higher infection rate of 38.5% compared to those with normal faeces, which stood at 9.9%, with a significance level of p<0.0001. These outcomes are in agreement with the results of several other studies (Fox and Jacobs, 1986; Tomczuk *et al.*, 2005; Al-Saffar *et al.*, 2010; Al-Saffar *et al.*, 2013).

Microscopic examination revealed that *B. sulcata* was more prevalent in young animals (< 3 years) at 40.62%, compared to adults (>3 years) at 30.0%. In contrast, the PCR method showed a prevalence of 42.96% in young animals and 32.59% in adults. Young cattle (<3 years) exhibited a significantly higher incidence of the infection compared to adults (>3 years), with the chi-square test demonstrating statistical significance (p=0.047) (Table 4). This finding supports the hypothesis that immunological immaturity makes young cattle more susceptible. Furthermore, management practices such as group housing of calves and increased exposure to contaminated environments may enhance transmission. Ganai *et al.* (2015) highlighted a significantly greater prevalence in young once (33.1%) as opposed to adults (13.9%). Similarly, Omeragic and Crnkic (2015) reported that the infection rate among young animals was higher (33.1%) compared to that of adults (13.9%). The most significant prevalence (P<0.05) occurred in animals younger than 1 year at 17.54%, with the next highest being in those older than 5 years at 12.33% and the 1-5-year age group at 5.71% (Khan *et al.*, 2024).

Among 398 animals, 124 were identified as male and 274 as female. The detection rate of *B. sulcata* in male animals was assessed at 37.09% using microscopic evaluation, while the PCR approach showed a prevalence of 39.51%. Microscopic analysis revealed a prevalence of 31.75% in female animals, while PCR testing indicated a prevalence of 34.30%. There was a slight increase in prevalence among male cattle in comparison to females, yet this was not statistically significant (p=0.351) (Table 5)

Table 3: The rate of prevalence according to the consistency of faecal matter.

Health status	Sample size	Microscopic positive	Microscopic %	PCR positive	PCR %
Diarrheic	108	54	50.00	56	51.85
Non-diarrheic	290	79	27.24	87	30.00
Total	398	133	33.41	143	35.92

Chi-square test: χ^2 =17.5 (p<0.0001).

Table 4: Age-related occurrence of *B. sulcata*.

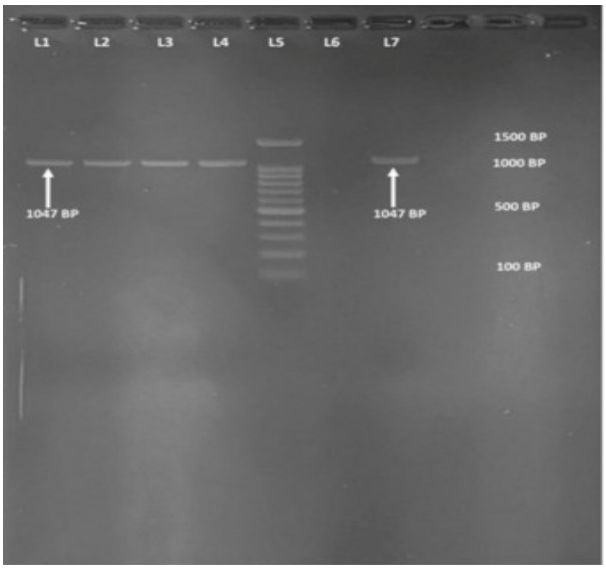
Age groups	Samples examined *via* microscopy	Positive samples *via* microscopy	Microscopic %	Samples examined *via* PCR	Positive samples *via* PCR	PCR %
<3 years	128	52	40.62	128	55	42.96
>3 years	270	81	30.00	270	88	32.59
Total	398	133	33.41	398	143	35.92

Chi-square test: χ^2 =3.94 (p=0.047).

Table 5: Sex-wise prevalence of *Buxtonella sulcata.*

Sex	Microscopically examined samples	Microscopic positive samples	Microscopic %	PCR examined samples	PCR positive samples	PCR %
Male	124	46	37.09	124	49	39.51
Female	274	87	31.75	274	94	34.30
Total	398	133	33.41	398	143	35.92

Chi-square test: χ^2 = NS (p=0.35).



L1-L4: Positive samples
L5: 100 bp plus DNA ladder
L6: Negative control
L7: Positive control

Fig 2: Amplified PCR product of 18S rRNA gene of *B. sulcata*.

and sex does not significantly affect the risk of infection. The minor difference noted may be attributed to management practices, as males are often utilized more for draught or transport and may receive lower quality feed and fodder, potentially leading to increased stress. Male buffaloes had an infection rate of 40%, which is higher than the 30% seen in females and there were no statistically significant differences between the two sexes (Al-Saffar *et al.,* 2013). Moreover, Al-Zubaidi and Al-Mayah (2011) reported a somewhat elevated prevalence of *Buxtonella* infection in males at 43.6%, in contrast to 42.8% in females in Baghdad.

Detection of *B. sulcata* through molecular method

A comprehensive examination of 398 faecal samples from cattle was conducted to detect *B. sulcata* via PCR, which involved amplifying a partial sequence of the 18S rRNA gene with the published primers, yielding a band size of 1040 bp (Fig 2). Microscopic examination, though widely used in parasitological diagnosis, has inherent limitations such as low sensitivity in cases of light infection, observer bias and the possibility of missing morphologically similar organisms (Soulsby, 1982). Similar findings have been noted in diagnostic studies of other protozoan parasites, indicating that molecular techniques exceeded microscopy in recognizing subclinical or chronic infections (Khare *et al.*, 2019; Singh *et al.*, 2021). This research illustrates that PCR is a more dependable and sensitive method than faecal smear analysis for diagnosing *B. sulcata*. However, considering resource-limited field conditions, faecal smear still holds significance as a rapid and cost-effective screening tool, while PCR can serve as a confirmatory diagnostic method for prevention and control of *B. sulcata* at field level.

CONCLUSION

The study revealed a notable presence of *B. sulcata* in cattle across Western Uttar Pradesh, with a positivity rate of 33.41% detected by microscopy and 35.92% through PCR. The rate of infection was markedly increased in young cattle and those suffering from diarrhoea. PCR revealed higher sensitivity relative to microscopy, stressing the requirement for ongoing screening and enhanced management strategies to ensure effective control.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Institutional Animal Ethics Committee, SVPUAT, Meerut (IAEC/SVPUAT/2025/168).

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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