Detection of intestinal ciliates in cattle, buffaloes and the environment

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Abstract

The problem of infection with *Buxtonella sulcata* in ruminants as a cause of diarrhea has not been considered until now. Therefore, the aims of this study came to determine the occurrence of the *Buxtonella sulcata* in cattle and buffaloes in Mosul city with study the level of contamination of water and soil with stages of Buxtonella.

Through examination of 200 fecal samples collected from both cattle (100 samples) and buffaloes (100 samples) which examined by direct smear and sedimentation techniques by water or formalin 1.5% and these methods are appropriate for detecting *Buxtonella sulcata* Cysts and trophozoites indicate that the overall infection rate with *Buxtonella sulcata* was 40%. The infection rate in cattle and buffaloes was 36% ,44% respectively with no statistically significant difference in the infection rate between cattle and buffaloes at a significance value $p \leq 0.05$.

Cattle and buffaloes fecal samples from several areas of the Nineveh Governorate were collected in this study to *identify B.sulcata*. Cattle in AL-Abbasiya region has the highest infection rate (50%), while the highest infection rate for *B.sulcata* in Buffaloes was found in the Hawi Al-Kanisa area (54.54%), It should be noted that, with regard to the various regions of the Nineveh Governorate, there was no significant variation in the infection rate in cattle and buffaloes. The current study's findings show that drinking water (25 samples) and soil samples (25 samples) intended for cattle and buffaloes is contaminated with *Buxtonella sulcata* cysts and trophozoites with percentages of 26.66% and 40%, respectively.

Key words: Buxtonella sulcata, ruminants, Diagnosis, water, soil

Introduction

Buxtonella sutcata is an opportunistic parasitic protozoan of the ciliate phylum that resides in the colon of ruminants. It is very similar to the *Balantidium coli* that is found in the large intestine of pigs and humans. Some researchers consider it to be within the same genus. [1,34,23]. This parasite is one of the causes of diarrhea in ruminants when the appropriate conditions are available for its reproduction inside the intestinal cavity [8,5].

Buxtonella sulcata has two stages: the vegetative stage, which is the first stage that lives in the colon of ruminants, and the cystic stage, which the parasite's life cycle goes through and is outside the host's body [14]. Infection with the *Buxtonella sulcata* occurs by ingesting cysts in feed or with contaminated drinking water [34].

Buxtonellosis is included in the list of protozoan diseases of cattle [2,26]. and the *Buxtonella* infection may be accompanied by diarrhea and other clinical signs in the

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host, although there is still no clear opinion on the pathogenicity of this parasite. It is possible that the pathogenicity of *Buxtonella* increases proportionally to the invasion intensity [2,9,26].

Studies of [3,15]. have revealed a strong relationship between the severity of infection with this parasite (the number of cysts per gram of feces and the occurrence of diarrhea in ruminants).

The first reported infection with *Buxtonella sulcata* in cattle in Iraq was in Al-Qadissiyah and Mosul province [1,3]. The problem of infection with *B. sulcata* has not yet been considered very important in ruminants, Therefore, this study aimed to identify *B.sulcata* in cattle and buffaloes fecal samples and to determine the level of contamination in drinking water and soil in the animal housing areas.

Material and Methods

1-Study Area

The study samples were collected from various areas of Mosul city\Nineveh governorate \ northern of Iraq, including cattle and Buffaloes feces, water and soil samples from the animal environment. Sampling took place from the beginning of August 2025 to February 2026.

The fecal samples were collected after obtaining official approval from the University of Mosul/College of Veterinary Medicine, based on the approval of the Scientific and Ethical Research Committee, as per letter dated July 9, 2025, and numbered UM.VET.2025.047.

Collection of the fecal samples

A total of 200 fresh fecal samples were collected randomly from 20 herds of cattle and buffaloes rom both one hundred fecal sample of local cattle and one hundred fecal sample of local Buffaloes from individual cases of various localities in Mosul city. included kokjali, shalalat, alabbasiya, bashiqa,,bartella, telkef and shekhan, then transferred to the laboratory of parasitology at the College of Veterinary Medicine/University of Mosul.

2-Study animals

Cattle and Buffaloes were (100 males and 100 females) with an age range from (less one year, 1-3years and above 3 years) and from animals suffering from diarrhea and with normal fecal consistency.

3- Sample collection

Approximately five to 10 grams of fecal sample was taken directly from the rectum by using (nylon gloves).

The samples are placed in clean plastic containers and labeled with data of samples (age, sex and consistency) and stored immediately at 4 C° until examination. The samples were examined in the laboratory of Parasitology, College of Veterinary Medicine - University of Mosul.

4- Environmental samples

Water samples were collected from the water troughs designated for drinking water by cattle and buffaloes. 25 water samples were collected from the trough using sterile, appropriately sized tubes, with an amount of 10 ml of water. Also, 25soil samples were collected from below the soil surface using a soil drill. The samples were placed in clean, sterile plastic containers, with an amount of five to 10 grams of soil. The samples were then transported, chilled, to the parasitology laboratory [30].

5-Laboratory examination

Fecal samples are examined by using The direct method, Tap Water Sedimentation Method according and Formalin-Ether Precipitation Method according to [1,33].

Examination of water samples**:** [30].

1-For the water sample, a 10 mL test tube was placed in it and centrifuged at least four times.

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2-The resulting filtrate was discarded, and 1 mL of distilled water was added to the precipitate until a homogeneous mixture was formed.
3-The mixture was then centrifuged again, and the remaining filtrate was discarded.
4- A drop of the precipitate was placed on a glass slide, a drop of iodine stain was added, and it was examined under a light microscope at 10X and 40X magnification.
The steps for examining soil samples to detect the parasite Buxtonella are as follows: [12].

1- The soil sample is filtered using several sieves of different mesh sizes to remove large particles.
2- 5 ml of distilled water is added and mixed with the soil sample until a homogeneous mixture is formed.
3- The mixture is then poured into test tubes and centrifuged.
4- A drop of the precipitate is taken and placed on a glass slide, a drop of iodine stain is added, and the sample is examined using the light microscope.

Study of the Morphological and Standard Characteristics of the Parasite *Buxtonella sulcata.*

The scientific morphological and standard characteristics (both vegetative and cystic stages) were studied and validated using the following sources: [11,34,1,27].

6-Statistical analysis

The results of this study analyzed statistically by using Chi-square.

Results

1-Results of microscopic Examination of fecal samples

The results of the current study, based on the examination of 200 fecal samples were collected from both cattle and buffalo (100 samples from each), indicate that the overall infection rate with reached 40%. The infection rate in cattle and buffaloes was 36% ,44% respectively. It should be noted that there was no statistically significant difference in the infection rate between cattle and buffaloes at a significance value $p \leq 0.05$ (Table 1).

Table (1): Shows the total percentage of infection with *Buxtonella sulcata* in total examine d animals (in cattle and buffaloes):

Animals	No. of examined animals	No. of positive animals	Percentage of infection	p-value
Cattle	100	36 a	36%	0.4495
Buffaloes	100	44 a	44%	
Total	200	80	40%	

The same letters mean no significant differences between cattle and buffaloes at $p \leq 0.05$

In this study, the parasite's cyst and trophozoites stages were identified using the direct method, sedimentation methods by using water or formalin 1.5%-ether. The cyst of *Buxtonella sulcata* appeared as circular to oval in shape, yellowish- green in color, showing macronucleus and contractile vacuoles, the cyst was surrounded by two layers' capsule) (Figure 1,2), while the trophozoites of *Buxtonella sulcata* appeared as oval in shape. It has a kidney-shaped nucleus with a nucleolus in its hollow section in the center, outside surface is coated in thick, short cilia. The anterior end is where the syncystoma is situated(Figure). In this study, the cyst measured 76.61 mμ (57-104.5) ±15.053 x75.64 mμ (57-104.5) ±15.299, and the trophozoite measured 106.428 ±7.40 (98-118 mμ) x75.642±8.139 (65-85 mμ).

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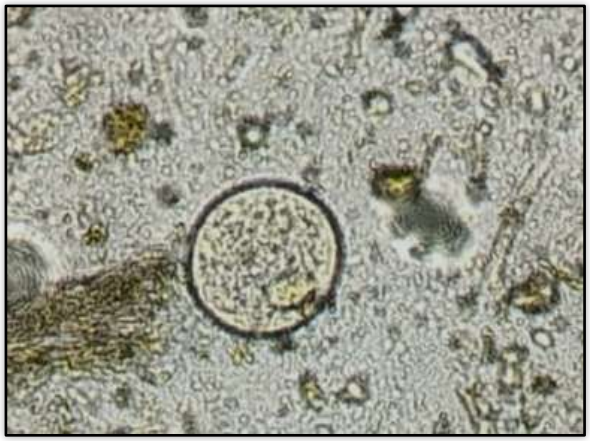

Figure (1): Cyst of Buxtonella sulcata in fecal sample of cattle .40X, by using digital camera.

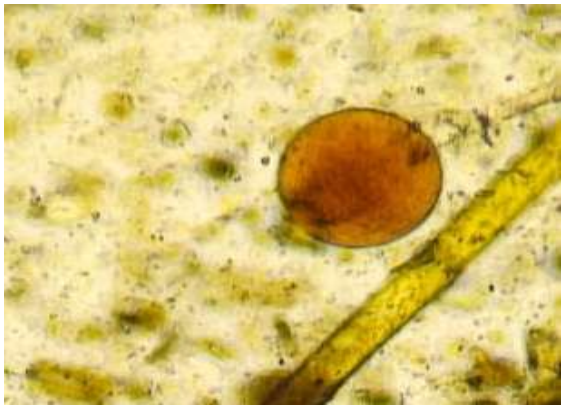

Figure (2) cyst of *Buxtonella sulcata* in fecal sample of buffalo stained with iodine stain 40 x by using digital camera.

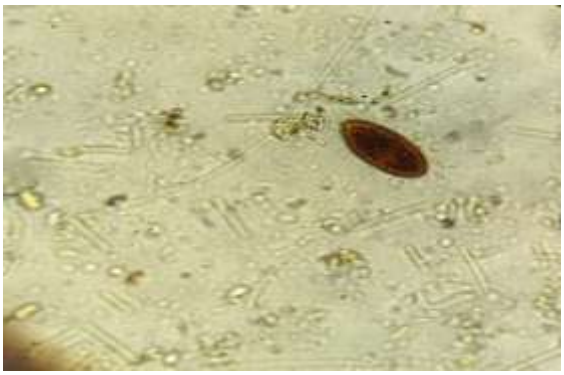

Figure (3) Trophozoite of *Buxtonella* with iodine stain 10x by using digital camera.

Cattle fecal samples from several areas of the Nineveh Governorate were collected in this investigation to *identify B.sulcata*. The AL-Abbasiya region has the highest infection rate (50%), followed by Kokjalil (40%). Infection rates in Sheikhan, Al-Shallalat, Bartella, Tel Keif, and Bashiqa were 38.46%, 35.71%, 33.33%, 28.57%, and 26.66%, respectively. The highest infection rate for B.sulcata in buffalo was found in the Hawi Al-Kanisa area (54.54%), followed by Al-Shalalt, AL-abbasiya, Al-Hamdaniya, Badosh, Hammam Al-Alil, and Tel Keif (50%, 45.45%, 42.85%, 40%, 38.88%, and 30%, respectively). It should be noted that, with regard to the various regions of the Nineveh Governorate, there was no discernible variation in the infection rate between cattle and buffalo (Table 2,3).

Table (2): Shows the incidence with *Buxtonella sulcata* in cattle according to the regions of the study:

Regions of study	Cattle		
	No. of examined animals	No. of positive animals	Percentage of infection
Kokjalil	15	6 a	40
Shallalat	14	5 a	35.71
AL-Abbasiya	14	7a	50
Bashiqa	15	4a	26.66

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Bartella	15	5a	33.33
Telkeif	14	4a	28.57
Shekhan	13	5a	38.46
Total	100	36	36

The same letters mean no significant differences between regions of study at p≤0.05

Table (3): Shows the incidence with *Buxtonella sulcata* in buffaloes according to the regions of the study:

Regions of study	Buffaloes		
	No. of examined animals	No. of positive animals	Percentage of infection
Hawi AL- kanisa	22	12 a	54.54
Hamam alil	18	7a	38.88
Shallalat	10	5a	50
AL-Abbasiya	11	5 a	45.45
Badosh	15	6 a	40
Telkeif	10	3 a	30
Humedanyia	14	6 a	42.85
Total	100	44	44

The same letters mean no significant differences between regions of study at p≤0.05

2-Result of the Microscopic examination for Environmental Samples (Water and Soil) collected from barns of cattle and buffalo

a-Drinking water samples

The current study's findings show that drinking water intended for cattle and buffalo is contaminated with *Buxtonella sulcata* cysts and trophozoites (Fig 3) with percentages of 26.66% and 40%, respectively. It also notes that there is no significant difference in the level of drinking water contamination in the pens of cattle and buffalo at p≤0.05 (Table 4).

Table (4) Shows the incidence of *Buxtonella sulcata* in drinking water in examined animal pens:

Water samples	No. of examined samples of water	No. of positive samples of water	Percentage of infection	p-value
Water samples from Pens of cattle	25	6 a	26.66	0.3832
Water samples from Pens of buffaloes	25	10 a	40	
Total	50	16	32	

b-Soil samples

The percentage of soil contamination with cysts and the parasite's trophozoites stage were discovered through microscopic examination of soil samples taken from cattle and buffalo pens. This percentage was 30% in soil samples taken from cattle pens, while it was higher in samples taken from buffalo pens (53.33%). It should be mentioned that the samples taken from buffalo and cattle pens did not significantly

differ in terms of pollution levels (Table 5).

Table (5) Shows the incidence of *Buxtonella sulcata* in soil examined animal pens

Soil of pens	No. of examined samples of soil	No. of positive samples of soil	Percentage of infection	p- value
Cattle pen soil	20	6	30	0.3654
Buffalo pen soil	15	8	53.33	
Total	35	14	40	

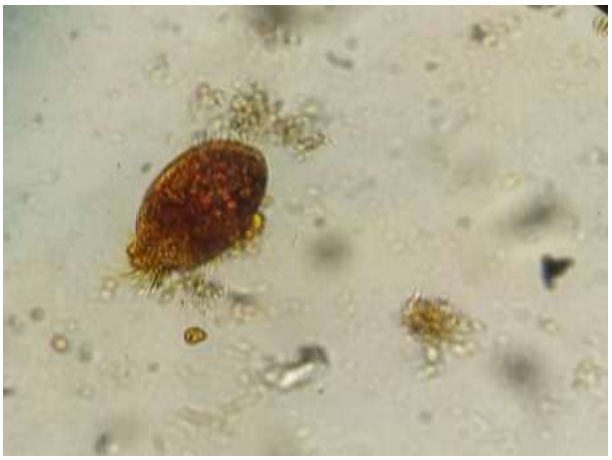

Figure (3): Trophozoite of *Buxtonella sulcata* in water sample examined with sedimentation method and stained with iodine, 10X, by using digital camera.

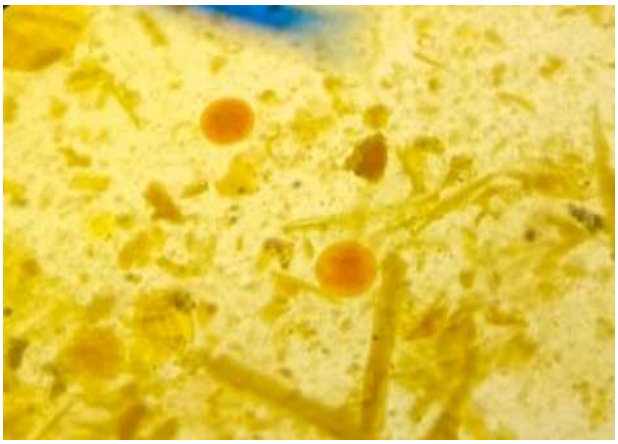

Figure (4): Cyst of *Buxtonella sulcata* in soil sample examined with sedimentation method and stained with iodine, 10X, by using digital camera.

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Discussion

Based on the analysis of 200 fecal samples taken from every 200 fecal samples of cattle and buffalo, the current study's findings showed an overall infection rate of 40%. Additionally, the study revealed that the infection rates in cattle and buffalo were 36% and 44%, respectively, with no discernible difference between the two species.

This study's infection rate is higher than that of cattle in Mosul [3] (24.16%) and buffalo in Mosul (35%) [4]. as well as dairy cattle in Sulaymaniyah province, Kurdistan region, Iraq (18.60%) [7]. Although this rate is lower than the 56% that [32]. found in buffalo in the city of Mosul, it is comparable to that found by [6]. in calves at the Al-Nasr cattle station in Baghdad (43.2%) and by [1] in the Al-Qadisiyah Governorate, Iraq (47%).

[8,21] found that *B.sulcata* has the highest prevalence rates of infection in Arab nations like Egypt, at roughly 32.86% and 69%, respectively.

Regarding nearby nations, [15] found that the overall infection rate in cattle in Iran's Sanandaj province was 45.63%. 9.5% of newborns and young calves in Turkey were reported by [13]. Furthermore, a number of studies on the significance of *Buxtonella sulcata* in ruminants in the occurrence of disease have been carried out in various countries around the world, including India, England, Thailand, Costa Rica, Bangalore, and Haryana. The infection rates have been reported to vary from 2 to 87% [25, 11, 20.17,19,24]. These variations in *B.sulcata* infection rates may be caused by various geographic locations, environmental conditions, farm management practices, and stressors.

The two stages of the parasite, the cyst stage and the trophozoite stage, are identified by the morphological analysis of *Buxtonella sulcata*. In this study, the cyst measured 76.61 mμ (57-104.5) ±15.053 x75.64 mμ (57-104.5) ±15.299, and the trophozoite measured 106.428 ±7.40 (98-118 mμ) x75.642±8.139 (65-85 mμ). The dimensions and form of these stages identified in this work are consistent with those identified by [27,1,3,28,21].

The parasite's life phases were measured by a number of researchers, and their measurements were somewhat similar such as [3] reported cyst diameter (68.6-107.8 μm) with a mean of 74.58 μm, and trophozoite size (107.8-137.2X 49-102.9 μm) with a mean of 121.25X94.06 μm. [6]. recorded the measuredof cyts wre 49.2-90.4 μm in diameter (mean 78.14 μm and the trophozoites of parasite measured 100.5 -108 x94.4 -102.2 μm (mean 105.12x98.44 μm), [21]. reported cyst size range μm d from 82.23×78.62 μm) with a mean of 58.78×4. Additionally, [31]. found that trophozoite sizes ranged from 84 x 60 to 120 x 90 μm, while cyst diameters ranged from 68 to 120 μm.

The results of this study agreed with [23,31]. that the use of iodine stain stain is very useful in diagnosing the *Buxtonella* parasite in fecal smear samples prepared by both the direct method and the sedimentation method and the parasite is easily observed and identified, in addition to distinguishing the internal structures of the parasite.

The present study demonstrated noticeable variation in the prevalence of *Buxtonella sulcata* infection among cattle and buffaloes across different regions of Nineveh governorate. Among cattle, the highest infection rate was recorded in the Al-Abbasiya area (50%), followed by Kokjalil (40%) and Al-Sheikhan (38.46%), whereas lower prevalence rates were observed in the remaining regions, ranging from 26.66% to 35.71%. Similarly, buffaloes showed the highest prevalence in Hawi Al-Kanisa (54.54%), followed by Shallalat (50%) and Al-Abbasiya (45.45%), while other areas exhibited moderate prevalence levels between 30% and 42.85%.

Management practices vary among regions and may contribute to the observed

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differences. Differences in housing systems, hygiene measures, feeding regimes, and access to veterinary care can influence the exposure risk and immune status of animals. Poor sanitation and irregular removal of fecal matter may lead to accumulation of infective cysts in barns and grazing fields, particularly in areas with traditional or extensive livestock management systems. Furthermore, nutritional deficiencies and stress associated with overcrowding or inadequate management may reduce host immunity, increasing susceptibility to parasitic infections.

Despite these apparent regional variations in prevalence, statistical analysis revealed no significant differences between the study areas. This suggests that *B. sulcata* infection is widely distributed throughout Nineveh Governorate and that the parasite is well adapted to a range of environmental and management conditions. The lack of statistical significance may also reflect similarities in climatic conditions and livestock management practices across the studied regions, which could contribute to a relatively uniform exposure risk.,this result was agreement [15]. who concluded that cattle are highly susceptible to B.sulcata infection under a variety of housing and environmental conditions inSanandaj province , Iran,[6]. also pointed out that the high incidence of the *Buxtonell sulcata* is related to the high density of calves within a limited breeding area, which facilitates the transmission of the parasite between animals

This study identified both the cysts and trophozoites of the *Buxtonella sulcata* in samples taken from the drinking water of cattle and buffaloes, as well as from the soil in their barns. The percentage of *Buxtonella sulcata* contamination in the water and soil samples collected from the buffaloes' pens was higher, at 40% and 53.33% respectively. No significant differences in the degree of contamination were observed between the cattle and Buffaloes pens, These findings were consistent with those of [22], who found a 40.62% infection rate with *Buxtonella sulcata* in sheep drinking water, and [23], who found a 45.45% contamination rate with *Buxtonella sulcata* in 11 sheep drinking (river) water from Al-Suwaira city and concluded that water contamination acts as a good source for the spread of *Buxtonella sulcata* infection.

Additionally, these findings were consistent with recent research done in Basrah and Baghdad to ascertain the drinking water contamination rate with various intestinal protozoa, which found contamination rates of 36.4% and 25.3%, respectively [18,10]. According to [29]. sewage, animal waste, and decaying animal corpses can contaminate drinking water. Additionally, humans and wildlife that live in this area have contributed parasites that contaminate open water resources including reservoirs, ponds, and water ways.

Conclusions

This study concluded that the intestinal parasite *Buxtonella sulcata* is a common parasite found in cattle and buffalo, and the degree of contamination of drinking water and soil is one of the most important sources of infection and spread of this parasite among different hosts.

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