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Research article

Identification of *Buxtonella sulcata* with phylogenetic analysis in cattle in Egypt

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Abstract

Protozoan diseases are of great significance in ruminants. In Egypt, the published data for *Buxtonella sulcata (B. sulcata)* in cattle is limited. This is the first study on *B. sulcata* in Assiut governorate via PCR and phylogenetic analysis. This study investigated the *B. sulcata* positive rate in cattle and its association with diarrhea. Out of 100 cattle fecal samples (50 males and 50 females) with different ages were collected from Assiut governorate, Egypt. Microscopically, the positive rate of *B. sulcata* was 69% (69/100). The cysts were oval or round with a size range of (82.23×78.63 to 53.65×48.62 µm) with a mean of (58.46×54.35 µm). While, its trophozoite appeared oval with characteristic grooves with a size range from (80.9×40.2 to 41.8×30.64 µm) with a mean of (58.78×41.26 µm). Females are slightly higher infected 72% (36/50) than male 66% (33/50). While, the highest positive rate recorded in age less than 1 year 78.6% (22/28), followed by age more than 3 years 68.4% (13/19), then the lowest in age 1-3 years 64.2% (34/53). The positive rate of *B. sulcata* was 92.2% (47/51) and 44.9% (22/49) of diarrheic and non-diarrheic feces, respectively, with very high significant differences. Diarrhea was established in all fecal samples with more than 1000 cysts per gram of faeces. Three fecal samples were positive for *B. sulcata* SSrDNA gene specific primer (1047 bp) by PCR. After DNA sequencing and phylogenetic analysis, all nucleotide sequences showed high similarity to *B. sulcata*. We advised regular examination for *B. sulcata* in the predominant areas with excessive studies to aid in planning effective control strategies against it.

Keywords: Buxtonella sulcata, Cattle, Microscopic, PCR, Phylogenetic Analysis

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INTRODUCTION

Parasitic diseases are one of the main livestock problems caused by frequent exposure to parasites with lack of knowledge about their transmission and poor management by the owners. Gastrointestinal parasites are prevalent worldwide, particularly in developing countries where the majority of the livestock is not achieving the acceptable yield level because of various parasitic diseases that play a significant role in poor performance (Ahmed et al., 2020; Mughal et al., 2022). In the developing world, parasitic diarrheal disease remains a significant cause of morbidity and mortality (Bhoi et al., 2021).

Buxtonella sulcata first named and reported by (Jameson, 1926). It belongs to the "Kingdom: Protozoa, Phylum: Ciliophora, Class: Kinetofragminophorea, Order: Trichostomatorida, Family: Pycnotrichidae and Genus: Buxtonella". It is an opportunistic protozoan parasite inhabiting the ruminants' alimentary tract "caecum and colon" worldwide (Huang et al., 2014; Kumar et al., 2017). Bovine is the reservoir for this zoonotic pathogen. Its morphology resembles that of *Balantidium coli* (*B. coli*) which found in humans and pigs. *Buxtonella* sulcata's life cycle consists of an intestinal cyst stage and a trophozoite stage. It possesses a resistance to chemical treatment and environmental stresses such as temperature and humidity outside the host (Göz et al., 2006; Al-Zubaidi and Al Mayah, 2011; Grim et al., 2015; Mughal et al., 2022). The cyst is stimulated by intestinal pH and proteases when it enters the small intestine after ingestion, resulting in the release of trophozoites that colonize and invade the wall of colon with its cytotoxic effects leading to diarrhea (El-Ashram et al., 2019; Mughal et al., 2022).

The buxtonellosis is caused by *B. sulcata* (Shibitov and Abdelhamid, 2022). An increase in the number of this protozoan parasite in bovine gastrointestinal tracts can result in poor condition of the animal and diarrhea particularly in immunocompromised animals. Untreated animals can lead to production losses and even death (Huang et al., 2014; Kumar et al., 2017). Diarrhea necessitates prompt diagnosis and effective treatment and management strategies (Grim et al., 2015). *Buxtonella sulcata* is a vital pathogenic element for causing diarrhea in suckling and post-weaning calves. The fundamental clinical sign of bovines with its infection is anorexia, lacrimation, abdominal pain, debilitating diarrhea, rough body coat and inanition as well as generalized weakness resulting in a decline in production and significant financial losses for farmers (Ganai et al., 2015; Köse and Zerek, 2018; Bhoi et al., 2021).

Pomajbíková et al. (2013) discovered the genetic and morphological similarity of *B. sulcata* cysts of cattle from *B. coli* cysts of monkeys. Therefore, the pathogenicity of *B. sulcata* for humans was not excluded. In addition, Dianso et al. (2018) stated that *B. sulcata* can be detected using microscopy and molecular methods (PCR) that exhibit high specificity and sensitivity which can be verified by the DNA sequencing that confirms the parasites identification that are still unclear after microscopy. Microscopic examination shown a cyst which had a clear wall, round micronucleus, macronucleus "kidney shaped" and ciliated trophozoite (Bhoi et al., 2021).

Wang et al. (2020) mentioned that nuclear small subunit ribosomal DNA (nSSU rDNA) is the most common gene marker for studying phylogeny, ecology and biodiversity of eukaryotes. Also, Pomajbíková et al. (2013) found

that *Balantidium entozoon* is believed to be different phylogenetically from *Balantidium coli* based on the polymorphism of SSrDNA sequences.

The current study investigated the positive rate and intensity of cattle buxtonellosis in Assiut governorate, Egypt. As there is lack of published data on its molecular detection in Egypt, it is still considered a neglected parasite in bovine indicating a dire need for conducting this study using conventional method and confirms the parasite through PCR and sequence analysis.

MATERIALS AND METHODS

Collection of the samples

A total of 100 fresh fecal samples were collected from cattle from individual cases of various localities in Assiut that were admitted to the Veterinary Teaching Hospital, Faculty of Veterinary Medicine at Assiut University during the period from April to September 2023. Cattle were (50 male and 50 female) with an age range from (2 months to 4 years). Approximately 5 grams of fecal sample was taken directly from each animal. 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.

Laboratory examination

Each fecal sample was inspected macroscopically and microscopically (Soulsby, 1986). Direct fecal smear was examined microscopically and morphologically by staining it with Lugol's iodine 5% at magnification (x100 and x400) (Sultan et al., 2013). Identification of *B. sulcata* cysts and trophozoites was based on definite morphological feature such as described by (Al-Zubaidi and Al-Mayah, 2011; Kalkal and Sangwan, 2019). The intensity of the infection was determined by counting the cysts per gram of feces (CPG) using a modified McMaster technique (Tomczuk et al., 2005; Shibitov and Abdelhamid, 2022). Positive samples were kept at − 20 °C for DNA extraction and PCR.

DNA extraction

The QIAamp DNA stool Mini Kit (QIAGEN, Hilden, Germany) was used to extract DNA from 220 mg fecal samples. The fecal samples were added to 1.4 ml buffer ASL, and then incubated at 70°C for 5 minutes. After homogenizing the samples for 6 minutes with the QIAGEN Tissue Lyser, they were centrifuged at 14000 rpm for 1 minute. After adding one InhibitEx tablet to 1.2 ml of the supernatant and vortexed, incubated at room temperature for 1 minute. The samples were centrifugated for 3 minutes at 14000 rpm and then added 200 µl of the supernatant to 200 µl of lysis buffer AL and 15 µl of proteinase K and incubated for 10 min at 70˚C. 200 µl of absolute ethanol was added to the lysate after incubation. The lysate was transported to the column, centrifuged for 1 minute at 14000 rpm, then washed and centrifuged in accordance with the manufacturer's recommendations. Elution buffer AE (100 µl) was used to nucleic acid elution.

PCR assays

Primers for amplification and sequencing of SSrDNA used in this study were provided from Metabion (Germany) and used in a 25 µl reaction containing 12.5 µl of "EmeraldAmp Max PCR Master Mix (Takara, Japan)", 1 µl of each primer of 20 pmol concentrations, 5.5 µl of water and 5 µl of DNA template. The extracted DNA was amplified through the PCR reaction cycles that consisted of "Primary denaturing step at 94°C for 5 min followed by 35 cycles at 94°C for 30 sec., 53°C for 40 sec. and 72°C for 1 min. with a final extension at 72°C for 10 min" by means of a T3 Biometra thermal cycler (Pomajbíková et al., 2013) (Table 1).

Table 1 Primers sequences of *B. sulcata* SSrDNA specifically designed for the PCR (Pomajbíková et al., 2013)

Target agent	Primers sequences (5′–3′)		Amplified segment (bp)
	Forward	Reverse	
Buxtonella sulcata SSrDNA	CGCAAATCGCGATTTTGTCGCG	AAATACATAGTCCCTCTAAGAAGTC	1047

Analysis of the PCR Products

The PCR products were separated using electrophoresis on a 1.5% agarose gel (Applichem, Germany, GmbH) in a 1x TBE buffer at room temperature applying gradients of 5V/cm. For gel analysis, 20 µl of the products was loaded in each gel slot. The fragment sizes were determined using Gelpilot 100 bp plus DNA Ladder (Qiagen, Gmbh, Germany). A gel documentation system (Alpha Innotech, Biometra) was used to photograph the gel and the data was analyzed with computer software.

Phylogenetic analysis

PCR products were purified by means of "QIAquick PCR Product extraction kit (Qiagen, Valencia)". "Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer)" was used for the sequence reaction and it was purified by Centrisep spin column. DNA sequences were gained by "Applied Biosystems 3130 genetic analyzer (HITACHI, Japan), a BLAST® analysis (Basic Local Alignment Search Tool)" (Altschul et al., 1990) was initially done to create sequence identity to GenBank accessions. The phylogenetic tree was made via "the MegAlign module of LasergeneDNAStar version 12.1" (Thompson et al., 1994) and Phylogenetic analyses was done using "maximum likelihood, neighbour joining and maximum parsimony in MEGA6" (Tamura et al., 2013). Statistical analysis: The data was analyzed using "SPSS, 16.0", a statistical program. Chi square test (χ^2) which applied to compare the *B. sulcata* infection rate between sex, age and fecal consistency of cattle. The results were deemed significant when ($P < 0.05$) (Makawi, 2019).

RESULTS

In the present study, the positive rate of *B. sulcata* infection in cattle was 69% (69/100) by microscopic examination in Assiut Governorate (Table 2). The cysts of *B. sulcata* were oval or round in shape, yellow in color with clear wall with kidney shaped macronucleus and a smaller micronucleus. The cyst diameter ranged from (82.23×78.63 to 53.65×48.62 µm) with a mean of (58.46×54.35 µm) (Figure 1). While, the trophozoite appeared oval in shape with a cytostome at its anterior end, cytopyge at posterior end, a curved groove which extend throughout the length of the body, macronucleus and contractile vacuoles. Its diameter ranged from (80.9×40.2 to 41.8×30.64 µm) with mean (58.78×41.26 µm) (Figure 2, Table 3).

Table 2 Positive rate of *B. sulcata* among cattle in relation to gender

Sex	Examined number	Number of Positive samples	Prevalence (%)	Chi Square value	P value
Male	50	33	66	0.421	0.517
Female	50	36	72		
Total	100	69	69		

Insignificant differences (P> 0.05)

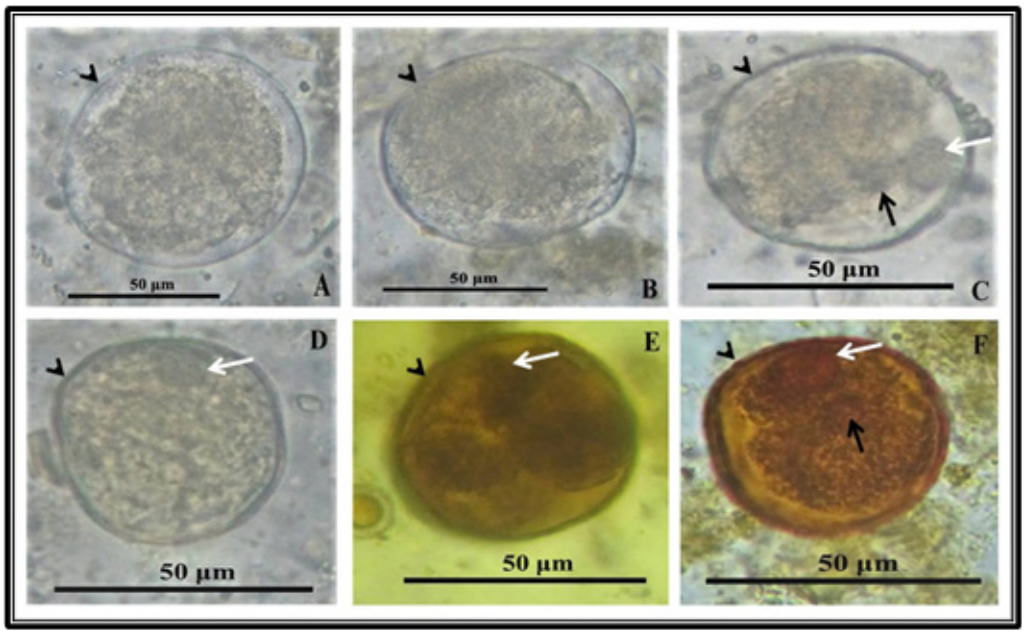

Figure 1 Microscopic fecal smears of cattle showing: *B. sulcata* cyst with clear wall "arrow head", macronucleus "white arrow" and round micronucleus "Black arrow" (X400). (A), (B), (C) and (D): unstained smears. (E) and (F): stained smears with iodine.

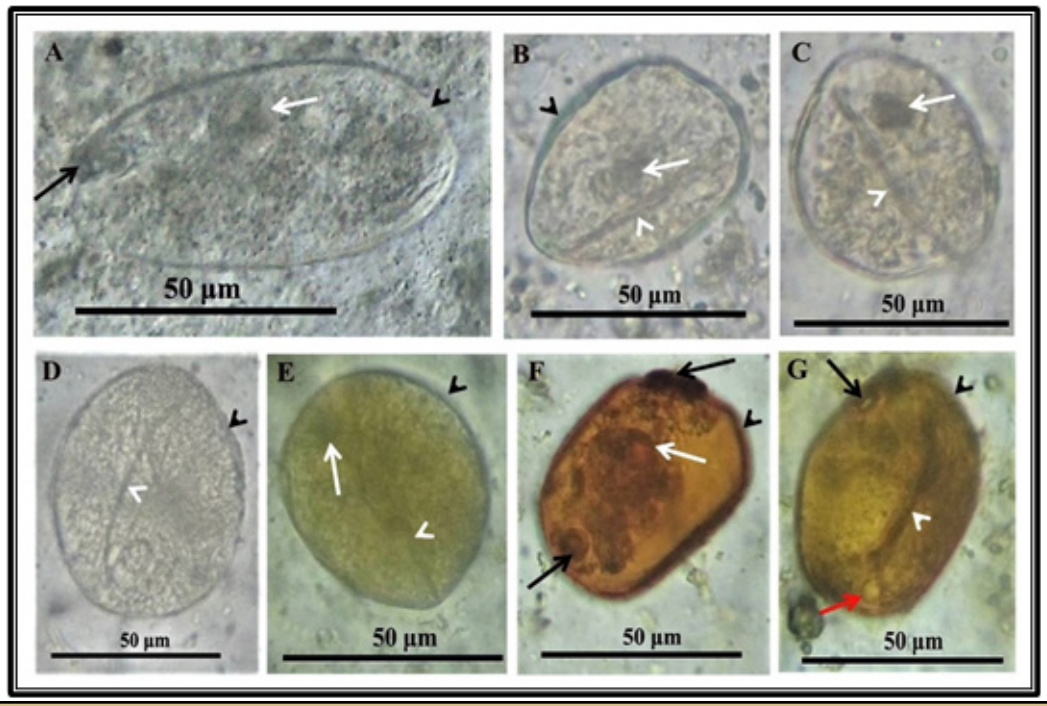

Figure 2 Microscopic fecal smears of cattle showing: *B. sulcata* trophozoite with cilia "black arrow head"; macronucleus "white arrow"; cytostome and cytopyge "black arrow"; a curved groove "white arrow head" and contractile vacuoles "red arrow". (X400). (A), (B), (C) and (D): unstained smears. (E) and (F): stained smears with iodine.

Table 3 Size range of *B. sulcata* in cattle

Parameter	Number	*B. sulcata* (Mean ± Standard error)	Minimum	Maximum	Standard Deviation	Variance
Trophozoite length (µm)	8	58.78±4.5	41.8	80.9	12.83	164.77
Trophozoite width (µm)	8	41.26±2.77	30.64	57.02	7.83	61.46
Cyst length (µm)	8	58.46±3.51	53.21	82.23	9.92	98.33
Cyst width (µm)	8	54.35±3.78	46.44	78.63	10.68	114.17

In present study, the positive rate of cattle buxtonellosis according to sex was slightly higher in female animals (72%) as compared to male (66%) (Table 2). According to age, the highest positive rate of cattle buxtonellosis was (78.6%) with age less than 1 year, followed by more than 3 years (68.4%), then 1-3 years (64.2%). No significant difference regard to sex and age was recorded (Table 2 and 4). The positive rate of *B. sulcata* of cattle according to the feces consistency was (92.2%) of diarrheic feces and was 44.9% of non-diarrheic feces. There were very high significant differences in positive rate between diarrheic and non-diarrheic feces (Table 5).

Table 4 Positive rate of *B. sulcata* among cattle according to age

Age	Examined number	Number of Positive samples	Prevalence (%)	Chi Square value (χ^2)	P value
<1 year	28	22	78.6	1.785	0.41
1-3 years	53	34	64.2		
> 3 years	19	13	68.4		
Total	100	69	69		

Insignificant differences (P> 0.05)

Table 5 Positive rate of *B. sulcata* among cattle according to feces consistency

Fecal consistency	Examined number	Number of Positive samples	Prevalence (%)	Chi Square value (χ^2)	P value
Diarrhoeic	51	47***	92.2	26.1	0.000
Non-diarrhoeic	49	22	44.9		
Total	100	69	69		

*** Very high significant p values (p< 0.001).

The intensity between the *B. sulcata* infection and the diarrhea occurrence was 41.7% of positive animals with less than 100 *B. sulcata* cysts per gram of faeces, 76% of positive animals with less than 500 *B. sulcata* cysts per gram of faeces, 75% of positive animals with less than 1000 *B. sulcata* cysts per gram of faeces, While the diarrhea were proven in all of the fecal samples 100% which had more than 1000 *B. sulcata* cysts per gram of faeces (Table 6).

Table 6 The relation between the occurrence of diarrhea and the intensity of infection with *B. sulcata*

The number of *B. sulcata* cysts per 1 gram of faeces	**The number of infected animals**	**The number of animals have diarrhea (%)**	**Chi Square value (χ^2)**	**P value**
50–99	24	10 (41.7)	14.238	0.007
100–499	25	19 (76)		
500–999	8	6 (75)		
1000–1499	4	4** (100)		
>1500	8	8** (100)		
total	69	47 (68.1)		

** High significant p values (p< 0.01)

Three cattle fecal samples were positive for *B. sulcata* -specific primer (1047 bp PCR product) by PCR (Figure 3). After DNA sequencing, all PCR products showed high homology to *B. sulcata* (Figure 4). The three nucleotide sequences of *B. sulcata* have been obtained (available in the GenBank/EMBL/DDBJ databases under the accession numbers OR395410, OR395411, OR395412). Upon phylogenetic analysis of the present study, three sequences isolated was recognized as *B. sulcata* belonged to the first clade and showed 100% similarity with *B. sulcata* of buffalo in Nebal (Accession No. AB786848) while it showed 99.9% similarity with isolated from Tišnov (Accession No. KP016717) and with isolated from Japan (Accession No. AB794979) and 99.8% similarity with isolated from Tišnov (Accession No. KP016718) and with isolated from Belgium (Accession No. JQ073337). The three sequences isolated were showed 99.4% similarity with *Infundibulorium cameli* isolated from camel in Oman (Accession No. OQ696204 and OQ696205) while it showed 96.3% similarity with *Balantioides coli* isolated from pig in Germany (Accession No. MK801496 and MK801497) and 96.6% similarity with *Neobalantidium coli* isolated from Chimpanzee in United Kingdom (Accession No. JQ073318) (Figure 5, 6).

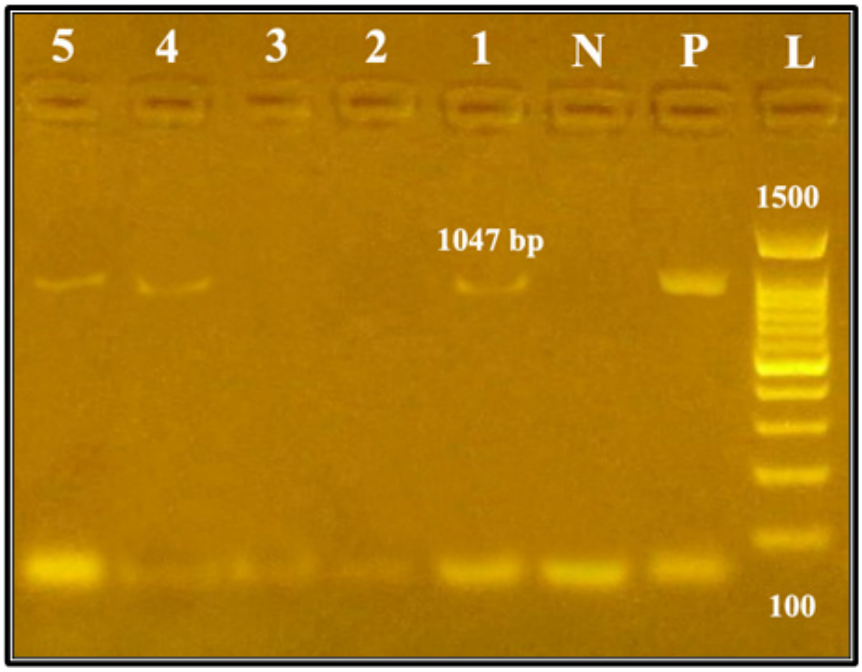

Figure 3 Gel electrophoresis pattern of PCR amplicon on cattle fecal samples using *B. sulcata* SSrDNA gene specific primer showing: L: 100 bp DNA ladder; 1,4,5: Positive samples (1047 bp PCR product); 2,3: Negative samples.

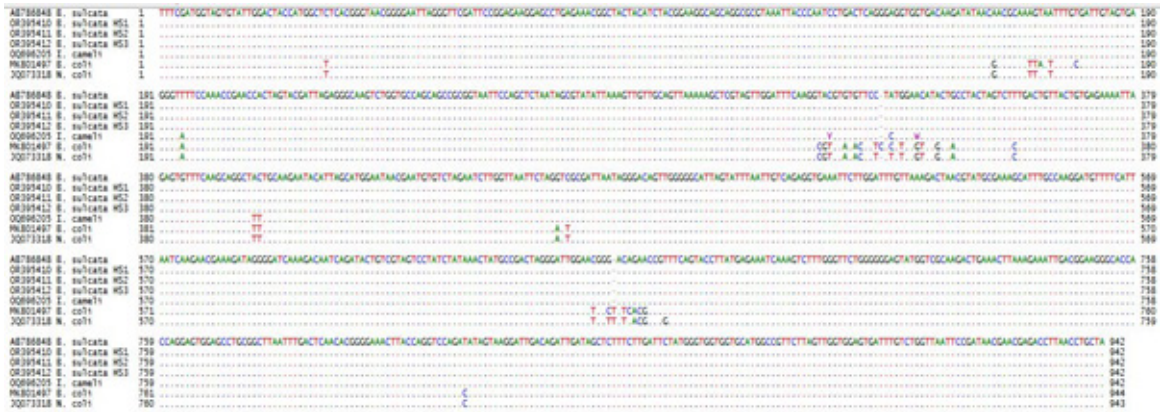

Figure 4 Nucleotides alignment of three isolates identified in present study of *B. sulcata* SSrDNA gene in cattle "OR395410 *B. sulcata* HS1, OR395411 *B. sulcata* HS2 and OR395412 *B. sulcata* HS3" with closely related protozoa (*I. cameli*, *B. coli* and *N. coli*) previously recorded in the GenBank.

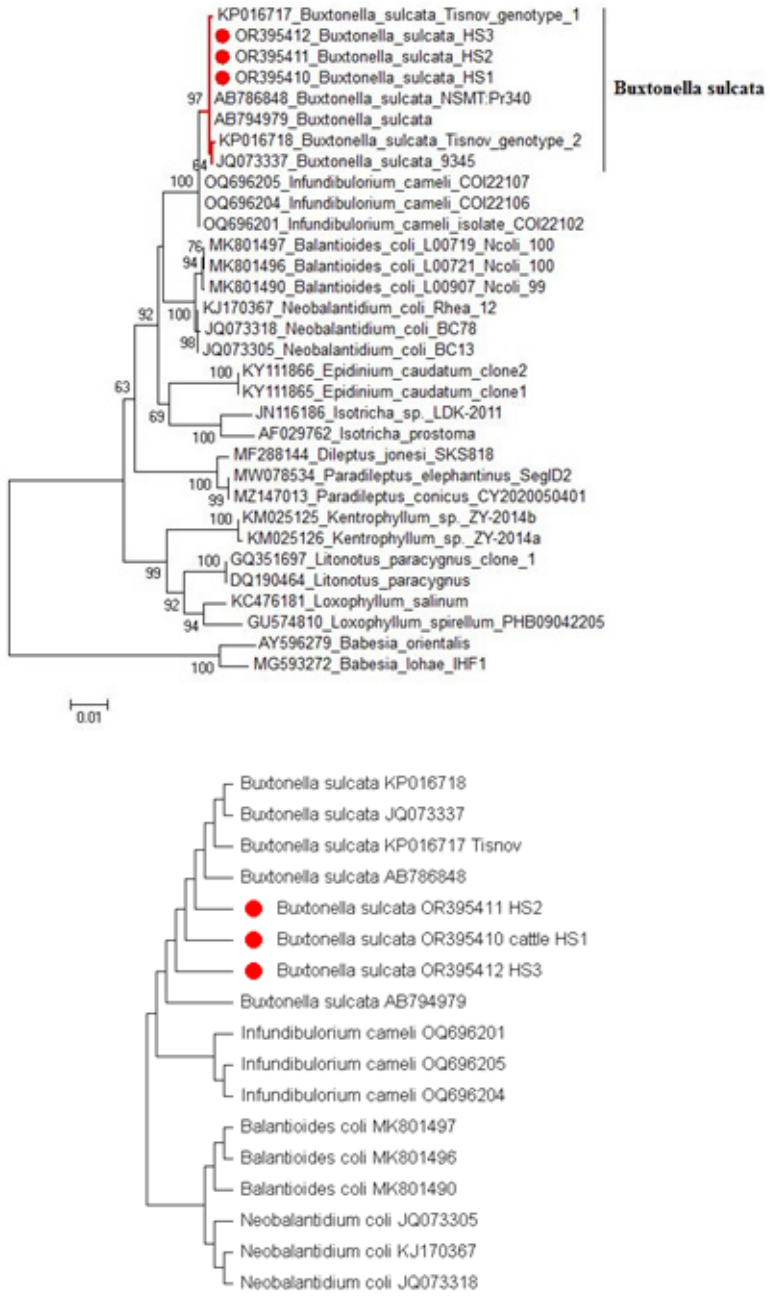

Figure 5 Maximum likelihood phylogenetic tree of *B. sulcata* of SSrDNA gene. Three isolates identified in present study with closely related protozoa previously recorded in the GenBank worldwide. Isolates were OR395410 *Buxtonella sulcata* HS1, OR395411 *Buxtonella sulcata* HS2 and OR395412 *Buxtonella sulcata* HS3. New sequences are marked with red circles.

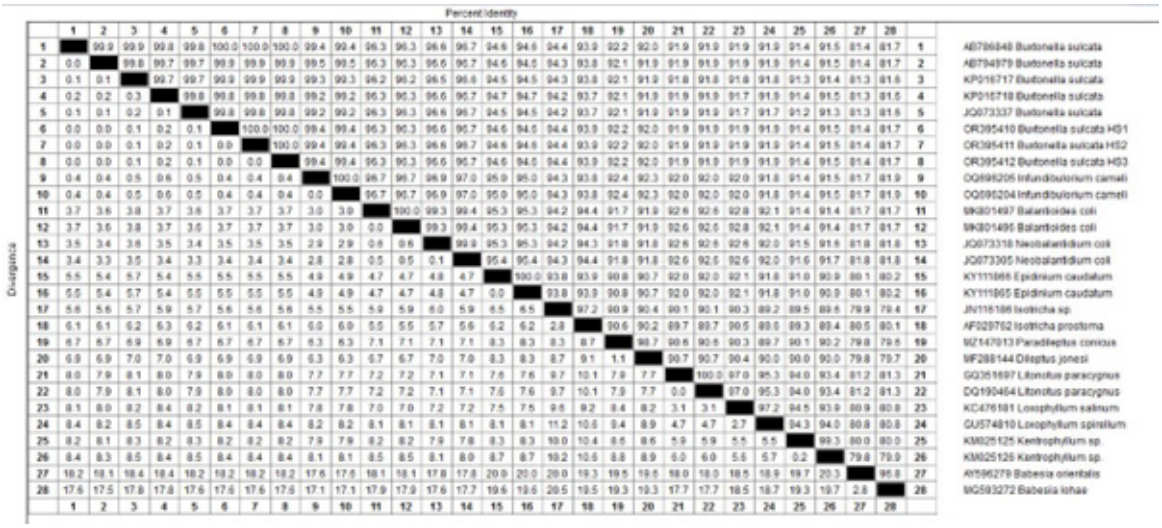

Figure 6 Distance matrix computed sequence distance for *B. sulcata* SSrDNA gene. The upper part of the matrix provides the (percent identity) between the alignments scaled as percentages. The lower part provides the alignments divergence.

DISCUSSION

Kočiš et al. (2014) reported that protozoan infections lead to high morbidity and mortality in cattle, resulting in economic losses. Amongst all parasitic infections, *B. sulcata* was the most common bovine gastrointestinal protozoa (Maharana et al., 2016). Levine (1985) added that the ciliates found in ruminants such as (cattle, buffaloes, camels) are in fact *B. sulcata*. The host is infected by intestinal protozoa through contaminated water or food that has protozoan cysts (Kalkal and Sangwan, 2019).

In the present study, the positive rate of *B. sulcata* infection in cattle was 69% (69/100) by microscope. Our result was higher than that detected in Egypt (Sultan et al., 2013) 48.2% from Al-Gharbiya province and (El-Ashram et al., 2019) 30.15% in El-Minia Province. Also, it was higher than that described globally by Göz et al. (2006) 9.5% in Turkey, Al-Zubaidi and Al-Mayah (2011) 43.2% in Baghdad, Adhikari et al. (2013) 27% in Nepal, Al-Saffar et al. (2013) 24.16% in Iraq, Huang et al. (2014) 61.7% in Taiwan, Ganai et al. (2015) 23.6% in RS Pura, Jammu, Hasheminasab et al. (2015) 45.63% in Iran, Omeragić and Crnkić (2015) 27% in Sarajevo area, Kumar et al. (2017) 35% in India and Mughal et al. (2022) 20.31% in Pakistan. While it was lower than that recorded by Tomczuk et al. (2005) 87.9% in Poland. These discrepancies in the percentage of *B. sulcata* infection could be due to different geographical regions, environmental situations, farm management and stress factors (Al-Saffar et al., 2010; Grim et al., 2015; Bhoi et al., 2021).

The morpho-metrical results of the *B. sulcata* cysts in this study are round or oval with macronucleus and a smaller micronucleus and were surrounded by a double-layered capsule which agrees with Shibitov and Abdelhamid (2022). Cyst size ranged (82.23×78.63 to 53.65×48.62 µm) with a mean of (58.46×54.35µm). This size are comparatively close to that prior studies reported by Jameson (1926) who recorded that *B. sulcata* was (54 to 124µm length and 40 to 72 µm breadth), Mehlhorn (2016) who mentioned that it was (50–130 µm×60 µm). Also, Shibitov and Abdelhamid (2022)

found that the diameter of *B. sulcata* cysts were (54.8–96.2 µm) with a mean of (67.3±11.1 µm). While, it is smaller than that recorded by Al-Saffar et al. (2010) who described cysts diameter (68.6-107.8 µm) with a mean of (74.58 µm) which surrounded by a two layered capsule. Also, Sultan et al. (2013) observed cysts diameter ranged between (68-120 µm). While, the most apparent morphological feature of the living trophozoite observed in our study appeared oval in shape with the presence of contractile vacuoles and a curved groove which extend throughout the length of the body. Similarly to (Jameson, 1926; Rees, 1930; Taylor et al., 2007; Sultan et al., 2013). In current work the trophozoite size ranged from (80.9×40.2 to 41.8×30.64 µm) with mean (58.78×41.26 µm) which similar to Levine (1961) who detected trophozoite size (60-138µ X 46-100 µm). But its smaller than reported by Al-Saffar et al. (2010) who found the vegetative forms were (107.8-137.2X 49-102.9 µm) with a mean of (121.25X94.06 µm). Also, Sultan et al. (2013) recorded its size were (84 x 60 to 120 x 90 µm) and (Grim et al., 2015) the trophozoites were (103.2 X 66.1 µm).

In present study, the positive rate of cattle buxtonellosis according to sex was slightly higher in female animals (72%) as compared to male (66%). Similarly, Hasheminasab et al. (2015) detected higher buxtonellosis infection rate in female animals (47.32%) as compared to male (38.46%) in Snandaj province, Iran and Mughal et al. (2022) found infection was higher in female (25.21%) as compared to male (12.33%). On the other hand, Al-Zubaidi and Al-Mayah (2011) found slightly higher rate in male (43.6%) as compared to female's animals (42.8%) in Baghdad and Adhikari et al. (2013) reported higher incidence in males (5.52%) than females (3.23%) cattle. Our findings revealed non-significant difference in infection rate between males and females, similar to (Al-Saffar et al., 2010; Al-Zubaidi and Al-Mayah, 2011). On contrary, Mughal et al. (2022) found that infection was significantly higher in females than males. While, Adhikari et al. (2013) reported higher significant incidence in males than females. The non-significant difference in the infection rate between genders due to both are grazed and subjected to the same environmental conditions (Al-Seady and Kawan, 2014).

El-Ashram et al. (2019) mentioned that the age is one of the main risk issues in the spread of parasitic infections and *B. sulcata* is the most common reason of cattle calves' diarrhea. Moreover, younger animals are at a higher risk of morbidity and infection than older animals (Trotz-Williams et al., 2011).

The highest positive rate of cattle buxtonellosis according to age was (78.6%) with age less than 1 year, followed by more than 3 years (68.4%), then 1-3 years (64.2%). Similarly, Ganai et al. (2015) found the higher infection rate of *B. sulcata* in younger animals (33.1%) as compare to adult (13.9%) in RS Pura, Jammu and Mughal et al. (2022) found the highest prevalence in less than 1 year age group (29.82%) followed by the animals greater than 5 year of age (21.92%) and 1 to 5 years age group (7.86%). Also, El-Ashram et al. (2019) mentioned that *B. sulcata* was (36.73%) in post-weaning cattle calves; Omeragić and Crnkić (2015) found higher percentage rate in calves (57.1%) contrary to adults and Shibitov and Abdelhamid (2022) stated that the *B. sulcata* prevalence was 63.4% and 60% in one and two years old, respectively. These findings confirmed by El-Ashram et al. (2019) who discovered that the high infection rate in calves may be a result of their immature immune system, which makes them more susceptible to parasitic diarrhea than adults. While

Constable (2009) added that calves diarrhea is due to changes in the parasite microenvironment in the gastrointestinal tract, especially pH changes after milk feeding by two hours, the intestinal pH falls under 6.6 which helps in the multiplication of *B. sulcata* of calves. No significant difference in infection rate between different age groups was recorded similar to (Al-saffar et al., 2013; Al-Seady and Kawan, 2014). In contrast to, Hasheminasab et al. (2015) who showed significant difference between different ages.

In present study, the positive rate of *B. sulcata* according to the feces consistency of cattle revealed very high positive rate of *B. sulcata* in diarrheic feces 92.2% than non-diarrheic feces 44.9%. These results are in line with those of prior studies (Tomczuk et al., 2005; Göz et al., 2006; Al-Saffar et al., 2010; Al Saffar et al., 2013; Sultan et al., 2013; Al-Zubaidi and Al-Mayah, 2011; Kočiš et al., 2014; Hasheminasab et al., 2015; Omeragić and Crnkić, 2015; Maharana et al., 2016; Köse and Zerek 2018; Shibitov and Abdelhamid 2022) who registered that cattle with diarrheic feces had a higher prevalence of *B. sulcata* compared to non-diarrheic feces, which indicating that *B. sulcata* was one of a primary causes of cattle diarrhea. This is explained by Makawi (2019) who concluded that contamination of food and water is a good source for spreading of *B. sulcata* infection.

The intensity between the *B. sulcata* infection and the diarrhea occurrence was 41.7% of positive animals with less than 100 *B. sulcata* cysts per gram of faeces, 76% of positive animals with less than 500 *B. sulcata* cysts per gram of faeces, 75% of positive animals with less than 1000 *B. sulcata* cysts per gram of faeces, While the diarrhea were proven in all of the fecal samples 100% which had more than 1000 *B. sulcata* cysts per gram of faeces. Likewise, Kočiš et al. (2014) found 50% of the positive animals for more than 500 *B. sulcata* cysts in gram of faeces showing diarrhea, 71.43% of examined animals had diarrhea in which more than a 1000 cysts were found in gram of faeces. In addition, diarrhea was found in 100% of the animals with more than 1.500 cysts were found in gram of faeces in Serbia. Also, the present finding are in accordance with Hong and Youn (1995); Tomczuk et al. (2005); Göz et al. (2006) and Al-Saffar et al. (2010) who reported a strong association between the intensity of *B. sulcata* infection (cysts number per gram of feces) and diarrhea. The number of *B. sulcata* in the feces was more than (500 cysts per gram). The frequency of diarrhea increases significantly, reaching 100% of cases when the intensity exceeds 2000 cysts per gram of faeces. This indicated an increase in the invasion of *B. sulcata*, which could cause diarrhea due to the acceleration of the passage of alimentary content in the intestine.

Three cattle fecal samples were positive for *B. sulcata* specific primer (1047 bp PCR product) by PCR in current study. After DNA sequencing, all PCR products showed high homology to *B. sulcata.* The three nucleotide sequences of *B. sulcata* have been obtained (Accession numbers OR395410, OR395411 a OR395412). Upon phylogenetic analysis of the current study, three sequences isolated were recognized as *B. sulcata* belonged to the first clade and showed 100% similarity with *B. sulcata* (Accession No. AB786848) while it showed 99.9% similarity with isolated (Accession No. KP016717 and AB794979) and 99.8% similarity with (Accession No. KP016718 and JQ073337). Similar to that observed by Grim et al. (2015) who showed that in evolution, *Buxtonella* is located in an ancestral derivative lineage which also comprises part of the Balantidiidae; in addition, *B. sulcata* is located in the same evolutionary branch

as *B. coli* in all the phylogenetic trees. Also, Pomajbíková et al. (2013) recorded that *B. sulcata* has been placed nearby to *B. coli.*

CONCLUSIONS

In conclusion, this is the first work to emphasize the situation of *B. sulcata* in Assiut, Egypt. Our results showed high positive rate of *B. sulcata* infection in cattle with great relation between the intensity of infection and diarrhea. The use of molecular methods using sequencing has permitted precise diagnosis and recognition of *B. sulcata.* So, we advised that cattle be regularly inspected for *B. sulcata* in the predominant areas. Further and excessive large-scale studies are required in the upcoming to aid in planning effective control plans against it.

AUTHOR CONTRIBUTIONS

Kuraa, H.M. and Malek, S.S. designed the study and helped in the microscopic examination, data analysis, and interpretation and reviewing the final version. Malek, S.S. collected samples. Kuraa, H.M. wrote the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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