Diarrhoea in cattle caused by *Buxtonella sulcata* in Sarajevo area

Jasmin Omeragić 1*, Ćazim Crnkić 2

**Abstract**
Recent studies showed that *Buxtonella sulcata* (*B. sulcata*) may be a potential causative agent of periodical recurrent diarrhoea of unknown etiology in cattle. This study aimed to investigate the prevalence and intensity of infection of *B. sulcata* and its role in diarrhoea in cattle in Sarajevo area. A total of 412 faecal samples were collected from cattle of different ages (189 young and 223 adults). The overall rate of infection was 27.2%, with a significant difference between young and adults (33.3% vs 21.9%). In animals infected with *B. sulcata* diarrhoea was present in 57.1% young and 51.0% adults, with no significant difference between them. The frequency of diarrhoea incidence increased proportionally to the intensity of infection with *B. sulcata*, either young or adults, in a typical logarithmic trend in both age groups (R²=0.97). In samples with more than 2000 cysts per 1g of faeces diarrhoea was found in almost 80% of adults, and in 100% of young animals. The results obtained here indicate that young animals are more prone to the infection with *B. sulcata*, but regardless of age the cattle similarly responds to the infection.

**Keywords**
cattle — protozoa — *Buxtonella sulcata* — diarrhoea — Sarajevo

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**Introduction**
In Bosnia and Herzegovina (B&H) diarrhoea caused by protozoan parasites in cattle, especially in young animals, was mainly attributed to the parasite phylum *Apicomplexa*, such as species of the genus *Eimeria* and *Cryptosporidium* (13). In the past, investigation on protozoan phylum *Ciliophora* did not attract special attention. It is known that some ciliates are localized in the gastrointestinal tract of ruminants, but their role has not been fully clarified. There is a general opinion that the protozoans in the gastrointestinal tract are involved in the process of digestion of plant feeds as a commensal organism. However, high incidence of infection in ruminants with *Buxtonella sulcata* (*B. sulcata*) and changes that it causes, points out that in some cases this ciliate may be responsible for the incidence and intensification of diarrhoea symptoms (18). Urman and Kelliky (20) and Skotarczak (15) suggested that *B. sulcata* can lead to pH changes of large intestinal content of cattle. Also, the multiplication of the parasite causes a cytotoxic effect in the large intestine which is manifested as lesions of the intestinal mucosa followed by secondary bacterial infections.

Protozoan parasite *B. sulcata* (Jameson, 1926) has been classified in the Kingdom: *Protozoa*, Phylum: *Ciliophora*, Class: *Kinetofragminophorea*, Order: *Trichostomatorida*, Family: *Pycnostichidae*, Genus: *Buxtonella* (17). Predilection site of *B. sulcata* is in the large intestine (colon) of cattle (11). Parasites of the phylum *Ciliophora*, including *B. sulcata* developed cilia to move, which are actually short growths of plasma.

The first written document on *B. sulcata* was given by Jameson (9). He reported heavy intestinal infection with *B. sulcata* in 5 out of 8 cattle from Cambridge. The most prominent character of this new ciliate was a dorsal ridge running in a wide sweeping curve from one end of the body to the other with a groove running down the middle. Roundish oval cysts of this ciliate were 80 to 100 µm in length by 60 to 80 µm in width.

Rees (14) studied morphology and behaviour of *B. sulcata* from cattle and *Balantidium coli* from pig. A ciliate occurring in the large intestine in about 25% of the cattle was detected in slaughtered animals by examination of the faecal material in Syracuse watch glasses under the binocular microscope. Henriksen (7) in Denmark, examined fecal samples of 762 cows and found cysts of *B. sulcata* in 547 (71.8%) cases, and Fox and Jacobs (5) from UK found cysts in 44.6% of fecal samples. Hong and Youn (8) reported prevalence of *B. sulcata* in 33.6% cattle in 1984 and 34.5% in 1994. In infected cattle diarrhoea symptoms have been present in 184 (69.1%) animals from 1984 and in 56 (55.5%) animals from 1994. Wacker et al. (21) reported presence of *B. sulcata* in cattle in Germany and clinical symptoms accompanied by diarrhoea in 73% of infected animals. The lowest incidence of *B. sulcata* in cattle (7.5%) in European countries was found in Greece (3).

Tomczuk et al. (18) examined fecal samples of 116 dairy cattle in Poland from 19 farms where they observed recurrent diarrhoea of unknown etiology. *B. sulcata* cysts were found in 102 (87.9%) animals, and diarrhoea was present in 35 (34.3%) animals examined. The authors also found that the diarrhoea intensity was proportional with the
Diarrhoea in cattle caused by *Buxtonella sulcata* in Sarajevo area — 51/54

*B. sulcata* infection intensity. With the infection intensity of over 1000 cysts per gram (CPG) of feces, diarrhoea was found in 90% of animals tested, and in all animals with more than 2000 CPG. By improving zoohygienic conditions and parenteral treatment with 5% and 20% glucose, vitamin C and vitamin B, along with a balanced mineral-vitamin dietary supplement, the number of cysts was drastically reduced and the diarrhoea ceased in all treated animals.

In Iraq, Al-Saffar et al. (1) conducted examination of 120 cattle fecal samples in the city of Mosul. Infection with *B. sulcata* was present in 29 animals (24.2%), and the cysts were found in 27 of 86 (31.3%) animals with diarrhoea symptoms. In dairy cows of Costa Rica, Jimenez et al. (10) have found *B. sulcata* in 38%, and 21.6% of beef cattle. Tung et al. (19) examined 310 fecal samples of cattle in Taiwan where *B. sulcata* was found in 26 (8.4%) animals. Ganai et al. (6) reported the incidence of *B. sulcata* in 20.9% bovines (n=374) of R.S. Pura, Jammu (India) where cattle showed higher infection rate (23.6%) than the buffaloes (18.5%). The infection was higher in young animals (33.1%) than the adults (13.9%). Animals with diarrhoeic feces had higher infection rate (38.5%) than the animals with normal feces (9.9%), suggesting the influence of *B. sulcata* on the incidence of diarrhoea.

Only one isolated study on *B.sulcata* was conducted in cattle in B&H (13) where 2019 fecal samples were examined and the cysts of *B. sulcata* were found in 464 samples (22.9%). However, only 93 samples from Sarajevo area were examined and 37 (39.8%) were positive, more than double percentage when compared to north-western part of the country (17.2%). Due to small number of samples from the Sarajevo area it was difficult to conclude that incidence of *B. sulcata* was so much different between investigated regions. Therefore, the aim of this study was to investigate presence on *B. sulcata* in a larger group of animals in Sarajevo area to obtain more correct data about the prevalence of infection.

**Material and Methods**

**Study area.** Sarajevo, the capital of B&H and its largest urban, cultural, economic and transportation center, is located almost in the geographical center of B&H (coordinates N 43° 52′ 0″, E 18° 25′ 0″, average altitude is 511 m). Based on the data of the Federal Office of Statistics of B&H (16), the climate for the area of Sarajevo is characterized by moderate and mild continental. The average annual temperature is 9.5 °C; precipitation is approximately 900 l/m².

**Sample collection and examination.** During the two-year study (2012 and 2013) 412 fecal samples of cattle of different age were collected. All the samples were taken directly from the animal rectum, and were classified according to age and diarrhoea symptoms. Samples were stored at 4 °C into an isothermic box and sent to the Laboratory of Parasitology, Veterinary Faculty of Sarajevo, B&H. Coprological examination was carried out using standard sedimentation and flotation techniques. Determination of cysts *B. sulcata* was conducted on the basis of characteristic morphological features and measurement of the parasite’s diameter (Figure 1).

**Statistical analysis.** The data were recorded in two groups according to the age of the cattle: young (below 1 year of age) and adults (above 1 year of age). The fecal samples were divided into two groups: non-infected and infected. The feces of infected animals were further subdivided either as diarrhoeic and non-diarrhoeic, or as four groups according to the number of cysts in 1 g (CPG) of feces. Results were analyzed using Chi square test with a p < 0.05 considered as significant. Fisher’s exact test was used in the case of 2×2 tables when any expected frequency was too low for Chi square test. Regression analysis was performed to estimate the relationships between the frequency of diarrhoea incidence and intensity of infection with *B. sulcata*. All statistics were performed using Minitab 15 statistical software (12).

**Results**

Coprological examination of cattle feces in this study showed the presence of *B. sulcata* cysts in 112 (27.2%) animals (Table 1). Significantly higher prevalence of infection (p=0.010) was found in young compared to adults (33.3% vs 21.9%). The infection was associated with diarrhoea in more than a half of the infected animals, either young (57.1%) or adults (51%), with no significant difference between them (p=0.519).

**Table 1. Infection rate with *B. sulcata* and diarrhoea incidence in infected animals**

<table>
<thead>
<tr>
<th>Age of cattle</th>
<th>Examined</th>
<th>Infected (%)</th>
<th>Animals with diarrhoea (% of infected animals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>189</td>
<td>63 (33.3)*</td>
<td>36 (57.1)</td>
</tr>
<tr>
<td>Adults</td>
<td>223</td>
<td>49 (21.9)</td>
<td>25 (51.0)</td>
</tr>
<tr>
<td>Total</td>
<td>412</td>
<td>112 (27.2)</td>
<td>61 (54.5)</td>
</tr>
</tbody>
</table>

*Significantly higher than in adults (p=0.010)

The increasing frequency of diarrhoea incidence in infected animals was proportional to the *B. sulcata* infection intensity (Table 2) having a logarithmic trend (R²=0.98; y = 45.963Ln(x) + 26.93) and the frequency was significantly different depending on the infection intensity (p<0.001). The relationship was evident even when viewed separately for young (p<0.001) and adults (p=0.018), with the same type of logarithmic trend for both age groups (R²=0.97). Logarithmic growth of diarrhoea frequency was described by equation y = 52.141Ln(x) + 25.99 or y = 39.711Ln(x) + 27.474, for young and adults, respectively. However, separate analysis of each infection intensity in Table 3 showed no significant differences between young and adult animals.

**Table 2. The effect of *B. sulcata* infection intensity on frequency of diarrhoea incidence**

<table>
<thead>
<tr>
<th>Infection intensity (CPG)</th>
<th>Number of infected animals</th>
<th>Animals with diarrhoea (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;500</td>
<td>45</td>
<td>11 (24.4)</td>
</tr>
<tr>
<td>500 - 1000</td>
<td>28</td>
<td>18 (64.3)</td>
</tr>
<tr>
<td>1000 - 2000</td>
<td>21</td>
<td>16 (76.2)</td>
</tr>
<tr>
<td>&gt;2000</td>
<td>18</td>
<td>16 (88.9)</td>
</tr>
<tr>
<td>Total</td>
<td>112</td>
<td>61 (54.5)</td>
</tr>
</tbody>
</table>

*Significantly different depending on infection intensity (p< 0.001)
Discussion and conclusion

The overall percentage of B. sulcata infection in cattle was lower in this study in comparison to the percentages of infection in Denmark, Britain, and South Korea (5, 7, 8), but was higher compared with the results of similar studies conducted in India, Greece, Iraq, and Taiwan (1, 3, 6, 19). However, the obtained result are much lower if compared with the results obtained for Sarajevo area in our previous study, but still higher than those results for north-western part of the country (13).

More than a half of the infected animals in the present study showed diarrhoea symptoms which is similar to the results obtained in our earlier work (13). Compared to results reported here, the diarrhoea incidence was higher in infected cattle in South Korea and Germany (8, 21), while in Poland and Iraq have been lower (1, 18).

The diarrhoea incidence increased proportionally with the results shown in Table 1, we suggest that cattle similarly respond to B. sulcata infection regardless of age. However, in routine practice we still can expect more frequent incidence of diarrhoea caused by B. sulcata in young animals, bearing in mind that they have much higher prevalence of infection compared to adults as shown in this (Table 1) and other studies (6).

The results obtained here, as well as the findings from other studies (5, 8, 18, 20) suggest that at low infection intensity (<500 CPG) a relatively small number of animals showed clinical signs, such as diarrhoea. However, in animals with the infection intensity of >500 CPG diarrhoea incidence was markedly increased, reaching 100% in cases with the highest infection intensity (>2000 CPG).

Besides the number of cysts in the gastrointestinal tract, there are many other factors influencing the course of buxtonelosis, including virulence of the pathogens, host susceptibility, environmental conditions, etc. Important for the parasite-host impact are the pH changes in the colon. Acidity stimulates growth and multiplication of parasites and increases the cytotoxic effects on host tissue. Detrimental effect on the intestinal mucosa is exacerbated and the resulting inflammatory lesions are suitable places for the development of secondary bacterial infections that further complicates the pathological changes (18). It is known that decrease in colonic pH may be a consequence of feeding regimen. When high concentrate diets are fed, some starch escapes ruminal degradation and passes to the hindgut where it undergoes fermentation resulting in higher concentration of volatile fatty acids (2). The acids decrease colonic pH creating environment that is favorable for growth of some pathogens. Low colonic pH also makes mucosal tissues more vulnerable, regardless of the pathogens action (4). Finally, all feeding situations when intestinal pH is moving toward acidic conditions can complicate protozoal infection. Even when intestinal pH is not compromised, increased infection with the protozoan parasites may result in the accelerated passage of alimentary contents in the gut causing disorders such as diarrhoea or poor condition of animals (18). If the primary infection with B. sulcata is not complicated by disruption of bacterial flora, then the dietary changes and pH regulation in large intestine with probiotics and symptomatic therapy would normally satisfy therapeutic measures.

The results of this study showed that the prevalence of B. sulcata infection in Sarajevo area is lower than previously showed (13). Results confirm that young animals are more prone to the B. sulcata infection but also indicate similar infection responsiveness of cattle regardless of age.
References

Dijareja goveda uzrokovana sa *Buxtonella sulcata* na području Sarajeva

**Sažetak**

**Uvod**

Ranija istraživanja su pokazala da *Buxtonella sulcata* (*B. sulcata*) može biti potencijalni uzročnik periodičnih povratnih dijareja nepoznate etiologije u goveda. U BiH je do sada provedeno samo jedno takvo istraživanje, a prisustvo cisti *B. sulcata* utvrđeno je u 22,9% uzoraka fecesa. Međutim, na području Sarajeva 39,8% uzoraka bilo je pozitivno, što je dvostruko više u odnosu na sjeverozapadni dio zemlje, gdje je stopa infestacije bila 17,2%. Zbog relativno malog broja uzoraka sa područja Sarajeva (n=93), bilo je teško pouzdano zaključiti da su razlike između dva navedena područja uzorkovanja toliko različite. Cilj ovog rada bio je ispitati učestalost i intenzitet zaraze sa *B. sulcata* na većem broju uzoraka i utvrditi njenu ulogu u nastanku dijareje u goveda na području Sarajeva.

**Materijal i metode**

Ukupno 412 uzoraka fecesa prikupljeno je od goveda različite dobi (189 mladih i 223 odraslih). Koprološka ispitivanja su provedena standardnim parazitološkim metodama sedimentacije i flotacije. Determinacija cisti *B. sulcata* provedena je na bazi morfoloških karakteristika i mjerenjem dijametra parazita.

**Rezultati i interpretacija**

Ukupna stopa infekcije bila je 27,2%, sa značajnom razlikom između mladih i odraslih (33,3% nasuprot 21,9%). U životinja zaraženih *B. sulcata* dijareja je bila prisutna u 57,1% mladih i 51% odraslih, bez značajne razlike među njima. Učestalost pojave dijareje povećavala se proporcionalno intenzitetu zaraze sa *B. sulcata*, bilo da su mlade ili odrasle životinje u pitanju, u tipičnom logaritamskom trendu rasta u obje dobne skupine ($R^2 = 0,97$). U uzorcima sa više od 2000 cista po 1 g fecesa, dijareja je ustanovljena u gotovo 80% odraslih i 100% mladih životinja.

**Zaključci**

Dobiveni rezultati su pokazali da je stopa infekcije sa *B. sulcata* na području Sarajeva manja od one koja je utvrđena u ranijem istraživanju. Rezultati također ukazuju na to da su mlade životinje više podložne infekciji sa *B. sulcata*, ali i da goveda slično reagiraju na infekciju bez obzira na dob.