First evidence of *Blastocystis* spp. in livestock animals: An emerging zoonosis from Sulaymaniyah Province

Shadan Hassan Abdullah 1*

1 Department of Microbiology, College of Veterinary Medicine, University of Sulaimani, Sulaimani City, Kurdistan Region, Iraq

*Corresponding email: shadan.abdullah@univsul.edu.iq

**Table:**

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<th>Article info</th>
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<td>Original: 05/08/2023</td>
<td>Blastocystosis is an infection with zoonotic parasite <em>Blastocystis</em> spp. commonly habitat the intestinal tract of wide range of hosts including human, animals and birds. The study conducted during June to November 2022 in Sulaymaniyah province for detection of <em>Blastocystis</em> spp. from livestock animals. For this purpose, a total of 250 fecal samples were collected randomly from cattle, sheep, and goats of different age groups. Based on microscopic examination of the examined fecal smears the overall prevalence rate of <em>Blastocystis</em> spp. was 24.4% among examined ruminant hosts. Higher prevalence rate has been reported from cattle 29% followed by sheep 27%, and goats 16%, with no significant differences. The study data confirmed <em>Blastocystis</em> colonization of livestock animals in the study area, further study for detection of various subtypes by applying of molecular techniques is essential to find out the infected subtypes and define their impact on public health.</td>
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**Introduction**

*Blastocystis* is a cosmopolitan distributed enteric protozoan parasite [1]. It was first described in 1912 from humans and was named as Blastocystis hominis, but later on its name was changed to Blastocystis spp. [2], since its presence in human, the parasite has been detected habitat within the intestinal tract of wide range of hosts including various animal species, ruminants, rodents, and birds [3], which can also live in the intestine of reptile, fish and even invertebrates like cockroaches [4]. Blastocystis is a unicellular eukaryote parasite belonging to the class Blastocystea [5]. The organism is polymorphic and has distinct morphological forms such as vacuolar, granular, cyst, amoeboid, a vacuolar and multivacuolar forms [6]. The isolated Blastocystis spp. from human and animals are indistinguishable based on the morphological characters [7], while host specificity and pathogenic possibility of different isolates corelated with diversity in small subunit rRNA [8]. Based on the phylogeny of rRNA, 22 sub types have been isolated from human and animals throughout the world [9], of these 17 sub types (ST1- ST17) have been recognized in a wide range of hosts including human, mammals, birds, reptiles and insects [10]. As a result of the low host specificity of Blastocystis spp. and their zoonotic potential, animals were considered as a possible host for transmission of Blastocystis infection to human [11].

Transmission occurs through the fecal–oral route, with consumption of cysts either via direct contact with infected hosts or indirectly by ingestion of contaminated food or water [12]. The pathogenic effects of Blastocystis spp. are ongoing doubtful due to its higher prevalence rate in asymptomatic individuals, although identification of virulence factors such as cysteine proteases possibly involved in pathogenicity [13], beside that the pathogenicity is related to the involved subtype, and the parasitic burden, in which infected person with few numbers might be asymptomatic [14].
Infection by Blastocystis spp. in human would be associated with a variety of non-specific intestinal disorders, such as diarrhea and abdominal pain, as well as skin rash or urticarial [15]. Unlike human, animal particularly cattle that infected with Blastocystis spp. normally appear as healthy carriers and serve as a main reservoir in transmitting the infection to human [10, 16]. Infection of farm animals with mixed of zoonotic and enzootic Blastocystis subtypes have been reported [17].

To date, several diagnostic methods including direct smear examination, staining procedures by Giemsa stain and modified acid-fast stain (Ziehl-Neelsen stain) [6], other methods like formalin-ether concentration techniques, iodine or trichrome stained smear, in vitro cultivation, Molecular diagnosis (PCR and sequencing) have been used for detection of Blastocystis spp. [18]. Previous reports highlighted variation in prevalence rates in different geographical areas, with a higher reported prevalence rate in developing countries that possibly associated with variances in the standards of sanitation, waste disposal, contact with animals, and consumption of contaminated food or water [19].

The objective of the current study was an investigation of Blastocystis spp. from livestock animals in Sulaymaniyah province, based on the previous studies knowledge the parasite has been reported from human in the study area, while the status of Blastocystis in animals have not been studied yet, although the defined parasitological study is not adequately characterized distinct prevalence of Blastocystis subtypes, but provide a preliminary data about it, and necessitate the applying of epidemiological study on Blastocystis spp. in different animal hosts using advance molecular techniques.

Materials and Methods

During June to November 2022. A total of 250 animals were selected randomly for fecal sample collection, sheep (n= 100), goat (n= 75), and cattle (n=75) from ruminant flocks belonging to Sulaymaniyah province districts, the selected animals were from different age groups.

Fecal samples were collected directly from rectal using disposable gloves and stored in a plastic container, labeled and transported to the laboratory at Veterinary Medicine College, University of Sulaimani.

Samples were fixed and preserved in 10% formalin, all collected samples were screened for detection of Blastocystis parasite stages via direct wet mounting microscopy [20]. Concentration sedimentation technique was also applied for detection of parasitic stages, and the slides were evaluated based on morphological structure under 20X and 40X objective lenses.

For further confirmation, the suspected positive smears were also stained by Giemsa stain and modified acid-fast stain (Ziehl-Neelsen stain), and examined under 100X objective lens [21]. Samples were declared positive if one of the parasitic stages have been observed by examination methods. The Chi-square test of significance was used for data study using statistical analysis system SPSS version 20.

Results

Through microscopical examination of obtained fecal samples, the observed prevalence rate was 24.4% via morphological observation of Blastocystis stages. The prevalence rate of blastocytosis among different animals were 29%, 27% and 16% in cattle, sheep and goats respectively, no significant difference was found (Table 1).

Table 1: Frequency of Blastocystis spp. in ruminants from Sulaymaniyah province.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Total examined samples</th>
<th>No. of infected</th>
<th>% of infected</th>
<th>Chi-square value</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Cattle</td>
<td>75</td>
<td>22</td>
<td>29</td>
<td>4.224</td>
<td>0.12</td>
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<tr>
<td>Sheep</td>
<td>100</td>
<td>27</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goat</td>
<td>75</td>
<td>12</td>
<td>16</td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
<td>250</td>
<td>61</td>
<td>24.4</td>
<td></td>
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Figure 1: *Blastocystis* spp. Vacuolated form, wet mount (20X).

Figure 2: *Blastocystis* spp. Granular form, wet mount (40X)

Figure 3: *Blastocystis* spp. Vacuolated form A. Giemsa stain. B. Ziehl-Neelsen stain (X100).
Discussion

The Current study highlights the existence of Blastocystis spp. in livestock animals with prevalence rate of 24.4%. Recently, higher prevalence rate of Blastocystis infection in cattle than the current finding, 77.6% and 54.5% were reported in Colombia [22], and in Turkey [3], respectively, moreover the recorded 71% and 39.2% from France [23], and Lebanon [24] were disagree with current data. Moderate prevalence of 22.7% and 25% were reported from United Arab Emirates and Malaysia by [25] and [26], respectively. As well as lower prevalence rate (9.6%) has been reported from Iran [27]. To our knowledge in previous studies, molecular techniques were applied for detection of Blastocystis spp. which considered as an accurate diagnostic technique. The frequency of blastocystosis in current study signified higher colonization of 29% among cattle, similarly 29.34% and 25% were reported [28, 26] from Malaysia, contrary to the current data higher prevalence rate of 50.6% from Iran and lower prevalence rate of 9.5% from China were reported [28, 18] respectively. It was denoted by Cian et al. [23] that bovines have the ability to carry pathogenic Blastocystis spp. from human, ruminants and rodents, and increased the likelihood of zoonotic transmission. Recently, molecular findings represented higher prevalence rates of Blastocystis colonization in sheep 43.07% and 32.0% from Malaysia and Iran [28, 29], furthermore 63.6% from United Arab Emirates [25] were disagree with current findings, however lower prevalence rate of 6% was reported from China [30]. In regard to the occurrence of blastocystosis in goats, contrary to the current data higher prevalence rate of 94.7% from
Thailand and 29.16% from Malaysia were reported [31, 28] respectively, although low prevalence rate of 5% from Indonesia was reported [32]. Variation in prevalence rate from different geographical area might related to factors such as the applied diagnostic method for detection of the parasite, and differences in animals’ husbandry conditions.

Analysis of sequence isolates denoted similarly in Blastocystis subtypes among different hosts, and the reported subtypes in livestock animals have been found as a potential pathogen for human and indicate zoonotic transmission, a previous study stated that the reported ST1 from farm animals was correlated to zoonosis [6, 8]. It has been defined [16] that higher infection rates of Blastocystis spp. have been found among animal handlers in zoo, abattoir staffs and researchers than other peoples who are not in contact with animals. In Sulaymaniyah city, the prevalence rate of Blastocystis infection in human has been studied, with a prevalence rate of 22% [33]. Concerning the public health hazardous of blastocystosis, resent findings observed the positive associations between irritable bowel syndrome (IBS) patients and the presence of Blastocystis infection detected Blastocystis parasite in 60% of IBS patients [34].

In current study, the obtained samples for detection of Blastocystis spp. were collected from non-diarrheic animals, such results harmony with the reported findings by [16], and indicted that infection with Blastocystis spp. might not associated with diarrhea and remain asymptomatic and verified that in spite of the parasitic existence, the wellbeing of the cattle was not affected and could be considered as a natural host of Blastocystis spp. [26].

In spite of livestock animals, Blastocystis spp. have also been reported from other animal hosts with various prevalence rates, which might be sources for human infection by harboring zoonotic subtypes, in pet animals 50% was reported in dogs from Colombia [22], and 17% in cats from Iran [35]. In birds, 42.9% was reported in pigeon from Iran [4], and 32% in chicken from Lebanon [24], additionally [22] was observed Blastocystis infection in horses with prevalence rate of 54.5%. Blastocystis spp. were also reported [25] with various frequency of 33.3%, and 37.5% in rabbit and rodents respectively from United Arab Emirates.

Conclusions

The current study providing a new insight into the prevalence of blastocystosis in livestock animals, and the result demonstrate moderate colonization of Blastocystis for ruminant hosts with higher prevalence rate in cattle. Occurrence of Blastocystis spp. in cattle, sheep, and goats is illustrative for their role in the epidemiology of the parasite. Although the applied diagnostic procedures are reliable for diagnosis of Blastocystis infection, a comprehensive study by applying of advanced molecular techniques are required to investigate Blastocystis spp. from different animal hosts for determination of infected subtypes. Moreover, adequate estimation of each subtype prevalence rate in different hosts with their potential zoonosis are another important issue.

Conflict of interest

The authors confirm that they are not affiliated with or involved in any organization or entity with financial interests.

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Blastocystis Subtypes in Domestic Animals from Colombia Using Amplicon-Based Next Generation Sequencing Front. Veterinary Sciences ;24 (8):732129.


