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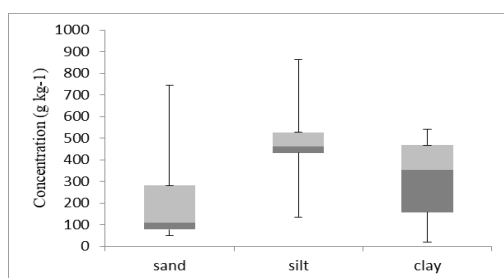
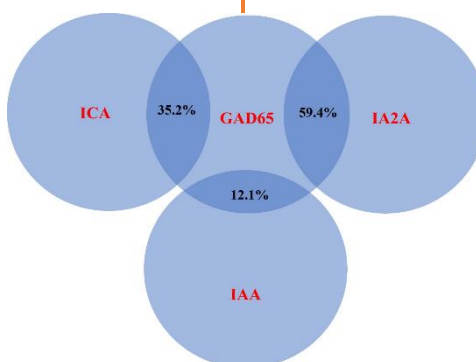
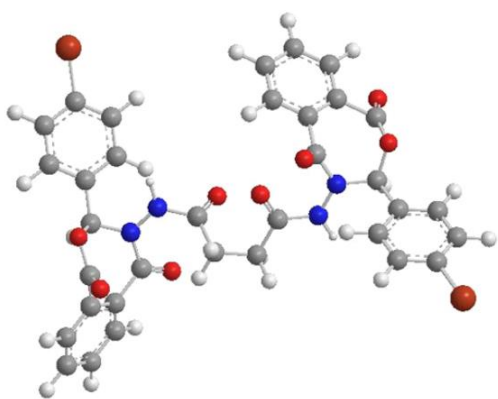
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First evidence of *Blastocystis* spp. in livestock animals: An emerging zoonosis from Sulaymaniyah Province

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Abstract

Blastocystosis is an infection with zoonotic parasite *Blastocystis* 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 *Blastocystis* 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 *Blastocystis* 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 *Blastocystis* 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.

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 correlated 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.

Animals	Total examined samples	No. of infected	% of infected	Chi-square value P-value
Cattle	75	22	29	4.224
Sheep	100	27	27	0.12
Goat	75	12	16	
Total	250	61	24.4	

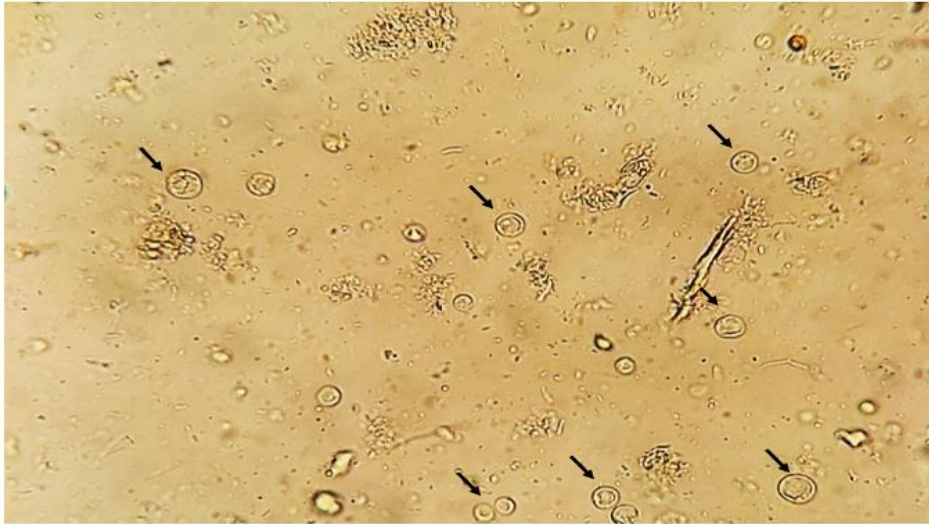


Figure 1: *Blastocystis* spp. Vacuolated form, wet mount (20X).

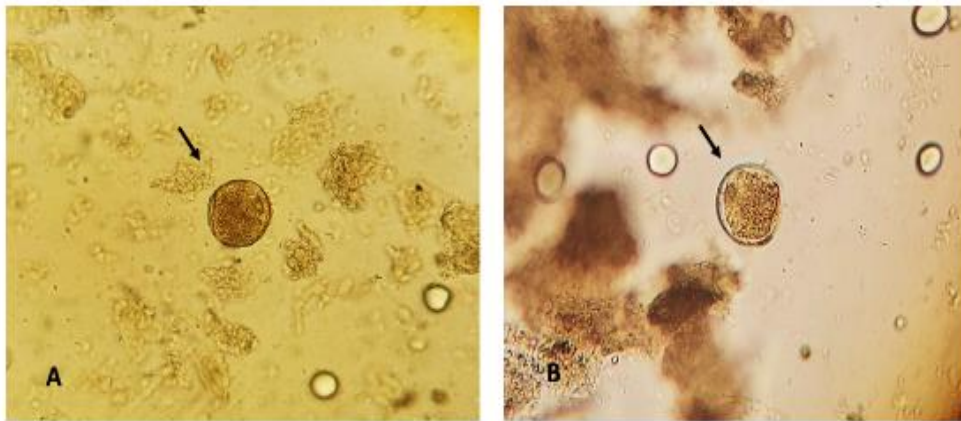


Figure 2: *Blastocystis* spp. Granular form, wet mount (40X)

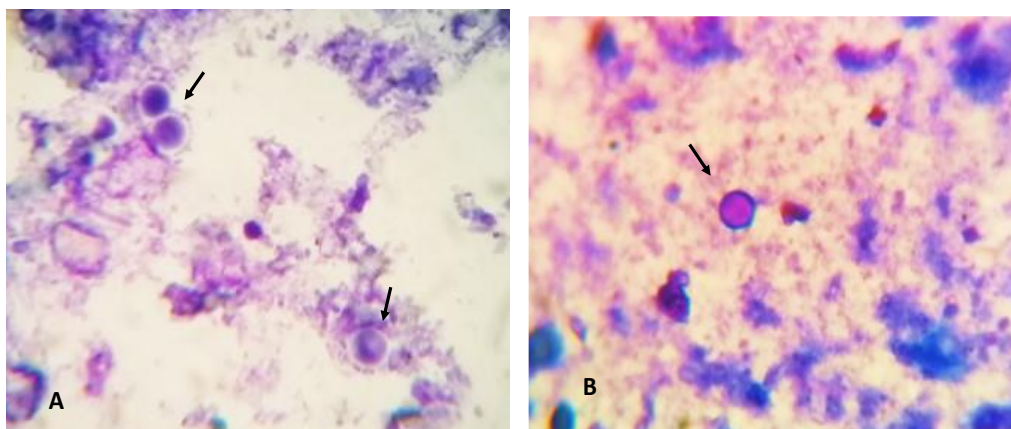


Figure 3: *Blastocystis* spp. Vacuolated form A. Giemsa stain. B. Ziehl- Neelsen stain (X100).

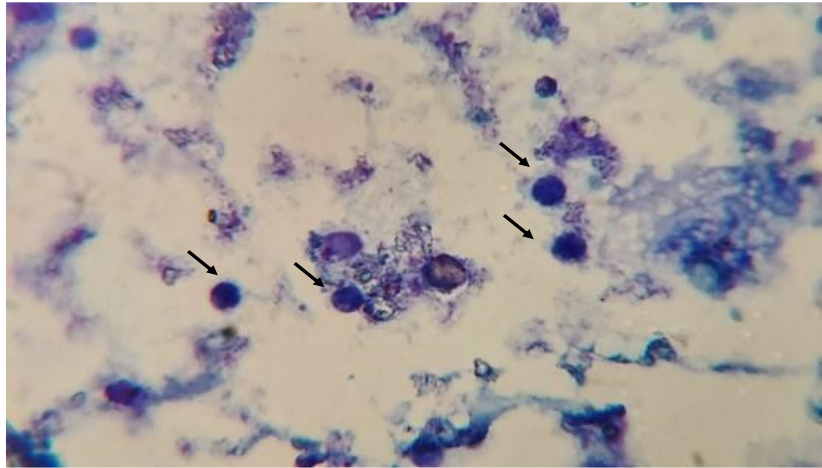


Figure 4: *Blastocystis* spp. Cystic form using Giemsa stain (100X).

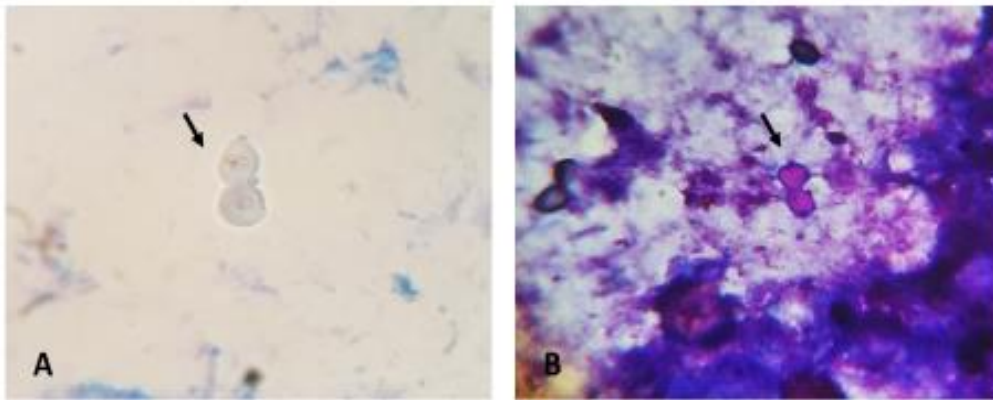


Figure 5: *Blastocystis* spp. During division, A. Giemsa stain, B. Ziehl- Neelsen stain (100X).

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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References

1. Barbosa CV, do Bomfim TCB, Teixeira BR, Gentile R, da Costa Neto SF, Magalhães BSN, Balthazar DA, da Silva FA, Biot R, Levy CMA, Santos HLC. (2019). Molecular epidemiology of *Blastocystis* isolated from animals in the state of Rio de Janeiro, Brazil. Plos One; 14(1): e0210740.

2. Silberman JD, Sogin ML, Leipe DD, Clark CG. (1996). Human parasite finds taxonomic home. *Nature*; 380(6573): 398–398.
3. Oner TO, Karakavuk M, Doskaya AD, Güvendi M, Gül A, Koseoglu AE, Alak SE, Gürüz AY, Cemal Ün C, Mert Doskaya M, Hüseyin Can H. (2022). Molecular prevalence of *Blastocystis* sp. and subtype diversity in fecal samples collected from cattle in dairy farms in Turkey. *Comparative Immunology, Microbiology and Infectious Diseases* 87:101850.
4. Asghari A, Sadraei J, Pirestani M, Mohammadpour M. (2019). First molecular identification and subtype distribution of *Blastocystis* sp. isolated from hooded crows (*Corvus cornix*) and pigeons (*Columba livia*) in Tehran Province, Iran. *Comparative Immunology, Microbiology and Infectious Diseases*; 62: 25-30.
5. Cavalier-Smith T. (1998). A revised six-kingdom system of life. *Biol. Rev. Camb. Philos. Soc*; 73:203-266.
6. Tan KS. (2008). New insights on classification, identification, and clinical relevance of *Blastocystis* spp. *Clinical Microbiology Reviews*; 21:639–665.
7. Gentekaki E, Curtis BA, Stairs CW, Klimeš V, Eliáš M, Salas-Leiva DE, Herman EK, Eme L, Arias MC, Henrissat B, et al. (2017). Extreme Genome Diversity in the Hyper-Prevalent Parasitic Eukaryote *Blastocystis*. *PLOS Biology*; 15: e2003769.
8. Noël C, Dufernez F, Gerbod D, Edgcomb VP, Delgado-Viscogliosi P, Ho LC et al. (2005). Molecular phylogenies of *Blastocystis* isolates from different hosts: Implications for genetic diversity, identification of species, and zoonosis. *Journal of Clinical Microbiology*; 43: 348–55.
9. Stensvold CR, Clark CG. (2020). Pre-empting Pandora’s Box: *Blastocystis* subtypes revisited. *Trends in Parasitology*; 36(3): 229–232.
10. Alfellani MA, Taner-Mulla D, Jacob AS, Imeede CA, Yoshikawa H, Stensvold CR, Clark, CG (2013). Genetic diversity of *Blastocystis* in livestock and zoo animals. *Protist*; 164:497–509.
11. Ahmed SA, Karanis P. (2018). *Blastocystis* spp., ubiquitous parasite of human, animals and environment. 2nd edn. Elsevier Inc;.
12. Hublin JSY, Maloney JG, Santin M. (2021). *Blastocystis* in domesticated and wild mammals and birds. *Research in Veterinary Science*; 135: 260–282.
13. Ajjampur SS, Tan KS. (2016). Pathogenic mechanisms in *Blastocystis* spp. interpreting results from in vitro and in vivo studies. *Parasitology International Journal*; 65:772–779.
14. Coyle CM, Varughese J, Weiss LM, Tanowitz HB. (2012). *Blastocystis*: To Treat or Not to Treat. *Clinical Infectious Diseases*; 54(1):105–10.
15. Andersen LOB, Stensvold CR. (2016). *Blastocystis* in health and disease: Are we moving from a clinical to a public health perspective? *Journal of Clinical Microbiology*; 54: 524-528.
16. Lee H, Lee SH, Seo MG, Kim HY, Kim JW, Lee, YR, Kim JH, Kwon OD, Kwak D. (2018). Occurrence and genetic diversity of *Blastocystis* in Korean cattle. *Veterinary Parasitology*; 258: 70-73.
17. Hublin JS, Maloney JG, Santin M. *Blastocystis* in domesticated and wild mammals and birds. *Research in Veterinary Science* 2020; 135: 260–82.
18. Wang J, Gong B, Yang F, Zhang W, Zheng, Y, Liu A. (2018). Subtype distribution and genetic characterizations of *Blastocystis* in pigs, cattle, sheep and goats in northeastern China’s Heilongjiang Province. *Infection, Genetics and Evolution Journal*; 57:171–176.
19. Clark CG, van der Giezen M, Alfellani MA, Stensvold CR. (2013). Recent developments in *Blastocystis* research. *Advances in Parasitology*; 82:1–32.
20. Elghareeb AS, Younis MS, El Fakahany AF, Nagaty IM, Nagib MM. (2015). Laboratory diagnosis of *Blastocystis* spp. in diarrheic patients. *Tropical Parasitology*;5: 36–41.
21. Arpitha GM, Sreekumarb C, Lathaa BV, Bharathi MV. (2018). Prevalence and staining characteristics of *Blastocystis* isolates from food animals in Tamil Nadu Veterinary. *Parasitology: Regional Studies and Reports*;11: 61–65.
22. Higuera A, Herrera G, Jimenez P, García-Corredor D, Pulido-Medellín M, Bulla-Castañeda DM, Carlos Pinilla J, Moreno-Pérez DA, Maloney JG, Santín M, Ramírez JD. (2021). Identification of Multiple

- Blastocystis* Subtypes in Domestic Animals from Colombia Using Amplicon-Based Next Generation Sequencing Front. Veterinary Sciences ;24 (8):732129.
23. Cian A, El Safadi D, Osman M, Moriniere R, Gantois N, Benamrouz-Vanneste S, Delgado-Viscogliosi P, Guyot K, Li L, Monchy S, Noel C, Poirier P, Nourrisson C, Wawrzyniak I, Delbac F, Bosc S, Chabé M, Petit T, Certad G, Viscogliosi E. (2017). Molecular epidemiology of *Blastocystis* sp. In various animal groups from two French zoos and evaluation of potential zoonotic risk. PLoS ONE; 12: e0169659.
24. Greige S, El Safadi D, Bécu N, Gantois N, Pereira B, Chabé M, et al. (2018). Prevalence and subtype distribution of *Blastocystis* sp. isolates from poultry in Lebanon and evidence of zoonotic potential. Parasites & Vectors; 11(1–10): 389.
25. AbuOdeh R, Ezzedine S, Mohamed M, Stensvold CR, Samie A, Nasrallah G, Al Absi E, El Bakri A. (2019). Molecular Subtyping of *Blastocystis* from Diverse Animals in the United Arab Emirates. Protist; 170(5): 125679.
26. Kamaruddin SK, Mat Yusof A, Mohammad M. (2020). Prevalence and subtype distribution of *Blastocystis* sp. in cattle from Pahang, Malaysia. Tropical Biomedicine; 37(1): 127–141.
27. Badparva E, Sadraee J, Kheirandish F. (2015). Genetic diversity of *Blastocystis* isolated from cattle in Khorramabad, Iran. Jundishapur Journal of Microbiology; 8(3): e14810.
28. Abd Razak N, Mohammad M. (2022). Prevalence of *Blastocystis* sp. in cattle, goat and sheep reared by different farm management system in Pahang, Malaysia. Malaysian Applied Biology; 51(3):47–55.
29. Salehi R, Rostami A, Mirjalali H, Stensvold CR, Haghighi A. (2022). Genetic characterization of *Blastocystis* from poultry, livestock animals and humans in the southwest region of Iran-Zoonotic implications. Transboundary and Emerging Diseases; 69(3):1178-1185.
30. Li WC, Wang K, Gu Y. (2018). Occurrence of *Blastocystis* sp. and *Pentatrichomonas hominis* in sheep and goats in China. Parasites & Vectors;11: 93.
31. Udonsom R, Prasertbun R, Mahittikorn A, Mori H, Changbunjong T, Komalamisra C, et al. (2018). *Blastocystis* infection and subtype distribution in humans, cattle, goats, and pigs in central and western Thailand. Infection, Genetics and Evolution Journal; 65:107–11.
32. Abdillah MF, Lastuti NDR, Utama S, Suprihati E. (2021). Identification and prevalence of digestive tract endoparasites of goats in Ujungpangkah, Gresik District. Journal of Parasite Science; 5: 61–64.
33. Mohammed RM, Ali SA. (2015). A Study of *Blastocystis hominis* Infection in Sulaimani Pediatric Teaching Hospital. International Journal of Current research and academic review; 3(8): 290-299.
34. Ismail MH, Abbas SK, Molan A. (2022). Prevalence and subtype diversity of *Blastocystis* sp. in an Iraqi population with and without irritable bowel syndrome (IBS). Annals of Parasitology; 68(2): 275-286.
35. Mohammadpour I, Bozorg-Ghalati F, Gazzonis AL, Manfredi MT, Motazedian MH, Mohammadpour N. (2020). First molecular subtyping and phylogeny of *Blastocystis* sp. isolated from domestic and synanthropic animals (dogs, cats and brown rats) in southern Iran. Parasites & Vectors; 13:365.