

Prevalence of *Blastocystis* infection in free-range Tibetan sheep and Tibetan goats in the Qinghai-Tibetan Plateau in China

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ABSTRACT

Blastocystis is one of the most common enteric parasites in humans and domestic animals. For Tibetan sheep and Tibetan goats, the traditional grazing methods still occupy a dominant position, and the close contact between humans and domestic animals increases the risk of infection by *Blastocystis* between herdsman and livestock. However, less pertinent information is available for Tibetan sheep or Tibetan goats. In this study, 880 fecal specimens from Tibetan sheep and Tibetan goats were collected from 6 sampling sites in Tibet to test for *Blastocystis* using the polymerase chain reaction and sequencing analysis of the partial SSU rRNA gene. The infection rate of *Blastocystis* was 8.55% for Tibetan sheep (53/620) and 8.46% for Tibetan goats (22/260). The genetic analysis of 53 positive samples from Tibetan sheep identified 4 known subtypes (ST4, ST5, ST10, and ST14). Four known subtypes (ST1, ST5, ST6, and ST10) were identified in Tibetan goats. ST10 was the dominant subtype in Tibetan sheep and Tibetan goats, accounting for 65.33% (49/75) of total subtypes. ST1, ST4, ST5, and ST6 were recognized as belonging to zoonotic subtypes. This report provides a detailed data on the prevalence and subtype distribution of *Blastocystis* in Tibetan sheep and Tibetan goats in Tibet, which enriches the epidemiological data of *Blastocystis* infection in Tibetan sheep and Tibetan goats in China. Our results indicated that Tibetan sheep and Tibetan goats can be infected with multiple *Blastocystis* subtypes, including zoonotic subtypes. More research is needed among humans, livestock and wild animals in Tibet to better understand their role in the spread of *Blastocystis*. And, One Health measures need to be taken to control and prevent its zoonotic transmission.

1. Introduction

Blastocystis is an intestinal non-fungal microeukaryote that colonizes the gastrointestinal tracts of vertebrates, including humans, birds, and mammals [1,2]. Transmission of *Blastocystis* cysts to humans occurs mainly via ingestion of contaminated food or water, exposure to fecal contaminated environments, and person-to-person contact [3,4]. Although the classification and pathogenicity of *Blastocystis* are still controversial, some studies have shown that gastrointestinal dysfunction, irritable bowel syndrome, and skin lesions are associated with *Blastocystis* infection [5,6]. Studies have confirmed that *Blastocystis* isolates from patients can cause damage and ulceration to the colon of experimental mouse [7]. Previous studies have shown that there is a higher infection rate in hosts with diarrhea or other gastrointestinal

symptoms and in young animals and immunocompromised individuals [8,9]. However, one recent study challenged this view, where *Blastocystis* appeared to be more common in healthy individuals [1]. Due to the low host specificity, genetic diversity, and zoonotic potential of *Blastocystis*, it is believed that animals may be potential hosts for the transmission of this organism [10].

Due to the development of molecular biotechnology, *Blastocystis* strains can be classified by different genes, which also can be used to identify and evaluate the infectivity, pathogenicity, and other properties [11–13]. Based on phylogenetic analysis using the small subunit ribosomal RNA (SSU rRNA) gene sequence, *Blastocystis* isolated from humans and animals has shown considerable genetic diversity [14–16]. Genetic characterization of *Blastocystis* isolates based on SSU rRNA gene sequences has revealed the existence of 22 subtypes (STs 1–17, ST21,

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and STs 23–26) that differ in their host distribution [17]. ST 1 to ST 9 and ST 12 are found in humans and in many other mammals; ST 10, ST 11, STs 13–17, and STs 23–26 occur in a variety of non-human hosts [18,19]. ST1–ST4 are more common in humans, accounting for more than 95% of the reported prevalence [20–22]. The prevalence of *Blastocystis* sp. in humans ranges from 0.5–30% in developed world and can reach 100% in developing countries [23]. Furthermore, animal-keepers and abattoir workers are at high risk of infection of *Blastocystis*, which means that *Blastocystis* are transmitted between animals and people [14,22,24]. Obviously, the high infection rate of *Blastocystis* in rural communities and animal-keepers should carriage doubts about its role in human health and disease. Notably, based on molecular detection methods (particularly PCR-based), mixed subtypes of infection were detected in a variety of animals [25,26]. Inter-subtype recombinant and transmission of infection between species are thought to be the major evolution sources of diversity in the zoonotic pathogen [27,28].

In Tibet, animal husbandry is one of the major activities and human-livestock contact occurs daily. With close contact with herders, the role of livestock in the infection of zoonotic pathogens is getting more traction in Tibet [29,30]. Tibetan sheep and Tibetan goats share habitat with wildlife on the plateau and are the major livestock breeds in the Qinghai-Tibet Plateau region. Both are well adapted to the local environment, and there is extensive feeding and management in alpine pastoral areas. The genetic performance of sheep and goats in this area is stable, and the region is famous for its high-quality cashmere [31,32]. However, there are no available reports on conditions of *Blastocystis* infection in Tibetan sheep or Tibetan goats under the grazing conditions in Tibet. Efforts have been made to study livestock parasitic infection in some districts in Tibet [29,30,33]. Investigations are needed to facilitate improved interventions and prevention to minimize the burden of zoonotic *Blastocystis* infection associated Tibetan sheep and Tibetan goats. The aim of this research is to understand the prevalence of *Blastocystis* infection in Tibetan sheep and Tibetan goats in Tibet. And the impact of this organism on One Health is also discussed.

2. Materials and methods

2.1. Sample collection

A total of 880 fresh fecal samples from Tibetan sheep and Tibetan goats were collected from sites randomly located in Gongbo'gyamda, Nedong, Chanang, Konggar, Namling, and Xietongmen during June to July 2016. All the animals are raised on rangeland by natural grazing, and sharing the Tibetan Plateau habitat with other animals including humans. Prior to sample collection, the appropriate permission was obtained from the herdsmen. Fecal samples were collected from the ground after natural defecation of the animals, therefore, no permission regarding laws on animal protection was required. To avoid repeated sampling as well as avoiding sampling bias, the following sampling strategy was used: (1) random sampling of herds including Tibetan sheep and Tibetan goats; (2) up to 20 samples was collected from each flock; (3) the herd size in this study was 200–1600 sheep/goats; (4) all the samples were collected irrespective of any other symptoms or sign. Each specimen (about 10 g) was collected immediately after being defecated onto the ground by the animal, and samples were individually placed into clean plastic bags, then stored in 2.5% (w/v) potassium dichromate solution at 4 °C prior to further analysis. Sampling was conducted according to the animal welfare guidelines of the OIE.

2.2. DNA extraction and PCR amplification

Approximately 200 mg of stool per sample was placed in a clean microcentrifuge tube, and sterile water was used to remove potassium dichromate in the sample. Total DNA was extracted from fecal samples using an E.Z.N.A. Stool DNA Kit (D4015–02, Omega Bio-tek, Inc., Norcross, GA), according to the manufacturer's instructions. Detection of

Blastocystis subtypes by PCR used the previously reported primers [34] (RD5: 5'-ATC TGG TTG ATC CTG CCA GT-3' and BhrDr: 5'-GAG CTT TTTA ACT GCA ACA ACG-3'). The PCR reaction conditions were performed as previously reported [35]. For each PCR, three biological repetitions were performed, including for negative and positive controls. The products were screened by 1% agarose gel electrophoresis with SYBR Green (TIANDZ Inc., Beijing, China) staining.

2.3. Sequencing and phylogenetic analysis

Direct bidirectional sequencing of objective fragments (~600 bp) was performed using an ABI3730xl by SinoGenoMax (SinoGenoMax Inc., Beijing, China). The sequencing peaks obtained were viewed using Chromas software. Mixed infections yield overlapping peaks in the chromatogram peaks when they are sequenced directly. The sequencing results were analyzed by the NCBI BLAST program, and a set of associated SSU rRNA gene sequences was downloaded from the GenBank database, and all raw sequences were assembled and aligned using DNASTAR software (DNASTAR Inc., Madison, WI, USA) to determine the subtypes. The neighbor-joining (NJ) method in the MEGA7 software (<http://www.megasoftware.net/>) was used to construct the phylogenetic tree, and 1000 bootstrap repetitions were used to evaluate the reliability of the branches.

2.4. Availability of data and materials

The nucleotide sequences in this study have been submitted to the GenBank database (GenBank Accession No. MW713070–MW713084).

2.5. Statistical analysis

Statistical comparisons were performed with SPSS version 17.0 (IBM SPSS Inc., Chicago, IL, USA). The Pearson's chi-squared test (χ^2) were used for comparisons between two groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Prevalence of *Blastocystis*

The overall infection rate of *Blastocystis* in 880 fecal samples was 8.52% (75/880), with infection rates of 8.55% (53/620) in Tibetan sheep and 8.46% (22/260) in Tibetan goats (Table 1). The results from different sampling sites showed that the positive rate differed according to site; Chanang was highest at 12.3%, and Gongbo'gyamda was lowest at 2.15%. Significant difference ($P > 0.05$) of *Blastocystis* infection were detected among six study sites, ranging from 2.15% to 12.3%. There was no significant difference ($P > 0.05$) in the rate between Tibetan sheep and Tibetan goats.

3.2. Distribution of *Blastocystis* subtypes

In order to determine the subtypes of *Blastocystis*, we analyzed the genotypes of these isolates based on the sequence of SSU rRNA. A total of six subtypes were detected (Table 1), ST1(1/75), ST4(6/75), ST5(6/75), ST6(3/75), ST10(49/75), ST14(10/75); four belonged to zoonotic subtypes (ST1, ST4, ST5, ST6), and two were classified as animal-specific subtypes (ST10, ST14). Four (ST4, ST5, ST10, ST14) of these subtypes were represented among the 53 *Blastocystis* samples from Tibetan sheep, and four subtypes (ST1, ST5, ST6, ST10) detected in Tibetan goats. In this study, ST10 was the preponderant subtype in Tibetan sheep and Tibetan goats, 62.3% (33/53) and 72.7% (16/22), respectively. ST5 and ST10 were present both in Tibetan sheep and Tibetan goats. Zoonotic subtype ST4 was only detected in Tibetan sheep, and Zoonotic subtypes ST1, ST5, and ST6 were only detected in Tibetan goats. No mixed subtypes infection was detected in this study.

Table 1

The infection rate and subtype distribution of *Blastocystis* in Tibetan sheep and Tibetan goats at different locations in Tibet, China.

Species	State	Positive No	Sample No	Positive rate%	Subtype (No.)
Sheep	Gongbo'gyamda	2	93	2.15%	ST10A(n = 2)
	Nedong	2	34	5.88%	ST10A(n = 2)
	Chanang	35	290	12.07%	ST4A(n = 4), ST5A(n = 3), ST10A(n = 11), ST10B(n = 3), ST10C(n = 5), ST14A(n = 6), ST14B(n = 2), ST14C(n = 1)
	Konggar	9	160	5.63%	ST4B(n = 2), ST5A(n = 1), ST10A(n = 4), ST10D(n = 2)
	Namling	5	43	11.63%	ST10A(n = 3), ST10D(n = 1), ST14A(n = 1)
Total		53	620	8.55%	ST4A(n = 4), ST4B(n = 2), ST5A(n = 4), ST10A(n = 22), ST10B(n = 3), ST10C(n = 5), ST10D(n = 3), ST14A(n = 7), ST14B(n = 2), ST14C(n = 1)
Goats	Xietongmen	22	260	8.46%	ST1(n = 1), ST5B(n = 2), ST6A(n = 3), ST10A(n = 9), ST10E(n = 1), ST10F(n = 3), ST10G(n = 3)
	Totals	75	880	8.52%	ST1(n = 1), ST4A(n = 4), ST4B(n = 2), ST5A(n = 4), ST5B(n = 2), ST6A(n = 3), ST10A(n = 31), ST10B(n = 3), ST10C(n = 5), ST10D(n = 3), ST10E(n = 1), ST10F(n = 3), ST10G(n = 3), ST14A(n = 7), ST14B(n = 2), ST14C(n = 1)

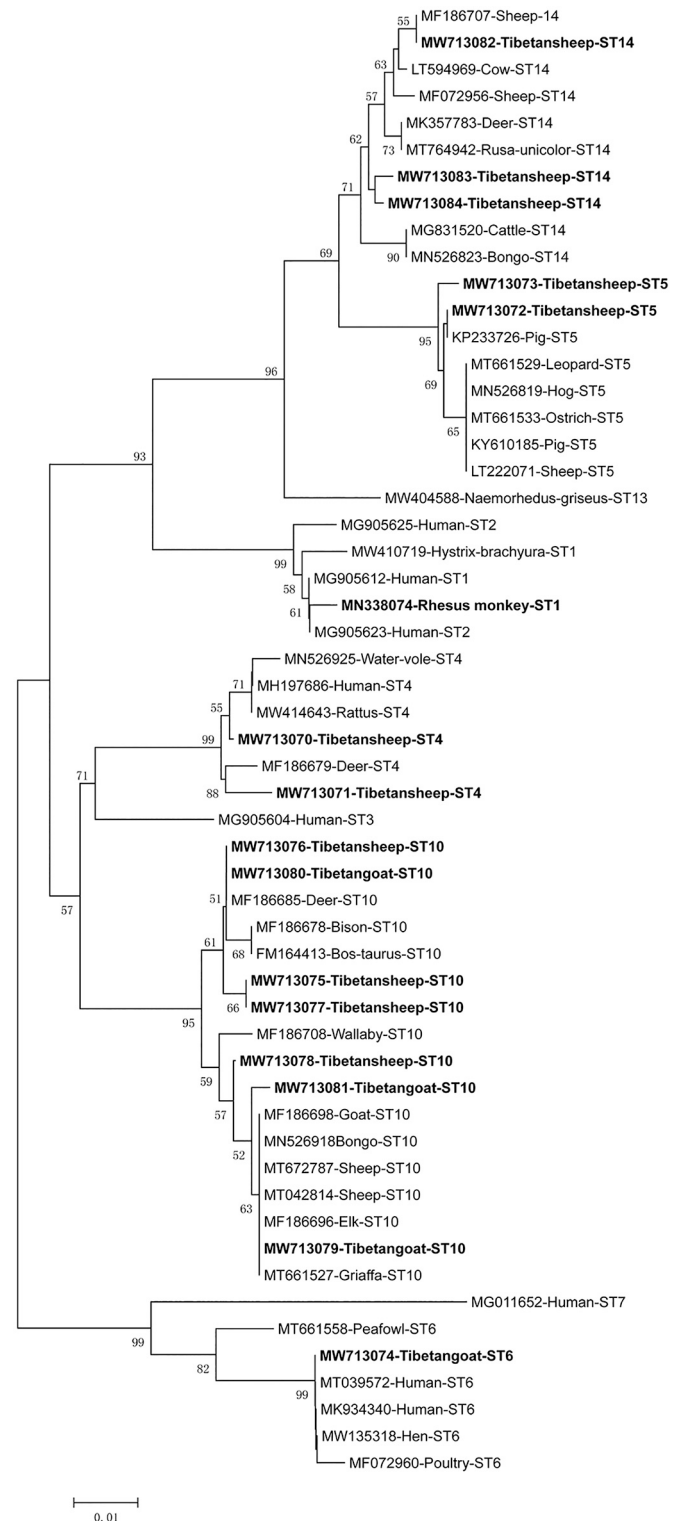


Fig. 1. Phylogenetic relationships using neighbor-joining analysis among *Blastocystis* isolates based on SSU rRNA nucleotide sequences. The self-test value was 1000 repetitions. Isolates from this study are in bold.

3.3. Phylogenetic analysis of *Blastocystis* sp.

In this study, 75 positive isolates were sequenced, and 15 representative sequences were obtained. The newly obtained sequences have been uploaded to NCBI (GenBank Accession No. MW713070-MW713084). The sequences obtained in this study have high homology with the reference sequence of *Blastocystis* sp. in the GenBank database (Fig. 1). The newly obtained sequences belong to ST4, ST5, ST6, ST10, and ST14. Two variations were identified in ST4 and ST5, respectively. Only one variation was identified in ST6. 49 ST10 isolates produced seven variations (ST10A to ST10G), with a similarity of 97.9–99.8%. ST10A, comprising 31 out of 49 ST10 subtypes, was the dominant subtype. Three variations of the subtype ST14 were identified by sequence alignment analysis, and subtype ST14A(n = 7) was the dominant subtype. The SSU rRNA gene-based sequence of the ST1

isolate is consistent with that from the Rhesus monkey in Bangladesh, and the GenBank sequence accession number is MN338074. Phylogenetic analysis also revealed the polymorphism of the SSU rRNA gene of *Blastocystis* isolates in this study.

4. Discussion

Blastocystis is the most common intestinal organism in humans. The transmission route of *Blastocystis* similar to those of other intestinal protozoans, and transmission occurs fecal-orally through contaminated water and food. In addition, people who are working in farms and zoos have a much higher risk of infections than those who do not [36]. As an important ecological security barrier area in China, Tibet has high biodiversity with many species of livestock, and animal husbandry plays an important role in social, economic, political, and cultural life [37]. The traditional grazing methods still occupy a dominant position, and these practices carry the risk of zoonotic transmission [38]. The close contact between humans and domestic animals increases the risk of infection by *Blastocystis* between herdsman and livestock.

Results of the study indicate that *Blastocystis* are common in Tibetan sheep and Tibetan goats in Tibet, China. In the present study, *Blastocystis* was detected in all six collecting areas. The 8.55% infection rate observed in Tibetan sheep was lower than the infection rates previously described in United Arab Emirates (63.64%) [25] and in China Jiangsu (24%) and Shandong (16.67%) [39] but higher than that in China Anhui (3.16%) [39] and Heilongjiang (5.5%) [40]. In Tibetan goats, the infection rate of *Blastocystis* was 8.46%, which was lower than those in China Shanxi (58.05%) [41], Malaysia (30.9%) [42], Thailand (94.74%) [43], Brazil (33.33%) [44] and USA (75%) [45], but higher than those in China Anhui (0.35%) [39] and Nepal (0.75%) [46]. The detected differences in infection rate of *Blastocystis* between different surveys may be due to many factors, including regional disparity, rearing conditions, detection method, and host health status.

In this study, subtypes ST1 (1), ST4 (6), ST5 (6), ST6 (3), ST10 (49), and ST14 (10) were detected by comparing the sequence of the SSU rRNA gene. ST10 was the dominant subtype in this study and was observed in 62.26% of Tibetan sheep and 72.73% of Tibetan goats. In addition, among the four zoonotic subtypes identified in this study, ST4 and ST5 were found in Tibetan sheep, while ST1, ST5, and ST6 were found in Tibetan goats. Some studies have shown that ST1 and ST4 are the main subtypes of human infection [17,47,48]. ST4 was also found in cattle and rodents [49,50]. Zoonotic subtype ST5 has been detected in a variety of animals [51,52], and an early investigation identified transmission from person to person [53]. In addition, ST6 was detected in cattle and birds [54,55]. ST10 and ST14 are common in domestic animals and are considered as animal-specific subtypes. ST10 is found predominately in Artiodactyls [19,41,56,57]. Factors such as animal age, season, and environmental differences may cause different subtype infection [11].

This survey did not detect the mixed infection of different subtypes. Some investigations have detected mixed infection of different subtypes [25,26], but infection with a single subtype is still the most common pattern. In addition, when zoonosis pathogen co-infection occurs, recombination and genetic admixture between genotypes is highly increases, indicating that the risk of continuing evolution and broadening its host range is increased too [27,28]. In addition, Tibetan sheep and Tibetan goats share habitat with wildlife on the plateau may increase the challenges of One Health measures.

As described in the text, sequencing revealed genetic variations among *Blastocystis* subtypes, as well as at the intra-subtype level. Furthermore, it is unknown whether the genetic variations are linked with clinical signs and pathogenicity disorders. Some investigations found that ST1 may contribute to clinical symptoms [58–60]. Similarly, several surveys also found that ST4 and ST6 were detected in symptomatic patients [61,62], although ST4 and ST6 appear to show some degree of host specificity [60]. And these data suggesting the possibility that these subtypes may be related to acute infection in humans.

5. Conclusion

We demonstrated the prevalence and subtype diversity of *Blastocystis*

in Tibetan sheep and Tibetan goats. Although the infection rate was different in each sampling area, the results show that *Blastocystis* is a common parasite in Tibetan sheep. Based on molecular analysis of the SSU rRNA gene, six known gene subtypes were detected, and four were zoonotic subtypes. *Blastocystis* is not only highly prevalent in Tibetan sheep and Tibetan goats but also carries the risk of animal-to-human zoonotic transmission. At present, infection of *Blastocystis* has been identified worldwide, and this has aroused the interest of many researchers. However, the source and transmission route of *Blastocystis* are still unclear, and further investigations are required to clarify its etiology and pathological mechanisms and One Health approaches are needed to prevent infection.

Ethics statement

The Research Ethics Committee of Henan Agricultural University reviewed and approved the experimental protocol. The collection of samples was performed in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Health, China). All samples tested in this experiment were collected with the permission of the herdsman, and no special permission was required for sampling locations.

Declaration of competing interest

None.

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References

- [1] R.Y. Tito, S. Chaffron, C. Caenepeel, G. Lima-Mendez, J. Wang, S. Vieira-Silva, G. Falony, F. Hildebrand, Y. Darzi, L. Rymenans, C. Verspecht, P. Bork, S. Vermeire, M. Joossens, J. Raes, Population-level analysis of *Blastocystis* subtype prevalence and variation in the human gut microbiota, *Gut* 68 (7) (2019) 1180–1189.
- [2] H. Yoshikawa, M. Tokoro, T. Nagamoto, S. Arayama, P.B. Asih, I.E. Rozi, D. Syafruddin, Molecular survey of *Blastocystis* sp. from humans and associated animals in an Indonesian community with poor hygiene, *Parasitol. Int.* 65 (6 Pt B) (2016) 780–784.
- [3] S. Balduvsson, P. Karanis, Waterborne transmission of protozoan parasites: review of worldwide outbreaks - an update 2004–2010, *Water Res.* 45 (20, 2011) 6603–6614.
- [4] F. Bahrami, E. Babaei, A. Badirzadeh, T.R. Riabi, A. Abdoli, *Blastocystis*, urticaria, and skin disorders: review of the current evidences, *Eur. J. Clin. Microbiol. Infect. Dis.* 39 (6) (2020) 1027–1042.
- [5] F. Cakir, M. Cicek, I.H. Yildirim, Determination the subtypes of *blastocystis* sp. and evaluate the effect of these subtypes on pathogenicity, *Acta Parasitol.* 64 (1) (2019) 7–12.
- [6] I. Wawrzyniak, P. Poirier, E. Viscogliosi, M. Dionigia, C. Texier, F. Delbac, H. E. Alaoui, *Blastocystis*, an unrecognized parasite: an overview of pathogenesis and diagnosis, *Ther. Adv. Infect. Dis.* 1 (5) (2013) 167–178.
- [7] J.A. Yason, Y.R. Liang, C.W. Png, Y. Zhang, K.S.W. Tan, Interactions between a pathogenic *Blastocystis* subtype and gut microbiota: in vitro and in vivo studies, *Microbiome* 7 (1) (2019) 30.
- [8] J. Yakoob, W. Jafri, N. Jafri, R. Khan, M. Islam, M.A. Beg, V. Zaman, Irritable bowel syndrome: in search of an etiology: role of *Blastocystis hominis*, *The Am. J. Trop. Med. Hyg.* 70 (4) (2004) 383–385.
- [9] F. Dogruman-Al, Z. Simsek, K. Boorum, E. Ekici, M. Sahin, C. Tuncer, S. Kustimur, A. Altinbas, Comparison of methods for detection of *Blastocystis* infection in routinely submitted stool samples, and also in IBS/IBD patients in Ankara, Turkey, *PLoS One* 5 (11, 2010), e15484.

- [10] A.A. Rauff-Adedotun, S.N. Mohd Zain, M.T. Farah Haziqah, Current status of blastocystis sp. in animals from Southeast Asia: a review, *Parasitol. Res.* 119 (11, 2020) 3559–3570.
- [11] M. Lepczyńska, J. Białkowska, E. Dzika, K. Piskorz-Ogórek, J. Korycińska, Blastocystis: how do specific diets and human gut microbiota affect its development and pathogenicity? *Eur. J. Clin. Microbiol. Infect. Dis.* 36 (9) (2017) 1531–1540.
- [12] C.G. Clark, Extensive genetic diversity in Blastocystis hominis, *Mol. Biochem. Parasitol.* 87 (1) (1997) 79–83.
- [13] H. Yoshikawa, Z. Wu, K. Pandey, B.D. Pandey, J.B. Sherchand, T. Yanagi, H. Kanbara, Molecular characterization of Blastocystis isolates from children and rhesus monkeys in Kathmandu, Nepal, *Vet Parasitol* 160 (3–4) (2009) 295–300.
- [14] C.R. Stensvold, G.K. Suresh, K.S.W. Tan, R.C.A. Thompson, R.J. Traub, E. Viscogliosi, H. Yoshikawa, C.G. Clark, Terminology for Blastocystis subtypes - a consensus, *Trends Parasitol.* 23 (3) (2007) 93–96.
- [15] P. Poirier, I. Wawrzyniak, C.P. Vivares, F. Delbac, H. El Alaoui, New insights into blastocystis spp.: a potential link with irritable bowel syndrome, *PLoS Pathog.* 8 (3) (2012) e1002545.
- [16] D. El Safadi, D. Meloni, P. Poirier, M. Osman, A. Cian, L. Gaayeb, I. Wawrzyniak, F. Delbac, H. El Alaoui, L. Delhaes, E. Dei-Cas, H. Mallat, F. Dabboussi, M. Hamze, E. Viscogliosi, Molecular epidemiology of Blastocystis in Lebanon and correlation between subtype 1 and gastrointestinal symptoms, *The Am. J. Trop. Med. Hyg.* 88 (6) (2013) 1203–1206.
- [17] C.R. Stensvold, C.G. Clark, Re-empting Pandora's box: blastocystis subtypes revisited, *Trends Parasitol.* 36 (3) (2020) 229–232.
- [18] R. Fayer, M. Santin, D. Macarasin, Detection of concurrent infection of dairy cattle with blastocystis, cryptosporidium, giardia, and enterocytozoon by molecular and microscopic methods, *Parasitol. Res.* 111 (3) (2012) 1349–1355.
- [19] M.A. Alfellani, D. Taner-Mulla, A.S. Jacob, C.A. Imeade, H. Yoshikawa, C. R. Stensvold, C.G. Clark, Genetic diversity of blastocystis in livestock and zoo animals, *Protist* 164 (4) (2013) 497–509.
- [20] C.R. Stensvold, C.G. Clark, Current status of Blastocystis: A personal view, *Parasitol. Int.* 65 (6 Pt B) (2016) 763–771.
- [21] C. Noël, F. Dufernez, D. Gerbod, V.P. Edgcomb, P. Delgado-Viscogliosi, L.C. Ho, M. Singh, R. Wintjens, M.L. Sogin, M. Capron, R. Pierce, L. Zenner, E. Viscogliosi, Molecular phylogenies of Blastocystis isolates from different hosts: implications for genetic diversity, identification of species, and zoonosis, *J. Clin. Microbiol.* 43 (1) (2005) 348–355.
- [22] U. Parkar, R.J. Traub, S. Vitali, A. Elliot, B. Leveck, I. Robertson, T. Geurden, J. Steele, B. Drake, R.C.A. Thompson, Molecular characterization of Blastocystis isolates from zoo animals and their animal-keepers, *Vet. Parasitol.* 169 (1–2) (2010) 8–17.
- [23] C. Audebert, G. Even, A. Cian, A. Loywick, S. Merlin, E. Viscogliosi, M. Chabé, Colonization with the enteric protozoa Blastocystis is associated with increased diversity of human gut bacterial microbiota, *Sci. Rep.* 6 (2016) 25255.
- [24] R. Salehi, A. Rostami, H. Mirjalali, C.R. Stensvold, A. Haghighi, Genetic Characterization of Blastocystis from Poultry, Livestock Animals and Humans in the Southwest Region of Iran-Zoonotic Implications, *Transboundary and Emerging Diseases*, 2021.
- [25] R. AbuOdeh, S. Ezzedine, M. Madkour, C.R. Stensvold, A. Samie, G. Nasrallah, E. AlBakri, Molecular subtyping of blastocystis from diverse animals in the United Arab Emirates, *Protist* 170 (5) (2019) 125679.
- [26] L. Deng, Y. Chai, Z. Zhou, H. Liu, Z. Zhong, Y. Hu, H. Fu, C. Yue, G. Peng, Epidemiology of Blastocystis sp. infection in China: a systematic review, *Parasite* 26 (2019) 41.
- [27] Z. Zhang, J. Ma, X. Huang, X. Wen, W. Jiang, L. Chen, N. Li, Y. Guo, L. Zhang, L. Xiao, Y. Feng, Population genetic analysis suggests genetic recombination is responsible for increased zoonotic potential of Enterocytozoon bienewsi from ruminants in China, *One Health (Amsterdam, Netherlands)* 11 (2020) 100184.
- [28] W.B. Karesh, A. Dobson, J.O. Lloyd-Smith, J. Lubroth, M.A. Dixon, M. Bennett, S. Aldrich, T. Harrington, P. Formenty, E.H. Loh, C.C. Machalaba, M.J. Thomas, D. L. Heymann, Ecology of zoonoses: natural and unnatural histories, *Lancet (London, England)* 380 (9857) (2012) 45–1936.
- [29] Y. Wu, Y. Chen, Y. Chang, X. Zhang, D. Li, L. Wang, S. Zheng, R. Wang, S. Zhang, J. Li, L. Zhang, Genotyping and identification of Cryptosporidium spp., Giardia duodenalis and Enterocytozoon bienewsi from free-range Tibetan yellow cattle and cattle-yak in Tibet, China, *Acta Trop.* 212 (2020) 105671.
- [30] S. Zheng, D. Li, C. Zhou, S. Zhang, Y. Wu, Y. Chang, Y. Chen, J. Huang, C. Ning, G. Zhang, L. Zhang, Molecular identification and epidemiological comparison of Cryptosporidium spp. among different pig breeds in Tibet and Henan, China, *BMC Vet. Res.* 15 (1) (2019) 101.
- [31] X.J. Hu, J. Yang, X.L. Xie, F.H. Lv, Y.H. Cao, W.R. Li, M.J. Liu, Y.T. Wang, J.Q. Li, Y.G. Liu, Y.L. Ren, Z.Q. Shen, F. Wang, E. Hehua, J.L. Han, M.H. Li, The genome landscape of Tibetan sheep reveals adaptive introgression from argali and the history of early human settlements on the Qinghai-Tibetan plateau, *Mol. Biol. Evol.* 36 (2) (2019) 283–303.
- [32] Y. Shi, S. Fan, M. Wu, Z. Zuo, X. Li, L. Jiang, Q. Shen, P. Xu, L. Zeng, Y. Zhou, Y. Huang, Z. Yang, J. Zhou, J. Gao, H. Zhou, S. Xu, H. Ji, P. Shi, D.D. Wu, C. Yang, Y. Chen, YTHDF1 links hypoxia adaptation and non-small cell lung cancer progression, *Nat. Commun.* 10 (1) (2019) 4892.
- [33] D. Li, S. Zheng, C. Zhou, M.R. Karim, L. Wang, H. Wang, F. Yu, J. Li, W. Wang, Y. Wang, S. Zhang, F. Jian, R. Wang, C. Ning, L. Zhang, Multilocus typing of enterocytozoon bienewsi in pig reveals the high prevalence, zoonotic potential, host adaptation and geographical segregation in China, *J. Eukaryot. Microbiol.* 66 (5) (2019) 707–718.
- [34] S.M. Scicluna, B. Tawari, C.G. Clark, DNA barcoding of blastocystis, *Protist* 157 (1) (2006) 77–85.
- [35] M. Qi, Z. Wei, Y. Zhang, Q. Zhang, J. Li, L. Zhang, R. Wang, Genetic diversity of Blastocystis in kindergarten children in southern Xinjiang, China, *Parasit. Vectors* 13 (1) (2020) 15.
- [36] N. Abe, M. Nagoshi, K. Takami, Y. Sawano, H. Yoshikawa, A survey of Blastocystis sp. in livestock, pets, and zoo animals in Japan, *Vet. Parasitol.* 106 (3) (2002) 203–212.
- [37] M.S. Wang, Y. Li, M.S. Peng, L. Zhong, Z.J. Wang, Q.Y. Li, X.L. Tu, Y. Dong, C. L. Zhu, L. Wang, M.M. Yang, S.F. Wu, Y.W. Miao, J.P. Liu, D.M. Irwin, W. Wang, D. D. Wu, Y.P. Zhang, Genomic analyses reveal potential independent adaptation to high altitude in Tibetan chickens, *Mol. Biol. Evol.* 32 (7) (2015) 1880–1889.
- [38] X. Feng, X. Qi, L. Yang, X. Duan, B. Fang, Q. Gongsang, B. Bartholomot, D. A. Vuitton, H. Wen, P.S. Craig, Human cystic and alveolar echinococcosis in the Tibet autonomous region (TAR), China, *J. Helminthol.* 89 (6) (2015) 671–679.
- [39] W.C. Li, K. Wang, Y. Gu, Occurrence of Blastocystis sp. and Pentatrichomonas hominis in sheep and goats in China, *Parasit. Vectors* 11 (1) (2018) 93.
- [40] J. Wang, B. Gong, F. Yang, W. Zhang, Y. Zheng, A. Liu, Subtype distribution and genetic characterizations of Blastocystis in pigs, cattle, sheep and goats in northeastern China's Heilongjiang Province, *Infect. Genet. Evol.* 57 (2018) 171–176.
- [41] J.K. Song, Y.L. Yin, Y.J. Yuan, H. Tang, G.J. Ren, H.J. Zhang, Z.X. Li, Y.M. Zhang, G.H. Zhao, First genotyping of Blastocystis sp. in dairy, meat, and cashmere goats in northwestern China, *Acta Trop.* 176 (2017) 277–282.
- [42] T.C. Tan, P.C. Tan, R. Sharma, S. Sugnaselan, K.G. Suresh, Genetic diversity of caprine blastocystis from Peninsular Malaysia, *Parasitol. Res.* 112 (1) (2013) 85–89.
- [43] R. Udonsom, R. Prasertbun, A. Mahittikorn, H. Mori, T. Changbunjong, C. Komalamisra, A.R. Pintong, Y. Sukthana, S. Popruk, Blastocystis infection and subtype distribution in humans, cattle, goats, and pigs in central and western Thailand, *Infect. Genet. Evol.* 65 (2018) 107–111.
- [44] R.G.F. Moura, M.B. Oliveira-Silva, A.L. Pedrosa, G.A.N. Nascentes, M. Cabrine-Santos, Occurrence of Blastocystis spp. in domestic animals in Triângulo Mineiro area of Brazil, *Rev. Soc. Bras. Med. Trop.* 51 (2) (2018) 240–243.
- [45] R. Gould, K. Boorom, Blastocystis surface antigen is stable in chemically preserved stool samples for at least 1 year, *Parasitol. Res.* 112 (7) (2013) 2469–2471.
- [46] T.R. Ghimire, N. Bhattarai, A survey of gastrointestinal parasites of goats in a goat market in Kathmandu, Nepal, *J. Parasit. Dis.* 43 (4) (2019) 686–695.
- [47] P.A. Jiménez, J.E. Jaimes, J.D. Ramírez, A summary of Blastocystis subtypes in North and South America, *Parasit. Vectors* 12 (1) (2019) 376.
- [48] C.R. Stensvold, M.A. Alfellani, S. Nørskov-Lauritsen, K. Prip, E.L. Victory, C. Maddox, H.V. Nielsen, C.G. Clark, Subtype distribution of Blastocystis isolates from synanthropic and zoo animals and identification of a new subtype, *Int. J. Parasitol.* 39 (4) (2009) 473–479.
- [49] W. Zhu, W. Tao, B. Gong, H. Yang, Y. Li, M. Song, Y. Lu, W. Li, First report of Blastocystis infections in cattle in China, *Vet. Parasitol.* 246 (2017) 38–42.
- [50] J. Wang, B. Gong, X. Liu, W. Zhao, T. Bu, W. Zhang, A. Liu, F. Yang, Distribution and genetic diversity of Blastocystis subtypes in various mammal and bird species in northeastern China, *Parasit. Vectors* 11 (1) (2018) 522.
- [51] J.G. Maloney, J.E. Lombard, N.J. Urie, C.B. Shively, M. Santin, Zoonotic and genetically diverse subtypes of Blastocystis in US pre-weaned dairy heifer calves, *Parasitol. Res.* 118 (2) (2019) 575–582.
- [52] F. Macchioni, H. Segundo, V. Totino, S. Gabrielli, P. Rojas, M. Roselli, G.A. Paredes, M. Masana, A. Bartoloni, G. Cancrini, Intestinal parasitic infections and associated epidemiological drivers in two rural communities of the Bolivian Chaco, *J. Infect. Develop. Countries* 10 (9) (2016) 1012–1019.
- [53] W.Q. Wang, H. Owen, R.J. Traub, L. Cuttlet, T. Inpankaew, H. Bielefeldt-Ohmann, Molecular epidemiology of Blastocystis in pigs and their in-contact humans in Southeast Queensland, Australia, and Cambodia, *Vet. Parasitol.* 203 (3–4) (2014) 264–269.
- [54] P.D. Scanlan, Blastocystis: past pitfalls and future perspectives, *Trends Parasitol.* 28 (8) (2012) 327–334.
- [55] E. Badparva, J. Sadraee, F. Kheirandish, Genetic diversity of blastocystis isolated from cattle in Khorramabad, Iran, *Jundishapur J. Microbiol.* 8 (3) (2015), e14810.
- [56] G.H. Zhao, X.F. Hu, T.L. Liu, R.S. Hu, Z.Q. Yu, W.B. Yang, Y.L. Wu, S.K. Yu, J. K. Song, Molecular characterization of Blastocystis sp. in captive wild animals in Qinling Mountains, *Parasitol. Res.* 116 (8) (2017) 2327–2333.
- [57] A. Masuda, T. Sumiyoshi, T. Ohtaki, J. Matsumoto, Prevalence and molecular subtyping of Blastocystis from dairy cattle in Kanagawa, Japan, *Parasitol. Int.* 67 (6) (2018) 702–705.
- [58] L.H. Li, X.P. Zhang, S. Lv, L. Zhang, H. Yoshikawa, Z. Wu, P. Steinmann, J. Utzinger, X.M. Tong, S.H. Chen, X.N. Zhou, Cross-sectional surveys and subtype classification of human Blastocystis isolates from four epidemiological settings in China, *Parasitol. Res.* 102 (1) (2007) 83–90.
- [59] C.R. Stensvold, M.C. Arendrup, C. Jespersgaard, K. Mølbak, H.V. Nielsen, Detecting Blastocystis using parasitologic and DNA-based methods: a comparative study, *Diagn. Microbiol. Infect. Dis.* 59 (3) (2007) 303–307.
- [60] K.F. Boorom, H. Smith, L. Nimri, E. Viscogliosi, G. Spanakos, U. Parkar, L.H. Li, X. N. Zhou, U.Z. Ok, S. Leelayoova, M.S. Jones, Oh my aching gut: irritable bowel

- syndrome, Blastocystis, and asymptomatic infection, *Parasit. Vectors* 1 (1) (2008) 40.
- [61] E.M. Hussein, A.M. Hussein, M.M. Eida, M.M. Atwa, Pathophysiological variability of different genotypes of human *Blastocystis hominis* Egyptian isolates in experimentally infected rats, *Parasitol. Res.* 102 (5) (2008) 853–860.
- [62] R. Stensvold, A. Brillowska-Dabrowska, H.V. Nielsen, M.C. Arendrup, Detection of *Blastocystis hominis* in unpreserved stool specimens by using polymerase chain reaction, *J. Parasitol.* 92 (5) (2006) 1081–1087.