The Presence of Blastocystis in Tibetan Antelope (Pantholops hodgsonii)

Hong-Li Geng†, Yu-Zhe Sun†, Jing Jiang*, He-Ting Sun, Yuan-Guo Li, Si-Yuan Qin, Zhen-Jun Wang, Tao Ma, Jun-Hui Zhu, Nian-Yu Xue and Hong-Bo Ni*

† College of Veterinary Medicine, Qingdao Agricultural University, Qingdao, China, * College of Life Sciences, Changchun Sci-Tech University, Shuangyang, China, ‡ General Monitoring Station for Wildlife-Borne Infectious Diseases, State Forestry and Grass Administration, Shenyang, China, § Key Laboratory of Zoonosis Research, Ministry of Education, College of Veterinary Medicine, Jilin University, Changchun, China, ¶ College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing, China

Blastocystis is a protozoan that parasitizes the intestines. A number of hosts of Blastocystis have been found, including human and animals. However, there has been no research on the prevalence of Blastocystis in Tibetan antelope. Here, a molecular test was performed using 627 Tibetan antelope fecal samples collected on Tibet in China from 2019 to 2020. The result showed that 30 (4.8%) samples were Blastocystis positive. The highest prevalence of Blastocystis was in Shuanghu County (25/209, 12.0%), followed by Shenza County (2/103, 1.9%), Nyima County (3/182, 1.6%), and Baigoin County (0/133, 0.0%). In addition, logistic regression analysis showed that the gender, sampling year, and area of Tibetan antelope were risk factors for Blastocystis prevalence. Three subtypes (ST10, ST13, and ST14) of Blastocystis were found in Tibetan antelope through a subtype sequence analysis, and ST13 was identified to be the dominant subtype. This is the first investigation for the infection of Blastocystis in Tibetan antelope. Collectively, the data in this study have expanded the host range of Blastocystis and provided basic information for the distribution of Blastocystis subtypes, which could support the prevention of Blastocystis infection in wild animals.

Keywords: Blastocystis, prevalence, subtypes, Tibetan antelope (Pantholops hodgsonii), PCR

INTRODUCTION

Blastocystis is a protozoan that parasitizes the intestines (Jiménez et al., 2019; Paik et al., 2019). It can infect a variety of hosts, such as mammals, amphibians, birds, and insects (Zhu et al., 2020). Blastocystis is transmitted through the fecal–oral route or water and food between susceptible hosts (Asghari et al., 2019; Deng et al., 2019). Hosts infected with Blastocystis could develop clinical signs, e.g., diarrhea, abdominal pain, and vomiting. Immunocompromised individuals are more susceptible to Blastocystis (Wang et al., 2018a; Paik et al., 2019).

Blastocystis was first isolated from animal feces in 1911 (Alexeieff, 1911). Since then, more and more animals and humans, such as cattle, deer, sheep, and goats, were identified to be the hosts of Blastocystis (Table 1). In China, Blastocystis was first reported in children in 1990 (Li et al., 1990). A large number of investigations regarding Blastocystis prevalence in different hosts were performed previously (Song et al.,
So far, the infection of *Blastocystis* has been reported in many animals, including domestic and wild animals (Zhao et al., 2017; Wang et al., 2018a). To date, approximately 29 proposed *Blastocystis* subtypes have been identified in a large number of literatures (Ma et al., 2020). ST1-9 and ST12 subtypes were identified in humans, while ST10-17 and ST21-28 subtypes were detected in animals (Stensvold and Clark, 2016; Ning et al., 2020; Hublin et al., 2021). Of note, some subtypes were identified in both humans and animals, such as ST1, ST3, and ST5 subtypes (Song et al., 2017; Wang et al., 2018a). ST4 was found in deer (Wang et al., 2018b; Kim et al., 2020; Shirozu et al., 2021), and ST12 was found in yaks in the plateau area (Ren et al., 2019).

Tibetan antelope (*Pantholops hodgsonii*) belongs to genus *Pantholops*, family Bovidae, order Cetartiodactyla according to the IUCN Red List in 2016 (IUCN SSC Antelope Specialist Group, 2016). Tibetan antelope is one of the most rare and endangered wild animals. There are approximately 100,000 to 150,000 Tibetan antelope in India and China (IUCN SSC Antelope Specialist Group 2016). Tibetan antelope can carry various pathogens, such as *Mycoplasma capricolum* subspecies, *capripneumoniae* (McCp) (Yu et al., 2012), and *Escherichia coli* (Bai et al., 2016). However, the existing data indicate that sheep may carry several potential *Blastocystis* subtypes, including ST1, ST3, ST4, ST5, ST6, and ST7 (Tan et al., 2013; Song et al., 2017; Wang et al., 2018a; Salehi et al., 2021). So far, studies on the prevalence and subtype diversity of *Blastocystis* in Tibetan antelope are unknown and the relevant public health impact is still unclear. This study provides important information on the diversity of *Blastocystis* subtypes in Tibetan antelope, and would help determine the role of Tibetan antelope in the transmission of *Blastocystis* to humans and other animals.

| Host         | No. tested | No. positive | Prevalence (%) | Location | Subtypes (STs) identified | Reference |
|--------------|------------|--------------|----------------|----------|--------------------------|-----------|
| Sheep        | 832        | 50           | 6.00%          | China    | ST5, ST10, ST14          | Li et al., 2018 |
| Sheep        | 109        | 6            | 5.50%          | China    | ST1, ST5, ST10, ST14     | Wang et al., 2018a |
| Sheep        | 100        | 32           | 32.00%         | Iran     | ST3, ST5, ST7, ST14      | Salehi et al., 2021 |
| Goat         | 751        | 2            | 0.30%          | China    | ST1                       | Li et al., 2018 |
| Goat         | 236        | 73           | 30.90%         | Malaysia | ST1, ST3, ST6, ST7       | Tan et al., 2013 |
| Goat         | 400        | 3            | 0.75%          | Nepal    | NA                       | Ghimire and Bhattarai, 2019 |
| Goat         | 38         | 36           | 94.70%         | Thailand | ST10, ST12, ST14         | Udonsom et al., 2018 |
| Goat         | 789        | 458          | 58.00%         | China    | ST1, ST3, ST4, ST5       | Song et al., 2017 |
| Cattle       | 147        | 14           | 9.52%          | China    | ST3, ST10, ST14          | Wang et al., 2018a |
| Cattle       | 22         | 5            | 22.70%         | UAE      | ST10                     | AbuOden et al., 2019 |
| Cattle       | 28         | 6            | 21.40%         | Brazil   | NA                       | Moura et al., 2018 |
| Cattle       | 42         | 21           | 50.00%         | Thailand | ST10, ST12               | Udonsom et al., 2018 |
| Cattle       | 80         | 9            | 11.30%         | Turkey   | ST10, ST14               | Aynur et al., 2019 |
| Cattle       | 526        | 54           | 10.30%         | China    | ST4, ST5, ST10, ST14     | Zhu et al., 2017 |
| Cattle       | 47         | 9            | 19.20%         | USA      | ST10, ST14               | Fayer et al., 2012 |
| Cattle       | 196        | 19           | 9.60%          | Iran     | ST3, ST5, ST6            | Bapardpa et al., 2015 |
| Cattle       | 31         | 7            | 22.60%         | England  | ST1, ST5, ST10           | Alfellani et al., 2013 |
| Cattle       | 25         | 20           | 80.00%         | Colombia | ST1, ST3                 | Ramirez et al., 2014 |
| Cattle       | 36         | 15           | 41.70%         | Libya    | ST5, ST10, ST14          | Sharifi et al., 2020 |
| Cattle       | 75         | 25           | 33.30%         | Iran     | ST5, ST10                | Hemalatha et al., 2014 |
| Cattle       | 29         | 10           | 34.50%         | Malaysia | NA                       | Rostami et al., 2020 |
| Cattle       | 40         | 14           | 35.00%         | Iran     | ST10, ST14               | Lee et al., 2018 |
| Cattle       | 1512       | 101          | 6.70%          | Korea    | ST1, ST10, ST14          | Masuda et al., 2018 |
| Cattle       | 133        | 72           | 54.10%         | Japan    | ST14                     | Greige et al., 2019 |
| Cattle       | 254        | 161          | 63.40%         | Lebanon  | ST1, ST3, ST5, ST10, ST14 | Abd et al., 2019 |
| Cattle       | 110        | 6            | 5.40%          | Malaysia | NA                       | Ren et al., 2019 |
| Cattle       | 1027       | 278          | 27.10%         | China    | ST10                     | Hastusiek et al., 2019 |
| Cattle       | 500        | 47           | 9.40%          | Indonesia | NA                       | Maloney et al., 2019 |
| Cattle       | 2539       | 73           | 2.90%          | USA      | ST3, ST4, ST5, ST10, ST14 | Kamaruddin et al., 2020 |
| Cattle       | 120        | 30           | 25.00%         | Malaysia | ST1, ST3, ST4, ST5, ST10, ST14 | Suvanti et al., 2020 |
| Cattle       | 108        | 108          | 100.00%        | Indonesia | ST10                     | Wang et al., 2018b |
| Reindeer     | 104        | 7            | 6.70%          | China    | ST10, ST13               | Wang et al., 2018b |
| Sika deer    | 82         | 12           | 14.60%         | China    | ST10, ST14               | Kim et al., 2020 |
| Water deer   | 125        | 51           | 40.80%         | Korea    | ST4, ST14                | Wang et al., 2018b |
| Red deer     | 428        | 54           | 10.30%         | China    | NA                       | Li et al., 2019 |
| Spotted deer | 30         | 1            | 3.30%          | Bangladesh | ST14                    | Shirozu et al., 2021 |
| Sika deer    | 132        | 60           | 45.50%         | Japan    | ST14                     | Maloney and Santin, 2021 |
| White tailed-deer | 80  | 71 | 88.80% | USA | ST1, ST3, ST4, ST10, ST14, ST21, ST23, ST24, ST25, ST26 | |

UAE, United Arab Emirates; NA, not available.
MATERIALS AND METHODS

Specimen Collection
From August 2019 to September 2020, the feces of 627 wild Tibetan antelope was collected in four areas in Tibet (Table 2 and Figure 1). This study randomly observed Tibetan antelope in the field. Fresh fecal samples were put into a PE glove immediately after defecation onto the ground, and then were placed into ice boxes and transported to the laboratory. This study was approved by the Ethics Committee of Jilin University. Appropriate permission was obtained from the General Monitoring Station for Wildlife-Borne Infectious Diseases, State Forestry and Grass Administration.

DNA Extraction and PCR Amplification
Genomic DNA was extracted using the E.Z.N.A.® Stool DNA Kit (Omega Biotek Inc., Norcross, GA, USA) according to the manufacturer’s instructions and stored at −20°C until PCR amplification. SSU rRNA gene was the target for PCR analysis using primers RD5 (5’-ATCTGGTTGATCCTGCCAGT-3’) and BhRDr (5’-GAGCTTTTTAACTGCAACAACG-3’) as described previously to amplify an approximately 600-bp region (Scicluna et al., 2006). Positive and negative controls were included in each test. PCR products were observed using UV light after electrophoresis at a 1.5% agarose gel containing ethidium bromide.

Sequence and Phylogenetic Analyses
The Blastocystis-positive PCR products were sent to Sangon Biotech Company (Shanghai, China) for sequencing. The sequence accuracy was confirmed by bidirectional sequencing. The sequencing was re-performed if variation was present in the previous result. Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/blast/) was employed to compare consensus sequences with the similar sequences on GenBank. The subtypes of Blastocyst isolates were determined through the online platform PubMLST (https://pubmlst.org/bigsdb?db=pubmlst_blastocystis_seqdef). The obtained sequences were aligned using ClustalX 1.83 program. The alignment was trimmed using the trimAI v1.2 software (http://trimal.cgenomics.org/downloads) (Li et al., 2019). All positions with

| Season          | Longitude | Latitude | Altitude | Temperature | Humidity | Climate             |
|-----------------|-----------|----------|----------|-------------|----------|---------------------|
| Summer (August) | 31°29’    | 92°04’   | 4,507 m  | 9.0°C       | 68 mm    | Plateau alpine climate |
| Autumn (September) | 6.1°C     | 70 mm    |

FIGURE 1 | A map of Tibet Autonomous Region, China, in which Tibetan antelope are sampled.
gaps were eliminated, and 104 unambiguously aligned sites were used for phylogenetic inference. The maximum likelihood (ML) method (Kimura two-parameter model) was employed to reconstruct phylogenetic trees by using MEGA X. Representative nucleotide sequences were submitted to GenBank under accession numbers: MZ444657–MZ444662.

Statistical Analysis

The variation of Blastocystis prevalence (y) in Tibetan antelope on the basis of sampling year (x1), gender (x2), and collecting region (x3) was analyzed with χ² test using SAS version 9.4 (SAS Institute Inc., USA). In the multivariable regression analysis, each of the variables was independently contained in the binary Logit model. The best model was judged by Fisher’s scoring algorithm. All tests were two-sided, and the results were considered statistically significant when p < 0.05. To explore the association between Blastocystis prevalence and the investigated factors, the odds ratios (ORs) and their 95% confidence intervals (95% CIs) were calculated.

RESULTS

Prevalence of Blastocystis sp.

In the present study, 30 out of 627 Tibetan antelope feces were identified to be Blastocystis positive (Figure 2). The infection rate of Blastocystis in Tibetan antelope in 2019 (0.6%, 2/322) was lower than that (9.2%, 28/305) in 2020. The prevalence of Blastocystis in different investigated counties ranged from 0% to 12% (Table 3). Blastocystis were detected in all three counties, except for Baigoin County. The highest prevalence of Blastocystis was in Shuanghu County (25/209, 12.0%), followed by Shenza County.

![Figure 2](image)

**Figure 2** The PCR amplification result of Blastocystis rRNA gene. "1–30": 30 positive samples of Blastocystis in TA25, TA26, TA72, TA100, TA103, TA105, TA107, TA108, TA111, TA118, TA124, TA128, TA130, TA131, TA147, TA148, TA156, TA157, TA169, TA175, TA214, TA216, TA228, TA230, TA232, TA235, TA238, TA252, TA291, and TA307; "+": negative control; "-": positive control; "M": DL2000 Marker.

| Factor          | Category          | No. tested | No. positive | Prevalence (%) (95% CI) | p-value | OR (95% CI) | Subtypes (no.) |
|-----------------|-------------------|------------|--------------|-------------------------|---------|-------------|----------------|
| Gender          | Female            | 300        | 8            | 2.7 (0.8–4.5)           | 0.017   | Reference   | ST10 (1); ST13 (6); ST14 (1) |
|                 | Male              | 327        | 22           | 6.7 (4.0–9.4)           | <0.01   | 2.63 (1.15–6.00) | ST10 (3); ST13 (18); ST14 (1) |
| Sampling year   | 2019              | 322        | 2            | 0.6 (0.0–1.5)           | <0.01   | Reference   | ST13 (2)     |
|                 | 2020              | 305        | 28           | 9.2 (5.9–12.4)          | <0.01   | 16.17 (3.82–68.50) | ST10 (4); ST13 (22); ST14 (2) |
| Region          | Nyima County      | 182        | 3            | 1.6 (0.0–3.5)           | <0.01   | Reference   | ST13 (3)     |
|                 | Shuanghu County   | 209        | 25           | 12.0 (7.6–16.4)         | <0.01   | Reference   | ST10 (3); ST13 (20); ST14 (2) |
|                 | Shenza County     | 103        | 2            | 1.9 (0.0–4.6)           | <0.12   | 1.18 (0.19–7.19) | ST10 (1); ST13 (11) |
|                 | Baigoin County    | 133        | 0            | 0.0 (0.0–0.0)           | –       | –           | ST10 (4); ST13 (24); ST14 (2) |
| Total           |                   | 627        | 30           | 4.8 (2.1–6.5)           | –       | –           | –              |
County (2/103, 1.9%) and Nyima County (3/182, 1.6%; Table 3). The infection rate of *Blastocystis* in Tibetan antelope in females (2.7%, 8/300) was significantly lower than that (6.7%, 22/327) in males ($p = 0.017$).

**Risk Factors of *Blastocystis* sp.**

To expose gender, sampling year, and collecting region of Tibetan antelope, and *Blastocystis* prevalence, univariate analysis was also conducted in the present study (Table 3). A Fisher’s scoring method-based positive stepwise logistic regression analysis was performed to estimate the influence of multiple variables on *Blastocystis* infection. Only one variable was found to have effects on the *Blastocystis* infection in the final model, as described by the equation: $y = 1.3138x_3 + 0.7097$. Collecting region had a positive impact on the risk of *Blastocystis* infection with the OR of 3.720 (95% CI 2.061–6.715). Nyima County (1.6%, 95% CI 0.0–3.5) was considered to have lower prevalence than Shuanghu County (OR = 8.11, 95% CI 2.41–27.33) and Shenza County (OR = 1.18, 95% CI 0.19–7.19) (Table 3).

**Distribution and Phylogenetic Analysis of *Blastocystis* Subtypes**

Three *Blastocystis* subtypes (ST10, ST13, and ST14) were detected in this study. Among them, the ST13 subtype was found in 24 individuals and was widely distributed in different gender subgroups, sampling years, and collecting regions. In the sampling years, all three *Blastocystis* subtypes appeared in Tibetan antelope in 2020, and only ST13 was found in the Tibetan antelope in 2019 (Table 3). In the gender subgroups, although all of the three subtypes appeared in the Tibetan antelope, the infection of ST10 and ST13 in males was higher than that in females. Tibetan antelope in Shuanghu County was found to be infected with three *Blastocystis* subtypes in the regional subgroups. However, only the ST13 subtype was found in the Tibetan antelope in Nyima County (Table 3).

The six representative sequences in this study and 49 sequences on GenBank were used to construct a phylogenetic tree. According to the phylogenetic tree analysis, the sequences of the three subtypes (ST10, ST13, and ST14) obtained from this study were clustered with their reference subtypes (Figure 3). The sequence of ST10 isolate has 99% homology with that of ST10 isolated from sika deer (MK930358) and sheep (MW850529). The sequence identified as ST13 in this study has a high degree of homology (99%) with the sequence identified in white Kangaroo (MT672637) and reindeer (MH325366). The ST14 sequence has 98% homology with the known reference sequence identified in sheep (MF186707).

**DISCUSSION**

The overall infection rate of *Blastocystis* in Tibetan antelope was 4.8% (30/627) in Tibet, which was lower than the prevalence of 5.5% (6/109) and 6.0% (50/832) identified in sheep (Wang et al., 2018a; Li et al., 2018) in China and 19.3% (9/150) and 32.0% (32/100) in sheep in Iran (Rostami et al., 2020; Salehi et al., 2021). The difference in the prevalence of *Blastocystis* may be related to the living environment and geographical factors of different countries (Tan, 2008). The infection rate of *Blastocystis* in different species is different. For example, the prevalence of Tibetan antelope in this study and sheep, goats, and cattle in other studies were 4.8% (30/627), 0.75% (3/400) (Ghimire and Bhattarai, 2019), and 14.43% (72/500) (Hastutiek et al., 2019), respectively. The results showed that the prevalence of *Blastocystis* might be related to the sensitivity of animals to *Blastocystis*. Therefore, future research should collect more samples to better understand the population characteristics of *Blastocystis* in Tibetan antelope.

The average temperature in Baingoin County is ~17.1°C annually, which is much lower than that of other counties. The survival of *Blastocystis* may be affected by the low-temperature environment in Baingoin County. *Blastocystis* might survive in warm and humid environment (Sari et al., 2021). This is probably the reason why the infection rate of *Blastocystis* in Baingoin County (0.0%, 0/133) was significantly lower than that of the other three counties.

Previous studies have shown that the infection rate of *Blastocystis* in males (4.8%, 25/517) was higher than that in females (3.1%, 9/291) in cattle (Lee et al., 2018) and in sambar (males: 38.2%, 21/55 vs. females: 23.3%, 7/30) (Kim et al., 2020). This study also found that the infection rate of *Blastocystis* (6.7%, 22/327) was higher in males than in females (2.7%, 8/300) of Tibetan antelope. This may be due to the fact that males have a wider range of activities than females, and have a relatively higher chance for contacting with cysts.

At present, 29 proposed *Blastocystis* subtypes have been identified (Maloney and Santin, 2021). Among them, ST1, ST3, ST5, ST10, and ST14 subtypes were detected in sheep and goats (Song et al., 2017; Li et al., 2018; Wang et al., 2018a), among which ST10 and ST14 were the most common subtypes (Fayer et al., 2012; Zhao et al., 2017; Hublin et al., 2021). ST10 and ST14 were also detected in Tibetan antelope. However, it is worth noting that ST10 (n = 4) and ST14 (n = 2) were not common in the samples of Tibetan antelope in this study. On the contrary, ST13 (n = 24) represented the infection trend of *Blastocystis* in Tibetan antelope. ST13 subtype is a relatively rare subtype. ST13 has been detected in deer, flying squirrels, kangaroo, monkeys, and other animals (Parkar et al., 2010; Alfellani et al., 2013; Wang et al., 2018b; Li et al., 2019; Xiao et al., 2019). Compared with domestic animals, ST13 may be more common in wild animals. Therefore, the follow-up research should focus on the distribution of *Blastocystis* genotypes in wild animals.

In summary, this is the first report of *Blastocystis* infection in Tibetan antelope in Tibet, China. The total prevalence of *Blastocystis* was 4.11% (30/627). Moreover, ST10, ST13, and ST14 subtypes were found in Tibetan antelope, among which ST13 was the dominant subtype. These results not only expanded the knowledge of hosts of *Blastocystis*, but also provided data for further studies on the distribution of *Blastocystis* subtypes in Tibetan antelope, and also provided data supporting for the prevention of *Blastocystis* infection in wild animals.
FIGURE 3 | Phylogenetic analyses of Blastocystis using (ML) method (Kimura two-parameter model). Bootstrap values below 50% from 1,000 replicates are not shown. Blastocystis isolates identified in the present study are indicated by solid circles.
DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

ETHICS STATEMENT

The animal study was reviewed and approved by the Ethics Committee of Jilin University.

AUTHOR CONTRIBUTIONS

H-BN and JJ conceived and designed the study and critically revised the manuscript. S-YQ, H-TS, J-HZ, Z-JW, and TM collected the samples. H-LG, Y-ZS, Y-GL, and N-YX performed the experiments. H-LG and Y-ZS analyzed the data and drafted the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

Abd, R. N. A., Yusof, A. M., and Mohammad, M. (2019). Identification of Blastocystis Sp. Infection Form Cattle, Goat and Sheep Isolated From Farms in Pahang, Malaysia. Int. J. Allied Health Sci. 3 (3), 810–810.
AbuOdeh, R., Ezzedine, S., Madkour, M., Stensvold, C. R., Samee, A., Nasrallah, G., et al. (2019). Molecular Subtyping of Blastocystis From Diverse Animals in the United Arab Emirates. Protistol 170 (5), 1256-79. doi: 10.1016/j.protis.2019.125679
Alexieff, A. (1911). Sur La Nature Des Formations Dites "Kystes De Trichomonas Intestinalis". Comptes Rendus Des. Seances la Sociite Biologie 71, 296298.
Alfellani, M. A., Taner-Mulla, D., Jacob, A. S., Imeede, C. A., Yoshikawa, H., Alexeieff, A. (1911). Sur La Nature Des Formations Dites "Kystes De Trichomonas Intestinalis". Comptes Rendus Des. Seances la Sociite Biologie 71, 296298.

ACKNOWLEDGMENTS

We thank Dr. Chuang Lyu (Shandong New Hope Liuhe Group Co., Ltd. and Qingdao Jiazi Biotechnology Co., Ltd., Qingdao, China) for critically revising the manuscript.
Ma, L., Qiao, H., Wang, H., Li, S., Zhai, P., Huang, J., et al. (2020). Molecular Prevalence and Subtypes of Blastocystis Sp. In Primates in Northern China. Transbound Emerg. Dis. 67 (6), 2789–2796. doi: 10.1111/tbed.13644
Masuda, A., Sumiyoshi, T., Ohtaki, T., and Matsumoto, J. (2018). Prevalence and Molecular Subtyping of Blastocystis From Dairy Cattle in Kanagawa, Japan. Parasitol Int. 67 (6), 702–705. doi: 10.1016/j.parint.2018.07.005
Moura, R. G. F., Oliveira-Silva, M. B., Pedrosa, A. L., Nascentes, G. A. N., and Masuda, A., Sumiyoshi, T., Ohtaki, T., and Matsumoto, J. (2018). Prevalence and Genetic Diversity of Caprine Blastocystis From Peninsular Malaysia. Parasitol Res. 112 (1), 85–89. doi: 10.1007/s00436-012-3107-3
Udonsom, R., Prasertbun, R., Mahittikorn, A., Mori, H., Changbunjong, T., Komalaimisra, C., et al. (2018). Blastocystis Infection and Subtype Distribution in Humans, Cattle, Goats, and Pigs in Central and Western Thailand. Infect. Genet. Evol. 65, 107–111. doi: 10.1016/j.meegid.2018.07.007
Wang, J., Gong, B., Liu, X., Zhao, W., Bu, T., Zhang, W., et al. (2018b). Distribution and Genetic Diversity of Blastocystis Subtypes in Various Mammal and Bird Species in Northeastern China. Parasit. Vectors 11 (1), 522. doi: 10.1186/s13071-018-3106-z
Wang, J., Gong, B., Yang, F., Zhang, W., Zheng, Y., and Liu, A. (2018a). Subtype Distribution and Genetic Characterizations of Blastocystis in Pigs, Cattle, Sheep and Goats in Northeast China’s Heilongjiang Province. Infect. Genet. Evol. 57, 171–176. doi: 10.1016/j.meegid.2017.11.026
Xiao, X., Zhou, S. H., Jiang, N., Tian, D. Z., Zhou, Z. M., Zhang, M., et al. (2019). First Record of Leptospira and Blastocystis Infections in Captive Flying Squirrels (Troglodytes Xanthipes) From Enshi County, China. Acta Trop. 197, 105065. doi: 10.1016/j.actatropica.2019.105065
Yu, Z., Wang, T., Sun, H., Xia, Z., Zhang, K., Chua, D., et al. (2012). Contagious Caprine Pleuropneumonia in Endangered Tibetan Antelope, Chin. Emerg. Infect. Dis. 19 (12), 2051–2053. doi: 10.3201/eid1912.130067
Zhao, G. H., Hu, X. F., Liu, T. L., Hu, R. S., Yu, Z. Q., Yang, W. B., et al. (2017). Molecular Characterization of Blastocystis Sp. In Captive Wild Animals in Qinling Mountains. Parasitol Res. 116 (8), 2327–2333. doi: 10.1007/s00436-017-5506-y
Zhu, W., Tao, W., Gong, B., Yang, H., Li, Y., Song, M., et al. (2017). First Report of Blastocystis Infections in Cattle in China. Vet. Parasitol. 246, 38–42. doi: 10.1016/j.vetpar.2017.09
Zhu, W., Wei, Z., Li, Q., Lin, Y., Yang, H., and Li, W. (2020). Prevalence and Subtype Diversity of Blastocystis in Human and Nonhuman Primates in North China. Parasitol Res. 119 (8), 2719–2725. doi: 10.1007/s00436-020-06761-w

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Geng, Sun, Jiang, Sun, Li, Zhu, Xia and Ni. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.