Occurrence and subtyping of *Blastocystis* in coypus (*Myocastor coypus*) in China

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**Abstract**

**Background:** *Blastocystis* is an anaerobic unicellular protist frequently detected in the gastrointestinal tracts of humans and animals worldwide. However, the prevalence and subtype distribution of *Blastocystis* in the coypu (*Myocastor coypus*) population have not been reported so far. The aim of this study was to determine the prevalence, genetic characteristics, and zoonotic potential of *Blastocystis* isolates detected in coypus in China.

**Results:** A total of 308 fecal samples were collected from coypus in seven regions across China and subsequently examined. *Blastocystis* was detected in 44 (14.3%) specimens by nested PCR amplification of the small subunit ribosomal RNA (SSU rRNA) gene. Further DNA sequencing and phylogenetic analyses resulted in the identification of two zoonotic known subtypes, ST4 and ST5, and an unknown subtype. ST4 was the most predominant subtype observed in the samples. ST5 infections were only observed in three coypus. Factors that were associated with prevalence of *Blastocystis* included age, geographical region and subtype. Interestingly, this is the first report about a potentially novel subtype infecting coypus.

**Conclusions:** This is the first comprehensive report of *Blastocystis* in *M. coypus* across a wide geographic range of China. A moderate degree of genetic divergence was observed. The presence of zoonotic subtypes in farmed *M. coypus* suggests that these animals have the potential to transmit blastocystosis to both humans and domestic animals. These findings provide a better understanding of the genetic diversity of *Blastocystis* in rodents and contribute towards the establishment of efficient blastocystosis control strategies in the investigated areas.

**Keywords:** *Blastocystis*, Prevalence, Subtype, Zoonotic potential, Coypus

**Background**

*Blastocystis* is a eukaryotic protozoan parasite that belongs to the stramenopile clade of heterokonts whose members are commonly observed in the intestinal tract of humans and animals worldwide [1, 2]. The pathogenicity of *Blastocystis* remains controversial because many infections produce subclinical and asymptomatic disease in humans and animals, but this parasite has been reported in patients with diarrhea and irritable bowel syndrome [2, 3]. The protist is believed to be transmitted via the fecal–oral route, involving direct contact with infected hosts and the ingestion of contaminated food or water [4, 5]. However, only a small number of published studies have reported zoonotic transmission between animals and their handlers [6]; therefore, a better understanding the zoonotic potential of *Blastocystis* is crucial. In addition, the identification of *Blastocystis* in a variety of pets, livestock and wildlife suggests widespread opportunities for these reservoir species to transmit the parasite to humans [7].
The genus *Blastocystis* exhibits a remarkable genetic diversity [8]. To date, a total of 32 *Blastocystis* subtypes have been reported based on polymorphisms within the small subunit ribosomal RNA (SSU rRNA) gene [4, 9, 10]. Subtypes ST1–ST32 have been described in various mammals and birds. However, based on the current criteria in place to qualify as a unique subtype, a total of 28 subtypes (ST1–ST17, ST21 and ST23–ST32) are generally widely recognized as being valid subtypes [4, 8–10]. In the past two decades, numerous epidemiologic studies on *Blastocystis* have demonstrated that most subtypes have a low host specificity. Infections have been associated with at least 14 subtypes (ST1–ST10, ST12, ST14, ST16 and ST24) in both humans and animals, indicating a strong potential for zoonotic transmission [11–13]. Moreover, > 95% of human *Blastocystis* cases have been attributed to ST1–ST4 [1, 2]. Thus, a variety of mammalian and avian hosts have been examined to determine the identity of *Blastocystis* subtypes carried by each species.

Wildlife, including animals in captivity, can serve as infection sources for zoonotic parasitic diseases [7]. Sub-type analysis of *Blastocystis* infection in wildlife is important to improve our understanding of the potential role of wildlife in the transmission dynamics of these protozoa to livestock and human hosts [14, 15]. While a few studies have been published on *Blastocystis* in some rodents [6, 7, 13, 16], these studies did not specifically examine the prevalence of subtypes or conduct subtype distribution analysis of *Blastocystis* in the large semi-aquatic rodent *Myocastor coypus*. In the present study, we evaluated the prevalence and subtypes of *Blastocystis* in *M. coypus* across China, with the overall aim to better understand the risk of zoonotic spread of this parasite.

**Methods**

**Source and sample collection**

A total of 308 fecal samples were collected between August 2018 and March 2019 from seven *M. coypus* farms in Hebei Province (Baoding), Henan Province (Anyang and Kaifeng), Sichuan Province (Chengdu), Hunan Province (Yongzhou), Jiangxi Province (Ganzhou) and Guangxi Zhuang Autonomous Region (Laibin), in China (Table 1). Each farm possessed anywhere from 500 to 2000 *M. coypus*, and all had active breeding programs in place. Approximately 10–15% of animals representing each age group were randomly sampled at each farm. All fecal samples were collected immediately after excretion and placed in a 50-ml Eppendorf tube using disposable polyethylene gloves; the collection date and site, and the age and health condition of the animal were recorded at the time of fecal sample collection. All samples were immediately placed in a cooler with ice packs, transported to the laboratory within 48 h and stored at 4 °C until the time of analysis.

**DNA extraction and PCR analysis**

Genomic DNA was extracted from 0.2 g of each fecal specimen using the E.Z.N.A.® Stool DNA Kit, D4015-2 (Omega Bio-Tek Inc., Norcross, GA, USA), according to the manufacturer’s protocol, with minor modifications. Each DNA sample was stored at −20 °C for subsequent molecular analysis.

Nested PCR amplification targeting the 479-bp fragment of the SSU rRNA gene was performed to screen for *Blastocystis* infection [17]. The primers RD3 (5′-GGGATCCTGTACCTTTCCGCAGGTTACCTAC-3′) and RD5 (5′-GGAAGCTTATCTGGATATCCTGC CAGTA-3′) that amplified a fragment of about 1780 bp were used in the primary PCR [18]. The primers Bla1 (5′-GGAGGTAGTGACATAAAATC-3′) and Bla2 (5′-TTCGCACTGTGTCATC-3′) that amplified a nested fragment of approximately 480 bp were used in the secondary PCR [17]. The PCR conditions were: 95 °C for 5 min; 35 cycles of denaturation at 95 °C/45 s, annealing at 54 °C or 65 °C/45 s and extension at 72 °C/1 min; with a final extension 72 °C for 8 min. Each PCR reaction was carried out in a total reaction volume of 25 μl containing 12.5 μl of 2× Taq PCR Master Mix (Sangon, Shanghai, China), 8.5 μl of deionized H2O, 1 μl of forward and reverse primers and 2 μl of genomic DNA or primary amplification products. Both positive (ST7 from quail) and negative (ultrapure H2O) controls were run in each PCR assay. All amplicons of the secondary PCR were resolved by electrophoresis in a 1% agarose gel with Golden View staining.

**Sequence and phylogenetic analyses**

All positive PCR products were sequenced in both directions on an ABI 3730 DNA Analyzer (Applied Biosystems, Thermo Fisher Scientific, Foster City, CA USA). Sequencing reactions were performed by the Sangon Biotech Company (Shanghai, China). Raw sequences were corrected and assembled manually using the DNAStar 7.1 (https://www.dnastar.com/) and BioEdit 7.0 (http:// www.mbio.ncsu.edu/BioEdit/bioedit.html) programs to guarantee the accuracy of the called nucleotides. To determine *Blastocystis* subtypes, each clean sequence was compared with GenBank sequences by BLAST (Basic Local Alignment Search Tool) analysis (http://www.ncbi.nlm.nih.gov/BLAST/) and the online platform of the definitions database of *Blastocystis* (https://pubmlst.org/bigsdb?db=pubmlst_blastocystis_seqdef), respectively. The reference sequences of known valid subtypes were downloaded from the GenBank database. All sequences obtained in this study were aligned with the reference
sequences using Clustal X (http://www.clustal.org). A phylogenetic relationship was constructed by the neighbor-joining (NJ) method using the Kimura 2-parameter model and the maximum parsimony (MP) method with 1000 bootstrap replicates using the MEGA 7.0 software (http://www.megasoftware.net/).

Representative unique nucleotide sequences obtained herein have been submitted to the GenBank database under the accession numbers OK235451–OK235463.

Statistical analysis
Differences in prevalence of Blastocystis subtypes between age groups and geographical locations were analyzed using a Chi-square ($\chi^2$) test and 95% confidence intervals (CIs) in the SPSS version 22.0 software package (www.ibm.com/products/spssstatistics). Differences were considered to be statistically significant at $P<0.05$.

Results and discussion
To the best of our knowledge, this study is the first report on the Blastocystis infection in M. coypus throughout China. Of the 308 fecal samples tested by nested PCR amplification of the SSU rRNA gene, 44 were positive for Blastocystis (prevalence: 14.3%; 95% Cl 10.4–18.2). Notably, the highest prevalence of Blastocystis was detected in fecal samples from M. coypus at the Laibin City farm (27.3%, 6/22), followed by those collected at the farms located in the cities of Anyang (22.8%, 23/101), Kaifeng (19.2%, 10/52), Baoding (11.4%, 4/35) and Yongzhou (13%, 3/23) (Table 1); in contrast, the parasite was not detected in any of the samples collected from farms in the cities of Chengdu and Ganzhou. In addition, higher infection rates were found at the Laibin and Anyang sites than at sites in the other five regions sampled, with the associations of infection rate with locality being statistically significant ($P=0.044$ and 0.034) (Table 1). It has been reported that geographical and environmental factors might influence the prevalence of Blastocystis in animals and humans [5, 19]. For all locations sampled in the present study, significant differences in the observed prevalence may be attributable to different sanitation practices and standards between the farms.

When the data were stratified by age, prevalence of Blastocystis was found to be inversely related to age. The highest rate of infection was reported in animals aged <3 months (24.6%, 16/65), followed those aged 3–6 months (19.1%, 9/47) and finally those aged >6 months (9.7%, 19/196) (Table 1). Evaluation of the correlation between age and infection rates based on the calculated odds ratios (ORs) and 95% CI values revealed a strong negative correlation, with an OR of 0.329 (95% CI 0.157–0.687; $P=0.003$) associated with the >6-month-old group. However, for the 3- to 6-month-old group, the correlation was statistically non-significant, with an OR of 0.725 (95% CI 0.289–1.820; $P=0.494$) (Table 1). These findings are in contrast with those reported on Blastocystis infection in Chinese and Korean cattle [5, 20] as well as in young children in Turkey [21], all of which reported that age was significantly associated with Blastocystis infection.

Abundant studies on the molecular epidemiology of Blastocystis in livestock and wildlife have been published, many of which have chronicled transmission dynamics in addition to the zoonotic significance [7, 22]. However, the incidence of Blastocystis in rodents has not been sufficiently investigated, and only a few reports have been

| Variables | No. of farmed M. coypus | No. of subtypes | Total prevalence (% positive/total) | P-value | Odds ratio (95% confidence interval) |
|-----------|-------------------------|----------------|-----------------------------------|---------|-------------------------------------|
| Location  |                         |                |                                   |         |                                     |
| Baoding, Hebei Province | 35 | 3 | 1 | 11.4 (4/35) | 0.347 | 2.839 (0.297–27.160) |
| Anyang, Henan Province | 101 | 14 | 1 | 22.8 (23/101) | 0.044 | 6.487 (0.829–50.759) |
| Kaifeng, Henan Province | 52 | 10 | | 19.2 (10/52) | 0.093 | 5.238 (0.629–43.611) |
| Chengdu, Sichuan Province | 40 | | 0.0 (0/40) | | 0.184 | -- |
| Yongzhou, Hunan Province | 23 | 1 | | 4.3 (1/23) | Reference | Reference |
| Ganzhou, Jiangxi Province | 35 | | 0.0 (0/35) | | 0.213 | -- |
| Laibin, Guangxi Autonomous Region | 22 | 6 | | 27.3 (6/22) | 0.034 | 8.250(0.903–75.414) |
| Age (months) | | | | | | |
| <3 | 65 | 8 | 2 | 6 | 24.6 (16/65) | Reference | Reference |
| 3–6 | 47 | 8 | 1 | 19.1 (9/47) | 0.494 | 0.725 (0.289–1.820) |
| >6 | 196 | 17 | 1 | 1 | 9.7 (19/196) | 0.003 | 0.329 (0.157–0.687) |
| Total | 308 | 33 | 3 | 8 | 14.3 (44/308) | | |

ST Subtype
Fig. 1  Phylogenetic relationship of the SSU rRNA genes (about 480 bp) of Blastocystis isolates from Myocastor coypus fecal samples. Relationships to other known Blastocystis subtypes were inferred by the MP and NJ methods based on evolutionary distances. Bootstrap values were obtained using 1000 pseudoreplicates, and values > 50% are shown. The filled triangles indicate potentially novel subtypes identified in the current study. 

Proteromonas lacertae was used as the outgroup for this tree. Abbreviations: no, not supported/lower bootstrap values.
A number of published studies have clearly demonstrated that ST4 has a global distribution, with its detection in a broad array of host species, including rodents, suggesting a high degree of promiscuity [7, 20]. The number of studies on Blastocystis infection in rodents are far fewer than those in livestock. Interestingly, approximately 30 species of rodents have been surveyed to date, and at least 11 subtypes (ST1–ST8, ST10, ST13, ST15 and ST17) have been detected in rodent hosts [6, 7, 16, 23]. Of these, ST4 infection remains the most prevalent subtype, having been reported in > 17 rodent species, indicating that it has become well adapted to infect rodents. Moreover, ST4 has been detected sporadically in pigs in most provinces of China [39, 40]. Based on this observation, it would be interesting to examine whether or not transmission occurs between pigs and coypus. In addition, both ST4 and ST5 have been found to also infect cattle in China [20]. Hence, the infection risk to other animals around these farms cannot be ignored in view of the various transmission pathways of Blastocystis.

Frequently detected in humans across a broad geographic range, ST4 is likely a significant concern for public health [11, 19, 41]. In contrast, infections with ST5 have been infrequently reported and only from a small number of countries [13, 42, 43]. Moreover, the potentially zoonotic ST4 and ST5 have been recently reported in patients in several provinces of China, including regions surveyed in the present study [42, 44–46]. Also notable it that transmission of Blastocystis between domesticated animals and their handlers has been demonstrated [6, 47]. Thus, there is a potential zoonotic threat to farmers/animal caretakers on M. coypus farms, but further study is required to determine the actual risk.

**Conclusions**

This is the first molecular study of prevalence and subtype distribution of Blastocystis in M. coypus. The known zoonotic ST4 and ST5 subtypes as well as a potentially novel subtype were detected. These findings add to our current understanding of the genetic features of Blastocystis in M. coypus.
**Blastocystis** in rodent populations and of the potential zoonotic risk to farmers/animal caretakers of *M. coypus*. Further studies to extend the surveyed region and to analyze genetic characteristics of the unknown subtype are required.

**Abbreviations**

BLAST: Basic local alignment search tool; MP: Maximum parsimony; NJ: Neighbor-joining; SSU rRNA: Small subunit ribosomal RNA.

**Authors’ contributions**

MQ and LXZ conceived and designed the experiments. XHL, FZN and JQJ collected the samples. RJW, YMG and XFY performed the experiments and data analyses. XHL, MQ and LXZ wrote and revised this manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

All data generated and analyzed during this study are included in the article as published. The representative unique nucleotide sequences obtained herein were submitted to the GenBank database under the accession numbers OK235451 through to OK235463.

**Declarations**

**Ethics approval and consent to participate**

This study was performed in strict compliance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the Ministry of Health, China. The Research Ethics Committee of Tarim University reviewed and approved the research protocol (approval no. ECTU 2018-0026). Permission was granted by the farmers to the study team for access to the farms and to collect samples from the animals. No specific permits were required for the described field studies, and no animals were harmed during the sampling process.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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**References**

1. Stensvold CR, Clark CG. Current status of Blastocystis: a personal view. Parasitol Int. 2016;65:763–71.
2. Salehi R, Rostami A, Mirjalali H, Rune Stensvold C, Haghighi A. Genetic characterization of Blastocystis from poultry, livestock animals, and humans in the southwest region of Iran—zoonotic implications. Transbound Emerg Dis. 2021. https://doi.org/10.1111/tbed.14078.
3. Mirjalali H, Abbasi MR, Naderi N, Hasanizadeh G, Mirsambadi ES, Stensvold CR, et al. Distribution and phylogenetic analysis of Blastocystis sp. subtypes isolated from IBD patients and healthy individuals in Iran. Eur J Clin Microbiol. 2017;36:2335–42.
4. Maloney JG, Jang Y, Molokan A, George NS, Santin M. Wide genetic diversity of Blastocystis in white-tailed deer (*Odocoileus virginianus*) from Maryland, USA. Microorganisms. 2021;9:1343.
5. Lee H, Lee SH, Seo MG, Kim HY, Kim JW, Lee YR, et al. Occurrence and genetic diversity of Blastocystis in Korean cattle. Vet Parasitol. 2018;258:70–3.
6. Koster PC, Dashiti A, Ballo B, Muadica AS, Maloney JG, Santin M, et al. Occurrence and genetic diversity of protist parasites in captive non-human primates, zookeepers, and free-living sympatric rats in the Cordoba Zoo Conservation Centre, southern Spain. Animals. 2021;11:700.
7. Hublin J SY, Maloney JG, Santin M. Blastocystis in domesticated and wild mammals and birds. Res Vet Sci. 2021;135:260–82.
8. Stensvold CR, Clark CG. Pre-empting Pandora’s box: Blastocystis subtypes revisited. Trends Parasitol. 2020;36:229–32.
9. Higuera A, Herrera G, Jiménez P, García-Corredor D, Pullido-Medellín M, Bulla-Castaneda DM, et al. Identification of multiple Blastocystis subtypes in domestic animals from Colombia using amplicon-based next generation sequencing. Front Vet Sci. 2021;8:73219.
10. Maloney J, Santin M. Mind the gap: new full-length sequences of Blastocystis subtypes generated via Oxford nanopore minicon sequencing allow for comparisons between full-length and partial sequences of the small subunit of the ribosomal RNA gene. Microorganisms. 2021;9:997.
11. Osorio-Pulgarín MI, Higuera A, Beltran-Alzate JC, Sanchez-Jimenez M, Ramirez JD. Epidemiological and molecular characterization of Blastocystis infection in children attending daycare centers in Medellín. Colombia Biology. 2021;10:699.
12. Khaled S, Santos N, Ly AT, Senghor S, Even G, Dautel E, et al. Prevalence and subtype distribution of Blastocystis sp. in Senegalese school children. Microorganisms. 2020;8:1408.
13. Jimenez PA, Jaimés JE, Ramírez JD. A summary of Blastocystis subtypes in North and South America. Parasit Vectors. 2019;12:376.
14. Kim KT, Noh G, Lee H, Kim SH, Jeong H, Kim Y, et al. Genetic diversity and zoonotic potential of Blastocystis in Korean water deer. *Hydropotes inermis* argypus. Pathogens. 2020;9:955.
15. Skotarczak B. Genetic diversity and pathogenicity of Blastocystis. Ann Agric Environ Med. 2018;25:411–6.
16. Liu X, Ge Y, Wang R, Dong H, Yang X, Zhang L. First report of Blastocystis infection in Pallas’s squirrels (*Callosciurus erythraeus*) in China. Vet Res Commun. 2021;45:441–5.
17. Santin M, Gomez-Munoz MT, Solano-Aguilar G, Fayer R. Development of a new PCR protocol to detect and subtype Blastocystis spp. from humans and animals. Parasitol Res. 2011;109:205–12.
18. Clark CG. Extensive genetic diversity in Blastocystis hominis. Mol Biochem Parasit. 1997;87:79–83.
19. Alfellani MA, Stensvold CR, Vidal-Lapiedra A, Oruocha ES, Fagbenro-Beyioku AF, Clark CG. Variable geographic distribution of Blastocystis subtypes and its potential implications. Acta Trop. 2013;126:11–8.
20. Zhu W, Tao W, Gong B, Yang H, Li Y, Song M, et al. First report of Blastocystis infections in cattle in China. Vet Parasitol. 2017;246:38–42.
21. Beyhan Y, Yilmaz H, Cengiz Z, Ecki A. Clinical significance and prevalence of Blastocystis hominis in Van, Turkey. Saudi Med J. 2015;36:1118–21.
22. Alfellani MA, Taner-Mulla D, Jacob AS, Immede CA, Yoshikawa H, Stensvold CR, et al. Genetic diversity of Blastocystis in livestock and zoo animals. Protist. 2013;164:497–500.
23. Mohammadpour I, Bozorg-Halati F, Gazzoni AL, Manfredi MT, Motazedian MH, Mohammadpour N. First molecular subtyping and phylogeny of Blastocystis sp. isolated from domestic and synanthropic animals (dogs, cats and brown rats) in southern Iran. Parasit Vectors. 2020;13:365.
24. Chai Y, Deng L, Liu H, Yao J, Zhong Z, Fu H, et al. First subtyping of Blastocystis sp. from pet rodents in southwestern China. Int J Parasitol Parasites Wildl. 2020;11:143–8.
25. Katsumata M, Yoshikawa H, Tokoro M, Mizuno T, Nagamato T, Hemandto J, et al. Molecular phylogeny of Blastocystis isolates from wild rodents captured in Indonesia and Japan. Parasitol Res. 2018;117:2841–6.
26. Matyalay E, Basaran G, Gokcimen A, Ertebaybak H, Ergut S. First molecular characterisation of Blastocystis from experimental rats in Turkey and comparison of the frequencies between obese and non-obese groups. *Turkiye Parazitol Derg*. 2021;45:165–70.
27. Betts EL, Gentekaki E, Tsacoussis AD. Exploring micro-eukaryotic diversity in the gut: co-occurrence of Blastocystis subtypes and other protists in zoo animals. Front Microbiol. 2020;11:288.

28. Farah Haziqah MT, Mohd Zain SN, Chandravathani P, Premalaitha B, Mohd Khairul Nizam IMK, Arutchelvan R, et al. Genetic diversity of rodent Blastocystis sp. from Peninsular Malaysia. Trop Biomed. 2018;35:586–92.

29. Cian A, El Safadi D, Osman M, Moriniere R, Gantois N, Benamrouz-Vanneste S, et al. Molecular epidemiology of Blastocystis sp. in various animal groups from two French zoos and evaluation of potential zoonotic risk. PLoS ONE. 2017;12:e0169659.

30. Martinez-Hernandez F, Martinez-Ibarra JA, Lopez-Escamilla E, Villanueva-Garcia C, Munoz-Garcia CI, Rendon-Franco E, et al. Molecular genotyping of Blastocystis spp. in wild mammals from Mexico. Parasitol Res. 2020;119:97–104.

31. Li XD, Zou Y, Pan J, Liang QL, Zeng Z, Meng YM, et al. Prevalence and subtypes of Blastocystis sp. infection in zoo animals in three cities in China. Parasitol Res. 2020;119:465–71.

32. Wang JG, Gong BY, Liu XH, Zhao W, Bu T, Zhang WZ, et al. Distribution and genetic diversity of Blastocystis subtypes in various mammal and bird species in northeastern China. Parasit Vectors. 2018;11:522.

33. Noël C, Peyronnet C, Gerbod D, Edgcomb VP, Delgado-Viscoiglosi P, Sogin ML, et al. Phylogenetic analysis of Blastocystis isolates from different hosts based on the comparison of small-subunit rRNA gene sequences. Mol Biochem Parasitol. 2003;126:119–23.

34. Yoshikawa H, Wu Z, Nagano I, Takahashi Y. Molecular comparative studies among Blastocystis isolates obtained from humans and animals. J Parasitol. 2003;89:85–94.

35. Noël C, Dufnernez F, Gerbod D, Edgcomb VP, Delgado-Viscoiglosi P, Ho LC, et al. Molecular phylogenies of Blastocystis isolates from different hosts: implications for genetic diversity, identification of species, and zoonosis. J Clin Microbiol. 2005;43:348–55.

36. AbuOdeh R, Ezzedine S, Madkour M, Stensvold CR, Samie A, Nasrallah G, et al. Molecular subtyping of Blastocystis from diverse animals in the United Arab Emirates. Protist. 2019;170:125679.

37. Betts EL, Gentekaki E, Thomasz A, Breakell V, Carpenter AI, Tsaousis AD. Genetic diversity of Blastocystis in non-primate animals. Parasitology. 2018;145:1228–34.

38. Goe AM, Heard DJ, Easley JR, Weeden AL, Childress AL, Wellehan JF. Blastocystis sp. and Blastocystis Ratti in a Brazilian porcupine (Coendou Prehensilis) with diarrhea. J Zoo Wildl Med. 2016;47:640–4.

39. Rauff-Adedotun AA, Mohd Zain SN, Farah Haziqah MT. Current status of Blastocystis sp. in animals from Southeast Asia: a review. Parasitol Res. 2020;119:3559–70.

40. Asghari A, Sadjebbazzar A, Shamsi L, Shams M. Global prevalence, subtypes distribution, zoonotic potential, and associated risk factors of Blastocystis sp. in domestic pigs (Sus domesticus) and wild boars (Sus scrofa) in United Arab Emirates. Protist. 2019;170:125679.

41. Lepczynska M, Dzikza E, Chen W. Prevalence of Blastocystis subtypes in healthy volunteers in northeastern Poland. J Parasitol. 2021;107:684–8.

42. Ma L, Qiao H, Wang H, Li S, Zhai P, Huang J, et al. Molecular prevalence and subtypes of Blastocystis sp. in primates in northern China. Transbound Emerg Dis. 2020;67:2789–96.

43. Cabrine-Santos M, Moura RGF, Pedrosa AL, Correia D, Oliveira-Silva MB. Molecular characterization of Blastocystis subtypes isolated in the city of Uberaba, Minas Gerais state, Brazil. Rev Soc Bras Med Trop. 2020;54:e03052021.

44. Deng Y, Zhang SX, Ning CQ, Zhou YK, Teng XJ, Wu XP, et al. Molecular epidemiology and risk factors of Blastocystis sp. infections among general populations in Yunnan Province, southwestern China. Risk Manag Healthc Policy. 2020;13:1791–801.

45. Ning CQ, Hu ZH, Chen JH, Ai L, Tian LG. Epidemiology of Blastocystis infection from 1990 to 2019 in China. Infect Dis Poverty. 2020;9:168.

46. Zhang SX, Carmena D, Ballesteros C, Yang CL, Chen JX, Chu YH, et al. Symptomatic and asymptomatic protist infections in hospital inpatients in southwestern China. Pathogens. 2021;10:684.

47. Oliveira-Arbex AP, David EB, Tenorio MDS, Cicchi PJF, Patti M, Coradi ST, et al. Diversity of Blastocystis subtypes in wild mammals from a zoo and two conservation units in southeastern Brazil. Infect Genet Evol. 2020;81:104053.