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Short communication

First report of *Blastocystis* infections in cattle in China

Weining Zhu[a,1], Wei Tao[a,1], Binbin Gong[b], Hang Yang[a], Yijing Li[a], Mingxin Song[a], Yixin Lu[a], Wei Li[a,*]

[a] College of Veterinary Medicine, Northeast Agricultural University, Harbin, Heilongjiang 150030, China
[b] School of Chemical Engineering & Biotechnology, Xingtai University, Xingtai, Hebei 054001, China

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ABSTRACT

Blastocystis is one of the most common intestinal protists of humans and can also infect a variety of other mammals and birds. *Blastocystis* infections and subtype distribution in cattle have been documented, while nothing is known about those in China. Herein, a total of 526 dairy cattle from northeast China were sampled and investigated for the prevalence and genetic characteristics of *Blastocystis* and the potential role of bovine animals in zoonotic transmission of *Blastocystis*. The parasite was identified in 54 (10.3%) fecal specimens by nested PCR and DNA sequencing of the small subunit ribosomal RNA gene. Sequence analysis enabled identification of four *Blastocystis* subtypes (STs). Among those, subtype ST10 (75.9%, 41/54) has the highest frequency, followed by ST14 (18.5%, 10/54), ST4 (3.7%, 2/54), and ST5 (1.9%, 1/54). High prevalence and widespread distribution of ST10 and ST14 in cattle observed herein, together with analysis of their host distribution patterns in earlier studies, indicated some host-adapted potential in the two subtypes. The identification of human-pathogenic subtypes ST4 and ST5 might imply a potential zoonotic risk of cattle origin. This is the first study exploring the prevalence and genetic characteristics of *Blastocystis* in cattle in China. The host range of subtype ST4 was extended. The findings of this study should be helpful for a better understanding of the epidemiology and public health potential of *Blastocystis*.

1. Introduction

Blastocystis, one of the most ubiquitous parasites of mammalian intestinal tracts, was reported in a variety of vertebrate hosts including humans (Fayer et al., 2012; Parkar et al., 2010; Roberts et al., 2013). The pathogenicity of *Blastocystis* is related to such factors including subtype variation and host immune status, while the exact mechanism remains controversial (Cirioni et al., 1999; Elwakil and Hewedi, 2010). *Blastocystis* is commonly transmitted through sewage-contaminated water and food that contains cysts and the fecal-oral route is a major mode of transmission (Leelayoova et al., 2004; Leelayoova et al., 2008; Yoshikawa et al., 2004b). Close contacts with infected animals may constitute risks of zoonotic transmission of *Blastocystis* (Osman et al., 2015; Wang et al., 2014).

Genetic polymorphisms of the small subunit ribosomal RNA (SSU rRNA) gene enabled identification of 17 distinct *Blastocystis* subtypes (STs) in different human and animal species (Alfellani et al., 2013b; Stensvold et al., 2009; Wang et al., 2014). Nine subtypes (ST1–ST9) have been reported in human *Blastocystis* infections, with ST3 and ST1 most frequently examined. The variation in human *Blastocystis* subtype by geographical region has been analyzed, with predominance of ST1 (followed by ST2 and ST3) in America, ST3 (ST4 and ST1) in Europe, ST3 (ST1 and ST6) in Africa, ST3 (ST1 and ST4) in Australia, and ST3 (ST1 and ST2) in Asia (Alfellani et al., 2013a). The host specificity and pathogenic potential among the various *Blastocystis* subtypes differ considerably as well, which might represent contributors to the symptom variability in patients with *Blastocystis* (Dominguez-Marquez et al., 2009; Khademvatan et al., 2017; Mohamed et al., 2017; Souppart et al., 2010). The rest eight subtypes such as ST10 and ST14 usually circulate in specific animal hosts and have never appeared in human infections. Most of the animal species surveyed can harbor potentially zoonotic *Blastocystis* subtypes and thus can be potential reservoirs of human infections (Khademvatan et al., 2017; Stensvold and Clark, 2016; Wang et al., 2014).

In recent decades, *Blastocystis* infections have been repeatedly reported in a wide range of domestic animals and some factors associated with zoonotic transmission have been evaluated as well (Cian et al., 2017; Osman et al., 2015). Several reports have indicated the prevalence and distribution of *Blastocystis* subtypes ST1, ST3, ST5, ST6, ST10, and ST14 in cattle from Iran, USA, UK, Libya, Denmark, Japan,

* Corresponding author.
E-mail address: neaulw@gmail.com (W. Li).
[1] These authors contribute equally to this work.

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and Colombia (Alfellani et al., 2013b; Badparva et al., 2015; Ramirez et al., 2014; Santin et al., 2011; Stensvold et al., 2009; Yoshikawa et al., 2004a). This study was conducted to investigate 526 dairy cattle of various ages in northeast China for the prevalence and genetic characteristics of *Blastocystis* as well as to assess the potential role of bovine animals in zoonotic transmission of *Blastocystis*.

2. Materials and methods

2.1. Ethics statement

The protocol of the current study was reviewed and approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University (no. SRM-08). Before the experiment, we contacted the farm owners for their permissions. No specific permits were required for the described field studies. And the locations where we sampled are not privately-owned or protected in any way.

2.2. Specimen collection

A total of 526 fecal specimens were collected from cattle in cities Harbin (n = 196) in October 2013, Qiqihar (n = 190) in April 2014, and Daqing (n = 140) in July 2014. The three cities are closely linked and located in northeast China. Three age categories of animals were seen in Harbin, with preweaned animals aged < 3 months (n = 69), weaned animals aged 3–12 months (n = 61), and yearlings and adults aged > 12 months (n = 66) (Table 1). Specimens from Harbin were transferred individually into 50-ml bottles filled with 2.5% (m/v) potassium dichromate and stored at 4 °C. Only preweaned calves were kept on the farm in Qiqihar, and weaned cattle in Daqing likewise (Table 1). Specimens from the two locations were stored frozen in disposable plastic bags at −20 °C. All the specimens were collected as quickly as possible after defecation. One specimen per animal was used in this study.

2.3. DNA extraction and PCR

Prior to DNA extraction, fecal specimens stored in potassium dichromate were washed twice in double-distilled H_2O by centrifugation at 12,000 × g for 5 min at room temperature. Genomic DNA was extracted from 0.2 g of feces using Stool DNA rapid extraction kits (TIANGEN, China) as instructed by the manufacturer. Nested PCR based on the SSU rRNA gene was performed using the primers RD3 (5′-GGGATCCTGATCCTTCCGCAGGTTCACCTAC-3′) and RD5 (5′-GGAAGCTTATCTGGTTGATCCTGCCAGTA-3′) that amplified a fragment of about 1780 bp in length in the first round PCR (Parkar et al., 2010) and the primers Blasto 2F (5′-TCTGGTTGATCCTGCCAGT-3′) and Blasto 2R (5′-AGCTTTTTAACTGCAACAACG-3′) that amplified a fragment of about 600 bp in length in the second round PCR (Souppart et al., 2009). PCR reaction systems used herein were exactly the same as those described (Souppart et al., 2009). Two parallel PCRs were conducted for each specimen using 2 µl DNA extract per reaction. Suitable positive and negative controls were placed with each plate. All PCRs were conducted in a GeneAmp PCR System 9700 (Applied Biosystems) thermal cycler. Agarose gel electrophoresis (1.5%) was performed to screen positive PCRs.

2.4. Sequence analysis and phylogeny

PCR amplicons of expected size were sequenced in both directions at the Beijing Genomics Institute. Raw sequences were proofread and edited manually with Chromas Pro 1.33 (Technelysium, Queensland, Australia) and BioEdit 7.0 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). To determine *Blastocystis* subtypes, each of the corrected sequences was compared with GenBank sequences by BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST/). All the sequences obtained in this study were aligned with the reference sequences of known *Blastocystis* subtypes using MAFFT 7 (http://mafft.cbrc.jp/alignment/software/). Their phylogenetic relationships were analyzed by the neighbor-joining (NJ) method under the Kimura 2-parameter model and the maximum parsimony (MP) method with bootstrap values out of 1000 replicates, using the MEGA 5.0 (http://www.megasoftware.net/). Unique SSU rRNA sequences gained herein were deposited in GenBank under accession numbers of MF573940 to MF573945.

2.5. Statistical analysis

We used chi-square test in SPSS 17.0 (SPSS Inc., Chicago, IL, USA) to analyze the prevalence difference between animal groups. P values of < 0.05 were considered statistically significant.

3. Results

3.1. Prevalence of Blastocystis in cattle

Nested PCRs and sequence analysis of the SSU rRNA gene identified *Blastocystis* in 54 of 526 (10.3%) cattle fecal specimens, with a prevalence of 23.0% (45/196) in Harbin and 6.4% (9/140) in Daqing (Table 1). The prevalence difference between the two cities was significant ($p < 0.01$, $\chi^2 = 16.5$) (Table 1). In addition, weaned cattle (36.1%, 22/61) from Harbin had a significantly higher prevalence than those (6.4%, 9/140) within the same age range from Daqing ($p < 0.01$, $\chi^2 = 28.6$) (Table 1). There were no *Blastocystis*-positive isolates found in preweaned cattle from Qiqihar (Table 1). In Harbin, the prevalence of *Blastocystis* in weaned cattle (36.1%, 22/61) was significantly higher than that in preweaned calves (18.8%, 13/69; $p < 0.05$, $\chi^2 = 4.9$) and that in yearlings and adults (15.2%, 10/66; $p < 0.01$, $\chi^2 = 7.4$) (Table 1). When the other two study areas were considered, the differences in the overall prevalence between weaned and preweaned cattle (15.4%, 31/201 versus 5.0%, 13/259) and between adult and preweaned cattle (15.2%, 10/66 versus 5.0%, 13/259) were significant ($p < 0.01$, $\chi^2 = 14.2$ and 6.7, respectively) (Table 1).

3.2. Subtype distribution

Sequencing is available for all 54 *Blastocystis*-positive isolates. Four *Blastocystis* subtypes were identified by sequence polymorphisms of the SSU rRNA gene (Table 1). Among those, subtype ST10 (75.9%, 41/54) has the highest frequency, followed by ST14 (18.5%, 10/54), ST4

Table 1
Prevalence and subtype distribution of *Blastocystis* in dairy cattle from northeast China.

City	No. of cattle	No. of subtype				Prevalence in age groups (% [positive no./total no.])			Total prevalence (% [positive no./total no.])
		ST4	ST5	ST10	ST14	< 3 months	3–12 months	> 12 months	
Harbin	196	2	1	38	4	18.8 (13/69)	36.1 (22/61)	15.2 (10/66)	23.0 (45/196)
Qiqihar	190	0	0	0	0	0.0 (0/190)			0.0 (0/190)
Daqing	140	0	0	3	6		6.4 (9/140)		6.4 (9/140)
Total	526	2	1	41	10	5.0 (13/259)	15.4 (31/201)	15.2 (10/66)	10.3 (54/526)