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Distribution and molecular characterization of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* amongst grazing adult sheep in Xinjiang, China

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ABSTRACT

To assess the prevalence and molecular characteristics of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* in grazing adult sheep from Xinjiang Uygur Autonomous Region, China, 318 fecal samples were collected and screened for the presence of these parasites by polymerase chain reaction. The overall infection rate for the three pathogens was 13.5% (43/318), with observed individual infection rates of 0.9% (3/318), 7.5% (24/318), and 6.3% (20/318) for *Cryptosporidium* spp., *G. duodenalis*, and *E. bieneusi*, respectively. Three *Cryptosporidium* species were identified amongst the samples, including *C. xiaoi* (n = 1), *C. ubiquitum* (n = 1), and *C. parvum* (n = 1), with *gp60*-based subtyping analysis identifying *C. parvum* as subtype IIdA15G1 and *C. ubiquitum* as subtype XIIa. Eight *E. bieneusi* genotypes were identified based on internal transcribed spacer region sequencing, including six known (BEB6, CHG1, CHG3, CHS3, CHS8, and COS-I) and two novel (designated XJS1 and XJS2) genotypes. All *G. duodenalis*-positive samples were identified as assemblage E based on small subunit rRNA (n = 24) and *gdh* (n = 10) gene sequence analysis. These data support the occurrence of host adaptation by *Cryptosporidium* spp., *G. duodenalis*, and *E. bieneusi* in sheep, and the zoonotic risk may posed by these parasites in Xinjiang, China.

1. Introduction

Cryptosporidium spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* are important intestinal zoonotic pathogens causing diarrhea and enteric disease in humans and animals [1–4]. Amongst the 37 valid *Cryptosporidium* species, *C. ubiquitum*, *C. xiaoi*, and *C. parvum* are the most commonly associated with infections in goats and sheep [1,3]. *G. duodenalis* consists of eight assemblages, designated A–H, with assemblages A and B found in both humans and animals and C—H demonstrating host specificity for non-human species. Sheep are often infected with assemblage E and infrequently with assemblage A strains, with assemblage B infections being very rare [2]. *E. bieneusi* is an obligate intracellular fungus, with > 250 human- and animal-associated genotypes identified to date based on sequence analysis of the internal transcribed spacer (ITS) region of the rRNA gene. Phylogenetic analysis showed that the *E. bieneusi* genotypes can be divided into at least ten large groups (Groups 1–9 and the so-called outlier in dog) [4]. Group 1 was considered to have zoonotic potential; some genotypes in groups 2 and 7 have been detected in human samples, suggesting these groups may be also have zoonotic potential; remaining groups include most host-specific genotypes found in specific animals hosts [4,5].

Several previous studies have examined the prevalence and species/genotypes of *Cryptosporidium* spp., *G. duodenalis*, and *E. bieneusi* in sheep from various regions of China. For example, a study by Wu et al. [5] revealed *Cryptosporidium* spp., *G. duodenalis*, and *E. bieneusi* infection rates of 4.5%, 1.7%, and 34.5%, respectively, amongst sheep in Gansu Province, with all *E. bieneusi* genotyped as Group 2 and *G. duodenalis* belonging to assemblage E. The prevalence of *Cryptosporidium* spp. in sheep from Inner Mongolia was 13.1%, with all *C. parvum* isolates characterized as subtype IIaA15G2R1, which is the dominant subtype amongst infected humans in developed countries [6]. Additionally, a study by Zhang et al. [7] showed that the prevalence of *E.*

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bieneusi amongst sheep in Tibet was 23.4%, with most isolates belonging to genotype Group 2. Importantly, all of these reports indicated that the three intestinal pathogens are prevalent amongst sheep in China, and have potential for zoonotic transmission.

In 2016, the total number of goats and sheep in China exceeded 150 million and 160 million, respectively (http://www.fao.org/faostat/zh/#data/QL). Sheep farming has always been a very important part of the economy of Xinjiang Uygur Autonomous Region (hereafter referred to as Xinjiang) because of the unique geography, climatic conditions, ethnic beliefs, and dietary habits of the region. There are > 10 native sheep breeds in Xinjiang, with grazing being the predominant feeding mode, which may contribute to the spread of zoonotic diseases. However, there have been no reports of *Cryptosporidium*, *E. bieneusi*, or *G. duodenalis* infection in sheep in Xinjiang.

This study aimed to determine the prevalence and molecular characterization of *Cryptosporidium*, *E. bieneusi*, and *G. duodenalis* in grazing adult sheep from Xinjiang to better understand the host specificity and zoonotic risk of these enteric pathogens in sheep in China.

2. Materials and methods

2.1. Ethics approval and consent to participate

This study was conducted in accordance with the Chinese Laboratory Animal Administration Act (1988). The study protocol was approved by the Institutional Animal Care and Use Committee of Henan Agricultural University (authorization number IACUC-henau-20130605). Appropriate permission was obtained from farmers prior to collection of fecal samples.

2.2. Sample collection

From August 2015 to September 2017, 318 individual fresh fecal samples were collected from 15 groups of sheep from different locations in Xinjiang, China (Fig. 1). The sheep were free-grazing and shared pastures with cattle and wild animals, with each herd containing between 100 and 400 animals. Fecal samples were collected directly from the rectum of each adult sheep using disposable gloves. No obvious diarrhea was observed in the sampled animals. The fecal samples were stored at 4 °C prior to DNA extraction.

2.3. DNA extraction

Aliquots (3–5 g) of each fecal sample was passed with sterile distilled water through a wire mesh sieve with a 250 μm pore size and then centrifuged at 5000 ×*g* for 3 min. Genomic DNA was extracted from approximately 200 mg of each fecal sample precipitate using an E.Z.N.A.R Stool DNA Kit (Omega Biotek Inc., Norcross, GA, USA) according to the manufacturer's instructions. Extracted DNA samples were used as template for polymerase chain reaction (PCR)-based analyses.

2.4. PCR amplification

Nested PCR was performed to identify *Cryptosporidium* spp. based on the small subunit rRNA (SSU rRNA) gene sequence [8]. *C. parvum* and *C. ubiquitum* were subtyped based on sequence analysis of the 60-kDa glycoprotein (*gp60*) gene following PCR amplification [8,9]. *E. bieneusi* was detected via nested PCR-based amplification of the entire internal transcribed spacer (ITS) region as described previously [10]. *G. duodenalis* was identified by nested PCR-based amplification of the SSU rRNA gene as described previously [11]. DNA from all SSU rRNA-positive samples was subjected to further PCR-based analysis to detect the presence of the glutamate dehydrogenase (*gdh*) gene [12]. The primers sequences, fragment length, and the annealing temperatures were listed in Table 1. Each sample was analyzed in duplicate using positive and negative controls. The secondary PCR products were examined by electrophoresis using a 1.5% agarose gel and staining with GelRed (Biotium Inc., Hayward, CA, USA).

2.5. Sequencing and phylogenetic analysis

PCR amplicons of the correct size were DNA sequenced by GENEWIZ (Suzhou, China). Sequence accuracy was confirmed by bidirectional sequencing. Resulting sequences were aligned with reference sequences downloaded from GenBank using ClustalX 2.1 (http://www.clustal.org/) for determination of genotypes. The *E. bieneusi* ITS genotypes from sheeps in this study were compared with previously reported reference sequences using Monte Carlo Markov Chain analysis in MrBayes v 3.2.6 (http://nbisweden.github.io/MrBayes) based on Bayesian inference (BI). FigTree v 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) was used to visualize and edit the

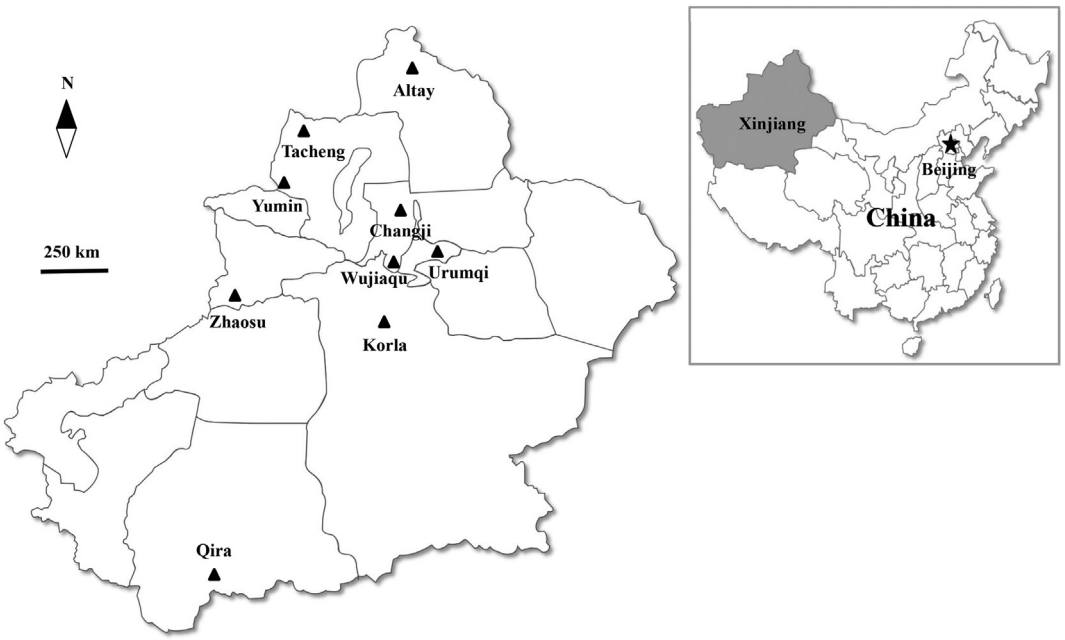

Fig. 1. Location of the study area in Xinjiang Uygur Autonomous Region, China. Filled triangles indicate sampling sites.

Table 1
The primers used in the characterization of *Cryptosporidium* spp., *E. bieneusi* and *G. duodenalis* in the present study.

Gene	Primer sequence (5′ to 3′)	Fragment length (bp)	Annealing temperature (°C)	Usage (s)	References
SSU rRNA	SSU-F2: TTCTAGAGCTAATACATGCG	~1325	55	Specific nested PCR of *Cryptosporidium* spp.	[8]
	SSU-R2: CCCATTTCCTTCGAAACAGGA				
	SSU-F3: GGAAGGGTTGTATTTATTAGATAAAG	~840	58		
	SSU-R4: CTCATAAGGTGCTGAAGGAGTA				
gp60	AL3531: ATAGTCTCCGCTGTATTC	~1280	55	Subtyping of *C. parvum* and *C. hominis*	[8]
	AL3535: GGAAGGAACGATGTATCT				
	AL3532: TCCGCTGTATTCTCAGCC	~850	58		
	AL3534: GCAGAGGAACCAGCATC				
gp60	Ubi-18S-F1: TTTACCCACACATCTGTAGCGTCG	~1044	58	Subtyping of *C. ubiquitum*	[9]
	Ubi-18S-R1: ACGGACGGAATGATGTATCTGA				
	Ubi-18S-F2: ATAGGTGATAATTAGTCAGTCTTTAAT	~948	55		
	Ubi-18S-R2: TCCAAAAGCGGCTGAGTCAGCATC				
ITS	AL4037: GATGGTCATAGGGATGAAGAGCTT	~410	55	Specific nested PCR of *E. bieneusi*	[10]
	AL4039: AATACAGGATCACTTGGATCCGT				
	AL4038: AGGGATGAAGAGCTTCGGCTCTG	~392	55		
	AL4040: AATATCCCTAATACAGGATCACT				
SSU rRNA	Gia2029: AAGTGTGGTGCAGACGGACTC	~497	55	Specific nested PCR of *G. duodenalis*	[11]
	Gia2150c: CTGCTGCCGTCCTTGGATGT				
	RH11: CATCCGGTCGATCCTGCC	~292	59		
	RH4: AGTCGAACCCTGATTCTCCGCCCAGG				
gdh	Ghd1: TTCCGTRTYCAGTACAACTC		50	Subtyping of *G. duodenalis*	[12]
	Gdh2: ACCTCGTTCTGRGTGGCGCA				
	Gdh3: ATGACYGAGCTYCAGAGGCACGT	~530	50		
	Gdh4: GTGGCGCARGGCATGATGCA				

maximum clade credibility tree generated by these analyses. Posterior probability values were estimated based on 1,000,000 generations with four simultaneous tree building chains, with trees being saved every 100th generation. A 50% majority rule consensus tree for each analysis was constructed based on the final 75% of trees generated by BI.

2.6. Nucleotide sequence accession numbers

The nucleotide sequences reported in this study have been deposited in the GenBank database at the National Center for Biotechnology Information under accession numbers MH794164–MH794180.

2.7. Statistical analysis

A chi-square test was used to compare the prevalence of pathogens infection between different sampling locations and breeds of animals. Statistical significance was set at a value of $p < .05$.

3. Results

3.1. Prevalence and molecular characterization of Cryptosporidium spp., E. bieneusi, and G. duodenalis

A total of 318 fecal samples from grazing adult sheep were screened for *Cryptosporidium* spp., *E. bieneusi*, and *G. duodenalis* using various PCR-based assays. The three intestinal pathogens were identified in 14 out of the 15 groups of sheep, with an overall infection rate of 13.5% (43/318). The infection rates of three pathogens in different sampling locations ranging from 0 to 33.3% (Table 1). Differences in the three pathogens infection rates were not significant amongst sampling locations ($p > .05$). The higher infection rates of three pathogens was found in Cele black sheep (33.3%, 6/18), followed by Hotan sheep (25.0%, 5/20), Altay sheep (20.0%, 2/10), Suffolk sheep (16.1%, 10/62), Bayinbuluk sheep (14.0%, 6/43), Small Tail Han sheep (14.0%, 6/43), Bashbay sheep (6.9%, 2/29) and native sheep (6.5%, 6/93). Differences in the three pathogens infection rates were significant amongst sheep breeds ($p < .05$) (Table 2).

The infection rates for each of the three pathogens across all 318 samples were 0.9% ($n = 3$), 6.3% ($n = 20$) and 7.5% ($n = 24$) for *Cryptosporidium* spp., *E. bieneusi* and *G. duodenalis* respectively. The mixed infection rate was 1.3% (4/318) (Table 1), with one sample showing a mixed infection of *Cryptosporidium* spp. and *G. duodenalis*, and the remaining three samples containing a mixture of *G. duodenalis* and *E. bieneusi*.

3.2. Distribution of species, genotypes, and subtypes

Sequence analyses of the SSU rRNA gene revealed the presence of three *Cryptosporidium* species, including *C. ubiquitum* ($n = 1$), *C. xiaoi* ($n = 1$), and *C. parvum* (n = 1). The *C. ubiquitum* and *C. parvum* samples were further subtyped based on *gp60* gene sequence analysis, with *C. parvum* identified as subtype IIdA15G1 and *C. ubiquitum* as subtype XIIa. The two *gp60* sequences showed 100% nucleotide sequence identity to the corresponding regions of *C. parvum* and *C. ubiquitum* isolates from a rhesus macaque (GenBank accession no. KJ917586) and a sika deer (KX259144), respectively, from China.

Eight genotypes were identified amongst the 20 *E. bieneusi*-positive samples based on ITS gene sequence analysis, including six known genotypes (BEB6, $n = 12$; CHG1, n = 1; CHG3, n = 1; CHS3, n = 1; CHS8, n = 1; and COS-I, $n = 2$) and two novel genotypes (designated XJS1 and XJS2) (Table 1). Novel genotype XJS1 differs from BEB6 at one nucleotide, while genotype XJS2 shows two nucleotide differences. Phylogenetic analysis of the ITS genotypes revealed that all genotypes identified in this study fell into Group 2 (Fig. 2).

SSU rRNA gene sequencing identified 24 *G. duodenalis*-positive samples. Ten of these SSU rRNA-positive samples were then successfully subtyped at the *gdh* gene, with all being identified as assemblage E. Amongst the resulting *gdh* sequences, six, two, one, and one were identical to the corresponding *gdh* sequences from *G. intestinalis* isolates derived from cattle in China (KY769099, KY710744, and KY769098) and a goat in Australia (KX813711), respectively.

4. Discussion

The observed *Cryptosporidium* spp. infection rate of 0.9% (3/318) in the current study. Previous studies have reported a strong association between prevalence of *Cryptosporidium* infection and age in sheep, with lambs generally exhibiting higher rates of infection than adult sheep. Santin et al. [13] recorded an infection rate of 76.2% in 2 week old lambs, which dropped to 64.4% by the third week of life and to 23.0%

Table 2
Prevalence and molecular characterization of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* in fecal samples.

Sampling location	Sheep breeds	No. of samples	No. of positives (%)	*Cryptosporidium* spp.		*E. bieneusi*		*G. duodenalis*	
				No. of positives (%)	SSU rRNA (no.), *gp 60* (no.)	No. of positives (%)	ITS (no.)	No. of positives (%)	SSU rRNA (no.), *gdh* (no.)
Wujiaqu	Native	9	0 (0.0)						
Changji	Native	35	2 (5.7) CI[a]:-2.0–13.4	1 (2.9) CI:-2.7–8.5	*C. xiaoi* (1)	1 (2.9) CI:-2.7–8.5	XJS1 (1)		
Urumqi	Native	27	3 (11.1) CI:-0.8–23.0			2 (7.4) CI:-2.5–17.3	BEB6 (1), CHG3 (1)	1 (3.7) CI:-3.4–10.8	E (1), E[b] (1)
Korla1	Bayinbuluk	14	2 (14.3) CI:-4.0–32.6			2 (14.3) CI:-4.0–32.6	BEB6 (1), CHG1 (1)		
Korla2	Bayinbuluk	19	3 (15.8) CI:-0.6–32.2			2 (10.5) CI:-3.3–24.3	BEB6 (1), COS-I (1)	1 (5.3) CI:-4.8–15.4	E (1)
Korla3	Bayinbuluk	10	1 (10.0) CI:-8.6–28.6	1 (10.0) CI:-8.6–28.6	*C. ubiquitum* (1), XIIa (1)				
Yumin	Bashbay	29	2 (6.9) CI:-2.3–16.1			1 (3.4) CI:-3.2–10.0	BEB6 (1)	1 (3.4) CI:-3.2–10.0	E (1)
Altay	Altay	10	2 (20.0) CI:-4.8–44.8			2 (20.0) CI:-4.8–44.8	BEB6 (1), CHS3 (1)		
Tacheng1	Small Tail Han	22	5 (22.7) CI:5.2–40.2	1 (4.5) CI:-4.2–13.2	*C. parvum* (1), IIdA15G1 (1)	1 (4.5) CI:-4.2–13.2	CHS8 (1)	4 (18.2) CI:2.1–34.3	E (4), E[b] (2)
Tacheng2	Suffolk	29	3 (10.3) CI:-0.8–21.4			1 (3.4) CI:-3.2–10.0	BEB6 (1)	2 (6.9) CI:-2.3–16.1	E (2), E[b] (1)
Zhaosu1	Native	22	1 (4.5) CI:-4.2–13.2			1 (4.5) CI:-4.2–13.2	COS-I (1)		
Zhaosu2	Suffolk	33	7 (21.2) CI:7.3–35.1			1 (3.0) CI:-2.8–8.8	BEB6 (1)	6 (18.2)	E (6), E[b] (2)
Zhaosu3	Small Tail Han	21	1 (4.7) CI:-4.4–13.8			1 (4.7) CI:-4.4–13.8	BEB6 (1)	1 (4.7) CI:-4.4–13.8	E (1)
Qira1	Hotan	20	5 (25.0) CI:6.0–44.0			1 (5.0) CI:-4.6–14.6	XJS2 (1)	5 (25.0) CI:6.0–44.0	E (5), E[b] (2)
Qira2	Cele black	18	6 (33.3) CI:11.5–55.1			4 (22.2) CI:3.0–41.4	BEB6 (4)	3 (16.7) CI:-0.5–33.9	E (3), E[b] (2)
Total		318	43 (13.5) CI:9.7–17.3	3 (0.9) CI:-0.1–1.9	*C. xiaoi* (1); *C. ubiquitum* (1), XIIa (1); *C. parvum* (1), IIdA15G1 (1)	20 (6.3) CI:3.6–9.0	BEB6 (12), CHG1 (1), CHG3 (1), CHS3 (1), CHS8 (1), COS-I (2), XJS1 (1), XJS2 (1)	24 (7.5) CI:4.6–10.4	E (24), E[b] (10)

[a] 95% CI: 95% confidence intervals.
[b] Successfully subtyped at the *gdh* gen.

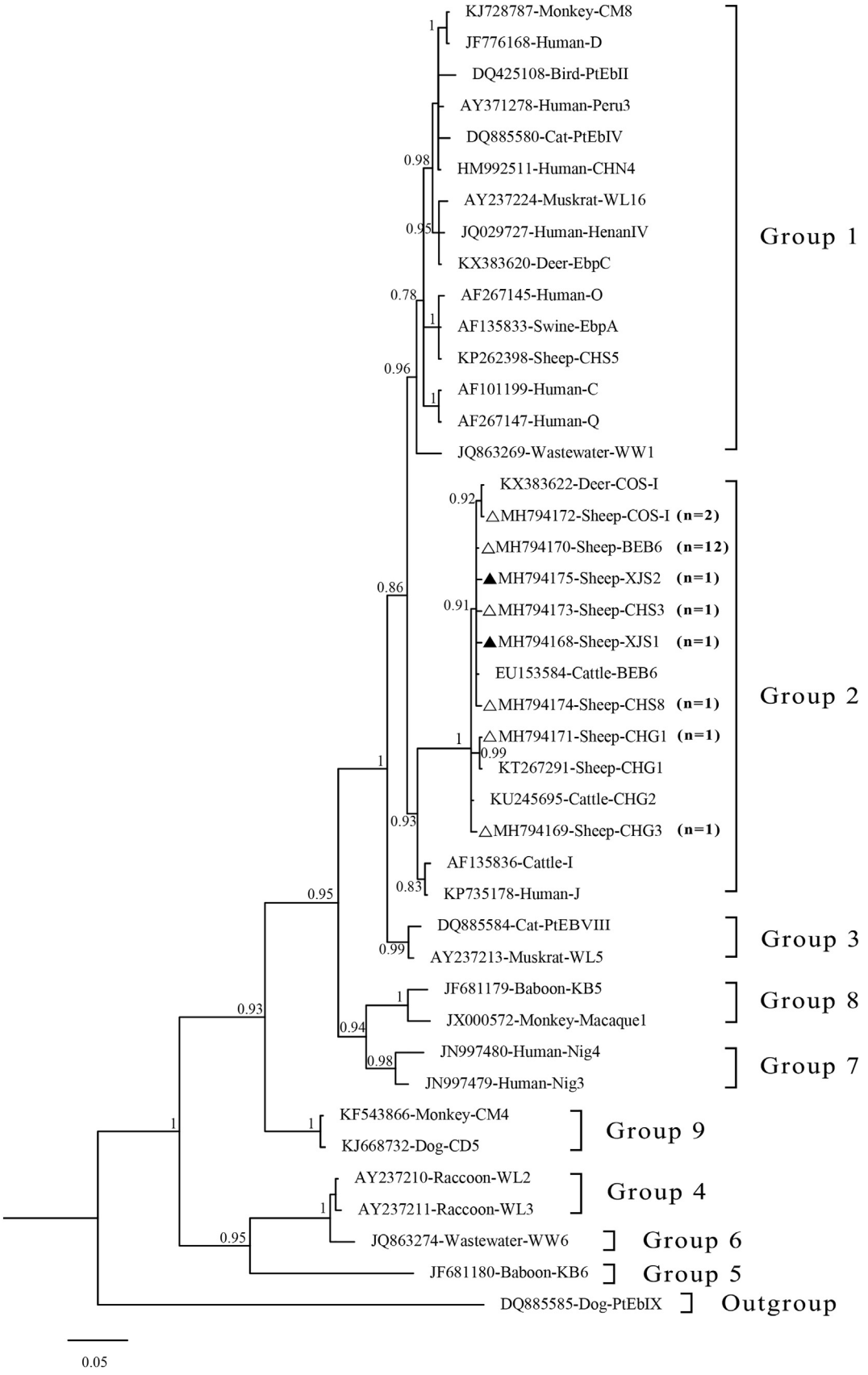

Fig. 2. Phylogenetic relationships amongst the *Enterocytozoon bieneusi* genotypes identified in this study and other reported genotypes. Bayesian phylogenetic analysis of *E. bieneusi* ITS gene sequences. Statistically significant posterior probabilities are indicated at branches. Sample names include GenBank accession number followed by host and then genotype designation. The *E. bieneusi* genotypes PtEbIX (DQ85585) from dogs were used as outgroup. Known and novel genotypes identified in this study are indicated by triangles and filled triangles, respectively.

in 1–3 month old lambs. Similar data was recorded in Serbia, with infection rates of 45.3% and 38.7% determined for lambs aged < 1 month and 1–3 months, respectively [14]. In addition, the prevalence of *Cryptosporidium* spp. in adult ewes from Brazil was 14.3% (6/42) but was 20.3% (12/59) in lambs [15], while 19.9% (235/1182) of weaning lambs, 16.2% (185/1141) of post-weaning lambs, and 14.3% (156/1089) of pre-slaughter lambs tested positive for *Cryptosporidium* spp. at eight farms across four regions of Australia [16]. In the current study, samples were only collected from adult sheep, most of which were reared in a free-range grazing mode. These two factors may contribute to the relatively low observed infection rates.

Three *Cryptosporidium* species, including *C. xiaoi*, *C. parvum*, and *C. ubiquitum*, were identified in the present study. *C. parvum* and *C. ubiquitum* have an extensive host range that includes humans, ruminants, and rodents [17]. *C. ubiquitum* appears to be the most common *Cryptosporidium* species infecting sheep in China, with the identification of subtype XIIa *C. ubiquitum* in the current study consistent with previous studies in sheep from Gansu and Qinghai, goats from Guangdong and Shanghai, and humans in the United Kingdom, Turkey, Peru, and Canada [5,9,18,19]. Further analysis revealed that sequences from the current study were associated with *C. parvum* subtype IIdA15G1, which is commonly found in animals (yaks, buffalos, rodents, and calves) in China [1]. IIdA15G1 was also the dominant subtype amongst calves in northwestern regions of China, including Xinjiang, and was responsible for an outbreak of lethal cryptosporidiosis in the Ningxia Hui Autonomous Region that resulted in the deaths of hundreds of pre-weaned calves following severe diarrhea [20]. In addition, *C. parvum* subtype IIdA15G1 strains have also been isolated from diarrheic lambs and goat kids from Spain and Greece, and from humans in Australia [21–23]. All of these findings indicate that sheep have played a role in the spread of *C. parvum* and *C. ubiquitum* and represent a risk for zoonotic transmission.

In the current study, 6.3% of the 318 sheep examined were positive for *E. bieneusi*. This prevalence rate amongst sheep from Xinjiang was higher than that recorded in Liaoning (4.7%, 3/64) but lower than rates reported in Tibet (23.4%, 73/312), Gansu (34.5, 61/177), Henan (25.2%, 78/310), and Heilongjiang (25.0, 10/40), China [5,24–26]. Genotyping of *E. bieneusi* based on sequence analysis of the ITS region revealed that BEB6 (60.0%, 12/20) was the predominant *E. bieneusi* genotype in the current study, which is mainly consistent with other relevant domestic reports of BEB6 in sheep [7]. In addition to their prevalence in sheep, genotype BEB6 strains have also been reported in red deer, captive non-human primates, non-human primates, and children [27]. Phylogenetic analysis of all *E. bieneusi* genotypes identified in the current study, including BEB6, CHG1, CHG3, COS-I, CHS3, CHS8, and the two novel genotypes (XJS1 and XJS2), showed that all of the genotypes belonged to Group 2, suggesting that these genotypes exhibit zoonotic potential.

G. duodenalis infection is relatively common in sheep, although its prevalence differs greatly across China. Previous studies have reported prevalence rates of 1.7% (3/117) in Gansu, 5.4% (39/716) in Henan, 3.5% (13/375) in Inner Mongolia, 4.6% (25/539) in Heilongjiang, and 12.6% (51/406) in Qinghai, China [5,28,29]. The prevalence of *G. duodenalis* in the current study was 7.5% (24/318), which is within the range reported in previous studies. Molecular typing assays based on the SSU rRNA and *gdh* genes showed that the 24 *G. duodenalis*-positive samples in the current study all belonged to assemblage E. Recent studies aimed at genetically characterizing *G. duodenalis* in sheep have shown that assemblage E is the predominant assemblage, with assemblages A and B being relatively rare [30]. Previous reports suggested that assemblage E was animal-specific and did not infect humans [2]. However, more recent studies have unexpectedly detected assemblage E *G. duodenalis* strains in humans from Brazil and Egypt [31,32], demonstrating a new anthropozoonotic route of *G. duodenalis* transmission.

5. Conclusions

In conclusion, the present study demonstrates that *Cryptosporidium* spp., *G. duodenalis*, and *E. bieneusi* are relatively common amongst grazing sheep in Xinjiang, with more detailed genotypic analysis suggesting that these three intestinal pathogens may pose a potential risk for zoonosis. Further study is needed to investigate the relationships between these pathogens and their ovine hosts.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] Y. Feng, L. Xiao, Molecular epidemiology of cryptosporidiosis in China, Front. Microbiol. 8 (2017) 1701.
[2] Y. Feng, L. Xiao, Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis, Clin. Microbiol. Rev. 24 (2011) 110–140.
[3] M. Kváč, G. Vlnatá, J. Ježková, M. Horčičková, R. Konečný, L. Hlásková, et al., *Cryptosporidium occultus* sp. n. (Apicomplexa: Cryptosporidiidae) in rats, Eur. J. Protistol. 63 (2018) 96–104.
[4] M. Santín, R. Calero-Bernal, D. Carmena, M. Mateo, A. Balseiro, M. Barral, et al., Molecular characterization of *Enterocytozoon bieneusi* in wild carnivores in Spain, J. Eukaryot. Microbiol. 65 (2018) 468–474.
[5] Y. Wu, Y. Chang, Y. Chen, X. Zhang, D. Li, S. Zheng, et al., Occurrence and molecular characterization of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* from Tibetan sheep in Gansu, China, Infect. Genet. Evol. 64 (2018) 46–51.
[6] J. Ye, L. Xiao, Y. Wang, L. Wang, S. Amer, D.M. Roellig, et al., Periparturient transmission of *Cryptosporidium xiaoi* from ewes to lambs, Vet. Parasitol. 197 (2013) 627–633.
[7] Q. Zhang, J. Cai, P. Li, L. Wang, Y. Guo, C. Li, et al., *Enterocytozoon bieneusi* genotypes in Tibetan sheep and yaks, Parasitol. Res. 117 (2018) 721–727.
[8] M. Alves, L.H. Xiao, I. Sulaiman, A.A. Lal, O. Matos, F. Antunes, Subgenotype analysis of *Cryptosporidium* isolates from humans, cattle, and zoo ruminants in Portugal, J. Clin. Microbiol. 41 (2003) 2744–2747.
[9] N. Li, L. Xiao, K. Alderisio, K. Elwin, E. Cebelinski, R. Chalmers, et al., Subtyping *Cryptosporidium ubiquitum*, a zoonotic pathogen emerging in humans, Emerg. Infect. Dis. 20 (2014) 217–224.
[10] I.M. Sulaiman, R. Fayer, A.A. Lal, J.M. Trout, F.W. Schaefer 3rd, L. Xiao, Molecular characterization of *microsporidia* indicates that wild mammals harbor host-adapted *Enterocytozoon* spp. as well as human-pathogenic *Enterocytozoon bieneusi*, Appl. Environ. Microbiol. 69 (2003) 4491–4501.
[11] A.J. Appelbee, L.M. Frederick, T.L. Heitman, M.E. Olson, Prevalence and genotyping of *Giardia duodenalis* from beef calves in Alberta, Canada, Vet. Parasitol. 112 (2003) 289–294.
[12] S.M. Caccio, R. Beck, M. Lalle, A. Marinculic, E. Pozio, Multilocus genotyping of *Giardia duodenalis* reveals striking differences between assemblages A and B, Int. J. Parasitol. 38 (2008) 1523–1531.
[13] M. Santin, J.M. Trout, R. Fayer, Prevalence and molecular characterization of *Cryptosporidium* and *Giardia* species and genotypes in sheep in Maryland, Vet. Parasitol. 146 (2007) 17–24.
[14] Z. Misic, S. Katic-Radivojevic, Z. Kulisic, *Cryptosporidium* infection in lambs and goat kids in Serbia, Acta Vet. Beograd. 56 (1) (2006) 49–54.
[15] L. Holsback, H.E. Lima, O. Vidotto, M.A. da Silva, T.H.C. Patelli, F.D.C. Martins, et al., *Cryptosporidium* occurrence in ruminants from the North Pioneer mesoregion of Parana, Brazil, Rev. Bras. Parasitol. Vet. 27 (2018) 248–253.
[16] R. Yang, C. Jacobson, G. Gardner, I. Carmichael, A.J. Campbell, J. Ng-Hublin, et al., Longitudinal prevalence, oocyst shedding and molecular characterisation of *Cryptosporidium* species in sheep across four states in Australia, Vet. Parasitol. 200

(2014) 50–58.
[17] J. Huang, Z. Zhang, Y. Zhang, Y. Yang, J. Zhao, R. Wang, et al., Prevalence and molecular characterization of *Cryptosporidium* spp. and *Giardia duodenalis* in deer in Henan and Jilin, China, Parasit. Vectors 11 (2018) 239.
[18] P. Li, J. Cai, M. Cai, W. Wu, C. Li, M. Lei, et al., Distribution of *Cryptosporidium* species in Tibetan sheep and yaks in Qinghai, China, Vet. Parasitol. 215 (2016) 58–62.
[19] R. Mi, X. Wang, Y. Huang, P. Zhou, Y. Liu, Y. Chen, et al., Prevalence and molecular characterization of *Cryptosporidium* in goats across four provincial level areas in China, PLoS One 9 (2014) e111164.
[20] Z. Cui, R. Wang, J. Huang, H. Wang, J. Zhao, N. Luo, et al., Cryptosporidiosis caused by *Cryptosporidium parvum* subtype IIdA15G1 at a dairy farm in Northwestern China, Parasit. Vectors 7 (2014) 529.
[21] V. Papanikolopoulou, D. Baroudi, Y. Guo, Y. Wang, E. Papadopoulos, S.Q. Lafi, et al., Genotypes and subtypes of *Cryptosporidium* spp. in diarrheic lambs and goat kids in northern Greece, Parasitol. Int. 67 (2018) 472–475.
[22] J. Quílez, E. Torres, R.M. Chalmers, S.J. Hadfield, E. Del Cacho, C. Sánchez- Acedo, *Cryptosporidium* genotypes and subtypes in lambs and goat kids in Spain, Appl. Environ. Microbiol. 74 (2008) 6026–6031.
[23] J. Ng, B. Mackenzie, U. Ryan, Longitudinal multi-locus molecular characterisation of sporadic Australian human clinical cases of cryptosporidiosis from 2005 to 2008, Exp. Parasitol. 125 (2010) 348–356.
[24] X. Zhang, Y. Jian, X. Li, L. Ma, G. Karanis, C. Qigang, et al., Molecular detection and prevalence of *Cryptosporidium* spp. infections in two types of domestic farm animals in the Qinghai-Tibetan Plateau Area (QTPA) in China, Parasitol. Res. 117 (2018) 233–239.
[25] W. Zhao, W. Zhang, D. Yang, L. Zhang, R. Wang, A. Liu, Prevalence of *Enterocytozoon bieneusi* and genetic diversity of ITS genotypes in sheep and goats in China, Infect. Genet. Evol. 32 (2015) 265–270.
[26] K. Shi, M. Li, X. Wang, J. Li, M.R. Karim, R. Wang, et al., Molecular survey of *Enterocytozoon bieneusi* in sheep and goats in China, Parasi. Vectors. 9 (2016) 23.
[27] W. Zhao, J. Wang, Z. Yang, A. Liu, Dominance of the *Enterocytozoon bieneusi* genotype BEB6 in red deer (*Cervus elaphus*) and Siberian roe deer (*Capreolus pygargus*) in China and a brief literature review, Parasite. 24 (2017) 54.
[28] Y. Jin, J. Fei, J. Cai, X. Wang, N. Li, Y. Guo, et al., Multilocus genotyping of *Giardia duodenalis* in Tibetan sheep and yaks in Qinghai, China, Vet. Parasitol. 247 (2017) 70–76.
[29] H. Wang, M. Qi, K. Zhang, J. Li, J. Huang, C. Ning, et al., Prevalence and genotyping of *Giardia duodenalis* isolated from sheep in Henan Province, Central China, Infect. Genet. Evol. 39 (2016) 330–335.
[30] Y. Jian, X. Zhang, X. Li, G. Karanis, L. Ma, P. Karanis, Prevalence and molecular characterization of *giardia duodenalis* in cattle and sheep from the Qinghai-tibetan plateau area (QTPA), northwestern China, Vet. Parasitol. 250 (2018) 40–44.
[31] M. Fantinatti, A.R. Bello, O. Fernandes, A.M. Dacruz, Identification of *Giardia lamblia* assemblage E in humans points to a new anthropozoonotic cycle, J. Infect. Dis. 214 (2016) 1256–1259.
[32] K.A. Abdel-Moein, H. Saeed, The zoonotic potential of *Giardia intestinalis* assemblage E in rural settings, Parasitol. Res. 115 (2016) 3197–3202.