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RESEARCH

Molecular characterization and prevalence of *Cryptosporidium* spp. in sheep and goats in western Inner Mongolia, China

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Abstract

Cryptosporidium spp. are zoonotic intestinal parasites that infect fish, birds, reptiles and mammals. *Cryptosporidium* spp. are common cause of diarrhea. In this study, a total of 1032 fecal samples were collected from the rectums of sheep and goats. The samples were analyzed using nested polymerase chain reaction (nested PCR) based on the small subunit ribosomal RNA (*SSU* rRNA) gene of *Cryptosporidium* spp. The average infection rate of *Cryptosporidium* spp. was 2.23% ($n=23$), and three *Cryptosporidium* species were identified, namely *Cryptosporidium ubiquitum* (8/23), *Cryptosporidium andersoni* (5/23) and *Cryptosporidium xiaoi* (10/23). Subtyping of *C. ubiquitum* and *C. xiaoi* was carried out by DNA sequence analysis of the 60-kDa glycoprotein (*gp60*) gene. Eight *C. ubiquitum* isolates were identified as zoonotic subtype XIIa. Nine *C. xiaoi* isolates were identified as subtypes XXIIIc ($n=1$), XXIIIf ($n=3$) and XXIIIg ($n=5$). Subtype XXIIIg was first found in Chinese sheep. *C. ubiquitum* subtype XIIa was found in both sheep and goats, suggesting that sheep and goats are important sources of *C. ubiquitum* infections.

Keywords *Cryptosporidium* · Sheep · Goats · *SSU rRNA* · *gp60* · Zoonotic

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The authors Jiashu Lang and Han Han contributed equally to this work.

Key findings
- The infection rate of *Cryptosporidium* spp. was 2.23% in sheep and goats.
- *Cryptosporidium ubiquitum*, *Cryptosporidium andersoni* and *Cryptosporidium xiaoi* were identified in sheep and goats.
- *Cryptosporidium ubiquitum* subtype XIIa, and *C. xiaoi* subtypes XXIIIc, XXIIIf and XXIIIg were found in this study.

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Introduction

Cryptosporidium spp. are zoonotic intestinal parasites that infect fish (Zahedi et al. 2021), birds (Holubová et al. 2019), reptiles (Jezkova et al. 2016), and mammals (Ryan et al. 2021), it spreads through fecal–oral transmission or by ingestion of contaminated food or water, and *Cryptosporidium* spp. are common cause of diarrhea in sheep and goats (Santín 2013). A total of 44 *Cryptosporidium* species and more than 120 genotypes have been described in various animals (Ryan et al. 2021).

Cryptosporidium parvum is one of the most widely transmitted zoonotic species, and it has major public health importance. Sheep and goats are important hosts of *C. parvum*, and they can also be infected by *Cryptosporidium ubiquitum*, *Cryptosporidium hominis*, *Cryptosporidium xiaoi*, *Cryptosporidium andersoni*, *Cryptosporidium scrofarum* and more than a dozen of other *Cryptosporidium* species (Fiuza et al. 2011; Zhang et al. 2020). *Cryptosporidium ubiquitum*, *C. xiaoi*, and *C. parvum* were commonly found in sheep and goats. However, in different countries or regions, the dominant species or distributions of *Cryptosporidium* spp. may vary. For example, in Kuwait (Majeed et al. 2018), Spain (Díaz et al. 2015), and Italy (Dessì et al. 2020), the infection rate of *C. parvum* was higher than *C. ubiquitum* and *C. xiaoi*, which were the most common *Cryptosporidium* species in Ningxia (Yang 2018), Anhui (Li et al. 2019) Xinjiang, Beijing (Mi et al. 2018) and other regions of China. In addition, *C. hominis*, *C. parvum* and *C. scrofarum* have also been detected in sheep and goats within Papua New Guinea (Koinari et al. 2014).

Inner Mongolia is located on the northern frontier of the People's Republic of China, with an average altitude of about 1000 m. The region encompasses 1,177,500 sq. km with varied vegetation patterns. Sheep farming is an important component of its economic development. *Cryptosporidium* is an important cause of lamb diarrhea (Fan et al. 2021), and it has caused economic losses to the sheep industry (Scallan et al. 2011). There are few studies of *Cryptosporidium* infection in sheep and goats within Inner Mongolia. Therefore, we studied the species and distribution of *Cryptosporidium*

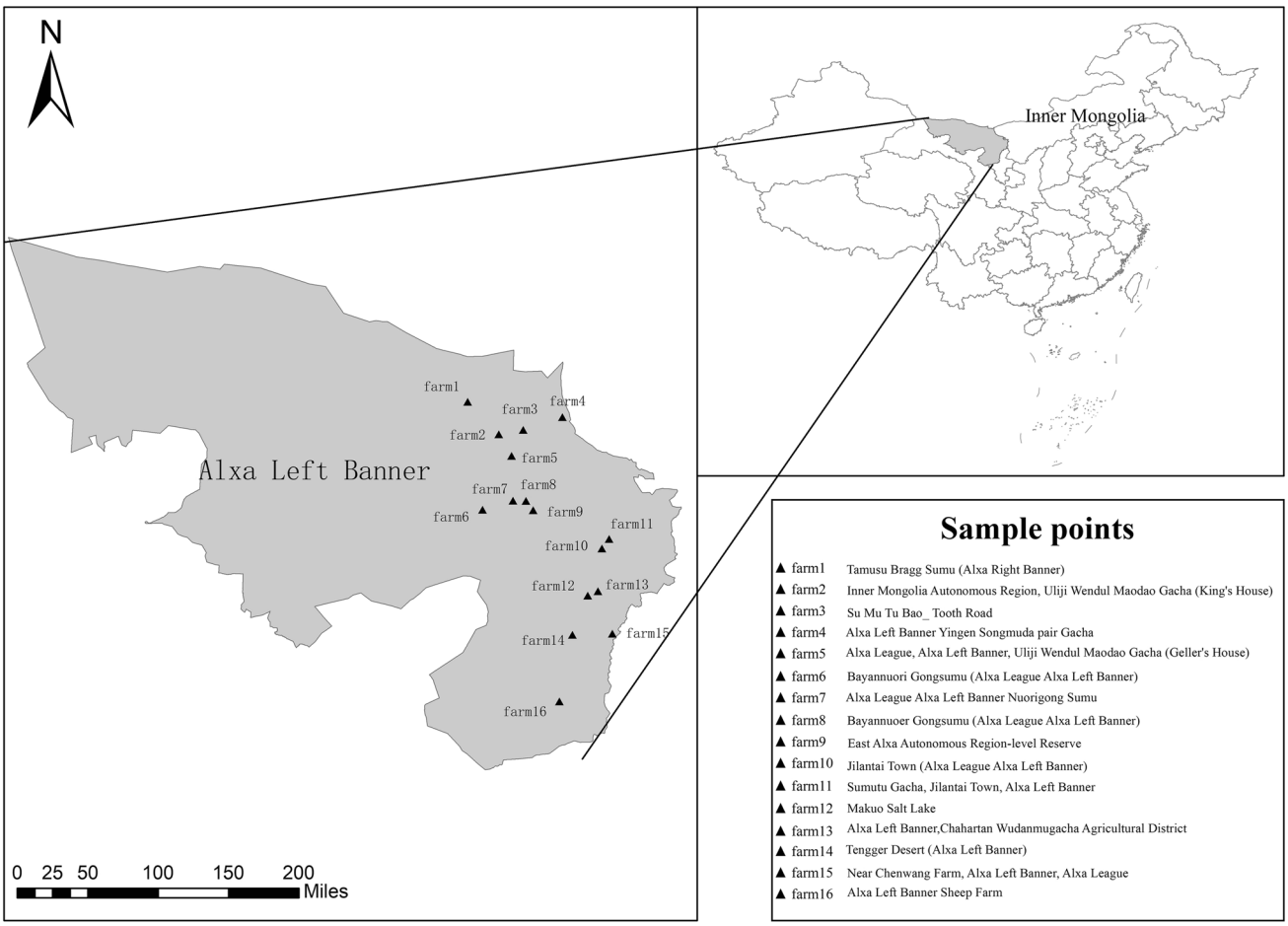

Fig. 1 Map of the sampling locations in Inner Mongolia, China. The figure was originally designed using ArcGIS 10.2 software. The original vector diagram imported in ArcGIS was adapted from Natural Earth (http://www.naturalearthdata.com)

in Alxa League, Inner Mongolia, to better understand the zoonotic potential of *Cryptosporidium* in sheep and goats, and to help reduce the economic losses caused by *Cryptosporidium* infection.

Materials and methods

Sample collection

A total of 1032 fecal samples were collected between October 2019 and December 2020 from 16 sheep and goats farm in Alxa, Inner Mongolia (Fig. 1). Rectal sampling was performed directly with a sterile swab, samples were put in clean sampling bags. Sample number, sampling time and place, animal species (sheep or goats), feeding models, age patterns, animal health status (diarrhea or not, the sampled animals were mostly healthy and no obvious phenomenon of diarrhea) and other important information were recorded. The samples were stored in an ice chest and returned to the laboratory of veterinary parasitology, at Henan Agricultural University. We took 0.5–1 g of sample in a 2 ml centrifuge tube, and used this for fecal DNA extraction. We then added feces to 2 ml centrifuge tubes, added 2.5% potassium dichromate, and stored the processed samples at 4℃ in refrigerators.

DNA extraction and PCR amplification

The genomic DNA was extracted from the fecal pellets with the E.Z.N.A Stool DNA Kit (Omefa Biotek Inc, Norcross, GA, USA), according to manufacturer's instructions. The extracted DNA was kept at − 20 °C before it was used for molecular analysis.

Cryptosporidium spp. were identified by nested PCR based on the small subunit (*SSU*) rRNA gene and *gp60* gene (Alves et al. 2003). The primer sequences used were chosen according to previous studies (Xiao et al. 2001; Li et al. 2014; Fan et al. 2021). The products of the secondary PCR were detected using 1% agarose gel electrophoresis containing DNAGREEN (Tiandz, Inc., Beijing, China). An Applied Biosystems 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA) was used to amplify the *Cryptosporidium SSU* rRNA gene. This was achieved in 25 µl volumes, including 1 µl template DNA or primary PCR product, 2.5 µl 10× KOD-Plus PCR buffer, 2.5 µl dNTPs (2 nM), 1.5 µl MgSO4 (25 nM), 0.5 µl of each primer (25 nM), 16 µl double distilled water, and 0.5 µl KOD-Plus amplification enzyme (1 unit/µl) (ToYoBo Co., Ltd., Osaka, Japan). A total of 35 cycles were carried out; each of these consisted of 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 1 min (90 s for *C. ubiquitum* and *C. xiaoi gp60* gene). There was also an initial hot start at 94 °C for 5 min and a final extension at 72 °C for 7 min (10 min for *C. ubiquitum* and *C. xiaoi gp60* gene). The secondary cycling conditions were identical to those used in the primary PCR. Both the positive and negative controls were included in each PCR amplification, positive controls were the genomic DNA of sheep fecal samples, these samples were PCR-positive for *Cryptosporidium* spp., negative controls were 24 µl volumes without 1 µl template DNA.

Sequence analysis

The secondary PCR products of the *SSU* rRNA gene were sequenced bidirectionally by SinoGenoMax Biotechnology CO., Ltd (Beijing, China). Sequence accuracy was confirmed by two-directional sequencing and by sequencing a new PCR product if necessary.

To infer the phylogenetic relationships of the detected samples, neighbor-joining (NJ) trees were constructed with the MEGA 7.0 software (http://www.megasoftware.net) based on evolutionary distances calculated with the Kimura 2-parameter model. The reliability of these trees was assessed with a bootstrap analysis of 1000 replicates.

Statistical analysis

The prevalence of parasitic infections, with a 95% confidence interval (CI), was calculated. The chi-square test was used to compare differences in infection rates between different age groups and clinical symptoms. A two-tailed p-value < 0.05 was considered statistically significant.

Results

Prevalence of *Cryptosporidium* species

A total of 1032 fecal samples (491samples of sheep, 541samples of goats) were collected from 16 sheep and goat farms. Of the total, 23 specimens were PCR-positive for *Cryptosporidium* spp., and the overall infection rate was 2.23%. The highest infection rate (20.00%, 4/20) was detected on site 1, followed by site 8 (7.84%, 4/51), site 13 (7.14%, 3/42) and site 5 (7.00%, 4/57), and the infection rates in site 7, site 3, site 2 and site 11 were 4.76% ($n=1$), 3.30% ($n=1$), 2.92% ($n=4$), 1.11% ($n=2$) respectively. There was no *Cryptosporidium* detected in the other 8 farms (Table 1).

Distribution and subtype of *Cryptosporidium*

Twenty-three isolates were identified as three *Cryptosporidium* species: *C. andersoni*, *C. xiaoi* and *C. ubiquitum* through DNA sequence analysis of the *SSU* rRNA gene in *Cryptosporidium* spp., and *C. xiaoi* was the predominant

Table 1 Sampling information and occurrence of *Cryptosporidium* spp. in ruminants in western Inner Mongolia, China

Region and seasons	Collection site	Sampling number	*Cryptosporidium*-positive NO. and rate	Animal species	Feeding models	*Cryptosporidium* species/subtypes (n)
Alxa Left Banner (Inner Mongolia) Autumn	1	20	20.00 (4/20)	Goats	Pastoral	*C. xiaoi* (3)/XXIIIf (3); *C. andersoni* ($n=1$)
Autumn	2	137	2.92 (4/137)	Sheep	Captive	*C. xiaoi* (1); *C. ubiquitum* (3)/XIIa (3)
Autumn	3	30	3.30 (1/30)	Goats	Pastoral	*C. xiaoi* (1)/XXIIIc (1)
Autumn	4	45	0	Goats	Pastoral	
Autumn	5	57	7.00 (4/57)	Goats	Pastoral	*C. andersoni* (4)
Alxa Left Banner (Inner Mongolia) Summer	6	21	0	Sheep	Pastoral	
Summer	7	21	4.76 (1/21)	Sheep	Pastoral	*C. xiaoi* (1)/XXIIIg (1)
Summer	8	51	7.84 (4/51)	Goats	Pastoral	*C. xiaoi* (4)/XXIIIg (4)
Alxa Left Banner (Inner Mongolia) Winter	9	104	0	Sheep	Pastoral	
Winter		22		Sheep	Captive	
Winter	10	120	0	Goats	Pastoral	
Winter		9		Sheep		
Winter	11	69	1.11 (2/180)	Sheep	Pastoral	*C. ubiquitum* (2)/XIIa (2)
Winter		111		Goats		
Winter	12	45	0	Goats	Pastoral	
Winter	13	42	7.14 (3/42)	Goats	Pastoral	*C. ubiquitum* (3)/XIIa (3)
Winter	14	29	0	Goats	Pastoral	
Winter		15		Sheep		
Winter	15	69	0	Sheep	Pastoral	
Winter	16	15	0	Goats	Pastoral	
Total		1032	2.23 (23/1032)			

species. It accounted for 43.47% of all *Cryptosporidium* positive specimens, and it was detected in four farms. *C. ubiquitum* and *C. andersoni* were detected in three farms, respectively. In the analysis of subtypes, the *C. ubiquitum* isolates were all identified as subtype family XIIa, and nine *C. xiaoi* isolates were identified as subtypes XXIIIc, XXIIIf and XXIIIg (Table 1).

Sequence analysis

From the *Cryptosporidium SSU* rRNAgene, eight isolates shared 100% homology to Indian cattle *C. ubiquitum* isolate, Chinese sheep *C. ubiquitum* isolate and Pacific Northwest mountain beaver isolate (GenBank Accession NO.MT044147, MH059802, MT524974). Ten isolates shared 100% homology to Chinese sheep and goat isolates (GenBank Accession NO.MH049731, MG 602,953), and five isolates shared 100% homology to Bangladeshi calf *C. andersoni* isolate (GenBank Accession NO.MK982465).

Nine *C. xiaoi* isolates and five *C. ubiquitum* isolates were successfully identified at the *gp60* locus: three isolates from site 1, one isolate from site 3, one isolate from site 2, one isolate from site 3, one isolate from site 7, and four isolates from site 8. Five isolates belonged to subtype XXIIIg, three isolates belonged to subtype XXIIIf, and one isolate belonged to subtype XXIIIc. Three *C. xiaoi* XXIIIg subtype isolates were found in this study, and they have 10 single nucleotide polymorphisms (SNP) in comparison with Chinese goat XXIIIg subtype isolate (GenBank Accession NO. MW815228), and two *C. xiaoi* XXIIIg subtype isolates found in this study have only one nucleotide difference in comparison with Chinese goat XXIIIg subtype isolates (GenBank Accession NO.MW815228). The *C. xiaoi* XXIIIc and XXIIIf subtypes isolates found in this study shared 100% homology to Chinese goat XXIIIc and XXIIIf subtypes isolates, respectively (GenBank Accession NOs. MW815204 and MW815225).

All of the eight *C. ubiquitum* sequences belonged to subtype XIIa, which shared 100% homology to Chinese goat, sheep and Czechic Mustela vison (GenBank Accession NOs.

Table 2 Species, genotype, infection rate and distribution of *Cryptosporidium* spp. among sheep and goats of different factors

Factors	Category	Infection rate	*Cryptosporidium* spp.	*P*-value
Seasons	Summer[a]	5.38% (5/93)	*C. xiaoi* (5)	0.728
	Autumn[a]	3.8% (13/289)	*C. andersoni* (5), *C. xiaoi* (5), *C. ubiquitum* (3)	0.001
	Winter[b]	0.77% (5/650)	*C. ubiquitum* (5)	0.001
Sheep or goats	Goats[a]	3.14% (19/541)	*C. andersoni* (5), *C. xiaoi* (8), *C. ubiquitum* (6)	0.003
	Sheep[b]	1.22% (4/491)	*C. xiaoi* (2), *C. ubiquitum* (2)	
Feeding models	Pastoral[a]	2.18% (19/873)	*C. andersoni* (5), *C. xiaoi* (9), *C. ubiquitum* (5)	0.790
	Captive[a]	2.52% (4/159)	*C. xiaoi* (1), *C. ubiquitum* (3)	
Ages	Lamb[a]	0 (0/63)		
	Adult sheep and goats[a]	2.37% (23/969)	*C. andersoni* (5), *C. xiaoi* (10), *C. ubiquitum* (8)	0.216

[a, b] indicates that the two different categories were significantly different

[a, a] indicates that the two different categories were not significantly different

KM199742, MH049733 and KY596689), and one nucleotide difference in comparison with the polish *Chinchilla lanigera* XIIa subtype isolate, Swedish *Homo sapiens* XIIa-1 subtype isolate, and Czechic Republic *Struthio camelus* (GenBank Accession NOs.KY596686, KU852740 and MN973963).

Correlation analysis

Four *Cryptosporidium* spp. positive samples were detected in 491 sheep, and 19 *Cryptosporidium* spp. positive samples were detected in 541 goats. *Cryptosporidium ubiquitum* and *C. xiaoi* were detected as positive in both sheep and goats, while *C. andersoni* was only found in goats (Table 1).

The *Cryptosporidium* spp. infection rate of goats (3.51%, 19/541) was higher than the infection rate in sheep (0.81%, 4/491), and the infection rates in goats and sheep were significantly different ($\chi^2 = 8.594$, $p = 0.003$) (Table 2).

A total of 873 fecal samples were collected from pastured sheep and goats, and 19 *Cryptosporidium* spp. positive samples were detected. A total of 159 fecal samples were collected from captive sheep and goats, and four *Cryptosporidium* spp. positive samples were detected (Table 2).

The *Cryptosporidium* spp. infection rate of pastured animals (2.18%, 19/873) was lower than the infection rate of captive animals (2.52%, 4/159), but the *Cryptosporidium* spp. infection rates in pasture and captive animals were not significantly different ($\chi^2 = 0.071$, $p = 0.790$) (Table 2). Considering the ages of sheep and goats, 969 samples were collected from adult sheep and goats, and 23 *Cryptosporidium* spp. positive samples were found. Among the 63 samples collected from lambs, *Cryptosporidium* spp. positive samples were not detected (Table 1). The *Cryptosporidium* spp. infection rate of adult sheep and goats (2.37%, 23/969) was higher than the infection rate of lambs (0, 0/63). The *Cryptosporidium* spp. infection rates of two age groups were not significantly different ($\chi^2 = 1.529$, $p = 0.216$) (Table 2).

A total of 63 samples were collected in summer, 289 samples were collected in autumn, and 650 samples were collected in winter. The *Cryptosporidium* spp. infection rate in summer (4.63%, 5/93) was higher than the rates in autumn (3.80%, 13/289) and winter (0.77%, 5/650). The *Cryptosporidium* spp. infection rates in different seasons were significantly different ($\chi^2 = 14.796$, $p = 0.001$) (Table 1 and 3).

Discussion

In this study, the overall infection rate of *Cryptosporidium* spp. was 2.23%, which was lower than the infection rate previously reported for *Cryptosporidium* spp. in sheep and goats, such as Spain (Díaz et al. 2015) (37.72%,109/289), and Turkey (Kabir et al. 2020) (25.6%,106/415), and higher than the infection rate in Italy (Dessì et al. 2020) (1.64%,15/915) and Brazil (Fiuza et al. 2011) (1.60%,2/125). In China, some higher infection rates of *Cryptosporidium* spp. in sheep and goats have been reported in previous studies of Shandong (Zhu et al. 2018) (6.76%, 15/222), Ningxia (Yang 2018) (28.33%, 136/480), and Sichuan (Zhong et al. 2018) (4.7%, 16/342).

Various *Cryptosporidium* species were found in sheep and goats in previous studies, including *C. xiaoi*, *C. ubiquitum*, *C. andersoni*, *C. parvum*, *C. bovis*, and *C. hominis*, and the distribution of *Cryptosporidium* spp. in sheep and goats was related to geographic locations. In sheep, from China, the dominant *Cryptosporidium* species were previously reported to be *C. ubiquitum* and *C. xiaoi* in Henan (Wang et al. 2010), Sichuan (Zhong et al. 2018), Anhui (Li et al. 2019), Inner Mongolia (Ye et al. 2013), and Ningxia (Yang 2018). The dominant *Cryptosporidium* species was *C. parvum* in Italy (Dessì et al. 2020), Spain (Díaz et al. 2015), the UK (Pritchard et al. 2007), and most other countries.

Table 3 *Cryptosporidium* spp. species/subtypes in sheep and goats in previous studies of other countries

Age group	Number of positive/sample	Country	Species/genotype	Reference
Lambs aged 5–30 days; sheep from parturition to 30 days	1.64% (15/915)	Italy 1.64% (15/915)	*C. parvum* (*n* = 11), *C. ubiquitum* (*n* = 4)	(Dessì et al. 2020)
Sheep	2.17% (6/276)	Papua New Guinea 3.17% (16/504)	*C. parvum* (*n* = 4), *C. andersoni* (*n* = 1), *C. scrofarum* (*n* = 1)	(Koinari et al. 2014)
Goats	4.39% (10/228)		*C. hominis* (*n* = 6), *C. parvum* (*n* = 2), *C. xiaoi* (*n* = 1), rat genotype II (*n* = 1)	
Sheep (< 3 months and > 3 months)	11.38% (38/334)	Kuwait 9.71% (54/556)	*C. parvum* (*n* = 16), *C. ubiquitum* (*n* = 3), *C. xiaoi* (*n* = 1)	(Majeed et al. 2018)
Goats (< 3 months and > 3 months)	7.21% (16/222)		*C. parvum* (*n* = 7), *C. ubiquitum* (*n* = 2), *C. xiaoi* (*n* = 1)	
Sheep(< 35 days)	25.15% (43/171)	Spain 37.72% (109/289)	*C. parvum* (*n* = 32), *C. ubiquitum* (*n* = 11),	(Díaz et al. 2015)
Goats(< 35 days)	55.93% (66/118)		*C. parvum* (*n* = 61), *C. xiaoi* (*n* = 5)	
Calves and lambs and goat kids(< 3 months)	25.6% (106/415)	Turkey 25.6% (106/415)	*C. parvum* (*n* = 105), *C. bovis* (*n* = 1)	(Kabir et al. 2020)
Sheep (2–6 months and > 12 months)	1.6% (2/125)	Brazil 1.6% (2/125)	*C. ubiquitum* (*n* = 2),	(Fiuza et al. 2011)
Old lambs and adult sheep (> 3 months)	10.1% (16/159)	Poland 10.1% (16/159)	*C. parvum* (*n* = 10)	(Majewska et al. 2000)
Sheep (1 day to 10 weeks)	13.1% (18/137)	Belgium 11.58% (33/285)	*C. parvum* (*n* = 1), Cervine genotype (*n* = 9)	(Geurden et al. 2008; Maurya et al. 2013)
Goats (1 day to 10 weeks)	9.5% (15/148)		*C. parvum* (*n* = 11)	
Sheep (< 3 months)	1.8% (1/55)	India 2.92% (5/171)	*C. parvum* (*n* = 1)	
Goats (< 3 months)	3.5% (4/116)		*C. parvum* (*n* = 4)	
Lambs (< 12 weeks)	36.13% (56/155)	UK 36.13% (56/165)	*C. parvum* (*n* = 16)	(Pritchard et al. 2007)

However, in goats, from China, the dominant *Cryptosporidium* species were previously reported to be *C. ubiquitum* and *C. xiaoi* (Wang et al. 2010; Yang 2018). In contrast, the dominant species in most other countries were *C. parvum*. In this study, *C. ubiquitum* was the dominant *Cryptosporidium* species in sheep, and *C. xiaoi* was the dominant species in goats. The results were similar to the results of previous studies in most regions of China, and different from the reports in most of other countries. A previous study (Ye et al. 2013), presented results that were partly identical to the results in this study, and no *C. parvum* was detected in this study. The difference in distribution of *Cryptosporidium* species between China and other countries may be attributed to a variety of factors: although Hulunbeier and Alxa were in the same autonomous region, they are exactly distant, and the time of sample collection were not identical in the two studies, or different feeding models and so on.

Cryptosporidium parvum has been detected in sheep and goats of lots of other countries and in some regions of China, such as Spain (Díaz et al. 2015), the UK (Pritchard et al. 2007), and India (Maurya et al. 2013), and Anhui (Li et al. 2019), Ningxia (Yang 2018) of China (Table 3 and Table 4). In addition, *C. andersoni*, *C. scrofarum*, *C. hominis* and *C. rat* genotype II were found in China and other countries, but their dominant hosts are not sheep and goats. Therefore, the infections of these *Cryptosporidium* species in sheep and goats may result from sharing feeding grounds with other animals or farmers (Koinari et al. 2014).

In this study, *Cryptosporidium* spp. in sheep and goats showed seasonal variation, *C. xiaoi* was only detected in summer and *C. ubiquitum* was only detected in winter. *Cryptosporidium andersoni*, *C. xiaoi* and *C. ubiquitum* were detected in autumn. The infection rate of *Cryptosporidium* spp. in summer was the highest and that in winter was the lowest among the three seasons, which was similar to the results reported in Henan (Wang et al. 2010), Shandong (Zhu et al. 2018), Jilin and Liaoning (Mi et al. 2014). In this study, the infection rate of *Cryptosporidium* spp. in summer was 6.4%, and there was no infection in winter. In India, the infection rate reached to the highest in post-monsoon

Table 4 *Cryptosporidium* spp. species/subtypes in sheep and goats in previous studies of China

Age group	Number of positive/sample	Region	Species/genotype	Reference
Sheep	5.77% (48/832)	Anhui Province and neighboring provinces 7.19% (116/1613)	*C. xiaoi* (*n* = 27), *C. ubiquitum* (*n* = 21)	(Li et al. 2019)
Goats	8.71% (68/781)		*C.parvum* (*n* = 68)	
Sheep (pre-weaned, post-weaned and adult)	33.8% (81/240)	Ningxia 28.33% (136/480)	*C. parvum* (*n* = 6) *C. ubiquitum* (*n* = 16) *C. xiaoi* (*n* = 59)	(Yang 2018)
goats (pre-weaned, post-weaned and adult)	22.9% (55/240)		*C. parvum* (*n* = 1) *C. ubiquitum* (*n* = 28) *C. xiaoi* (*n* = 26)	
Tibetan Sheep < 1 year, 1–2 years and > 2 years old)	12.29% (43/350)	Qinghai 12.29% (43/350)	*C. xiaoi* (*n* = 39), *C. ubiquitum* (*n* = 4)	(Li et al. 2016)
Sheep: Preweaned lamb, postweaned lamb, pregnant ewe, and postparturition ewe	4.82% (82/1701)	Henan 4.82% (82/1701)	Cervine genotype (*n* = 74), *C. andersoni* (*n* = 4), *C. xiaoi* (*n* = 4)	(Wang et al. 2010)
Sheep < 6 months, 6–12 months, and > 12 months)	7.84% (8/102)	Shandong 6.76% (15/222)	*C. parvum* (*n* = 4) *C. ubiquitum* (*n* = 1) *C. xiaoi* (*n* = 3)	(Zhu et al. 2018)
Goats < 6 months, 6–12 months, and > 12 months)	5.83%, (7/120)		*C. parvum* (*n* = 4) *C. ubiquitum* (*n* = 1) *C. xiaoi* (*n* = 3)	
Black goats (0–2 months,3–6 months, 7–12 months, and > 12 months)	14.02% (15/107)	Shanxi 14.02% (15/107)	*C. xiaoi* (*n* = 6), *C.bovis* (*n* = 7)	(Song et al. 2017)
Goats	4.7% (16/342)	Sichuan 4.7% (16/342)	*C. xiaoi* (*n* = 11), *C. suis* (*n* = 5)	(Zhong et al. 2018)
Goats	3.48% (44/1265)	Henan and Sichuan Province 3.48% (44/1265)	*C. andersoni* (*n* = 16) *C. ubiquitum* (*n* = 24) *C. xiaoi* (*n* = 4)	(Wang et al. 2014)
Sheep	13.1% (19/375)	China Inner Mongolia 13.1% (19/375)	*C. ubiquitum* (*n* = 17) *C. xiaoi* (*n* = 31) *C. parvum* (*n* = 1)	(Ye et al. 2013)

(Maurya et al. 2013). However, in Anhui (Li et al. 2019), Chongqing (Wang et al. 2014), Henan (Wang et al. 2010), and Inner Mongolia (Ye et al. 2013), the infection rate of *Cryptosporidium* spp. in autumn was higher than in other seasons, and there was no infection in summer. The variation illustrated in the results suggested that the infection of *Cryptosporidium* spp. was not directly related to different seasons.

In this study, the *Cryptosporidium* spp. infection rate of captive sheep and goats (2.52%) was slightly higher than that of pastoral sheep and goats (2.18%), and there was no significant difference between the two feeding models. The results were similar to those of the other nine regions (Table 4), which were probably correlated to the sanitary conditions and breeding density of sheep and goats in sheepfolds. In addition, the *Cryptosporidium* spp. infection rate of pastoral sheep and goats was higher in autumn and winter, because of the lack of forage grass (Gao et al. 2021).

In addition, different age patterns were related to the infection rate of *Cryptosporidium* spp. In this study, no *Cryptosporidium* spp. was detected in lambs and kid goats, and all 23 *Cryptosporidium* spp. positive samples were collected from adult sheep and goats. The infection rate of *Cryptosporidium* spp. (2.37%) in adult sheep and goats was lower than the infection rate of most provinces in China, and some other countries (Table 3 and Table 4). In previous studies, the infection rate of *Cryptosporidium* spp. declined with the increased age of sheep and goats. This finding was not consistent with the result of this study, probably related to the different breeding density, environments and seasons, or because the number of samples of lambs and kid goats was fewer than samples of adult goats and sheep (Table 2).

Cryptosporidium ubiquitum was named as cervine genotype several years ago, because of its great distribution range and various host species. *C. ubiquitum* has been detected in China, the USA, the UK, Canada, Spain and many other countries (Blanco

et al. 2016). In previous studies, the nucleotide sequence of *gp60* gene of *C. ubiquitum* formed six subtype families: XIIa to XIIf (Li et al. 2014). In this study, the eight *C. ubiquitum* isolates all belonged to subtype XIIa, which has been detected in ruminants, humans and other animals. A total of 59 *C. ubiquitum* infection cases in humans were all caused by subtypes XIIa to XIId, and mainly by XIIa. In the USA, the UK, Canada, Australia and Wales, *C. ubiquitum* subtypes XIIb and XIId were detected in water (Li et al. 2014). However, this may not entirely indicate the reason of *C. ubiquitum* infections in humans, which were caused by *C. ubiquitum* susceptible animals or contaminated water, because *C. ubiquitum* subtypes XIIa to XIId can also be found in other animals.

Cryptosporidium xiaoi was commonly found in sheep and goats, in previous studies, *C. xiaoi* was found in Papua New Guinea (Koinari et al. 2014), Kuwait (Majeed et al. 2018), and Spain (Díaz et al. 2015). In China, *C. xiaoi* was the dominant *Cryptosporidium* species in sheep of Guangdong, Hubei, Shandong and Shanghai (Mi et al. 2014). It was the dominant *Cryptosporidium* species of goats in Inner Mongolia (Ye et al. 2013), Anhui (Li et al. 2019), Ningxia (Yang 2018) and Qinghai (Li et al. 2016). The distribution of *C. xiaoi* subtypes was correlated with different hosts. In previous studies. *C. xiaoi* subtype families, XXIIIa, XXIIIc, XXIIIg and XXIIIj, were only detected in goats; the other eight subtype families were found in both goats and sheep (Fan et al. 2021). In this study, nine *C. xiaoi* isolates were successfully subtyped, and all belonged to XXIIIf ($n=3$), XXIIIg ($n=5$) and XXIIIc ($n=1$) subtypes. The three subtypes were found in sheep and goats, which differed from the results of previous studies, subtype family XXIIIg was only found in sheep. In this study, geographic locations were not a significant factor to the distribution of *C. xiaoi* subtypes. In addition, *C. xiaoi* was found in two AIDS patients in Ethiopia, suggesting that *C. xiaoi* presented a zoonotic risk (Adamu et al. 2014).

Cryptosporidium andersoni has a wide host range, cattle and Bactrian camels being major hosts, sheep and goats being minor hosts (Xiao et al. 2004). *Cryptosporidium andersoni* has been found in sheep from Henan Province of China (Wang et al. 2010), and Papua New Guinea (Koinari et al. 2014). It was found in goats in Henan and Sichuan (Zhong et al. 2018) Provinces of China. *Cryptosporidium andersoni* has been identified in seven sporadic cases (Xiao et al. 2004), with low public health risk, and its clinical symptoms were not obvious.

In conclusion, we detected *C. ubiquitum*, *C. andersoni*, and *C. xiaoi* in sheep and goats from Inner Mongolia, China. *Cryptosporidium ubiquitum* and *C. xiaoi* were the dominant *Cryptosporidium* spp., and *C. xiaoi* XXIIIc subtype was first detected in sheep. Moreover, *C. ubiquitum* belonged to the zoonotic XIIa subtype, which has the potential threat for human health in Inner Mongolia. More studies are required to understand the differences in the transmission and the human public health significance of cryptosporidiosis in sheep and goats.

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Author contribution Longxian Zhang contributed to the conception and design of the experiments. Jiashu Lang, Han Han and Heping Dong performed the experiments. Ziyang Qin and Huikai Qin helped in interpretation of data. Yin Fu and Junchen Zhang collected fecal samples. Junqiang Li, Xiaoying Li, Guanghui Zhao and Jinfeng Zhao interpreted the results and drafted the manuscript. All of the authors read and approved the final version of the manuscript.

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Data availability All of the data generated and analyzed during this study are included in this published manuscript. The nucleotide sequences of *C. xiaoi* for the *gp60* gene obtained in this study have been deposited in GenBank, GenBank accession numbers: ON809515—ON809517.

Declarations

Competing interests The authors declare no competing interests.

Ethics approval This study was conducted in accordance with the Chinese Laboratory Animal Administration Act of 1988. The research protocol was reviewed and approved by the Research Ethics Committee of Henan Agricultural University. Permission was obtained from farm owners before the collection of animal fecal samples.

Conflict of interest The authors declare no competing interests.

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