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RESEARCH

Prevalence and molecular characterization of *Cryptosporidium* spp. in dairy and beef cattle in Shanxi, China

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Abstract

Cryptosporidium spp. are key gastrointestinal protists in humans and animals worldwide. Infected cattle are considered the main source of cryptosporidiosis outbreaks in humans. However, little is known about the genetic makeup of *Cryptosporidium* populations in Shanxi province, China. We analyzed 858 fecal samples collected from farms in Shanxi. The presence of *Cryptosporidium* spp. was determined via polymerase chain reaction and subsequent sequence analysis of the small subunit rRNA gene as well as restriction fragment length polymorphism analysis. *Cryptosporidium parvum* was subtyped following sequence analysis of the 60 kDa glycoprotein gene (*gp60*). The overall prevalence of *Cryptosporidium* in cattle was 11.19%, with a prevalence of 13.30% and 8.67% in Lingqiu and Yingxian, respectively. The overall prevalence of *Cryptosporidium* in dairy and beef cattle was 10.78% and 11.50%, respectively. *Cryptosporidium* infection was detected across all analyzed age groups. The overall prevalence of *Cryptosporidium* in diarrhea and nondiarrhea samples was 18.24% and 9.72%, respectively, whereas that in intensively farmed and free-range cattle was 17.40% and 3.41%, respectively. We identified five *Cryptosporidium* species, with *C. andersoni* being the dominant species. Further, two cases of mixed infections of *Cryptosporidium* species were detected. All identified *C. parvum* isolates belonged to the subtype IIdA17G1.

Keywords *Cryptosporidium* · Prevalence · Molecular characterization · China · Shanxi

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Introduction

Cryptosporidium infects various vertebrates, including humans (Fayer 2010). It is one of the four major pathogens responsible for severe diarrhea in infants and young children (Kotloff et al. 2013), the second leading cause of diarrhea and death in children after rotavirus (Zhao et al. 2019), and the fifth most important foodborne parasite worldwide (Ryan et al. 2021). Individual animals with weakened immune function are more susceptible to *Cryptosporidium* infection than healthy adult animals (Feng et al. 2018), and *Cryptosporidium* infection in cattle may manifest as persistent watery diarrhea (Wang et al. 2018), significant weight loss (Sarkar et al. 2014), and decreased growth rate after recovery (Thomson et al. 2019). During the environmental stage, the oocyst is highly resistant and difficult to inactivate (Blanchard 2012), and no vaccine or effective therapeutic drug is currently available (Ashigbie et al. 2021).

Since the first report of *Cryptosporidium* in 1907 (Tyzzer 1907), at least 44 valid *Cryptosporidium* species and approximately 120 genotypes have been described globally (Uran-Velasquez et al. 2022). Cattle are an important host (Ryan et al. 2014), and at least 12 *Cryptosporidium* species (Wang et al. 2011b), predominantly *Cryptosporidium parvum*, *C. andersoni*, *C. ryanae*, and *C. bovis*, have been reported in cattle worldwide (Fayer 2010; Wang et al. 2018; Yang et al. 2020). *C. parvum* is the most important cause of zoonotic cryptosporidiosis (Feng et al. 2018). Over 20 subtype families of *C. parvum* gp60 have been identified, with IIa, IIc, and IId being the most predominant subtype families (Wang et al. 2022). Only *C. parvum* infections caused by the IId subtype family have been identified in cattle in China, with IIdA15G1 and IIdA19G1 being the most common subtypes (Cai et al. 2017; Wang et al. 2017).

The global prevalence of cryptosporidiosis in cattle ranges from 6.25 to 39.65% (Tarekegn et al. 2021), with a global pooled prevalence of 29.1% (Hatam-Nahavandi et al. 2019). In China, the pooled prevalence has been estimated as 14.50% (Wang et al. 2017) and 11.9% (Gong et al. 2017). To date, only the infection rate of *Cryptosporidium* in rodents (3.8%, 2/53) has been reported in Shanxi province, China (Ni et al. 2021). We performed the molecular epidemiological survey of cattle in Shanxi to determine the prevalence and molecular characterization of *Cryptosporidium*.

Materials and methods

Study areas and sample collection

From March 2021 to September 2022, 858 fresh stool samples (30 g each) were collected from Lingqiu and Yingxian in Shanxi province, China (37°27′–38°25′N, 111°30′–113°09′E). The samples were collected from 48 preweaned calves (0–60 days old), 128 postweaned calves (61–180 days old), 186 young cattle (181–450 days old), and 496 adult cattle (>450 days old). Overall, 148 cattle had diarrhea. Each stool sample was placed inside a clean, labeled, and sterile plastic zipper bag, and the information was recorded (sampling date, age, and fecal consistency). Then, all samples were transported back to the laboratory for storage at 4°C and were processed as soon as possible.

DNA extraction, polymerase chain reaction, and sequence analysis

Total genomic DNA was extracted from each sample using E.Z.N.A Stool DNA Kit (Omega Bio-Tek Inc., Norcross, GA, USA) according to the manufacturer's instructions. The final volume of each DNA sample was 200 µL. The samples were stored at −20°C until polymerase chain reaction (PCR) amplification.

To determine the prevalence of *Cryptosporidium* spp., a fragment of the small subunit (SSU) rRNA gene (~840 bp) was amplified via nested PCR (Feng et al. 2007). The 25-µL PCR mixture comprised 12.5 µL of Premix Taq (TaKaRa Taq Version 2.0 plus dye; TaKaRa Bio Inc., Kusatsu, Shiga, Japan), 0.5 µL each of forward and reverse primers, 1 µL of template DNA or first-round PCR product, and 10.5 µL of sterilized double distilled water. The first-round PCR parameters were as follows: predenaturation at 94°C for 5 min; followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C for 1 min; extension at 72°C for 7 min; and insulation at 4°C. The second-round PCR conditions were identical to those of the first-round PCR, except that the annealing temperature was 58°C. All rounds of PCR amplification included positive and negative controls. The products from the second-round PCR were analyzed via 1.5% agarose gel electrophoresis, and all positive PCR products were sent to a commercial company for bidirectional sequencing (Sangon Biotech, Shanghai, China). The sequencing results were assembled and corrected using SeqMan software (DNASTAR Inc., Madison, USA) for sequence analysis.

Subsequently, restriction fragment length polymorphism (RFLP) analysis of PCR-positive products was performed using *Ssp*I and *Mbo*II restriction endonucleases to further determine the species and status of mixed infections of *Cryptosporidium*. The presence of *C. parvum* DNA was confirmed via the abovementioned enzyme digestion and sequence analysis, and *gp60* nested PCR was performed (Alves et al. 2003) at annealing temperatures of 52°C and 50°C for the first- and second-round PCR, respectively. A PCR amplification product of

approximately 850 bp was identified as *C. parvum*, and the subtype was identified following sequencing (Sulaiman et al. 2005).

All sequences were aligned with reference sequences downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) using MEGA v11.0 software (http://www.megasoftware.net/). The sequencing results were analyzed using the BLAST online platform (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome). To comprehensively investigate the relationship among different isolates, we constructed phylogenetic trees using the neighbor-joining algorithm based on a matrix of evolutionary distances, which were calculated using the Kimura two-parameter model via MEGA. We performed bootstrap analysis (1000 replicates) to assess the robustness of the clusters.

Statistical analysis and GenBank accession numbers

Chi-square test was performed, and 95% confidence interval (CI) values were determined using SPSS Statistics v21.0 (IBM Corp., New York, NY, USA) to compare *Cryptosporidium* infection rates among different sampling sites, age groups, breeds, and feeding methods as well as between diarrheal and nondiarrheal groups. A two-tailed *P*-value of <0.05 was considered to indicate statistical significance.

Results

Prevalence of Cryptosporidium spp.

Based on SSU rRNA PCR results, we revealed that 11.19% (96/858) of the samples were positive for *Cryptosporidium*. The overall prevalence of *Cryptosporidium* in cattle was 13.30% (62/466) and 8.67% (34/392) in Lingqiu and Yingxian, respectively, with a statistically significant difference between the two regions (odds ratio [OR]: 1.616, 95% CI: 1.039–2.514, $P = 0.032$). The overall prevalence of *Cryptosporidium* in dairy and beef cattle was 10.78% (40/371) and 11.50% (56/487), respectively, with no significant difference between them (OR: 0.930, 95% CI: 0.605–1.430, $P = 0.741$). Among the four age groups, postweaned calves (25.78%, 33/128) had the highest infection rate, followed by preweaned calves (18.75%, 9/48). Young (14.52%, 27/186) and adult (5.44%, 27/496) cattle showed the lowest infection rates. The overall prevalence of *Cryptosporidium* in preweaned calves, postweaned calves, and young cattle was significantly higher than that in adult cattle (OR: 4.009, 95% CI: 1.762–9.120, $P < 0.001$; OR: 6.034, 95% CI: 3.466–10.504, $P < 0.001$; and OR: 2.950, 95% CI: 1.680–5.179, $P < 0.001$, respectively). The overall prevalence of *Cryptosporidium* in diarrhea and nondiarrhea samples was 18.24% (27/148) and 9.72% (69/710), respectively, with the infection rate being significantly higher in diarrhea samples (OR: 2.073, 95% CI: 1.276–3.368, $P = 0.003$). The overall prevalence of *Cryptosporidium* in intensively farmed and free-range cattle was 17.40% (83/477) and 3.41% (13/381), respectively, with the infection rate being significantly higher in intensively farmed cattle (OR: 5.963, 95% CI: 3.267–10.884, $P < 0.001$) (Table 1).

Distribution of Cryptosporidium species and its subtypes

Sequencing of 96 *Cryptosporidium* PCR-positive products yielded 95 sequences from 5 *Cryptosporidium* species (*C. andersoni*, 49; *C. bovis*, 30; *C. parvum*, 8; *C. ryanae*, 7; and *C. ubiquitum*, 1) (Table 1). Phylogenetic analysis of the PCR products revealed five branches (Fig. 1). *C. ubiquitum* was only identified in Yingxian, whereas the other four *Cryptosporidium* species were detected in both Lingqiu and Yingxian. *C. andersoni* was the dominant species in both regions. Further, *C. andersoni* and *C. parvum* were identified across all four age groups and were the dominant species in preweaned calves. *C. bovis* was the dominant species in postweaned calves, whereas *C. andersoni* was the dominant species in young and adult cattle. Furthermore, *C. andersoni* was the dominant species in dairy and beef cattle as well as diarrhea and nondiarrhea samples. Additionally, RFLP analysis of the PCR products revealed two cases of mixed infections in Lingqiu: *C. andersoni* + *C. bovis* in one sample from dairy cattle and *C. andersoni* + *C. parvum* in one sample from beef cattle.

The six *gp60* sequences obtained in our study were analyzed and successfully identified as *C. parvum* subtype IIdA17G1.

Discussion

In recent decades, an increasing number of epidemiological investigations have been conducted both domestically and internationally to assess the prevalence of cryptosporidiosis(Cai et al. 2019; Gong et al. 2017; Hatam-Nahavandi et al. 2019; Wang et al. 2017). The prevalence of bovine cryptosporidiosis varies considerably across different countries, which is associated with factors such as animal age, sampling season, sanitation conditions, total sample number, study design, diagnostic method, geographical conditions, and climate (Gong et al. 2017; Hatam-Nahavandi et al. 2019). The present study revealed that the overall prevalence of *Cryptosporidium* in cattle was 11.19%, which was higher than that reported in Bangladesh (5%, 31/623) (Ehsan et al. 2015) and Egypt (10.2%, 49/480) (Ibrahim et al. 2016) but lower than that reported in Italy (38.8%,

Table 1 *Cryptosporidium* prevalence and information regarding *Cryptosporidium* species

Factor		Sample size	No. of positive results	Positivity rate (%)	Species (*n*)	*P-value*	OR (95% CI)
Region	Lingqiu	466	62	13.30	*Cryptosporidium bovis* (18), *C. parvum* (3), *C. ryanae* (5), *C. andersoni* (34), *C. andersoni* + *C. bovis* (1)[a], and *C. andersoni* + *C. parvum* (1)[a]	0.032	1.616 (1.039–2.514)
	Yingxian	392	34	8.67	*C. parvum* (5), *C. andersoni* (14), *C. bovis* (12), *C. ubiquitum* (1), and *C. ryanae* (2)		
Total		858	96	11.19	*C. andersoni* (48), *C. parvum* (8), *C. bovis* (30), *C. ubiquitum* (1), *C. ryanae* (7), *C. andersoni* + *C. parvum* (1)[a], and *C. andersoni* + *C. bovis* (1)[a]		
Breed	Dairy cattle	371	40	10.78	*C. bovis* (12), *C. parvum* (6), *C. andersoni* (18), *C. andersoni* + *C. bovis* (1)[a], *C. ryanae* (2), and *C. ubiquitum* (1)	0.741	0.930 (0.605–1.430)
	Beef cattle	487	56	11.50	*C. ryanae* (5), *C. bovis* (18), *C. andersoni* (30), *C. parvum* (2), and *C. andersoni* + *C. parvum* (1)[a]		
Total		858	96	11.19	*C. andersoni* (48), *C. parvum* (8), *C. bovis* (30), *C. ubiquitum* (1), *C. ryanae* (7), *C. andersoni* + *C. parvum* (1)[a], and *C. andersoni* + *C. bovis* (1)[a]		
Age (days)	Preweaned calves (<60 days old)	48	9	18.75	*C. andersoni* (4), *C. parvum* (4), and *C. ryanae* (1)	<0.001	4.009 (1.762–9.120)
	Postweaned calves (61–180 days old)	128	33	25.78	*C. andersoni* (14), *C. bovis* (15), *C. parvum* (1), *C. ryanae* (2), and *C. andersoni* + *C. parvum* (1)[a]	<0.001	6.034 (3.466–10.504)
	Young cattle (181–450 days old)	186	27	14.52	*C. ryanae* (4), *C. andersoni* (15), *C. parvum* (2), *C. bovis* (5), and *C. andersoni* + *C. bovis*(1)[a]	<0.001	2.950 (1.680–5.179)
	Adult cattle (>450 days old)	496	27	5.44	*C. andersoni* (15), *C. parvum* (1), *C. ubiquitum* (1), and *C. bovis* (10)	-	-
Total		858	96	11.19	*C. andersoni* (48), *C. parvum* (8), *C. bovis* (30), *C. ubiquitum* (1), *C. ryanae* (7), *C. andersoni* + *C. parvum* (1)[a], and *C. andersoni* + *C. bovis* (1)[a]		
Symptom	Diarrhea	148	27	18.24	*C. andersoni* (10), *C. parvum* (4), *C. bovis* (9), *C. ubiquitum* (1), *C. ryanae* (2), and *C. andersoni* + *C. parvum* (1)[a]	0.003	2.073 (1.276–3.368)
	Nondiarrhea	710	69	9.72	*C. andersoni* (38), *C. parvum* (4), *C. bovis* (21), *C. ryanae* (5), and *C. andersoni* + *C. bovis* (1)[a]		
Total		858	96	11.19	*C. andersoni* (48), *C. parvum* (8), *C. bovis* (30), *C. ubiquitum* (1), *C. ryanae* (7), *C. andersoni* + *C. parvum* (1)[a], and *C. andersoni* + *C. bovis* (1)[a]		

Table 1 (continued)

Factor		Sample size	No. of positive results	Positivity rate (%)	Species (*n*)	*P-value*	OR (95% CI)
Feeding method	Intensively cattle	477	83	17.40	*C. andersoni* (46), *C. parvum* (7), *C. bovis* (22), *C. ubiquitum* (1), *C. ryanae* (5), *C. andersoni* + *C. parvum* (1)[a], and *C. andersoni* + *C. bovis* (1)[a]	<0.001	5.963 (3.267–10.884)
	Free-range cattle	381	13	3.41	*C. andersoni* (2), *C. parvum* (1), *C. bovis* (8), and *C. ryanae* (2)		
Total		858	96	11.19	*C. andersoni* (48), *C. parvum* (8), *C. bovis* (30), *C. ubiquitum* (1), *C. ryanae* (7), *C. andersoni* + *C. parvum* (1)[a], and *C. andersoni* + *C. bovis* (1)[a]		

[a]The superscript a indicates mixed infection

57/147) (Díaz et al. 2018), USA (24.2%, 60/248) (Peng et al. 2003), and Estonia (23.0% (112/486) (Santoro et al. 2019); further, it was lower than the global pooled prevalence in cattle (29.1%) (Hatam-Nahavandi et al. 2019). Regarding the domestic market, the prevalence of *Cryptosporidium* in cattle in the present study was lower than that in Taiwan (37.6%, 173/460) (Watanabe et al. 2005), Shaanxi (20.2%, 52/258) (Qi et al. 2015a), Hubei (15.6%, 53/339) (Fan et al. 2017), and Jiangxi (12.8%, 71/556) (Li et al. 2021); moreover, it was lower than that observed in two reports of pooled prevalence in Chinese cattle: 14.5% (5265/36316) (Wang et al. 2017) and 11.9% (2623/22,051) (Gong et al. 2017).

In the present study, the *Cryptosporidium* infection rate in dairy cattle was 10.78% (40/371), which was lower than that reported in Heilongjiang (47.68%, 72/151) (Zhang et al. 2013); Xinjiang (38.4%, 39/232 (Wu et al. 2020); 16.0%, 86/514 (Qi et al. 2015b)); Shanghai (37%, 303/818) (Cai et al. 2017); Henan (21.5%, 172/801) (Wang et al. 2011b); Anhui, Jiangsu, and Shanghai (18.82%, 387/2056) (Chen and Huang 2012); and Sichuan (14.4%, 40/278) (Zhong et al. 2018). Further, it was lower than the previously reported pooled prevalence of *Cryptosporidium* in Chinese dairy cattle of 11.7% (3901/33,313) (Cai et al. 2019) and 13.98% (4405/31,504) (Wang et al. 2017). The prevalence of *Cryptosporidium* in dairy cattle in the present study was higher than that in Ningxia (1.61%, 22/1366) (Huang et al. 2014), Beijing (2.55%, 21/822) (Li et al. 2016), Gansu (4.2%, 60/1414) (Wang et al. 2020b), Guangdong (4.38%, 63/1440) (Liang et al. 2019), Northwest China (Gansu and Ningxia; 5.09%, 150/2945) (Zhang et al. 2015), Henan (7.9%, 104/1315) (Wang et al. 2011a), and Shaanxi (2.61%, 32/1224) (Zhao et al. 2013); moreover, it was higher than the pooled prevalence of *Cryptosporidium* in Chinese dairy cattle (10.44%, 1330/12743) (Cai et al. 2019). The *Cryptosporidium* infection rate in beef cattle in our study was 11.50% (56/487), which was lower than the reported prevalence in beef cattle in Henan (26.5%, 44/166) (Ma et al. 2015) and Heilongjiang (17.53%, 71/405) (Zhao et al. 2014), and higher than the pooled prevalence in Chinese beef cattle (8.09%, 82/1013 (Gong et al. 2017); 10.47%, 122/1165 (Wang et al. 2017)). Thus, geographical differences exist in *Cryptosporidium* distribution.

Host age is an important factor affecting the pathogenicity of *Cryptosporidium* (Helmy et al. 2013), and the prevalence of *Cryptosporidium* in preweaned calves is generally higher than that in other age groups (Liang et al. 2019). Compared with calves, the incidence of cryptosporidiosis is several times lower in older cattle (Santín and Trout 2007). However, our study revealed that the *Cryptosporidium* infection rate of postweaned calves was higher than that of preweaned calves, possibly due to higher levels of maternal antibodies in preweaned calves (Cai et al. 2019). Additionally, the age groups varied among studies. In our study, the preweaned

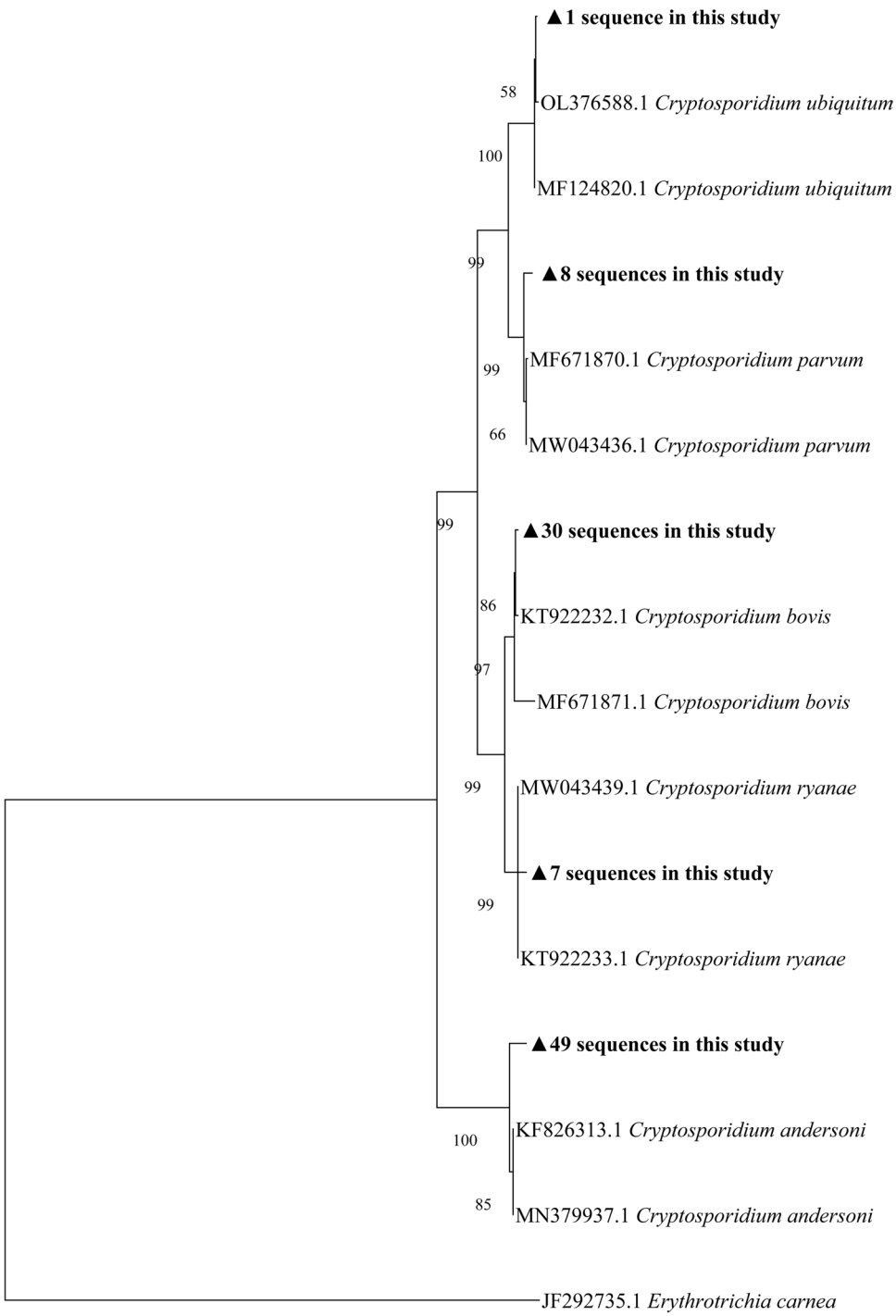

Fig. 1 Phylogenetic analyses of *Cryptosporidium* species in cattle in Shanxi, China. Species identified in the present study are indicated using black triangles (▲)

calves were <60 days old, whereas the preweaned calves in some studies were 0–90 days old (Watanabe et al. 2005; Liu et al. 2009; Zhao et al. 2013; Zhang et al. 2015; Wang et al. 2020b); thus, some of them would have been considered postweaned calves in our study.

In the present study, the overall prevalence of *Cryptosporidium* in bovine diarrhea samples (18.24%) was significantly higher than that in nondiarrhea samples (9.72%). This is consistent with a comprehensive report in China revealing that the prevalence of *Cryptosporidium* was higher in cattle with diarrhea than in those without diarrhea (Cai et al. 2019; Wang et al. 2017). In our study, the overall prevalence of *Cryptosporidium* (17.40%) in intensively farmed cattle was significantly higher than that in free-range cattle (3.41%), which might be attributed to a high feeding density. Several studies have reported that pathogen transmission is considerably more likely within a concentrated animal feeding operation than in extensive

farming systems (Hatam-Nahavandi et al. 2019). As reported in previous reviews in China, owing to the emergence of mega farms in some regions, the prevalence of *C. parvum* increased from 26.0% in 2011–2016 to 46.8% in 2017–2021 (Guo et al. 2022).

The *Cryptosporidium* species detected in our study were *C. andersoni*, *C. bovis*, *C. parvum*, *C. ryanae*, and *C. ubiquitum*, which included the top four species infecting cattle worldwide (Fayer 2010; Yang et al. 2020). *C. andersoni* was the dominant species in dairy and beef cattle in our study, consistent with reports from many regions of China and India (Liu et al. 2009; Paul et al. 2009; Wang et al. 2011b; Zhao et al. 2013). In contrast, *C. bovis* has been reported as the dominant species in beef cattle in Australia, Japan, and France (Abeywardena et al. 2013; Murakoshi et al. 2012; Rieux et al. 2013). *C. andersoni* usually infects adult cattle (Thomson et al. 2019), consistent with our study. This species is associated with gastritis, decreased milk production, and poor weight gain (Wang et al. 2020a). Additionally, although the *C. ubiquitum* infection rate in our study was low, its importance must not be neglected. In Ethiopia, *C. ubiquitum* was detected in a 12-year-old girl who had daily contact with adult cattle and sheep (Kifleyohannes et al. 2022), and investigations in the same study area revealed the presence of *C. ubiquitum* in calves, lambs, and goat kids (Kifleyohannes et al. 2021), indicating that *C. ubiquitum* poses a threat to human health. Additionally, *C. ubiquitum* has been detected in urban sewage in Harbin, China (Liu et al. 2011). Therefore, our study supports the fact that the impact of *C. ubiquitum* on humans cannot be neglected in China.

Several reports of age-related infections in different *Cryptosporidium* species have been published (Huang et al. 2014; Langkjær et al. 2007; Liang et al. 2019). In China, preweaned calves are mainly infected with *C. bovis* (Wang et al. 2017), as reported in Henan (Wang et al. 2011b), Heilongjiang (Zhang et al. 2013), and Shaanxi (Qi et al. 2015a). Some studies have suggested that *C. parvum* is the most common cause of *Cryptosporidium* infection in preweaned calves in various countries (Geurden et al. 2007; Plutzer and Karanis 2007). A similar phenomenon was observed in Ningxia (Cui et al. 2014; Huang et al. 2014) and Xinjiang (Qi et al. 2020) in China. In our study, the prevalence of *C. parvum* was similar to that of *C. andersoni* in preweaned calves. In the present study, *C. bovis* was the dominant species in postweaned calves, which differed from the findings of Wang et al. (2011a) and Qi et al. (2015b), who reported that *C. andersoni* was the dominant species in preweaned calves in China. Our study revealed that *C. andersoni* was the dominant species causing *Cryptosporidium* infections in both young and adult cattle, consistent with the finding of a previous study in Heilongjiang, China (Liu et al. 2009). Therefore, differences in the age distribution of samples from different studies might have led to differences in the identified dominant species.

In the present study, mixed infections were detected in Lingqiu. Mixed infections of different *Cryptosporidium* species are common, as observed in our previous study in Inner Mongolia (Zhao et al. 2023). The high prevalence of *Cryptosporidium* in Lingqiu also contributes to the emergence of mixed infections. A previous study stated that the first parasite to arrive can gain a marked reproductive advantage or induce cross-reaction immunity; however, it can also pave the way for subsequent infections by disrupting the associated defenses and through immunosuppression (Herczeg et al. 2021).

There are 14 subtype families of *C. parvum*, of which IIa and IId are the two main zoonotic subtype families in humans and animals (Wang et al. 2014), with other subtype families occasionally appearing in humans and animals (Wang et al. 2011b). In most industrialized countries, the IIa subtype family is relatively common (Holzhausen et al. 2019; Smith et al. 2014), particularly IIaA15G2R1 (Gharieb et al. 2019; Kváč et al. 2011), which is a common zoonotic subtype (Feng et al. 2013; Heckler et al. 2015; Imre et al. 2011). IIdA15G1 and IIdA19G1 are the dominant subtypes detected in dairy cattle infected with *C. parvum* in China (Feng and Xiao 2017; Wang et al. 2017). The *C. parvum* subtype in our study was identified as IIdA17G1, which was first detected in dairy cattle in Beijing, China, in 2016 (Li et al. 2016). Thus, our study verified the existence of this subtype in China. Additionally, IIdA17G1 was detected in British European hedgehogs (Sangster et al. 2016), preweaned lambs, and goat kids in northwestern Spain (Díaz et al. 2015) as well as raw and treated water in Portugal (Lobo et al. 2009). It has also been detected in humans in the UK (Chalmers et al. 2011), Qatar (Boughattas et al. 2017), Portugal (Alves et al. 2006), and Northern Spain (Azcona-Gutiérrez et al. 2017). Thus, this subtype is associated with a risk of zoonotic disease.

In summary, *Cryptosporidium* and *C. parvum* are widely distributed in cattle in China. Effective measures must be taken to prevent the spread of *Cryptosporidium* and *C. parvum* IId subtype in China as well as restrict the introduction of the *C. parvum* IIa subtype. Further biological and molecular epidemiological research should be conducted to improve our understanding of the host specificity and transmission of *Cryptosporidium*.

Abbreviations *gp60*: 60 kDa glycoprotein; *SSU rRNA*: small subunit ribosomal RNA; *RFLP*: restriction fragment length polymorphism; *PCR*: polymerase chain reaction; *CI*: confidence interval; *OR*: odds ratio

CRediT authorship contribution LZ, MYW, and YHL conceived and designed the study and critically revised the manuscript. LFW, LZ, MYW, YW, SZ, and YHL performed the samples collection. MYW and YHL prepared Fig. 1. MYW, LFW, YW, SZ, ZSZ, HLC, WJF, CY, YLD, JLW, and JS conducted the laboratory experiments. All the authors read and approved the final manuscript.

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Data availability All the sequences obtained in our laboratory have been uploaded to the GenBank database under the accession numbers OR460649 to OR460678, OR460682 to OR460689, OR460692 to OR460699, OR460744 to OR460792, and OR474477 to OR474482.

Declarations

Ethics approval Our study was performed in strict accordance with the international standards published in the Guide to the Feeding, Management and Use of Experimental Animals (8th Edition) and followed the Regulations on the Management of Experimental Animals and other relevant laws and regulations. The study was approved by the Biomedical Research Ethics Committee of Inner Mongolia Agricultural University (approval no. 2020 [081]). Additionally, permission was obtained from the farm owners prior to specimen collection, and all efforts were made to minimize animal suffering.

Competing interests The authors declare no competing interests.

Additional headings All authors consent to participate and consent to publish.

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