

## Original Article

# Prevalence and molecular characterization of *Cryptosporidium* spp. in dairy cattle from farms in China

Fu Chen<sup>1,2</sup>, Kehe Huang<sup>1,\*</sup>

<sup>1</sup>College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, China

<sup>2</sup>College of Animal Science and Veterinary Medicine, Qingdao Agricultural University, TsingTao 266109, China

Fecal samples of 2,056 dairy cattle from 14 farms were collected in three geographical regions of China and stained using a modified acid-fast staining technique to identify *Cryptosporidium* oocysts. A total of 387 (18.82%) positive samples were identified and further analyzed by polymerase chain reaction (PCR) using primers designed to amplify DNA fragments from the small subunit ribosomal RNA. The PCR products were sequenced and the sequences were deposited in the GenBank database under accession numbers EU369377-84 and GU070730-33. Phylogenetic analysis was performed and a distances matrix generated from these sequences confirmed the existence of *Cryptosporidium* (*C.*) *parvum* 'mouse' genotype, *C. bovis*, *C. andersoni*, *C. hominis*, and *C. serpentis* in cattle. These results represent the first report on the prevalence and genetic identification of *Cryptosporidium* species, and may contribute to a better understanding of the epidemiology of *Cryptosporidium* in cattle in China.

**Keywords:** *Cryptosporidium*, dairy cattle, modified acid-fast staining, phylogenetic analysis, polymerase chain reaction

## Introduction

Since the discovery of the enteric protozoan parasite *Cryptosporidium* in mice by Tyzzer [37], *Cryptosporidium* infections have been detected in a wide range of vertebrate hosts throughout the world including more than 170 animal species and humans [28]. Numerous case reports have described infections of the gastrointestinal and respiratory tract, and revealed that *Cryptosporidium* could cause a potentially severe infection in immunosuppressed and immunodeficient hosts [9,11,26,28]. At present, *Cryptosporidium* species have been found in over 90 countries and six

continents, including China [7,8], and 20 *Cryptosporidium* species are recognized [14].

Traditionally, cattle have been considered to be a primary reservoir for human *Cryptosporidium* infections and play a role in transmitting this parasite between humans and animals [25]. *Cryptosporidium* has been implicated as the cause of numerous outbreaks of watery diarrhea associated with contaminated food or water supplies [11,18,28], and some cases of water-borne transmission have been linked to domestic livestock, especially cattle [18]. Moreover, cryptosporidiosis has directly caused economic losses due to cattle death and treatment, and indirectly via decreased milk production capacity and increased production cost [28]. Although a large number of studies have demonstrated the efficacy of passive immunotherapy or chemotherapeutic agents for treating cryptosporidiosis, no significant clinical benefit has been demonstrated [8,18,38].

Nowadays, there are more than 13 million of dairy cattle in China [39], this figure places China in the front ranks of cattle-producing countries. To date, little information regarding the epidemiology and phylogeny of *Cryptosporidium* species in cattle from China has been reported [7,8]. Consequently, this study was designed to (a) assess the prevalence of *Cryptosporidium* in cattle in Anhui province, Jiangsu province and Shanghai city of eastern China, and (b) to precisely identify the species and genotypes of *Cryptosporidium* using molecular techniques.

## Materials and Methods

### Samples

This research was performed on four farms in Anhui province, six farms in Jiangsu province, and four farms in Shanghai city in eastern China. The 14 farms were randomly selected and visited between August 2007 and

\*Corresponding author: Tel: +86-25-84395507; Fax: +86-25-84398669; E-mail: khhuang@njau.edu.cn

December 2009. A total of 2,056 fecal samples from dairy cattle were collected directly using disposable gloves and immediately analyzed within 24 h after collection.

### Detection of *Cryptosporidium* oocysts

Triplicate fecal smears were prepared from each sample, and stained using the modified acid-fast staining technique (MAFS). Briefly, fecal smears were treated with a carbol-fuchsin solution (1 g fuchsin, 10 mL 95% ethanol, 5 g phenol, 95 mL distilled water) for 3 min, destained with a 1% hydrochloric acid-alcohol (70%) solution for 2 min, washed with running water, and counterstained with a 1% methylene blue solution for 1 min. After a final wash with water, the smears were dried at room temperature and examined by light microscopy using a 40× objective lens to verify the presence of *Cryptosporidium* oocysts. Oocyst size, shape, and staining characteristics were recorded. The average numbers of oocysts in 20 visual fields at a magnification of 400 × were classified as follows: '+' for 1 ~ 5 oocysts, '++' for 6 ~ 10, '+++ ' for 11 ~ 15, '++++' for 16 ~ 20, '+++++' for > 20, and '-' for none.

### Purification and morphologic evaluation of *Cryptosporidium* oocysts

Positive fecal samples were stored at 4°C in a 2.5% aqueous potassium dichromate solution (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>). Prior to purification, each sample was filtered through an 80-mesh sieve to remove large debris, and the filtrate was processed within 24 h of collection as follows: K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> was removed by three cycles of precipitation with phosphate buffered saline (PBS; 0.1 M, pH 7.4) and suspended in PBS. Oocysts were then isolated using the saturated sucrose floatation technique [33] and further purified by discontinuous sucrose gradient centrifugation [36]. Oocysts were counted with a hemocytometer (Great Apparatus, China) and the sizes of 50 oocysts were measured using digital image analysis software (Image-Pro Plus 5.1; Media Cybernetics, USA) and a DP12 digital camera (BX41 microscope; Olympus, Japan).

### DNA extraction

*Cryptosporidium* oocysts were washed three times with PBS and suspended in 0.2 mL sterile distilled H<sub>2</sub>O prior to DNA extraction. Genomic DNA was extracted using a DNA extraction kit (ver. 3.0; TaKaRa, Japan). The supernatant containing DNA was eluted in 50 µL of AE buffer (Qiagen, USA) and stored at -20°C before it was used for polymerase chain reaction (PCR) analysis. Genomic DNA was also extracted from a negative sample (no oocyst DNA present) as a negative control.

### Small subunit ribosomal RNA (SSU rRNA) gene amplification

A two-step nested PCR protocol was used to amplify a ~

430 bp fragment of the SSU rRNA gene using primers 5'-gtggcaatgacgggtaacgg-3' and 5'-caggacatctaaggcatca-3' for the primary PCR, and 5'-aagctcgtagtggatttctg-3' (CPB-DIAGF) and 5'-taagtgctgaaggagtaagg-3' (CPB-DIAGR) for the secondary PCR [22]. The PCR was carried out in 25 µL volume containing 2.0 µL (10 ng/µL) of genomic DNA, 2.5 µL 10× PCR buffer, 1.0 µL MgCl<sub>2</sub> (50 mM), 0.2 µL (5 IU/µL) Taq DNA polymerase, 0.5 µL dNTP (10 mM), 1.0 µL (20 pmol/µL) of each forward and reverse primer, and nuclease free water up to 25 µL. The first step PCR program included 35 cycles (94°C for 45 sec, 55°C for 30 sec and 72°C for 1 min), and the second PCR step included 40 cycles (94°C for 30 sec, 55°C for 30 sec and 72°C for 1 min) using the DNA product of the first step PCR as the template.

### Sequence and phylogenetic analysis

PCR products were analyzed by electrophoresis in a 1% (w/v) agarose gel using Tris-acetate-EDTA buffer (0.04 M Tris-acetate, 1 mM EDTA), purified using an agarose gel DNA purification kit (ver. 2.0; TaKaRa, Japan), and sequenced by TaKaRa (Japan). Sequence accuracy was confirmed by aligning the overlapping sequences obtained with an ABI 377 Automated Sequencer (Applied Biosystems, USA). Sequences from both orientations were aligned with reference sequences down-loaded from GenBank using the program Clustal V sequence alignment programs (DNASar, USA).

The nucleotide sequences of the SSU rRNA gene fragment from *Cryptosporidium* were deposited into the GenBank database and a search for highly similar sequences was performed using nucleotide blast (NCBI, USA) to confirm the species or genotype. Phylogenetic trees were constructed from the SSU rRNA using the SeqEd and Cluster V sequence alignment programs (DNASar, USA). In an initial neighbor-joining analysis, *Eimeria tenella* strain Wu (DQ136184) was used as an out-group. For comparative phylogenetic analysis, other sequences were retrieved from GenBank, including *Cryptosporidium* (*C.*) *hominis* strain H7 (AF108865), *C. hominis* strain HFL5: AF093492), *C. bovis* strain C1 (AF108864), *C. bovis* strain BOH6 (AF093490), *C. parvum* *monkey* strain CPRM1 (AF112569), *C. parvum* *mouse* M24 (AF108863), *C. parvum* strain RPHN (DQ898158), *C. parvum* strain CPM1 (AF112571), *C. ryanae* 2040 (AY120910), *C. parvum* *ferret* strain CPF (AF112572), *C. parvum* *koala* strain K2 (AF112570), *C. parvum* *rabbit* (AY120901), *C. suis* (AF108861), *C. wrairi* strain CWR (AF115378), *C. meleagridis* strain CMEL (AF112574), *C. felis* (AF108862), *C. canis* strain CPD1 (AF112576), *C. baileyi* *genotype* CBA01 (AF093495), *C. andersoni* strain bjcm (AY954885), *C. andersoni* IDVS-811 (AF093496), *C. serpentis* strain CSP01 (AF093499), *C. serpentis* isolate 119 (EU553560), *C. saurophilum*

**Table 1.** Specimens from each farm examined for *Cryptosporidium* by the modified acid fast staining technique

Region	Farm	No. of specimens	No. of positive samples	Positive (%)
Anhui	1	87	13	14.94
	2	108	19	17.59
	3	83	12	14.46
	4	72	8	11.11
Jiangsu	1	227	47	20.70
	2	225	45	20.00
	3	195	45	23.08
	4	191	34	17.80
	5	182	37	19.75
	6	195	43	20.33
Shanghai	1	79	11	13.92
	2	112	11	9.82
	3	98	12	12.24
	4	151	21	13.91

CSP06 (AF112573), and *C. muris* IDH-13 (AF093498), *C. fragile* clone A (EU162751).

### Data analysis

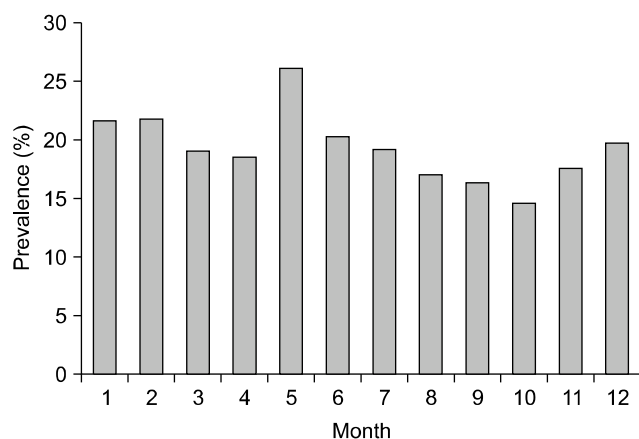
For statistical evaluation of the results, SPSS 13.0 (SPSS, USA) and Microsoft Office Excel 2003 (Microsoft, USA) were used. Prevalence of *Cryptosporidium* spp. in different groups of cattle was compared with a Chi-square test for independence ( $\alpha = 0.05$ ). A correlation coefficient ( $r$ ) was obtained and the significance of correlation between oocyst positivity and diarrhea was analyzed by Student's  $t$ -test.  $p$ -values  $< 0.05$  were considered statistically significant.

## Results

### Epidemiology analysis

A total of 387 out of 2,056 feces samples were positive for *Cryptosporidium* as determined by MAFS, representing a prevalence rate of 18.82%. This included 69 of 350 samples from the Anhui province, 251 of 1,315 samples from the Jiangsu province, and 67 of 491 samples from Shanghai city, corresponding to the prevalence rates of 19.71%, 19.09% and 13.65%, respectively (Table 1). There was a significant difference in prevalence among these districts ( $\chi^2 = 8.07$ ,  $p < 0.05$ ). The prevalence in Anhui and Jiangsu provinces was higher than that in Shanghai city ( $\chi^2 = 5.55$ ,  $7.30$ ,  $p < 0.05$ ), but no significant difference in prevalence between the Jiangsu and Anhui provinces was observed ( $\chi^2 = 0.07$ ,  $p > 0.05$ ).

Among the four farms in the Anhui province, the prevalence rates were 20.69% (18/87), 21.30% (23/108),

**Fig. 1.** Percentage of *Cryptosporidium*-positive cattle in China according to the month of diagnosis.

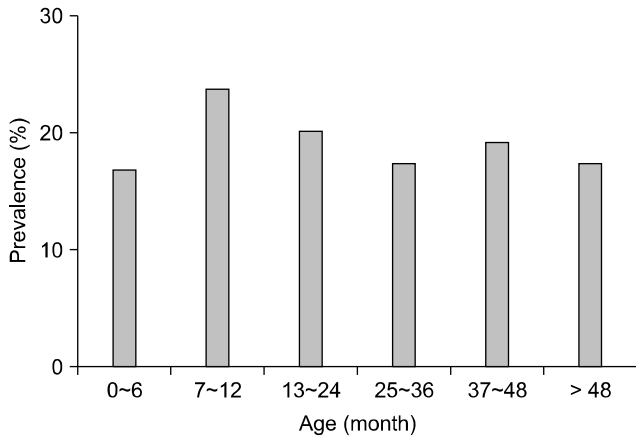
18.07% (15/83), and 15.28% (11/72). No significant difference in prevalence among these farms was observed ( $\chi^2 = 1.21$ ,  $p > 0.05$ ). The prevalence rates among the six farms in the Jiangsu province were 20.70% (47/227), 20.00% (45/225), 23.08% (45/195), 17.80% (34/191), 19.75% (37/182), and 20.33% (43/195). Among the four farms in Shanghai city, the prevalence rates were 14.62% (19/130), 9.82% (11/112), 12.24% (12/98), and 16.56% (25/151). No significant difference in prevalence among these farms was observed ( $\chi^2 = 2.74$ ,  $p > 0.05$ ).

Prevalence of *Cryptosporidium* oocysts in the feces (Fig. 1) was highest in May (21.88%, 42/192) and lowest in October (14.29%, 22/154). There was no significant difference between the two months ( $\chi^2 = 3.26$ ,  $p > 0.05$ ), and no significant difference among the 12 months was observed ( $\chi^2 = 7.15$ ,  $p > 0.05$ ). The rates of infection in spring, summer, autumn, and winter were 19.42% (81/417), 19.17% (107/558), 16.16% (96/594), and 17.54% (103/587), respectively. There was no a significant difference in prevalence among the four seasons ( $\chi^2 = 2.54$ ,  $p > 0.05$ ).

The prevalence of *Cryptosporidium* in cattle of different age ranged from 16.94% (41/242, 0~6 months old) to 23.81% (60/252, 7~12 months old) (Fig. 2). The youngest calf excreting oocysts was 6 days old and the oldest was 5 years old. Overall, the prevalence of *Cryptosporidium* infection did not vary significantly according to age ( $\chi^2 = 5.59$ ,  $p > 0.05$ ).

Out of the 387 positive samples, the intensity of infection ranged from '+' to '+++++', constituting 60.72%, 28.17%, 7.49%, 2.84%, and 0.78%, respectively. Cases of light infection, identified by scores of '+' and '++', represented 88.89% of the total.

The prevalence in cattle with and without diarrhea was also analyzed with a chi-square test. Out of the 245 samples from cattle with diarrhea and 1,811 samples from cattle without

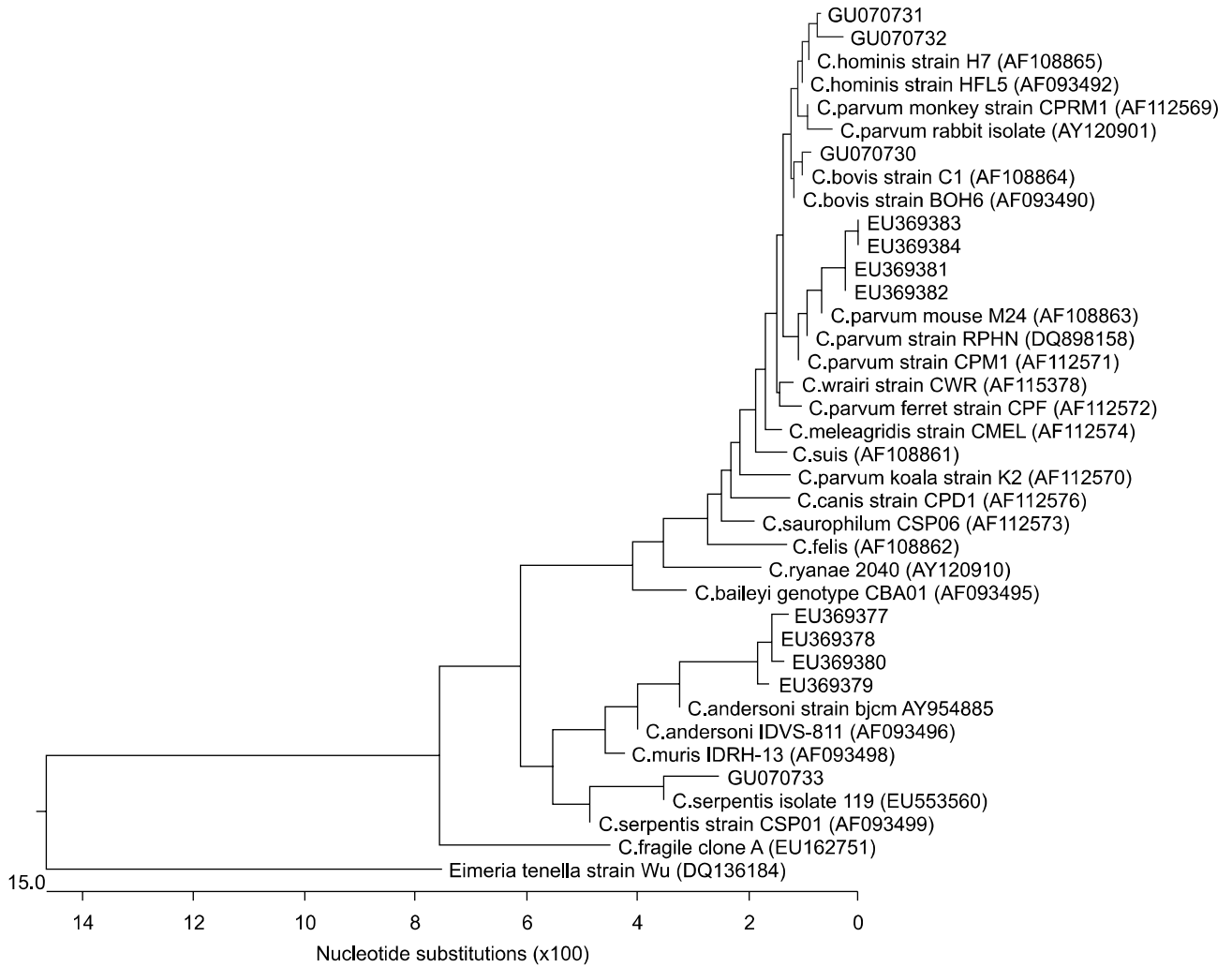


**Fig. 2.** Percentage of *Cryptosporidium*-positive cattle according to age.

diarrhea, 81 and 306 were detected positive, respectively. This indicated a significant association between positivity and diarrhea (33.06% and 19.90%;  $\chi^2 = 36.90, p < 0.01$ ).

**Oocyst characteristics**

*Cryptosporidium* oocysts, which were round or elliptic in shape, were observed by the contrast of their intense pink-rosy pigmentation against the yellow-green, blue, or glaucous background after staining with the MAFS technique. *Cryptosporidium* oocysts isolated by the saturated sucrose floatation technique and further purified by discontinuous sucrose gradient centrifugation showed different internal structures and cell size. One, two, or three sporozoites per oocyst were seen, but the total number in each oocyst could not be clearly visualized. Each oocyst also contained a residuum consisting of numerous small granules and a membrane-bound globule.



**Fig. 3.** Phylogenetic relationships among *Cryptosporidium* species and genotypes according to the neighbor-joining analysis of a fragment from the partial small subunit ribosomal RNA sequence. EU369377-84 and GU070730-33 were the *Cryptosporidium* isolates from cattle in China. Sequences of other *Cryptosporidium* species and genotypes were obtained from GenBank.

*Cryptosporidium* oocysts from different species varied in size and shape based on morphology and oocyst measurements. Oocysts (n = 50) from *C. parvum* ‘mouse’ measured 4.22~5.69  $\mu\text{m}$   $\times$  3.62~5.25  $\mu\text{m}$  with a mean size of 4.98  $\mu\text{m}$   $\times$  4.46  $\mu\text{m}$ , and had a shape index (the ratio of the length and width of oocysts) of 1.11. (1.05~1.32). Oocysts (n = 50) from *C. andersoni* measured 6.69~8.41  $\mu\text{m}$   $\times$  4.84~6.18  $\mu\text{m}$  with a mean size of 7.46  $\mu\text{m}$   $\times$  5.55  $\mu\text{m}$ , and a shape index of 1.34 (1.12~1.43). *C. bovis* oocysts (n = 50) measured 4.09~5.44  $\mu\text{m}$   $\times$  3.96~5.14  $\mu\text{m}$  with a mean size of 4.83  $\mu\text{m}$   $\times$  4.64  $\mu\text{m}$ , and a shape index of 1.06 (1.04~1.16). *C. hominis* oocysts (n = 50) measured 4.61~6.21  $\mu\text{m}$   $\times$  4.24~5.84  $\mu\text{m}$  with a mean size of 5.18  $\mu\text{m}$   $\times$  4.98  $\mu\text{m}$ , and had a shape index of 1.09 (1.02~1.18). Finally, oocysts (n = 50) from *C. serpentis* measured 5.73~6.49  $\mu\text{m}$   $\times$  4.96~5.38  $\mu\text{m}$  with a mean size of 6.17  $\mu\text{m}$   $\times$  5.16  $\mu\text{m}$ , and had a shape index of 1.18 (1.04~1.26).

### Sequence and phylogenetic analysis of the SSU rRNA gene

Nucleotide sequences of the SSU rRNA gene fragment from *Cryptosporidium* were deposited into the GenBank database under accession numbers EU369377-84 and GU070730-33. Using the BLAST sequence analysis services of GenBank, we found that EU369377-80 was most similar to the sequences of *C. andersoni* IDVS-811 (AF093496), isolate 21 (FJ608605), and isolates (FJ463171-87) with 99% sequence identity. EU369381-84 were most similar to sequences of the *C. parvum* ‘mouse’ genotype strain M24 (AF108863), the RPHN strain (DQ898158), and CPM1 strain (AF112571) with 99~100% sequence identity. GU070730 was most similar to sequences of *C. bovis* strain C1 (AF108864), the BOH6 strain (AF093490), GCH1 strain (AF093493), DRI strain (AF093494), IOWA strain (AF164102); and strains AB513870-81, AB513857-68, and AF161856-59 with 100% sequence identity. GU070731 and GU070732 were most similar (100%) to sequences of *C. hominis* strain HFL5 (AF093492), and had a 99% identity with the B1b- B3b strain (AY204231-33), CPRM1 strain (AF112569), and Sakha isolates (AB513857-68, AB513870-80). GU070733 were most similar to the sequences of *C. serpentis* (AF151376) and *C. serpentis* CSP01 isolate (AF093499) with 100% identity, *C. serpentis* CSP02 isolate (AF093500) with 99.5% identity, and *C. serpentis* 40 isolate (EU553553) and the EU-553558-83 isolate with 99% identity.

Phylogenetic (Fig. 3) and sequence homology analyses showed that EU369377-80 was placed in the *C. andersoni* branch with 97.9~100% homology, and adjacent to *C. muris*. EU369381-84 was placed in the *C. parvum* ‘mouse’ genotype branch with 98.4~100% homology, and adjacent to *C. hominis* and *C. bovis*. GU070730 was placed in the *C. bovis* branch with 100% homology, and adjacent to *C. hominis* and *C. parvum*. GU070731 and GU070732 were placed in the *C. hominis* branch with 98.2~100% homology,

and adjacent to *C. bovis*, *C. parvum* ‘rabbit’ genotype, and *C. parvum* ‘monkey’ and ‘mouse’ genotype. GU070733 was placed in the *C. serpentis* branch with 100% homology, and adjacent to *C. ryanae* and *C. baileyi*.

No cattle were detected with a mixture of *Cryptosporidium* species or genotypes. Of the 387 PCR-positive specimens characterized by gene sequencing, 185, 112, 62, 24, and 4 were infected with *C. parvum* ‘mouse’ genotype, *C. andersoni*, *C. bovis*, *C. hominis*, and *C. serpentis*, respectively. The percentages of each species or genotype of *Cryptosporidium* relative to the total number of *Cryptosporidium*-positive cattle were 47.80%, 28.94%, 16.02%, 6.20%, and 1.02%, respectively.

### Discussion

*Cryptosporidium* was first isolated in cattle from a case of calf diarrhea in 1971 [30]. Since then, there have been numerous reports of cryptosporidiosis in different countries and regions [5,6,17,19,20,27]. However, only a few publications have documented *Cryptosporidium* prevalence among animals in China [7,40]. Because cattle breeding in China is an important agricultural activity and given high potential for cattle-to-human transmission of *Cryptosporidium* [25], it is of interest to investigate *Cryptosporidium* epidemiology in these animals according to the set of the best means as described by other investigators [3,5,7,12,13]. In the present study, we systematically investigated the prevalence and genetic identification of *Cryptosporidium* species in dairy cattle in China. We found that 387 (18.82%) samples were positive for *Cryptosporidium* oocysts in 2,056 fecal samples of dairy cattle from 14 farms using MAFS. Phylogenetic and sequence analysis confirmed the existence of *C. parvum* ‘mouse’ genotype, *C. bovis*, *C. andersoni*, *C. hominis*, and *C. serpentis* in the fecal samples, and *C. parvum* ‘mouse’ and *C. andersoni* were the most general *Cryptosporidium* species. These results represent the first report on the prevalence and genetic identification of *Cryptosporidium* species in dairy cattle in China and may contribute to a better understanding of *Cryptosporidium* epidemiology in cattle.

In the current study, *Cryptosporidium* was not found only in specific farms or provinces (cities), but was present on all 14 farms in three districts of eastern China. The average rate of infection was 18.82%, and ranged from 9.82 to 23.08% on different farms. This level of infection was higher than that found in western Canada (1.1%) [17], Italy (4.99%) [10], Spain (8.4%) [6], Thailand (13% by the acid-fast staining technique and 9.63% by nested PCR) [27], Galicia (14.2%) [5], and Alberta (2.1% and 5.8%) [20]. The infection rate in our study was also similar to that reported in Canada (20%) [29] and Aragón (21.3%) [31], but was lower than that in California (28%) [21], Mongolia (26.4%) [4], and Malaysia (36%) [19]. Many factors may

contribute to different prevalence rates, including the climate and farm practices in different countries. It would be interesting to investigate whether cattle-to-human or cattle-to-other animal transmission of *Cryptosporidium* is relevant to public health.

Quilez *et al.* [31] reported that the prevalence of cryptosporidiosis is generally lower in neonatal animals due to maternal protection. Scott *et al.* [33] found no relation between the presence of oocyst-excreting adults and the development of infection in calves. de la Fuente *et al.* [9] reported that the detection rate of *Cryptosporidium* in animals 22~30 days old was significantly lower than those 1~21 days old in central Spain. In contrast, the present study found that the prevalence of *Cryptosporidium* was not different among cattle of different ages, suggesting that adult cattle might act as reservoirs for *Cryptosporidium* that infects neonatal and younger cattle.

Many authors have reported contradictory findings regarding the seasonal prevalence of *Cryptosporidium* on cattle farms [23,34]. Mohammed *et al.* [23] found that dairy cattle sampled in spring in southeastern New York State were significantly less likely to be positive for *Cryptosporidia* compared to cattle sampled in the winter. In contrast, Becher *et al.* [2] reported that there was no difference in seasonal prevalence on cattle farms in Western Australia. In the present study, we found no seasonality in *Cryptosporidium* prevalence, indicating high levels of humidity which frequently occur in all seasons in the study regions may favor transmission.

*Cryptosporidium* is considered to be one of the causative enteropathogens of diarrhea in cattle [9], although asymptomatic infection had also been described [26]. In the present study, the infection rate among cattle with diarrhea was higher than that among asymptomatic animals. Therefore, we concluded that cryptosporidiosis is an important cause of diarrhea in cattle.

Many studies have suggested that cattle are infected with at least four *Cryptosporidium* species (*C. parvum*, *C. bovis*, *C. andersoni*, and *C. ryanae*) in the United States [12,13,19,23,32]. Feng *et al.* [16] reported that *C. bovis* and *Cryptosporidium* deer-like genotype were found in all age groups of cattle from diverse geographic areas, and were much more prevalent in post-weaned cattle [12]. Fayer *et al.* [12,13] found that *C. andersoni* were found in more cows or cattle while *C. bovis*, *C. parvum*, and *Cryptosporidium* deer-like genotype were less prevalent [32]. Feltus *et al.* [15] reported that *C. bovis*, the deer-like genotype, and *C. andersoni* were identified in 9.4, 6.6, and 1.4% of the animals sampled, respectively. Zhou *et al.* [40] examined *C. andersoni* infection in dairy cattle in China. In this study, five *Cryptosporidium* species or genotypes were identified according to oocyst dimension and genetic analysis of SSU rRNA. *C. parvum* ‘mouse’, *C. hominis*, *C. serpentis*, and *C. bovis* were first found in cattle in China,

and the infection rates of *C. parvum* ‘mouse’ and *C. andersoni* were higher than those of other genotypes. *C. andersoni* and *C. bovis* were not the most general *Cryptosporidium* found in cattle. This finding was similar to other investigations showing that the infection rate of *C. andersoni* was lower among cattle [32], but was different from studies conducted in England and northern Ireland [3,36]. Based on these findings, it is possible to conclude that geographic condition, climate, feeding, and management may affect the prevalence of *Cryptosporidium* in cattle.

Morgan *et al.* [24] reported that *C. parvum* ‘mouse’ genotype was primarily isolated from wild rats and house mice, and wild rodents were thought to serve as an important reservoir. In the present study, *C. parvum* ‘mouse’ genotype was the most prevalent species in the 387 positive samples, constituted 47.8% of the positive samples. When sampling, we found that there were some mice and mouse feces in the farms, but we could not collect and detect those samples. Whether cryptosporidiosis is transmitted between rodents and cattle in eastern China remains to be investigated.

Morgan-Ryan *et al.* [25] reported that *C. hominis* was non-infectious in mice, rats, cats, dogs, and cattle, but infectious in calves, lambs, and piglets. *C. serpentis* was originally described as a gastric parasite found in lizards and other reptiles, but there are few reports of *C. serpentis* infection in other animals or humans [19]. Only Azami *et al.* [1] reported that *C. serpentis* can infect cattle. Similarly, *C. hominis* and *C. serpentis* infections were found in 6.20% and 1.02% of the cattle in the present study. Oocysts are transmitted from an infected host to a susceptible entity by the fecal-oral route, either through direct animal-to-human contact or water-borne routes via contaminated drinking water [11]. Although we did not confirm the transmission routes of *C. hominis* and *C. serpentis*, it is of great important to enhance the management of cattle and drinking water and to reduce transmission between cattle and humans or other animals and their feces in order to cut off the route of *Cryptosporidium* transmission to cattle.

In conclusion, there was a high prevalence of *Cryptosporidium* in dairy cattle on farms in China. *Cryptosporidium* species infecting the cattle in China belonged to *C. parvum* ‘mouse’ genotype, *C. andersoni*, *C. bovis*, *C. hominis*, and *C. serpentis*, and *C. parvum* ‘mouse’ and *C. andersoni* were the most general *Cryptosporidium* species.

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