Parasitology Research (2020) 119:105–114
https://doi.org/10.1007/s00436-019-06498-1

GENETICS, EVOLUTION, AND PHYLOGENY - ORIGINAL PAPER

Molecular detection of *Cryptosporidium* and *Enterocytozoon bieneusi* in dairy calves and sika deer in four provinces in Northern China

Wei-Fu Tao[1] · Hong-Bo Ni[1] · Hong-Feng Du[1] · Jing Jiang[2] · Jiao Li[3] · Hong-Yu Qiu[1] · Ye-Li[1] · Xiao-Xuan Zhang[1]

Received: 19 June 2019 / Accepted: 30 September 2019 / Published online: 26 November 2019

Abstract

The protistan pathogens *Cryptosporidium* and *Enterocytozoon bieneusi* can cause significant intestinal diseases in animals and humans. However, limited information is available regarding prevalence and molecular characterization of *Cryptosporidium* and *E. bieneusi* in ruminants in Northern China. In this study, the overall prevalence of *Cryptosporidium* and *E. bieneusi* was 19.3% (62/321) and 28.97% (93/321) in dairy calves and 1.10% (9/818) and 13.57% (111/818) in sika deer (*Cervus nippon*) in four provinces in Northern China, respectively. The prevalence of *Cryptosporidium* and *E. bieneusi* in different factor groups was various. Five *Cryptosporidium* species/genotypes were identified, of which *C. parvum*, *C. ryanae*, *C. bovis*, and *C. andersoni* were only found in dairy calves, and only *Cryptosporidium* deer genotype was found in sika deer. Moreover, J, I, and BEB4 ITS genotypes of *E. bieneusi* were found in dairy calves, and six known genotypes (JLD-III, JLD-IX, JLD-VII, EbpC, BEB6, and I) and ten novel genotypes (namely LND-I and JLD-XV to JLD-XXIII) were found in sika deer in this study. *Cryptosporidium parvum* and *E. bieneusi* genotype J were identified as the predominant species/genotypes in dairy calves, whereas the predominance of *Cryptosporidium* spp. and *E. bieneusi* in sika deer was *Cryptosporidium* deer genotype and BEB6, respectively. The present study reported the prevalence and genotypes of *Cryptosporidium* and *E. bieneusi* in dairy calves and sika deer in four provinces in northern China. The present findings also suggest that investigated dairy calves and sika deer may play an important role in the transmission of *E. bieneusi* and *Cryptosporidium* to humans and other animals, and also in an effort to better understand the epidemiology of these enteric pathogens in China.

Keywords *Cryptosporidium* · *Enterocytozoon bieneusi* · Dairy calves · Sika deer · Molecular detection · Northern China

Introduction

Cryptosporidium and *Enterocytozoon bieneusi* are the most important enteropathogens, which can infect a wide range of animals including cattle and sika deer (*Cervus nippon*) (Huang et al. 2014, 2018; Parsons et al. 2015; Ma et al. 2015; Naguib et al. 2015; Zhang et al. 2016; Baroudi et al. 2018; Wu et al. 2018; Amer et al. 2019; Lombardelli et al. 2019). Transmission of *Cryptosporidium* and *E. bieneusi* were mainly through ingestion of water and food contaminated by infective spores or oocysts, respectively (Wang et al. 2017). In general, the two pathogens infection in humans may cause acute or chronic diarrhea. However, their co-infection with immune-compromised individuals could cause death (Checkley et al. 2015). Moreover, they also have negative effects on the growth and cognitive functions of children (Pawlowic et al. 2017).

Wei-Fu Taon and Hong-Bo Ni contributed equally to this work.

Section Editor: Panagiotis Karanis

✉ Jing Jiang
jiangjingxiaoyao@163.com

✉ Xiao-Xuan Zhang
zhangxiaoxuan1988@126.com

[1] College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing, Heilongjiang Province 163319, People's Republic of China

[2] College of Life Sciences, Changchun Sci-Tech University, Shuangyang, Jilin Province 130600, People's Republic of China

[3] School of Life Sciences, Huzhou University, Huzhou, Zhejiang Province 313000, People's Republic of China

More than 96 *Cryptosporidium* species/genotypes and 240 distinct ITS *E. bieneusi* genotypes have been identified worldwide (Zhang et al. 2015a, 2016). *C. parvum*, *C. andersoni*, *C. ryanae*, and *C. bovis* are the four commonest species which are responsible for infections in cattle (Chalmers and Katzer 2013), and *C. parvum* mostly infects pre-weaned calves and causes diarrhea (Santín et al. 2004; Mercado et al. 2015). Only 12 *Cryptosporidium* species/genotypes (including *Cryptosporidium* deer genotype) have also been found in cervids around the world (Huang et al. 2018). *E. bieneusi* genotype group 1 were the zoonotic genotypes and group 2 were bovine-specific genotypes (Ma et al. 2015), but some genotypes (I, J, and BEB4) from group 2 also have recently been reported in humans (Li et al. 2016a). Of these, *C. hominis* and *C. parvum* are the commonest species for human cryptosporidiosis and group 1 genotypes of *E. bieneusi* are responsible for the majority of human microsporidiosis (Naguib et al. 2018; Zhang et al. 2018a). Sika deer and cattle are the important reservoir hosts of *Cryptosporidium* and *E. bieneusi* (Hu et al. 2017; Huang et al. 2018; Santin and Fayer 2015). To date, more than 10 *Cryptosporidium* species/genotypes and 35 distinct *E. bieneusi* genotypes have been found in cattle and sika deer (Wang et al. 2011a; Del Coco et al. 2014; Ma et al. 2014; Zhao et al. 2015; Jiang et al. 2015; Li et al. 2016b; Huang et al. 2017, 2018). Although some information regarding prevalence and molecular characterization of *Cryptosporidium* and *E. bieneusi* in dairy cattle and sika deer have been reported, the information regarding the two pathogens in dairy cattle and sika deer in China is also limited.

In this study, we further investigated the prevalence of *Cryptosporidium* and *E. bieneusi* and infection in dairy cattle and sika deer (*Cervus nippon*) in northern China and identified species/genotypes/subtypes of these pathogens. Moreover, the present study also aims to estimate their zoonotic potential for transmission to humans.

Materials and methods

Specimen collection and preparation

One thousand one hundred and thirty-nine fecal samples of dairy calves and sika deer (*Cervus nippon*) were randomly collected from farms from Heilongjiang Province (43° 26′ ~53° 33′ N, 121° 11′~135° 05′ E), Jilin Province (40° 50′ ~46° 19′ N, 121° 38′~131° 19′ E), Inner Mongolia (37° 24′ ~53° 33′ N, 97° 12′~126° 04′ E), Liaoning Province (38° 43′ ~43° 26′ N, 118° 53′~125° 46′ E), Northern China, between September 2017 and December 2018. All the sika deer are the same species. The pre-weaned sika deer was considered the young deer, the post-weaned sika deer was the adults. Every fresh fecal sample was collected from the rectum of each animal, and then placed into sterile gloves and transported back to the laboratory. The Stool DNA kit (OMEGA, USA) was used to extract the genomic DNAs of the fresh fecal samples. All the operations were strictly performed according to the manufacturer's instructions. Then, DNA were stored at − 20 °C and analyzed by PCR. Information regarding geographic origin, breed, and age was recorded and listed in Tables 1, 2, 3, and 4.

PCR amplification

The PCR based on the SSU rRNA gene was performed to determine the *Cryptosporidium* spp. species (Zhang et al. 2015a). The nested PCR of the ITS region of the ribosomal RNA (rRNA) gene was used to identify the *E. bieneusi* genotype (Wang et al. 2016). PCR reaction (25 μl) composed of 1× Ex *Taq* buffer (Mg^{2+} free), 2 mM $MgCl_2$, 200 μM each deoxy-ribonucleoside triphosphate (dNTP), 0.4 μM of each primer, 0.625 U of Ex *Taq* DNA polymerase (TAKARA, Japan), and 2 μl of DNA template. The cycling conditions were 5 min at 95 °C, 35 cycles of 45 s at 94 °C, 45 s at suitable temperature, and 1 min at 72 °C, followed by final extension at 72 °C for 10 min. Both positive and negative controls were included in each test. Amplification products were observed under UV light after electrophoresis in 1.5% agarose gel containing GoldView (Solarbio, China).

Sequencing and phylogenetic analyses

Positive secondary PCR products were sent to Sangon Biotech Company (Shanghai, China) for sequencing. The *Cryptosporidium* and *E. bieneusi* species/subtypes were determined by alignments with known reference sequences available in GenBank using the BLAST (http://www.ncbi.nlm.nih.gov/BLAST/), and the computer program ClustalX 1.83. The phylogenetic trees were re-constructed by using neighbor-joining (NJ) method (Kimura two-parameter model) in Mega 5.0 (http://www.megasoftware.net/), and bootstrapping was performed using 1000 replicates.

Statistical analysis

The variation in *Cryptosporidium* and *E. bieneusi* prevalence (y) of dairy calves and sika deer of different geographical location ($x1$), season ($x2$), and age ($x3$) were analyzed by Chi-square in Statistical Analysis System (SAS, Version 9.0), respectively. Results were considered statistically significant when $P < 0.05$. Odds ratios (ORs) and their 95% confidence intervals (95% CIs) were also provided.

Table 1 Distribution of *Cryptosporidium* species and *E. bieneusi* genotype in dairy calves in different farms from Heilongjiang Province

Cities	Farm IDs	No. tested	No. positive for *Cryptosporidium* (%)	*Cryptosporidium* species (No.)	No. positive for *E. bieneusi* (%)	*E. bieneusi* genotypes (No.)
Harbin	1	10	4 (13.33%)	*C. andersoni* (*n* = 1), *C. bovis* (*n* = 3)	4 (13.33%)	BEB4 (*n* = 1), J (*n* = 3)
	2	32	11 (34.36%)	*C. parvum* (*n* = 9), *C. bovis* (*n* = 1), *C. ryanane* (*n* = 1)	13 (40.63%)	BEB4 (*n* = 10), J (*n* = 3)
Tsitsihar	3	60	13 (21.67%)	*C. parvum* (*n* = 11), *C. andersoni* (*n* = 2)	9 (15%)	J (*n* = 2), I (*n* = 7)
Mudanjiang	4	3	0 (0%)	–	0 (0%)	–
Daqing	5	3	2 (66.67%)	*C. bovis* (*n* = 1), *C. andersoni* (*n* = 1)	0 (0%)	–
Suihua	6	12	0 (0%)	–	5 (41.67%)	J (*n* = 4), I (*n* = 1)
	7	40	2 (5%)	*C. ryanane* (*n* = 2)	26 (65%)	BEB4 (*n* = 3), J (*n* = 17), I (*n* = 6)
	8	24	0 (0%)	–	4 (16.67%)	BEB4 (*n* = 4)
	9	15	0 (0%)	–	0 (0%)	–
Hegang	10	9	0 (0%)	–	2 (22.22%)	I (*n* = 2)
Shuangyashan	11	50	1 (2%)	*C. parvum* (*n* = 1)	18 (36.00%)	BEB4 (*n* = 4), J (*n* = 10), I (*n* = 4)
Heihe	12	31	11 (35.48%)	*C. parvum* (*n* = 7), *C. bovis* (*n* = 2), *C. ryanane* (*n* = 2)	8 (25.81%)	I (*n* = 8)
	13	17	14 (82.35%)	*C. parvum* (*n* = 14)	1 (5.88%)	I (*n* = 1)
	14	15	4 (26.67%)	*C. parvum* (*n* = 4)	3 (20%)	J (*n* = 1), I (*n* = 2)
Total		321	62 (19.31%)	*C. parvum* (*n* = 46), *C. bovis* (*n* = 7), *C. ryanane* (*n* = 5), *C. andersoni* (*n* = 4)	93 (28.97%)	BEB4 (*n* = 22), J (*n* = 40), I (*n* = 31)

Data availability statement

Representative nucleotide sequences were submitted to GenBank under accession numbers: MH754165-MH754181 and MN056193-MN056201 for *Cryptosporidium* and MH732748-MH732751 and MN056202-MN056217 for *E. bieneusi*.

Committee of Ethics and Animal Welfare

This study was approved by the Animal Ethics Committee of Heilongjiang Bayi Agricultural University (registration protocol Mar 2017). The dairy calves and sika deer, which the feces were collected, were handled in accordance with good animal practices required by the Animal Ethics Procedures and Guidelines of the People's Republic of China.

Results

A total of 62 (19.31%) out of 321 examined fecal samples of dairy calves were test positive for *Cryptosporidium* (Table 1), whereas only 9 (1.10%) samples were *Cryptosporidium* positive among 858 sika deer samples (Table 2). All the *Cryptosporidium*-positive samples in sika deer were detected from farm 18 (*n* = 8) from Jilin Province and farm 25 (*n* = 1) from Heilongjiang Province. Pre-weaned dairy calves (22.75%, 38/167) had a higher *Cryptosporidium* infection rate than post-weaned dairy calves (15.58%, 24/154), but prevalence of *Cryptosporidium* in young sika deer (0/31) was lower than adults (9/787) (Table 3). The prevalence of *Cryptosporidium* in dairy calves collected in different seasons ranging from 1.03% (1/97) in summer to 33.67% (33/98) in spring, and the difference was statistically significant ($P < 0.0001$) (Table 3). Moreover, no *Cryptosporidium*-positive samples were found in female sika deer; whereas, 9 (1.29%) male sika deer were detected as *Cryptosporidium* positive. Sequencing and phylogenetic analysis suggested five *Cryptosporidium* species/genotypes were detected in the present study. Of which, *C. parvum* (*n* = 46), *C. ryanae* (*n* = 5), *C. bovis* (*n* = 7), and *C. andersoni* (*n* = 4) were only found in dairy calves (Table 1), and *Cryptosporidium* deer genotype (*n* = 9; 8 from farm 18 in Jilin Province and 1 from farm 25 in Heilongjiang Province) was only found in sika deer (Table 2; Fig. 1).

Of the 818 sika deer specimens analyzed, 111 (13.57%) were positive for *E. bieneusi* infection (Table 3). By sequence analysis, 6 known genotypes, namely JLD-III, JLD-IX, JLD-VII, EbpC, BEB6, and I) and 10 novel genotypes (namely LND-I and JLD-XV to JLD-XXIII) were identified (Table 2; Fig. 2). Prevalence of *E. bieneusi* in sika deer in different provinces ranges from 0% (0/106) in Inner Mongolia to 17.84% (96/538) in Jilin; the difference was statistically significant ($P < 0.0001$) (Table 3). Pre-weaned dairy calves

Table 2 Distribution of *Cryptosporidium* species and *E. bieneusi* genotype in sika deer in different farms from four provinces, northern China

Regions	Cities	Farm IDs	No. tested	No. positive for *E. bieneusi* (%)	*E. bieneusi* genotypes (No.)	No. positive for *Cryptosporidium* (%)	*Cryptosporidium* species/genotypes (No.)
Jilin	Shuangyang	17	34	0 (0%)	–	0 (0%)	–
		18	196	94 (47.96%)	BEB6 (*n* = 73), EbpC (*n* = 2), I (*n* = 1), JLD-III (*n* = 1), JLD-IX (*n* = 1), JLD-XV (*n* = 2), JLD-XVI (*n* = 1), JLD-XVII (*n* = 2), JLD-XVII (*n* = 2), JLD-XIX (*n* = 2), JLD-XX (*n* = 2), JLD-XXI (*n* = 2), JLD-XXII (*n* = 1), and JLD-XXIII (*n* = 2)	8 (4.08%)	*Cryptosporidium* deer genotype (*n* = 8)
	Changchun	19	59	0 (0%)	–	0 (0%)	–
		20	58	0 (0%)	–	0 (0%)	–
		21	60	0 (0%)	–	0 (0%)	–
		22	58	0 (0%)	–	0 (0%)	–
		23	29	2 (6.90%)	BEB6 (*n* = 1), EbpC (*n* = 1)	0 (0%)	–
		24	44	0 (0%)	–	0 (0%)	–
Heilongjiang	Yichun	25	81	13 (16.05%)	BEB6 (*n* = 10), JLD-VIII (*n* = 3)	1 (1.23%)	*Cryptosporidium* deer genotype (*n* = 1)
Liaoning	Shenyang	26	35	2 (5.71%)	LND-I (*n* = 1), JLD-XVI (*n* = 1)	0 (0%)	–
		27	58	0 (0%)	–	0 (0%)	–
Inner Mongolia	Chifeng	28	106	0 (0%)	–	0 (0%)	–
Total			818	111 (13.57%)	BEB6 (*n* = 84), EbpC (*n* = 3), I (*n* = 1), JLD-III (*n* = 1), JLD-VIII (*n* = 3), JLD-IX (*n* = 1), JLD-XV (*n* = 2), JLD-XVI (*n* = 2), JLD-XVII (*n* = 2), JLD-XVII (*n* = 2), JLD-XIX (*n* = 2), JLD-XX (*n* = 2), JLD-XXI (*n* = 2), JLD-XXII (*n* = 1), JLD-XXIII (*n* = 2), and LND-I (*n* = 1)	9 (1.1%)	*Cryptosporidium* deer genotype (*n* = 9)

(38.92%, 65/167) had a significant higher *E. bieneusi* infection rate than post-weaned dairy calves (18.18%, 28/154, $P<0.0001$), but prevalence of *E. bieneusi* in young sika deer (0%, 0/31) was lower than adults (14.10%, 111/787) (Table 3). Significant differences in infection rate were also observed among dairy calves collected during different seasons (Spring, 25.51%, 25/98; Summer, 20.62%, 20/97; Autumn, 45.35%, 39/86; Winter, 15.00%, 9/60; $P = 0.0001$; Table 3). In this study, prevalence of *E. bieneusi* in dairy calves was 28.97% (93/321), which was much higher than that in investigated sika deer (13.57%, 111/699), although only J, I, and BEB4 were identified in dairy calves (Table 1). Phylogenetic analysis revealed that EbpC was clustered into group 1d, and JLD-III, JLD-XIX, JLD-XVII, JLD-XXII, and JLD-XVI were classified in group 1a, and other 13 genotypes (JLD-IX, JLD-VII, BEB4, BEB6, I, J, LND-I, JLD-XV, JLD-IX, JLD-XXIII, JLD-XX, JLD-XVII, and JLD-XXI) were grouped in group 2 (Fig. 2).

In this study, 4 (0.49%, $n = 818$) samples were detected as co-infection of *E. bieneusi* and *Cryptosporidium* in sika deer. All the samples were adult sika deer collected from Shuangyang City. Moreover, 18 (5.61%) out of 321 dairy calves were positive to *E. bieneusi* and *Cryptosporodium* (Table 4).

Discussion

In this study, the overall prevalence of *Cryptosporidium* infection in sika deer was 1.1%, which was lower than that in sika deer in Henan (2.41%, 2/83) and Jilin (Wang et al. 2008) but higher than the 0% in red deer and Pere David's deer in Zhengzhou, China (Wang et al. 2008). It is also lower than

Table 3 Prevalence of *Cryptosporidium* and *E. bieneusi* infection in dairy calves and sika deer in different related factors

Host	Factor	Category	No. tested	*E. bieneusi*				*Cryptosporidium* spp.			
				No. positive	Prevalence (%) (95% CI)	*P* value	OR (95% CI)	No. positive	Prevalence (%) (95% CI)	*P* value	OR (95% CI)
Dairy calves	Season	Spring	98	25	25.51 (16.88–34.14)	0.0001	Reference	33	33.67 (24.32–43.03)	< 0.0001	Reference
		Summer	97	20	20.62 (12.57–28.67)		0.76 (0.39–1.48)	1	1.03 (0.00–3.04)		0.02 (0.003–0.15)
		Autumn	86	39	45.35 (34.83–55.87)		2.42 (1.30–4.51)	15	17.44 (9.42–25.46)		0.42 (0.21–0.84)
		Winter	60	9	15.00 (5.97–24.04)		0.52 (0.22–1.20)	13	21.67 (11.24–32.09)		0.55 (0.26–1.15)
	Age	Pre-weaned	167	65	38.92 (31.53–46.32)	< 0.0001	Reference	38	22.75 (16.40–29.11)	0.1040	Reference
		Post-weaned	154	28	18.18 (12.09–24.27)		0.35 (0.21–0.58)	24	15.58 (9.86–21.31)		0.63 (0.36–1.10)
	Sub-total		321	93	28.97 (24.01–33.94)			62	19.31 (15.00–23.63)		
Sika deer	Region	Jilin	538	96	17.84 (14.61–21.08)	< 0.0001	Reference	8	8.33 (2.80–13.86)	0.3968	Reference
		Liaoning	93	2	2.151 (0.00–5.10)		0.10 (0.02–0.42)	0			–
		Heilongjiang	81	13	16.05 (8.06–24.04)		0.88 (0.47–1.66)	1	1.24 (0.00–3.64)		0.83 (0.10–6.71)
		Inner Mongolia	106	0	0 (–)		–	0	0 (–)		–
	Age	Young	31	0	0 (–)	–	–	0	0 (–)	–	–
		Adult	787	111	14.10 (11.67–16.54)		–	9	1.14 (0.40–1.89)		–
	Gender	Female	119	0	0 (–)	–	–	0	0 (–)	–	–
		Male	699	111	15.88 (13.17–18.59)		–	9	1.29 (0.45–2.12)		–
	Sub-total		818	111	13.57 (11.22–15.92)			9	1.10 (0.39–1.82)		

that of the 1.3–80% prevalence of *Cryptosporidium* in other deer species in the USA, Nepal, Spain, Japan, Czech Republic, UK, Australia, and some regions of China (Deng and Cliver 1999; Feng et al. 2012; García-Presedo et al. 2013; Santin and Fayer 2015; Wells et al. 2015; Koehler et al. 2016; Kotkova et al. 2016; Kato et al. 2016; Huang et al. 2018). The overall 23.01% *Cryptosporidium* prevalence was found in dairy calves in this study, which was higher than the reported infection rates in Heilongjiang (19.15%, 158/825) (Zhao et al. 2014a), Anhui (14.9%, 52/350), Shanghai (12.5%, 55/440), Jiangsu (20.7%, 251/1215) (Chen and Qiu 2012), Henan (21.5%, 172/801) (Wang et al. 2011b), Ningxia (5.5%, 92/1688; 1.69%, 23/1366) (Huang et al. 2014; Zhang et al. 2015a), Gansu (4.6%, 58/1257) (Zhang et al. 2015a), and Shaanxi (20.2%, 52/258) (Qi et al. 2015) but lower than that in Heilongjiang (47.62%, 72/151) (Zhang et al. 2013) and Shanghai (37%, 303/818) (Cai et al. 2017). The differences in prevalence might partially be related to the timing of specimen collection, the different susceptibility of different animal species, different sample sizes, the ecological conditions, the different susceptibility of domesticated deer and wild deer, and host health status. In addition, geographical and seasonal differences can also affect the prevalence of *Cryptosporidium*.

Since *Cryptosporidium* infection in dairy cattle and sika deer were recorded, 13 genotypes/genotypes (*C. parvum*, *C. andersoni*, *C. suis*-like, *C. hominis*, *C. bovis*, *C. ryanae*, *C. ubiquitum*, *C. muris*, *Cryptosporidium* deer genotype, *Cryptosporidium* muskrat II genotype, *Cryptosporidium suis*-like genotype, *Cryptosporidium* hominis-like genotype, *Cryptosporidium* caribou genotype) have been found in sika deer worldwide (Jellison et al. 2009; Perz and Le Blancq 2001; García-Presedo et al. 2013; Kváč et al. 2014; Wells et al. 2015; Koehler et al. 2016; Kotkova et al. 2016), and at least 9 *Cryptosporidium* species/genotypes, namely *C. andersoni*, *C. bovis*, *C. parvum*, *C. ryanae*, *C. ubiquitum*, *C. meleagridis*, *C. xiaoi*, *C. serpentis*, and *C. suis-like* (Liu et al. 2009; Wang et al. 2011b; Chen and Huang 2012; Zhang et al. 2013; Cui et al. 2014; Ma et al. 2014, 2015) have been detected in cattle in China. However, only *Cryptosporidium* deer genotype was found in sika deer (Table 2) and only four *Cryptosporidium* spp., namely *C. parvum* ($n = 71$), *C. ryanae* ($n = 7$), *C. bovis* ($n = 7$), and *C. andersoni* ($n = 4$) were found in dairy calves in the present study (Table 1), which indicated that the five *Cryptosporidium* spp. were endemic in sika deer and dairy calves in investigated areas, respectively. The results of the present study also indicated that *C. parvum* (in 6 farms) was the commonest species on dairy cattle farms, which were similar with previous observations in pre-weaned calves in Ningxia (Huang et al. 2014), Henan (Wang et al. 2011b), and Xinjiang. Because *C. parvum* was frequently found in humans (Naguib et al. 2018), dairy calf has been indicated as one of the most important potential resources for human cryptosporidiosis. *Cryptosporidium* deer genotype was only

Table 4 Prevalence of *Cryptosporidium* and/or *E. bieneusi* in dairy calves and sika deer

Hosts	Agent(s)	Number positive	% positive	Total positive for any listed agent	% coinfected out of hosts with any listed agent
Dairy calves	*Cryptosporidium*	62	19.31	62	NA
	E. bieneusi	93	28.97	93	NA
	Cryptosporidium and *E. bieneusi*	18	5.61	155	11.61
Sika deer	*Cryptosporidium*	9	1.10	9	NA
	E. bieneusi	111	13.57	111	NA
	Cryptosporidium and *E. bieneusi*	4	0.49	116	3.45

identified in deer, including Red deer (UK), Roe deer (UK), White-tailed deer (USA, Czech Republic), and sika deer (Japan, China) (Robinson et al. 2011; Santin and Fayer 2015; Wells et al. 2015; Kotkova et al. 2016; Kato et al. 2016; Huang et al. 2018), which suggested that it seems to be a host-adapted genotype. Other deer-derived *Cryptosporidium* species/genotypes (including *C. parvum*) were not found in this study, which suggested that more sample size should be collected for further studies.

Presently, the prevalence of *E. bieneusi* in sika deer was 13.57% (111/818), which was higher than that in white-tailed deer in New York, USA (12.2%, 6/49) (Guo et al. 2014), sika deer in Jilin Province (7.1%, 23/326), China (Zhang et al. 2016), sambar deer (*Rusa unicolor*) (0%, 0/3), fallow deer

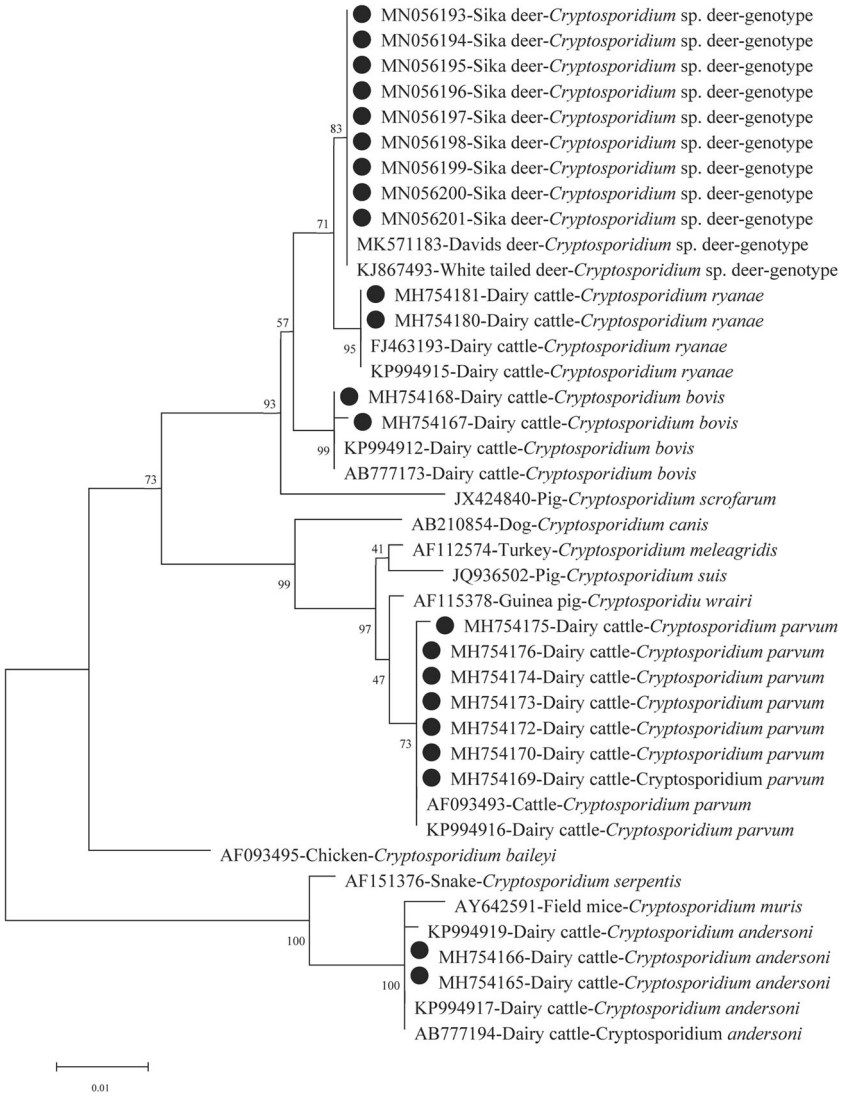

Fig. 1 Neighbor-joining (NJ) phylogenetic analyses of *Cryptosporidium* spp. based on the 830-bp sequence of the small subunit (SSU) rRNA gene (Kimura two-parameter model, 1000 replicates). *Cryptosporidium* isolates identified in this study are pointed out by a *solid circle*

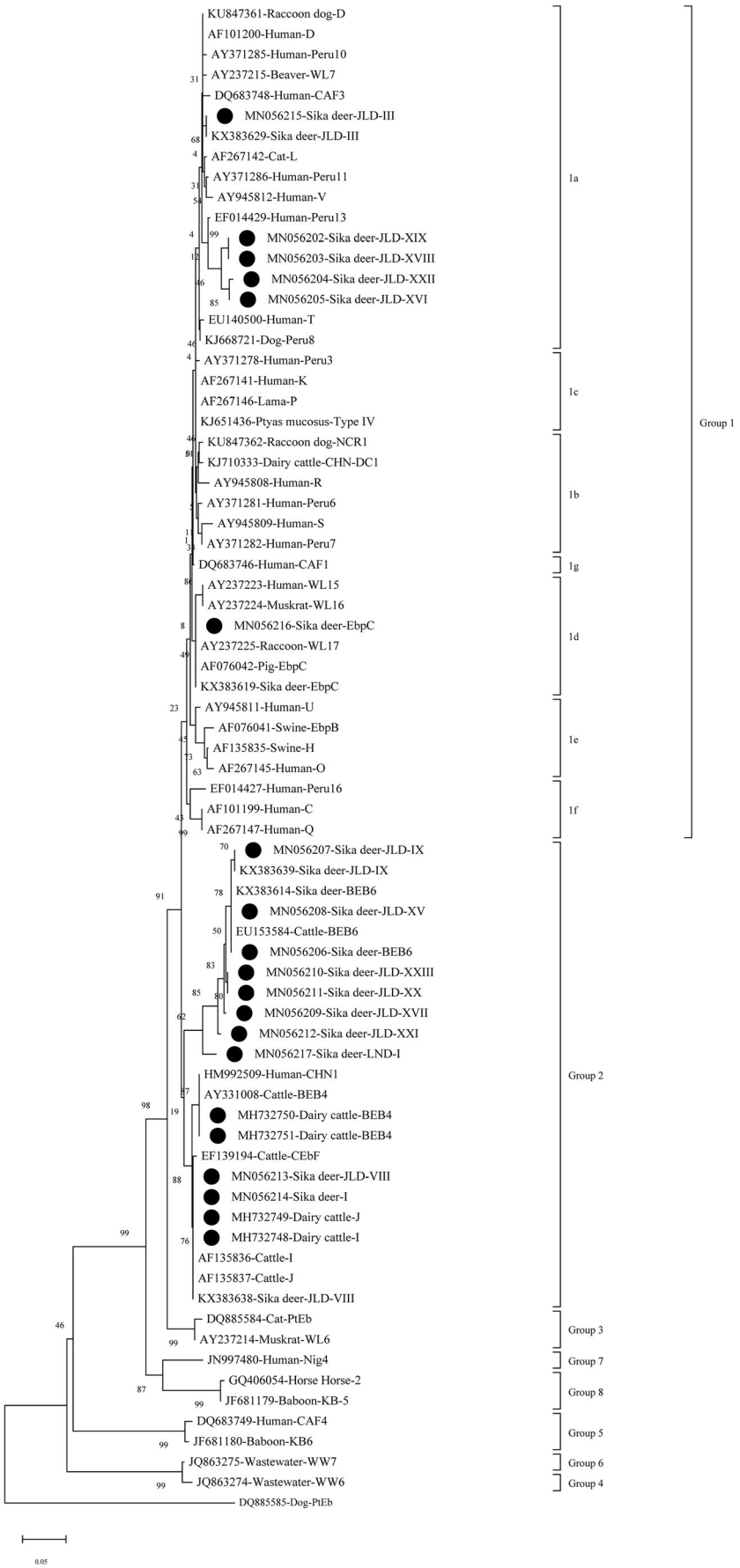

Fig. 2 Phylogenetic analyses of *Enterocytozoon bieneusi* using neighbor-joining (NJ) method (Kimura two-parameter model, 1000 replicates). *E. bieneus* identified in this study are indicated by a *solid circle*

(*Dama dama*) (0%, 0/7), and Pere David's deer (*Elaphurus davidianus*) (0%, 0/3) in Sichuan, China (Li et al. 2016b), and sambar deer (*Rusa unicolor*) (4.1%, 25/516), red deer (*Cervus elaphus*) (0%, 0/77), and fallow deer (*Dama dama*) (0%, 0/17) in Australia (Zhang et al. 2018b) but lower than the 17.04–75% prevalence of *E. bieneusi* in various species of deer in the USA (Santin and Fayer 2015), Korea (Amer et al. 2019), and China (Zhao et al. 2014b; Zhang et al. 2015b; Li et al. 2016b; Huang et al. 2017; Song et al. 2018). In the present study, only four farms were positive for *E. bieneusi*, with infection rates ranging between 5.71 and 47.96%. The highest infection rate in sika deer was found in farm 18 (47.96%, 94/196), while the lowest (0%) was in farms 17 (0/34), 19 (0/59), 20 (0/58), 21 (0/60), 22 (0/58), 24 (0/44), 27 (0/58), and 28 (0/106) (Table 2). The overall infection rate of *E. bieneusi* was 30.16% (122/386) in dairy calves, which was lower than that in cattle in Jilin (37.6%, 35/93) (Zhang et al. 2011), but it was higher than that in cattle in Shandong (2.0%, 3/148) (Ma et al. 2015) and Heilongjiang (30.1%, 40/133 and 5.89%, 31/526) (Zhao et al. 2015; Jiang et al. 2015) and calves in Shanghai (26.5%, 214/809) (Tang et al. 2018) and Shaanxi 19.68% (73/371) (Wang et al. 2016). The infection rate in this study was higher than in most parts of China, which may be due to the difference in detection methods, the different susceptibility of different animal species, diagnostic methods, study design, and climate. *E. bieneusi*-positive samples were detected in 13 dairy cattle farms (all in Heilongjiang) and 4 sika deer farms (2 from Jilin, 1 from Heilongjiang, 1 from Liaoning) in this study, which may be due to worse livestock management, host health status, and animal welfare in these farms. Moreover, all the investigated sika deer were collected in farms in the present study which differ from most samples of deer reported previously which are collected from wild deer. In addition, the breeding periods of these deer in each farm may also affect the pathogen transmission in the farm, which will be investigated in further studies.

Sequence analysis revealed that three known genotypes were detected in dairy calves, including J (n = 63), I (n = 36), and BEB4 (n = 23) (Table 1). We found genotype J was predominant in dairy calves in this study. Genotype J was also the dominant genotype found in dairy cattle in Xinjiang (Qi et al. 2017), Henan, and Ningxia (Li et al. 2016a). Meanwhile, similar results have been reported in pre-weaned calves in Shanghai (Tang et al. 2018). In contrast, other results reported genotype I was the dominant genotype in dairy cattle in Henan and Shandong (Ma et al. 2015) and in beef and dairy cattle in Shaanxi (Wang et al. 2016), genotype O was the dominant genotype in dairy cattle in Heilongjiang (Zhao et al. 2015), and genotype CHN3 was the dominant genotype in cows in Jilin (Zhang et al. 2011). The differences in the distribution of *E. bieneusi* genotypes in cattle from different areas in China may be because of geographical and host segregation. More than 60 *E. bieneusi* genotypes have been found in deer worldwide (Guo et al. 2014; Zhao et al. 2014b; Santin and Fayer 2015; Zhang et al. 2015b, 2016, 2018b; Li et al. 2016b; Huang et al. 2017; Song et al. 2018; Amer et al. 2019). However, only 16 genotypes, including 6 known genotypes (JLD-III, JLD-IX, JLD-VII, EbpC, BEB6, and I) and 10 novel genotypes (namely LND-I and JLD-XV to JLD-XXIII), were identified in this study. Genotypes BEB4, J, I, and BEB6, clustered in group 2, were widely found in lots of animals; however, all of them have also been found in humans (Huang et al. 2017; Yang et al. 2018). Genotype EbpC, known to cause human microsporidiosis (Liu et al. 2017; Huang et al. 2017), and genotypes JLD-III, JLD-XIX, JLD-XVII, JLD-XXII, and JLD-XVI clustered in group 1, have zoonotic potential. The findings suggested that the investigated dairy calves and sika deer may play an important role in the transmission of *E. bieneusi* to humans and other animals. Further studies will investigate the molecular characterization of *E. bieneusi* in these animals' feeders in order to further prove the evidence of transmission of *E. bieneusi* between humans and dairy calves or sika deer.

Conclusion

Cryptosporidium and *E. bieneusi* infection frequently occurred in dairy cattle and sika deer in Northern China. Co-infection of *Cryptosporidium* and *E. bieneusi* was present in investigated dairy calves and sika deer. Five *Cryptosporidium* species/genotypes were identified, of which *C. parvum*, *C. ryanae*, *C. bovis*, and *C. andersoni* were only found in dairy calves, and only *Cryptosporidium* deer genotype was found in sika deer. Moreover, J, I, and BEB4 ITS genotypes of *E. bieneusi* were found in dairy calves; 6 known genotypes (JLD-III, JLD-IX, JLD-VII, EbpC, BEB6, and I) and 10 novel genotypes (namely LND-I, JLD-XV to JLD-XXIII) were found in sika deer in this study. *C. parvum* and *E. bieneusi* genotype J were identified as the predominant species/genotypes in dairy calves, whereas the predominance of *Cryptosporidium* spp. and *E. bieneusi* in sika deer was *Cryptosporidium* deer genotype and BEB6, respectively. Genotypes EbpC, JLD-III, JLD-XIX, JLD-XVII, JLD-XXII, and JLD-XVI were clustered in group 1; the members of which have zoonotic potential, which also suggested that the investigated dairy calves and sika deer may play an important role in the transmission of *Cryptosporidium* and *E. bieneusi* to humans.

Funding information Project support was provided by the Heilongjiang Provincial Key Laboratory of Prevention and Control of Bovine Diseases (PCBD201706). No competing financial interests exist. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the funding agencies.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Amer S, Kim S, Han JI, Na KJ (2019) Prevalence and genotypes of *Enterocytozoon bieneusi* in wildlife in Korea: a public health concern. Parasit Vectors 12:160

Baroudi D, Zhang H, Amer S, Khelef D, Roellig DM, Wang Y, Feng Y, Xiao L (2018) Divergent *Cryptosporidium parvum* subtype and *Enterocytozoon bieneusi* genotypes in dromedary camels in Algeria. Parasitol Res 117:905–910

Cai M, Guo Y, Pan B, Li N, Wang X, Tang C, Feng Y, Xiao L (2017) Longitudinal monitoring of *Cryptosporidium* species in preweaned dairy calves on five farms in Shanghai, China. Vet Parasitol 241:14–19

Chalmers RM, Katzer F (2013) Looking for *Cryptosporidium*: the application of advances in detection and diagnosis. Trends Parasitol 9: 237–251

Checkley W, White AC Jr, Jaganath D, Arrowood MJ, Chalmers RM, Chen XM, Fayer R, Griffiths JK, Guerrant RL, Hedstrom L, Huston CD, Kotloff KL, Kang G, Mead JR, Miller M, Petri WA Jr, Priest JW, Roos DS, Striepen B, Thompson RC, Ward HD, Van Voorhis WA, Xiao L, Zhu G, Houpt ER (2015) A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for *Cryptosporidium*. Lancet Infect Dis 15:85–94

Chen F, Qiu H (2012) Identification and characterization of a chinese isolate of *Cryptosporidium serpentis* from dairy cattle. Parasitol Res 111:1785–1791

Chen F, Huang K (2012) Prevalence and molecular characterization of *Cryptosporidium* spp. in dairy cattle from farms in China. J Vet Sci 13:15–22

Cui Z, Wang R, Huang J, Wang H, Zhao J, Luo N, Li J, Zhang Z, Zhang L (2014) Cryptosporidiosis caused by *Cryptosporidium parvum* subtype IIdA15G1 at a dairy farm in Northwestern China. Parasit Vectors 7:529

Del Coco VF, Córdoba MA, Bilbao G, de Almeida CP, Basualdo JA, Santín M (2014) First report of *Enterocytozoon bieneusi* from dairy cattle in Argentina. Vet Parasitol 199:112–115

Deng MQ, Cliver DO (1999) Improved immunofluorescence assay for detection of *Giardia* and *Cryptosporidium* from asymptomatic adult cervine animals. Parasitol Res 85:733–736

Feng Y, Karna SR, Dearen TK, Singh DK, Adhikari LN, Shrestha A, Xiao L (2012) Common occurrence of a unique *Cryptosporidium ryanae* variant in zebu cattle and water buffaloes in the buffer zone of the Chitwan National Park, Nepal. Vet Parasitol 185:309–314

García-Presedo I, Pedraza-Díaz S, González-Warleta M, Mezo M, Gómez-Bautista M, Ortega-Mora LM, Castro-Hermida JA (2013) The first report of *Cryptosporidium bovis*, *C. ryanae* and *Giardia duodenalis* sub-assemblage A-II in roe deer (*Capreolus capreolus*) in Spain. Vet Parasitol 197:658–664

Guo Y, Alderisio KA, Yang W, Cama V, Feng Y, Xiao L (2014) Host specificity and source of *Enterocytozoon bieneusi* genotypes in a drinking source watershed. Appl Environ Microbiol 80:218–225

Hu S, Liu Z, Yan F, Zhang Z, Zhang G, Zhang L, Jian F, Zhang S, Ning C, Wang R (2017) Zoonotic and host-adapted genotypes of *Cryptosporidium* spp., *Giardia duodenalis* and *Enterocytozoon bieneusi* in dairy cattle in Hebei and Tianjin, China. Vet Parasitol 248:68–73

Huang J, Yue D, Qi M, Wang R, Zhao J, Li J, Shi K, Wang M, Zhang L (2014) Prevalence and molecular characterization of *Cryptosporidium* spp. and *Giardia duodenalis* in dairy cattle in Ningxia, northwestern China. BMC Vet Res 10:292

Huang J, Zhang Z, Yang Y, Wang R, Zhao J, Jian F, Ning C, Zhang L (2017) New genotypes of *Enterocytozoon bieneusi* isolated from sika deer and red deer in China. Front Microbiol 8:879

Huang J, Zhang Z, Zhang Y, Yang Y, Zhao J, Wang R, Jian F, Ning C, Zhang W, Zhang L (2018) Prevalence and molecular characterization of *Cryptosporidium* spp. and *Giardia duodenalis* in deer in Henan and Jilin, China. Parasit Vectors 11:239

Jellison KL, Lynch AE, Ziemann JM (2009) Source tracking identifies deer and geese as vectors of human-infectious *Cryptosporidium* genotypes in an urban/suburban watershed. Environ Sci Technol 43:4267–4272

Jiang Y, Tao W, Wan Q, Li Q, Yang Y, Lin Y, Zhang S, Li W (2015) Zoonotic and potentially host-adapted *Enterocytozoon bieneusi* genotypes in sheep and cattle in Northeast China and an increasing concern about the zoonotic importance of previously considered ruminant-adapted genotypes. Appl Environ Microbiol 81:3326–3335

Kato S, Yanagawa Y, Matsuyama R, Suzuki M, Sugimoto C (2016) Molecular identification of the *Cryptosporidium* deer genotype in the Hokkaido sika deer (*Cervus nippon yesoensis*) in Hokkaido, Japan. Parasitol Res 115:1463–1471

Koehler AV, Haydon SR, Jex AR, Gasser RB (2016) *Cryptosporidium* and *Giardia* taxa in faecal samples from animals in catchments supplying the city of Melbourne with drinking water (2011 to 2015). Parasit Vectors 9:315

Kotkova M, Nemejc K, Sak B, Hanzal V, Kvetonova D, Hlaskova L, Condlova S, McEvoy J, Kvac M (2016) *Cryptosporidium ubiquitum*, *C. muris* and *Cryptosporidium* deer genotype in wild cervids and caprines in the Czech Republic. Folia Parasitol (Praha) 25:63

Kváč M, McEvoy J, Stenger B, Clark M (2014) Cryptosporidiosis in other vertebrates. In: Cacciò SM, Widmer G (eds) *Cryptosporidium*: parasite and disease. Springer, Vienna, pp 237–323

Li J, Luo N, Wang C, Qi M, Cao J, Cui Z, Huang J, Wang R, Zhang L (2016a) Occurrence, molecular characterization and predominant genotypes of *Enterocytozoon bieneusi* in dairy cattle in Henan and Ningxia, China. Parasit Vectors 9:142

Li W, Deng L, Yu X, Zhong Z, Wang Q, Liu X, Niu L, Xie N, Deng J, Lei S, Wang L, Gong C, Zhou Z, Hu Y, Fu H, Xu H, Geng Y, Peng G (2016b) Multilocus genotypes and broad host-range of *Enterocytozoon bieneusi* in captive wildlife at zoological gardens in China. Parasit Vectors 9:395

Liu A, Wang R, Li Y, Zhang L, Shu J, Zhang W, Feng Y, Xiao L, Ling H (2009) Prevalence and distribution of *Cryptosporidium* spp. in dairy cattle in Heilongjiang Province, China. Parasitol Res 105:797–802

Liu H, Jiang Z, Yuan Z, Yin J, Wang Z, Yu B, Zhou D, Shen Y, Cao J (2017) Infection by and genotype characteristics of *Enterocytozoon bieneusi* in HIV/AIDS patients from Guangxi Zhuang autonomous region, China. BMC Infect Dis 17:684

Lombardelli JA, Tomazic ML, Schnittger L, Tiranti KI (2019) Prevalence of *Cryptosporidium parvum* in dairy calves and GP60 subtyping of diarrheic calves in central Argentina. Parasitol Res 118:2079–2086

Ma J, Cai J, Ma J, Feng Y, Xiao L (2014) Occurrence and molecular characterization of *Cryptosporidium* spp. in yaks (*Bos grunniens*) in China. Vet Parasitol 202:113–118

Ma J, Li P, Zhao X, Xu H, Wu W, Wang Y, Guo Y, Wang L, Feng Y, Xiao L (2015) Occurrence and molecular characterization of Cryptosporidium spp. and Enterocytozoon bieneusi in dairy cattle, beef cattle and water buffaloes in China. Vet Parasitol 207:220–227

Mercado R, Peña S, Ozaki LS, Fredes F, Godoy J (2015) Multiple *Cryptosporidium parvum* subtypes detected in a unique isolate of a Chilean neonatal calf with diarrhea. Parasitol Res 114:1985–1988

Naguib D, El-Gohary AH, Roellig D, Mohamed AA, Arafat N, Wang Y, Feng Y, Xiao L (2018) Molecular characterization of *Cryptosporidium* spp. and *Giardia duodenalis* in children in Egypt. Parasit Vectors 11:403

Naguib D, El-Gohary AH, Roellig D, Mohamed AA, Arafat N, Wang Y, Feng Y, Xiao L (2015) Epidemiology and molecular characterization of *Cryptosporidium* spp. in humans, wild primates, and domesticated animals in the Greater Gombe Ecosystem, Tanzania. PLoS Negl Trop Dis 9:e0003529

Parsons MB, Travis D, Lonsdorf EV, Lipende I, Roellig DM, Collins A, Kamenya S, Zhang H, Xiao L, Gillespie TR (2015) Epidemiology and molecular characterization of Cryptosporidium spp. in humans, wild primates, and domesticated animals in the Greater Gombe Ecosystem, Tanzania. PLoS Negl Trop Dis 9:e0003529

Pawlowic MC, Vinayak S, Sateriale A, Brooks CF, Striepen B (2017) Generating and maintaining transgenic *Cryptosporidium parvum* parasites. Curr Protoc Microbiol 46:20B.2.1–20B.2.32

Perz JF, Le Blancq SM (2001) *Cryptosporidium parvum* infection involving novel genotypes in wildlife from lower New York State. Appl Environ Microbiol 67:1154–1162

Qi MZ, Fang YQ, Wang XT, Zhang LX, Wang RJ, Du SZ, Guo YX, Jia YQ, Yao L, Liu QD, Zhao GH (2015) Molecular characterization of *Cryptosporidium* spp. in pre-weaned calves in Shaanxi Province, north-western China. J Med Microbiol 64:111–116

Qi M, Jing B, Jian F, Wang R, Zhang S, Wang H, Ning C, Zhang L (2017) Dominance of *Enterocytozoon bieneusi* genotype J in dairy calves in Xinjiang, Northwest China. Parasitol Int 66:960–963

Robinson G, Chalmers RM, Stapleton C, Palmer SR, Watkins J, Francis C, Kay D (2011) A whole water catchment approach to investigating the origin and distribution of *Cryptosporidium* species. J Appl Microbiol 111:717–730

Santín M, Trout JM, Xiao L, Zhou L, Greiner E, Fayer R (2004) Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. Vet Parasitol 122:103–117

Santin M, Fayer R (2015) *Enterocytozoon bieneusi*, *Giardia*, and *Cryptosporidium* infecting white-tailed deer. J Eukaryot Microbiol 62:34–43

Song Y, Li W, Liu H, Zhong Z, Luo Y, Wei Y, Fu W, Ren Z, Zhou Z, Deng L, Cheng J, Peng G (2018) First report of *Giardia duodenalis* and *Enterocytozoon bieneusi* in forest musk deer (*Moschus berezovskii*) in China. Parasit Vectors 11:204

Tang C, Cai M, Wang L, Guo Y, Li N, Feng Y, Xiao L (2018) Genetic diversity within dominant *Enterocytozoon bieneusi* genotypes in pre-weaned calves. Parasit Vectors 11:170

Wang R, Wang J, Sun M, Dang H, Feng Y, Ning C, Jian F, Zhang L, Xiao L (2008) Molecular characterization of the *Cryptosporidium* cervine genotype from a sika deer (*Cervus nippon Temminck*) in Zhengzhou, China and literature review. Parasitol Res 103:865–869

Wang R, Wang H, Sun Y, Zhang L, Jian F, Qi M, Ning C, Xiao L (2011a) Characteristics of *Cryptosporidium* transmission in preweaned dairy cattle in Henan, China. J Clin Microbiol 49:1077–1082

Wang R, Ma G, Zhao J, Lu Q, Wang H, Zhang L, Jian F, Ning C, Xiao L (2011b) *Cryptosporidium andersoni* is the predominant species in post-weaned and adult dairy cattle in China. Parasitol Int 60:1–4

Wang R, Zhao G, Gong Y, Zhang L (2017) Advances and perspectives on the epidemiology of bovine *Cryptosporidium* in China in the past 30 years. Front Microbiol 8:1823

Wang XT, Wang RJ, Ren GJ, Yu ZQ, Zhang LX, Zhang SY, Lu H, Peng XQ, Zhao GH (2016) Multilocus genotyping of *Giardia duodenalis* and *Enterocytozoon bieneusi* in dairy and native beef (Qinchuan) calves in Shaanxi province, northwestern China. Parasitol Res 115: 1355–1361

Wells B, Shaw H, Hotchkiss E, Gilray J, Ayton R, Green J, Katzer F, Wells A, Innes E (2015) Prevalence, species identification and genotyping *Cryptosporidium* from livestock and deer in a catchment in the Cairngorms with a history of a contaminated public water supply. Parasit Vectors 8:66

Wu J, Han JQ, Shi LQ, Zou Y, Li Z, Yang JF, Huang CQ, Zou FC (2018) Prevalence, genotypes, and risk factors of *Enterocytozoon bieneusi* in Asiatic black bear (*Ursus thibetanus*) in Yunnan Province, Southwestern China. Parasitol Res 117:1139–1145

Yang H, Mi R, Cheng L, Huang Y, An R, Zhang Y, Jia H, Zhang X, Wang X, Han X, Chen Z (2018) Prevalence and genetic diversity of *Enterocytozoon bieneusi* in sheep in China. Parasit Vectors 11:587

Zhang X, Wang Z, Su Y, Liang X, Sun X, Peng S, Lu H, Jiang N, Yin J, Xiang M, Chen Q (2011) Identification and genotyping of *Enterocytozoon bieneusi* in China. J Clin Microbiol 49:2006–2008

Zhang XX, Tan QD, Zhou DH, Ni XT, Liu GX, Yang YC, Zhu XQ (2015a) Prevalence and molecular characterization of *Cryptosporidium* spp. in dairy cattle, northwest China. Parasitol Res 114:2781–2787

Zhang Z, Huang J, Karim MR, Zhao J, Dong H, Ai W, Li F, Zhang L, Wang R (2015b) Zoonotic *Enterocytozoon bieneusi* genotypes in Pere David's deer (*Elaphurus davidianus*) in Henan, China. Exp Parasitol 155:46–48

Zhang XX, Cong W, Liu GH, Ni XT, Ma JG, Zheng WB, Zhao Q, Zhu XQ (2016) Prevalence and genotypes of *Enterocytozoon bieneusi* in sika deer in Jilin province, Northeastern China. Acta Parasitol 61: 382–388

Zhang Y, Koehler AV, Wang T, Robertson GJ, Bradbury RS, Gasser RB (2018a) *Enterocytozoon bieneusi* genotypes in people with gastrointestinal disorders in Queensland and Western Australia. Infect Genet Evol 65:293–299

Zhang Y, Koehler AV, Wang T, Haydon SR, Gasser RB (2018b) First detection and genetic characterisation of *Enterocytozoon bieneusi* in wild deer in Melbourne's water catchments in Australia. Parasit Vectors 11:2

Zhang W, Wang R, Yang F, Zhang L, Cao J, Zhang X, Ling H, Liu A, Shen Y (2013) Distribution and genetic characterizations of *Cryptosporidium* spp. in pre-weaned dairy calves in Northeastern China's Heilongjiang Province. PLoS ONE 8:e54857

Zhao W, Wang R, Zhang W, Liu A, Cao J, Shen Y, Yang F, Zhang L (2014a) MLST subtypes and population genetic structure of *Cryptosporidium andersoni* from dairy cattle and beef cattle in northeastern China's Heilongjiang Province. PLoS ONE 9:e102006

Zhao W, Zhang W, Wang R, Liu W, Liu A, Yang D, Yang F, Karim MR, Zhang L (2014b) *Enterocytozoon bieneusi* in sika deer (*Cervus nippon*) and red deer (*Cervus elaphus*): deer specificity and zoonotic potential of ITS genotypes. Parasitol Res 113:4243–4250

Zhao W, Zhang W, Yang F, Zhang L, Wang R, Cao J, Shen Y, Liu A (2015) *Enterocytozoon bieneusi* in dairy cattle in the Northeast of China: genetic diversity of ITS gene and evaluation of zoonotic transmission potential. J Eukaryot Microbiol 62:553–560