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Prevalence and diversity of *Cryptosporidium* spp. in bamboo rats (*Rhizomys sinensis*) in South Central China

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ABSTRACT

Cryptosporidium is one of the most prevalent zoonotic parasites and is responsible for the high burden of diarrheal disease across the globe. Rodents are globally overpopulated and are reservoirs for a variety of zoonotic pathogens. Bamboo rats are a common species of rodent that are bred for meat and wool in China. However, the genetic characterization of *Cryptosporidium* in bamboo rats in China is limited. The aim of this study was to determine the occurrence and genetic characterization of *Cryptosporidium* in bamboo rats from South Central China. From February2017to February 2018, 435 fecal samples were collected from bamboo rats in 13 farms located in 12 cities in South Central China. All fecal specimens were examined for *Cryptosporidium* by PCR, and through sequencing the partial small subunit of ribosomal DNA (SSU rRNA). *C. parvum*-positive samples were further subtyped through analysis of the 60-kDa glycoprotein (*gp60*) gene sequence. Meanwhile, all the new *Cryptosporidium* genotypes samples were selected for further sequence characterization at the 70-kDa heat shock protein (*HSP70*) gene and oocyst wall protein (*COWP*) gene as well as *gp60* gene. Infection rates of 2.1% (9/435) were recorded for *Cryptosporidium*. Sequence analysis confirmed the presence of two *Cryptosporidium* species including *C. parvum* (n = 2), *C. occultus* (n = 1) and two new *Cryptosporidium* genotypes termed *Cryptosporidium* bamboo rat genotype I (n = 5) and *Cryptosporidium* bamboo rat genotype II (n = 1). Two subtypes of *C. parvum* were identified including IIdA15G1 and IIpA19 (one each).The discovery of zoonotic *Cryptosporidium* species/genotypes in bamboo rats suggests they have significant zoonotic potential and pose a threat to human health. The novel sequences discovered provide new insight into genotypic variations in *Cryptosporidium* in bamboo rats.

1. Introduction

Cryptosporidium spp. are one of the most important intestinal protozoan in humans (Ryan et al., 2014). The parasite causes diarrhea in children, the elderly, and AIDS patients, and is considered a neglected disease by the World Health Organization (WHO) (Abdoli et al., 2018). In addition to infecting humans, *Cryptosporidium* is prevalent in a variety of animal species, including domestic and wild animals (Pumipuntu and Piratae, 2018). *Cryptosporidium* is transmitted in humans and animals through the fecal-oral route either directly through person-to-person, though contact with infected animals, or indirectly via food-borne or waterborne transmission following ingestion of contaminated food or water (Efstratiou et al., 2017; Ryan et al., 2018). Although the epidemiology of the disease in humans and animals in developing countries is poorly understood, *Cryptosporidium* has been suggested as an important diarrheal pathogen and a global public health concern (Checkley et al., 2015).

Understanding the host-adaptive nature of different species/genotypes of these parasites can benefit our understanding of the potential infection sources and routes of transmission (Feng et al., 2018). To date, 39 valid *Cryptosporidium* species and approximately 70 *Cryptosporidium* genotypes have been reported (Feng et al., 2018; Ryan et al., 2014; Holubová et al., 2019). Amongst them, 21 *Cryptosporidium* species/genotypes have been isolated in humans, five of which (*C. hominis*, *C.*

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parvum, *C. meleagridis*, *C.ubiquitum* and *C. cuniculus*) are commonly found in humans (Khan et al., 2017). Whilst molecular epidemiological investigations of animal cryptosporidiosis have gained interest, studies have focused on farm animals (pigs, sheep and cattle) or pets (dogs and cats) (Ryan et al., 2016). Molecular epidemiological data revealed the presence of at least 11 species and 20genotypes of *Cryptosporidium* in rodents, with 17 species or genotypes found in rats, including *C. parvum*, *C. ubiquitum*, *C. muris*, *C. andersoni*, *C. proliferans*, *C. scrofarum*, *C. meleagridis*, *C. occultus*, *C. viatorum*and *C. tyzzeri*, *C. canis*and *Cryptosporidium* rat genotypes I to IV, *Cryptosporidium* pika genotype and *Cryptosporidium* Qinghai vole genotype (Koehler et al., 2018; Zhao et al., 2018; Zhang et al., 2018; Kimura et al., 2007). Among them, *C. parvum*, *C. muris* and rat genotype III are most frequent in rats, implicating them as major sources of human infection (Koehler et al., 2018). Despite this knowledge, only a limited number of species of farmed rats have been surveyed for *Cryptosporidium* spp. at the molecular level (Koehler et al., 2018; Feng, 2010). It is unclear whether farmed rats can be infected with *Cryptosporidium* and whether zoonotic species or genotypes exist in these animals.

Currently, bamboo rats have been bred in South Central China to provide meat and wool. No information on *Cryptosporidium* infection is available in bamboo rats excluding the Sichuan Province of China which was limited to a small geographical area (a pet market in Ya'an, Sichuan Province) (Liu et al., 2015). It is unclear whether bamboo rats are infected with *Cryptosporidium* and whether zoonotic species or genotypes exist in these animals or in other areas of China. The aim of this study was to reveal the infection status of *Cryptosporidium* spp. In farmed bamboo rats from 13 farms in 12 cities of 5 provinces in China. We aimed to determine the prevalence of natural infections and genetically characterize *Cryptosporidium* spp. Using PCR and sequence analysis, and assess the risk of zoonotic transmission at the species/genotype or subtype level.

2. Materials and methods

2.1. Ethical approval

Prior to the studies, farm owners were contacted for permission to obtain their animals. The study was conducted in accordance with the Chinese Laboratory Animal Administration Act (1988). Protocols were reviewed and approved by the Research Ethics Committee of Tarim University.

2.2. Sample collection

From February2017to February 2018, 435 fecal samples were collected from bamboo rats in 13 farms located in 12 cities in South Central China (Table 1). All bamboo rat samples were maintained in small concrete tanks. Fecal samples were collected from tanks housing 1–6 bamboo rats. To minimize the chance of duplicate sampling of animals, only one fecal specimen was collected at one location of the ground in each tank. Approximately 30% of the total fecal samples were collected. One fresh fecal specimen (approximately 15 g) from each concrete tank was collected and transferred into 2.5% potassium dichromate solution. Tubes were labeled according to date, site, and number. DNA was extracted from the samples within one week.

2.3. DNA extraction

To remove potassium dichromate from the fecal samples, 3–5 mL of each sample was washed with distilled water and passed through a wire mesh sieve with a pore size of 250 μm. Samples were washed a further three times in distilled water and centrifuged at 5000 × *g* for 1 min. Genomic DNA was extracted from approximately 200 mg of each sample using the E.Z.N.A.® Stool DNA Kits (Omega Biotek Inc, Norcross, USA) according to the manufacturer's instructions. Samples were stored at −20 °C prior to PCR amplification.

2.4. Cryptosporidium genotyping and subtyping

All genomic DNA samples were subjected to nested PCR targeting *Cryptosporidium* by amplification of an 830 bp nucleotide fragment of the small subunit (SSU) rRNA of *Cryptosporidium.* Primers were designed as previously described (Xiao et al., 1999). *C. parvum* was further subtyped by nested PCR amplification of an 800–850 bp fragment of the 60-kDa glycoprotein (*gp60*) (Alves et al., 2003). All the isolates of *Cryptosporidium*-positive samples were selected for further sequence characterization at the 70-kDa heat shock protein (*HSP70*) gene, oocyst wall protein (*COWP*) gene and actin gene (Sulaiman et al., 2000, 2002; Xiao et al., 2000). PCR mixtures (25 μL total volume) were prepared for amplification of the SSU rRNA, *HSP70*, *COWP* and *gp60* genes. PCRs consisted of 1 μL of genomic DNA for primary PCRs, and 1 μL of amplification product for secondary PCRs, 12.5 μL of 2 × Easy Taq PCR SuperMix (Trans Gene Biotech Co. Ltd., Beijing, China), 0.3 μM forward and reverse primers, and 10.9 μL of deionized water. All PCRs included positive controls (chicken-derived *C. bailey* DNA) and negative controls (containing no template DNA). Secondary PCR products were visualized on 1.5% agarosegels stained with GelRed (Biotium Inc., Hayward, CA, USA) prior to sequencing.

2.5. Sequencing and phylogenetic analysis

Positive secondary PCR products forSSU rRNA and *gp60*of *Cryptosporidium* spp. Were commercially sequenced (GENEWIZ, Suzhou, China).Sequence accuracy wasconfirmed by bi-directional sequencing. Sequences were compared with reference sequences downloaded from the National Center for Biotechnology (https://www.ncbi.nlm.nih.gov/) with Clustal X 2.0 (http://www.clustal.org/) to determine the species/subtype of *Cryptosporidium* spp. Phylogenetic analyses of the four loci were performed using the neighbor joining model in MEGA 6. Bootstrap analysis was used to assess the robustness of the clusters using 1000 replicates.

2.6. Nucleotide sequence accession numbers

Representative sequences of *Cryptosporidium* were deposited into GenBank under accession numbers MK731960 to MK731963 (SSU rRNA), MK731964 to MK731966 (*gp60*), MK731967 (*COWP*), MK731968 and MK731969 (*HSP70*) and MN065774 (actin).

3. Results

3.1. Infection rates of cryptosporidium

A total of 435 fecal samples were subjected to nested-PCR to determine the presence of *Cryptosporidium* spp. Based on the SSU rRNA genes. Nine samples were positive for *Cryptosporidium* spp., with an infection rate of 2.1%. *Cryptosporidium* was found in three farms, with infection rates ranging from 4.2 to 13.6%. The farms were in the Hunan (two farms) and Guangdong Province (one farm) (Table 1).

3.2. Cryptosporidium species/genotypes

Sequence analysis of the SSU rRNA products of nine *Cryptosporidium* isolates, four *Cryptosporidium* species, or genotypes including *C. parvum* (2; 22.2%), *C. occultus* (1; 11.1%) and two new genotypes named *Cryptosporidium* bamboo rat genotype I (5; 55.5%) and *Cryptosporidium* bamboo rat genotype II (1; 11.1%) were identified (Table 1). *Cryptosporidium* bamboo rat genotype I was dominant in bamboo rats, and was identified in two farms from the Yongzhou, Hunan Province. A single *C. parvum* isolate was found in the Luoding farm, whilst another was identified in Yongzhou farm 2. *C. occultus* was found in a single bamboo

Table 1
Prevalence and distribution of*Cryptosporidium* species/genotypes and subtypes in bamboo rats in China.

Province of China	Farms	No. samples	*Cryptosporidium*		
			No. positive samples (%)	Species/genotype(s) (n)	Subtype(s) (n)
Chongqing	Jijiang	50	0	-	-
Guangdong	Luoding	24	1 (4.2)	*C. parvum* (1)	IIpA9 (1)
	Huazhou	22	0	-	-
Guangxi	Hechi	34	0	-	-
	Fusui	60	0	-	-
	Chongzuo	48	0	-	-
Hunan	Yueyang	29	0	-	-
	Yongzhou1	34	2 (5.9)	Bamboo rat genotype (2)	-
	Yongzhou2	44	6 (13.6)	*C. parvum* (1); Bamboo rat genotype I (3); Bamboo rat genotype II (1); *C. occultus* (1)	IIdA15G1 (1)
Jiangxi	Ji'an	18	0	-	-
	Yugan	24	0	-	-
	Shinao	21	0	-	-
	Pingxiang	27	0	-	-
Total		435	9 (2.1)	Bamboo rat genotype I (5); Bamboo rat genotype II (1); *C. parvum* (2); *C. occultus* (1)	IIdA15G1 (1); IIpA9 (1)

rat also from Yongzhou farm 2 (Table 1).

At the SSU rRNA locus, two sequences of *C. parvum* (rats 139 and 416) were identical and had 100% similarity with (KC885893) from bamboo rats in Sichuan of China. The sequence of *C. occultus* revealed 100% similarity with MG699177 in *Rattus norvegicus* from the Czech Republic. Five sequences of *Cryptosporidium* bamboo rat genotype I were identical and shared 97.7% identity with the MH794165 sequence of *C. ubiquitum* in sheep from Xinjiang of China. The Sequence of the *Cryptosporidium* bamboo rat genotype II isolate (bamboorat 421; Genebank number MK731962) have not been previously reported and had a homology of 99.3% to the MK241967 isolate in cattle from India.

Two *C. parvum*-positive and the new genotypes isolates were further subtyped by sequence analysis of the *gp60* gene, and the two *C. parvum* and the *Cryptosporidium* bamboo rat genotype II were successfully amplified. However, the five isolates of *Cryptosporidium* bamboo rat genotype I were amplification failure. Phylogenetic analysis of the *gp60* sequences suggested that they belong to three subtype families (Fig. 1). Sequences of the two *C. parvum* isolates had 100% similarity with subtype IIpA9 (KC885904) which was isolated from a bamboo rat from Sichuan, and IIdA15G1 (KJ917586) which was found in a monkey from Shaanxi of China, respectively. The *gp60* sequence of the *Cryptosporidium* bamboo rat genotype II (Genebank number MK731966) had a maximum nucleotide identity of 84.1% to *C. parvum* subtype IIaA15G2R1 (KX507244), which had extensive sequence polymorphisms in the non-repeat regions.

None of the five isolates of *Cryptosporidium* bamboo rat genotype I were successfully amplified at the *COWP* gene but successfully amplified at the *HSP70* gene, and the five *HSP70* gene sequences (MK731968) of *Cryptosporidium* bamboo rat genotype I were identical to each other and they had 96.4% similarity with that of *C. parvum* from human in Canada (DQ389176). Meanwhile, the *Cryptosporidium* bamboo rat genotype II-positive isolate was successfully amplified at the both *COWP* and *HSP70* genes, which were not been reported previously based on BLAST search results. The *COWP* gene sequence (MK731967) of isolate of *Cryptosporidium* bamboo rat genotype II has nine nucleotide substitutions with *C. parvum* in mouse from China (AB469366). The *HSP70* gene sequence (MK731969) of *Cryptosporidium* bamboo rat genotype II had 96.1% similarity with that of *C. parvum* from human in Canada (DQ389176). For actin gene only two bamboo rat genotype I isolates were successfully amplified, and the two isolates shared a same sequence which had 95.5% similarity with that of *C. ubiquitum* from human in USA (XM_029019099). Phylogenetic analysis of the actin, *gp60*, *HSP70*, *COWP* and SSU rDNA gene sequences showed in Fig. 1.

4. Discussion

In this study, 2.1% of the bamboo rats examined were infected with *Cryptosporidium* spp. To date, only one study reported the prevalence of *Cryptosporidium* in bamboo rats, with infection rates of 3.2% (Liu et al., 2015). These were higher than those observed in this study. *Cryptosporidium* have been detected in various rodent species with variable prevalence rates, including 8.0–31.4% in mice, 0.8–73.0% in voles and 2.1–63.0% in rats (Zhao et al., 2018). For rats, the invasive wild brown rats, black rats, and Asian house rats account for 95% of those sampled for *Cryptosporidium* with a 19.3% average infection rate (Koehler et al., 2018). In general, farmed bamboo rats have lower infection rates of *Cryptosporidium* compared to other wild rats. The lower infection rates of the parasite in farmed bamboo rats may be due to their limited mobility range. The infection rates of *Cryptosporidium* and other pathogens in farmed bamboo rats remain undefined and require further investigation.

According to previous epidemiological reports, a total of 17 *Cryptosporidium* species or genotypes have been observed in rats (Koehler et al., 2018; Zhao et al., 2018; Zhang et al., 2018; Feng, 2010; Liu et al., 2015). *C. parvum*, *C. muris* and rat genotype III were amongst the most frequent (Koehler et al., 2018). In this study, four *Cryptosporidium* species/genotypes were identified, including *C. parvum*, *C. occultus* and *Cryptosporidium* bamboo rat genotypes I and II. *C. parvum* is the most important zoonotic *Cryptosporidium* species with a broad host range that includes humans, farmed animals, and wild animals (Ryan et al., 2016). In China, 16.7% (44/263) of human cases of cryptosporidiosis were caused by *C. parvum* (data not show) which is common in farmed animals such as cattle, golden takins, sheep, goats, yaks, horses, donkeys, and some rodents (Feng and Xiao, 2017). *C. occultus* previously known as *Cryptosporidium* suis-like genotype, was found in cattle from Denmark, India, and China; yaks from China; and rats from the Philippines and China (Zhao et al., 2018). This suggests that *C. occultus* is common in China and is a threat to human health. *C. occultus* accounted for only 11.1% of all *Cryptosporidium* isolates in the investigated bamboo rats. The *Cryptosporidium* bamboo rat genotype I and II detected is possibly endemic, although this was the first report of its discovery. The number of bamboo rats screened for *Cryptosporidium* were low (n = 527) (Liu et al., 2015). In the future, more systematic molecular epidemiological investigations of *Cryptosporidium* in more number of farmed bamboo rats need to be carried out. This will permit more accurate assessments of the role of farmed bamboo rats in the transmission of *Cryptosporidium* to humans and other animals.

Subtyping tools are now widely used in the characterization of *C.*

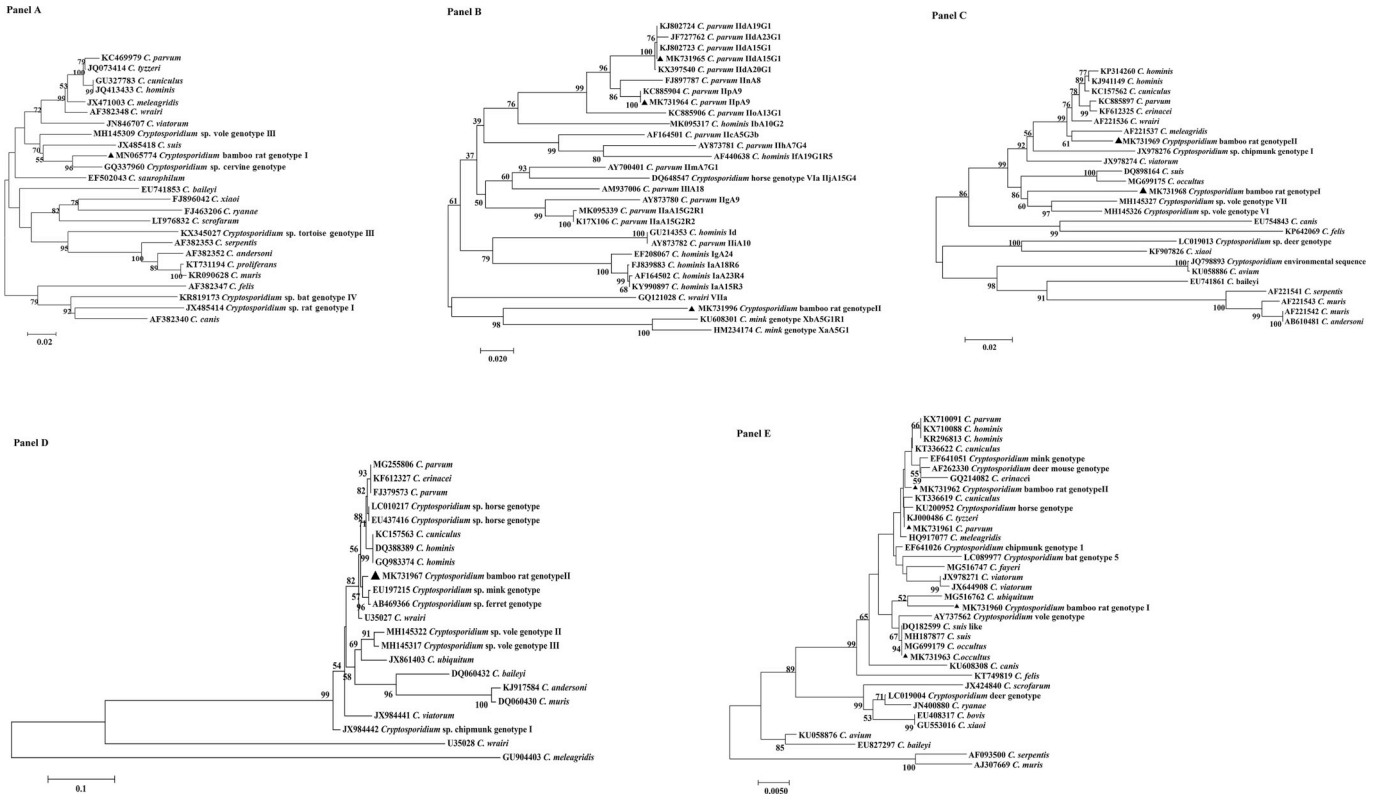

Fig. 1. Phylogenetic relationship of the five gene loci of *Cryptosporidium* species/genotypes. The Panel A to E are represent the *actin*, *gp60*, *HSP70*, *COWP* and SSU rDNA gene, respectively. The numbers on the branches are percent bootstrapping values from 1000 replicates. Each sequence is identified by its accession number and *Cryptosporidium* species/genotypes designation. The triangle filled in black indicate the subtypes identified in this study.

parvum in humans and animals (Šlapeta, 2017). The use of molecular diagnostic tools at the subtype level has increased our understanding of *C. parvum* transmission between humans and animals (Ryan et al., 2016). Some zoonotic outbreaks of cryptosporidiosis caused by *C. parvum* have been confirmed at the subtype level, including calf-derived IIaA15G2R1 in the UK, and sheep-derived IIaA17G1R1and IIaA15G2R (Chalmers et al., 2011; Cacciò et al., 2013). In China, only three subtypes (IIa, IId and IIp) have been identified and only IIdA19G1 was found in humans (Feng and Xiao, 2017).In this study, two subtype families were identified in bamboo rats, including IIdA15G1 and IIpA9. Cross-species transmission of IIdA15G1 subtypes are common in China, occurring in cattle, yaks, goats and rodents, including hamsters, chipmunks, and brown rats (Feng and Xiao, 2017; Zhao et al., 2015). More importantly, the subtypes were further identified in humans and non-human primates in China, suggesting the potential occurrence of zoonotic transmission (Du et al., 2015). Thus, bamboo rats infected with IIdA15G1are a potential threat to humans, as well as other animals.

To date, IIpA9 has been identified in bamboo rats and the potential of this subtype to cause disease in humans and livestock remains unknown (Liu et al., 2015). Likewise, whether the new *Cryptosporidium* bamboo rat genotype II displays host-specificity in bamboo rats remains unverified. Systematic molecular epidemiological investigations of *Cryptosporidium* spp. In a broader range of hosts must be performed to fully understand the host range of the newly identified genotypes.

In conclusion, this study demonstrated the occurrence of *Cryptosporidium* in bamboo rats in South Central China. The fact that zoonotic *C. parvum* and *C. occultus,* particularly subtype IIdA15G1 in bamboo rats suggesting that bamboo rats infected with *Cryptosporidium* had significant zoonotic potential. The novel sequences identified provide information on the genotypic variation of *Cryptosporidium* in bamboo rats. To better evaluate the transmission of *Cryptosporidium* from bamboo rats to humans, further studies investigating the biology, population genetics, and transmission dynamics of these parasites in bamboo rats throughout different geographical regions are now required.

Declaration of interest

We have no conflict of interest to declare with this work.

Conflicts of interest

The authors declared that they have no conflicts of interest to this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2019.06.010.

References

Abdoli, A., Ghaffarifar, F., Pirestani, M., 2018. Neglected risk factors of childhood morbidity and mortality caused by Cryptosporidium infection. Lancet Glob. Health 6, e1068.

Alves, M., Xiao, L., Sulaiman, I., Lal, A.A., Matos, O., Antunes, F., 2003. Subgenotype analysis of *Cryptosporidium* isolates from humans, cattle, and zoo ruminants in Portugal. J. Clin. Microbiol. 1, 2744–2747 2003.

Cacciò, S.M., Sannella, A.R., Mariano, V., Valentini, S., Berti, F., Tosini, F., Tosini, F., Pozio, E., 2013. A rare *Cryptosporidiumparvum* genotype associated with infection of lambs and zoonotic transmission in Italy. Vet. Parasitol. 191, 128–131.
Chalmers, R.M., Smith, R.P., Hadfield, S.J., Elwin, K., Giles, M., 2011. Zoonotic linkage and variation in *Cryptosporidium parvum* from patients in the United Kingdom. Parasitol. Res. 108, 1321–1325.
Checkley, W., White Jr., A.C., Jaganath, D., Arrowood, M.J., Chalmers, R.M., Chen, X.M., Fayer, R., Griffiths, J.K., Guerrant, R.L., Hedstrom, L., Huston, C.D., Kotloff, K.L., Kang, G., Mead, J.R., Miller, M., Petri Jr., W.A., Priest, J.W., Roos, D.S., Striepen, B., Thompson, R.C., Ward, H.D., Van Voorhis, W.A., Xiao, L., Zhu, G., Houpt, E.R., 2015. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for cryptosporidium. Lancet Infect. Dis. 15, 85–94.
Du, S.Z., Zhao, G.H., Shao, J.F., Fang, Y.Q., Tian, G.R., Zhang, L.X., Wang, R.J., Wang, H.,Y., Qi, M., Yu, S.K., 2015. *Cryptosporidium* spp., *Giardia intestinalis*, and *Enterocytozoon bieneusi* in captive con-cuman primates in Qinling mountains. Korean J. Parasitol. 53, 395–402.
Efstratiou, A., Ongerth, J.E., Karanis, P., 2017. Waterborne transmission of protozoan parasites: review of worldwide outbreaks - an update 2011-2016. Water Res. 114, 14–22.
Feng, Y., Ryan, U.M., Xiao, L., 2018. Genetic diversity and population structure of *Cryptosporidium*. Trends Parasitol. 34, 997–1011.
Feng, Y., Xiao, L., 2017. Molecular epidemiology of cryptosporidiosis in China. Front. Microbiol. 8, 1701.
Feng, Y., 2010. *Cryptosporidium* in wild placental mammals. Exp. Parasitol. 124, 128–137.
Holubová, N., Zikmundová, V., Limpouchová, Z., Sak, B., Konečný, R., Hlásková, L., Rajský, D., Kopacz, Z., McEvoy, J., Kváč, M., 2019. *Cryptosporidium proventriculi* sp. n. (apicomplexa: cryptosporidiidae) in psittaciformes birds. Eur. J. Protistol. 69, 70–87.
Khan, A., Shaik, J.S., Grigg, M.E., 2017. Genomics and molecular epidemiology of *Cryptosporidium* species. Acta Trop. 184, 1–14.
Kimura, A., Edagawa, A., Okada, K., Takimoto, A., Yonesho, S., Karanis, P., 2007. Detection and genotyping of *Cryptosporidium* from brown rats (*Rattus norvegicus*) captured in an urban area of Japan. Parasitol. Res. 100, 1417–1420.
Koehler, A.V., Wang, T., Haydon, S.R., Gasser, R.B., 2018. *Cryptosporidium viatorum* from the native Australian swamp rat *Rattus lutreolus* - an emerging zoonotic pathogen? Int. J. Parasitol. Parasites Wildl. 7, 18–26.
Liu, X., Zhou, X., Zhong, Z., Zuo, Z., Shi, J., Wang, Y., Qing, B., Peng, G., 2015. Occurrence of novel and rare subtype families of *Cryptosporidium* in bamboo rats (*Rhizomys sinensis*) in China. Vet. Parasitol. 207, 144–148.
Pumipuntu, N., Piratae, S., 2018. Cryptosporidiosis: a zoonotic disease concern. Vet. World 11, 681–686.
Ryan, U., Fayer, R., Xiao, L., 2014. *Cryptosporidium* species in humans and animals: current understanding and research needs. Parasitology 141, 1667–1685.
Ryan, U., Hijjawi, N., Xiao, L., 2018. Foodborne cryptosporidiosis. Int. J. Parasitol. 48, 1–12.
Ryan, U., Zahedi, A., Paparini, A., 2016. *Cryptosporidium* in humans and animals-a one health approach to prophylaxis. Parasite Immunol. 38, 535–547.
Šlapeta, J., 2017. *Cryptosporidium*: identification and genetic typing. Curr. Protoc. Microbiol. 6, 44 20B.1.1-20B.1.17.
Sulaiman, I.M., Morgan, U.M., Thompson, R.C., Lal, A.A., Xiao, L., 2000. Phylogenetic relationships of *Cryptosporidium* parasites based on the 70-kilodalton heat shock protein (HSP70) gene. Appl. Environ. Microbiol. 66, 2385–2391.
Sulaiman, I.M., Lal, A.A., Xiao, L., 2002. Molecular phylogeny and evolutionary relationships of *Cryptosporidium* parasites at the actin locus. J. Parasitol. 88, 388–394.
Xiao, L., Limor, J., Morgan, U.M., Sulaiman, I.M., Thompson, R.C., Lal, A.A., 2000. Sequence differences in the diagnostic target region of the oocyst wall protein gene of *Cryptosporidium* parasites. Appl. Environ. Microbiol. 66, 5499–5502.
Xiao, L., Escalante, L., Yang, C., Sulaiman, I., Escalante, A.A., Montali, R.J., Fayer, R., Lal, A.A., 1999. Phylogenetic analysis of *Cryptosporidium* parasites based on the small-subunit rRNA gene locus. Appl. Environ. Microbiol. 65, 1578–1583.
Zhang, X., Jian, Y., Li, X., Ma, L., Karanis, G., Karanis, P., 2018. The first report of *Cryptosporidium* spp. in *Microtus fuscus* (Qinghai vole) and *Ochotona curzoniae* (wild plateau pika) in the Qinghai-Tibetan Plateau area, China. Parasitol. Res. 117, 1401–1407.
Zhao, Z., Wang, R., Zhao, W., Qi, M., Zhao, J., Zhang, L., Li, J., Liu, A., 2015. Genotyping and subtyping of *Giardia* and *Cryptosporidium* isolates from commensal rodents in China. Parasitology 142, 800–806.
Zhao, W., Wang, J., Ren, G., Yang, Z., Yang, F., Zhang, W., Xu, Y., Liu, A., Ling, H., 2018. Molecular characterizations of *cryptosporidium* spp. and *Enterocytozoon bieneusi* in brown rats (*Rattus norvegicus*) from Heilongjiang province, China. Parasites Vectors 11, 313.