



Molecular Epidemiology of Cryptosporidiosis in China

Yaoyu Feng^{1*} and Lihua Xiao^{2*}

¹ College of Veterinary Medicine, South China Agricultural University, Guangzhou, China, ² Division of Foodborne, Waterborne and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States

OPEN ACCESS

Edited by:

Wei Hu,
Fudan University, China

Reviewed by:

Mingbo Yin,
Fudan University, China
Jiaxu Chen,
National Institute of Parasitic
Diseases, China

*Correspondence:

Yaoyu Feng
yyfeng@scau.edu.cn
Lihua Xiao
lxiao@cdc.gov

Specialty section:

This article was submitted to
Infectious Diseases,
a section of the journal
Frontiers in Microbiology

Received: 28 April 2017

Accepted: 23 August 2017

Published: 06 September 2017

Citation:

Feng Y and Xiao L (2017) Molecular
Epidemiology of Cryptosporidiosis in
China. *Front. Microbiol.* 8:1701.
doi: 10.3389/fmicb.2017.01701

Molecular epidemiology of cryptosporidiosis is an active research area in China. The use of genotyping and subtyping tools in prevalence studies has led to the identification of unique characteristics of *Cryptosporidium* infections in humans and animals. Human cryptosporidiosis in China is exemplified by the high diversity of *Cryptosporidium* spp. at species and subtype levels, with dominant *C. hominis* and *C. parvum* subtypes being rarely detected in other countries. Similarly, preweaned dairy calves, lambs, and goat kids are mostly infected with non-pathogenic *Cryptosporidium* species (*C. bovis* in calves and *C. xiaoi* in lambs and goat kids), with *C. parvum* starting to appear in dairy calves as a consequence of concentrated animal feeding operations. The latter *Cryptosporidium* species is dominated by IId subtypes, with IIa subtypes largely absent from the country. Unlike elsewhere, rodents in China appear to be commonly infected with *C. parvum* IId subtypes, with identical subtypes being found in these animals, calves, other livestock, and humans. In addition to cattle, pigs and chickens appear to be significant contributors to *Cryptosporidium* contamination in drinking water sources, as reflected by the frequent detection of *C. suis*, *C. baileyi*, and *C. meleagridis* in water samples. Chinese scientists have also made significant contributions to the development of new molecular epidemiological tools for *Cryptosporidium* spp. and improvements in our understanding of the mechanism involved in the emergence of hyper-transmissible and virulent *C. hominis* and *C. parvum* subtypes. Despite this progress, coordinated research efforts should be made to address changes in *Cryptosporidium* transmission because of rapid economic development in China and to prevent the introduction and spread of virulent and zoonotic *Cryptosporidium* species and subtypes in farm animals.

Keywords: cryptosporidiosis, *Cryptosporidium*, molecular epidemiology, zoonosis, China

INTRODUCTION

Cryptosporidiosis is a major cause of diarrhea and enteric disease in humans and farm animals. It has long been known as a primary cause of watery diarrhea in pre-weaned calves and lambs, responsible for significant morbidity and mortality (Holland, 1990; Naciri et al., 1999; Blanchard, 2012; Cho et al., 2013; Meganck et al., 2014). Even subclinical infection in older animals has been associated with reductions in growth, carcass weight and dressing efficiency (Jacobson et al., 2016). In zoo and pet snakes, gastric infection with *Cryptosporidium serpentis* is chronic and often fatal (Paiva et al., 2013). In birds, *C. baileyi* can cause respiratory and renal infections, resulting in high mortality (Santin, 2013). Recent human studies have implicated cryptosporidiosis as the second

most important cause for moderate-to-severe diarrhea in young children in developing countries (Kotloff et al., 2013; Platts-Mills et al., 2015). Several *Cryptosporidium* species from mammals and birds, such as *C. parvum*, *C. meleagridis*, *C. canis*, *C. felis*, and *C. ubiquitum*, are important zoonotic pathogens, causing animal contact-associated or waterborne and foodborne cryptosporidiosis in humans (Xiao, 2010).

Because of the clinical, economic, and public health importance of *Cryptosporidium* spp., cryptosporidiosis has attracted the attention of many Chinese scientists, especially in recent years. They have made significant contributions to improved characterizations of *Cryptosporidium* spp. at species and subtype levels and understanding of their biology and transmission. These studies have led to the identification of unique characteristics of *Cryptosporidium* transmission in humans and animals in China.

MOLECULAR EPIDEMIOLOGIC TOOLS

Molecular epidemiology of cryptosporidiosis in humans and animals is the most active area of *Cryptosporidium* research within China. In these studies, genotyping and subtyping tools are widely used in the identification of infection sources and assessment of cross-species transmission of *Cryptosporidium* spp. (Xiao, 2010). Chinese scientists, in collaborations with scientists in other countries, have played a major role in developing some recent molecular epidemiologic tools for *Cryptosporidium* spp. For example, the PCR-RFLP analysis of the small subunit (SSU) rRNA gene using SspI and MboII developed by Chinese scientists (Feng et al., 2007b) has become the most popular genotyping tool for rapid differentiation of common *Cryptosporidium* species in ruminants (*C. parvum*, *C. bovis*, *C. ryanae*, and *C. andersoni* in cattle and *C. parvum*, *C. ubiquitum*, and *C. xiaoi* in sheep and goats). Several subtyping tools targeting the 60 kDa glycoprotein (gp60) gene have been developed for the assessment of the importance of zoonotic infections with several emerging human-pathogenic *Cryptosporidium* species such as *C. ubiquitum* and *Cryptosporidium* chipmunk genotype I (Li et al., 2014; Guo et al., 2015a). Their use in comparative analyses of human, animal, and water samples has led to the identification of host adaptation in *C. ubiquitum* and differences in the role of ruminants and rodents in human infections among geographic areas (Li et al., 2014). This was substantiated recently by multilocus sequence type (MLST) analysis of *C. ubiquitum* isolates (Tang et al., 2016). An MLST tool has also been developed for *C. andersoni* and *C. muris* (Feng et al., 2011a), and used by Chinese scientists in population genetic characterizations of gastric *Cryptosporidium* species in various areas (Wang et al., 2012; Zhao et al., 2013, 2014; Du et al., 2015; Zhao G. H. et al., 2015; Qi et al., 2016; Deng et al., 2017). It was shown that while the population structure of *C. andersoni* was clonal in Shaanxi and Heilongjiang provinces (Zhao et al., 2013, 2014), it was epidemic in Xinjiang and other regions (Wang et al., 2012; Qi et al., 2016).

More recently, procedures have been developed to facilitate whole genome sequencing (WGS) and advanced typing of human-pathogenic *Cryptosporidium* spp. (Guo et al., 2015b).

The WGS and MLST tools have been used by Chinese scientists in studies of virulent and hypertransmissible *C. hominis* and *C. parvum* subtypes. Genetic recombination has been identified as a major mechanism for the emergence of these subtypes (Feng et al., 2013, 2014; Li et al., 2013; Guo et al., 2015c). WGS analysis of *Cryptosporidium* spp. has further indicated that copy number variation in two subtelomeric gene families encoding secreted MEDLE proteins and insulinase-like peptidases is involved in differences in host specificity between *C. parvum* and *C. hominis* and among host-adapted *C. parvum* subtype families (Guo et al., 2015c; Liu et al., 2016; Feng et al., 2017).

Although, molecular characterization of *Cryptosporidium* spp. is a recent development in China (Chen and Huang, 2007; Wang et al., 2008a,b,c), genotyping and subtyping tools are now widely used in the characterization of *Cryptosporidium* spp. in various animals (Karim et al., 2014; Liu et al., 2014a,b, 2015a,b; Ma et al., 2014, 2015; Qi et al., 2014, 2015a,b,d, 2016; Wang L. et al., 2014; Ye et al., 2014; Zhao et al., 2014; Du et al., 2015; Li J. et al., 2015, 2017; Liu A. et al., 2015; Li W. et al., 2015, 2017; Qi, M. Z., et al., 2015; Wang et al., 2015a,b; Zhang et al., 2015a,b; Zhao G. H. et al., 2015; Zhao Z. et al., 2015; Jian et al., 2016; Li F. et al., 2016; Li P. et al., 2016; Li Q. et al., 2016; Peng et al., 2016; Taylan-Ozkan et al., 2016; Xu et al., 2016; Yang et al., 2016; Zhang S. et al., 2016; Deng et al., 2017; Gong et al., 2017; Zou et al., 2017). The use of molecular diagnostic tools in prevalence studies has led to the identification of significant differences in the transmission of *Cryptosporidium* spp. between China and other countries.

CHARACTERISTICS AND DISTRIBUTION OF CRYPTOSPORIDIUM HOMINIS SUBTYPES IN CHINA

Molecular surveillance of *Cryptosporidium* spp. in untreated urban wastewater indicates that like other developing countries, *C. hominis* is the major *Cryptosporidium* species in humans in China (Feng et al., 2009; Li et al., 2012; Huang et al., 2017). The common *C. hominis* subtype families found in humans in developing countries, including Ia, Ib, Id, Ie, and If, are present in wastewater in Shanghai (Feng et al., 2009). However, several Ib subtypes that are rarely found elsewhere in the world, including IbA19G2, IbA20G2, and IbA21G2, are dominant *C. hominis* subtypes in wastewater. The occurrence of diverse *C. hominis* subtype families and divergent Ib subtypes in humans in China has been confirmed by subtype analysis of clinical specimens from pediatric patients (Table 1). Elsewhere in developing countries, humans are commonly infected with two *C. hominis* subtypes of the virulent Ib subtype family: IbA10G2 and IbA9G3, which have not been found in China (Xiao, 2010).

In addition to Ib subtypes, Ia and Id appear to be common in China (Table 1), and two of the Ia and Id subtypes, IaA14R4 and IdA19, were the cause of a cryptosporidiosis outbreak in a pediatric ward in Shanghai that lasted >14 months (Feng et al., 2012). In this outbreak, only IaA14R4 was associated with the occurrence of diarrhea. As these patients were orphans from a welfare institute, poor hygiene by patients and caregivers was implicated as the cause of the outbreak. This was supported

TABLE 1 | *Cryptosporidium* species and subtypes in humans and nonhuman primates in China.

Host	Location	Sample size	<i>Cryptosporidium</i> positive (%)	<i>C. hominis</i>		<i>C. parvum</i>		Other species (No.)	References
				No. positive	Subtype (No.)	No. positive	Subtype (No.)		
Human	Tianjin	–	5	5	IbA22G2 (1), IdA14 (1), IeA13G3T3 (1)	–	–	–	Peng et al., 2001
Human	Shanghai	6,284	102 (1.6%)	92	IaA14R4 (36), IaA18R4 (1), IdA19 (37), IdA14 (1), IbA19G2 (1), IgA14 (1)	–	–	<i>C. meleagridis</i> (6), <i>C. canis</i> (2), <i>C. felis</i> (2)	Feng et al., 2012
Human	Shanghai	252	34 (13.5%)	–	–	–	–	<i>C. andersoni</i> (34)	Liu H. et al., 2014
Human	Jiangsu	232	23 (9.9%)	2	–	–	–	<i>C. andersoni</i> (21)	Jiang et al., 2014
Human	Henan	1,366	11 (0.8%)	3	IbA19G2 (2), IeA12G3T3 (1)	2	IIdA19G1 (2)	<i>C. meleagridis</i> (5), <i>C. suis</i> (1)	Wang et al., 2013b
Human	Henan	–	10	9	IbA20G2 (3), IbA19G2 (2), IbA16G2 (1), IdA21 (2), IaA9R3 (1)	–	–	<i>C. felis</i> (1)	Wang et al., 2011c; Zhu et al., 2012
Human	Hubei	500	10 (2.0)	–	–	–	–	<i>C. meleagridis</i> (10)	Wang et al., 2017
Rhesus monkey	Guizhou	411	45 (10.9)	39	IdA20 (13), IeA11G3T3 (13), IaA13R8 (8), IaA13R7 (3), IaA14R7 (2), IfA16G2 (1)	5	IIdA5G3a (5)	<i>C. felis</i> (1)	Ye et al., 2012
Rhesus monkey	Shaanxi	86	6 (7.0)	–	–	1	IIdA15G1* (1)	<i>C. andersoni</i> (5)	Du et al., 2015
Crab-eating macaques	Guangxi	205	1	1	IdA14 (1)	–	–	–	Ye et al., 2014
Nonhuman primates	Guangdong, Guangxi, Shanghai, Henan	266	19 (0.7)	14	IbA12G3 (7), IiA17 (1)	–	–	<i>C. muris</i> (5)	Karim et al., 2014
Squirrel monkey	Sichuan	–	1	1	IkA7G4 (1)**	–	–	–	Liu et al., 2015a

*Misidentified as IIdA15G2R1 in the report (KJ917586). **This subtype was assigned to the wrong subtype family, as it differed significantly from KJ941148 (IkA15G1), which has priority over this sequence (KP314263).

by concurrent augment in the transmission of several other enteric pathogens, including *Giardia duodenalis*, *Enterocytozoon bieneusi*, and *Clostridium difficile* (Wang et al., 2013a).

The occurrence these common *C. hominis* subtype families in humans in China is also supported by studies of *Cryptosporidium* spp. in captive nonhuman primates. Four of the five *C. hominis* subtype families, including Ia, Id, Ie, and If, have been found at high frequency in rhesus monkeys in a popular park in Guizhou, where humans and animals interact with each other closely

(Ye et al., 2012). Divergent Ib subtypes, have been found in nonhuman primates in other studies (Table 1). Although, the source of *C. hominis* in nonhuman primates is not clear, these animals clearly can serve as potential reservoirs for this pathogen, and some of the *C. hominis* subtypes in nonhuman primates have been found in lake water frequented by these animals (Ye et al., 2012).

C. hominis has been recently found as a dominant *Cryptosporidium* species in horses and donkeys in China

(Jian et al., 2016; Deng et al., 2017). The subtypes involved, however, mostly belong to the rare *C. hominis* subtype family Ik (Jian et al., 2016). Elsewhere in the world, Ik subtypes have been identified in horses in Algeria and Brazil (Laatamna et al., 2015; Inacio et al., 2017), indicating this subtype family is a host-adapted *C. hominis* with only limited public health significance. This was supported recently by WGS analysis of a human Ik isolate from Sweden, which showed that it has a much more divergent genome compared with common *C. hominis* subtypes (Sikora et al., 2016).

CHARACTERISTICS OF *CRYPTOSPORIDIUM PARVUM* TRANSMISSION IN CHINA

There are some substantial differences in the transmission of *C. parvum* between China and other countries, especially in preweaned dairy calves. Two large scale studies in Henan showed that although the distribution of *Cryptosporidium* species in postweaned dairy cattle was similar to the one observed in industrialized nations, preweaned dairy calves were mainly infected with *C. bovis* instead of *C. parvum* (Wang et al., 2011a,b). This was supported by subsequent studies in Heilongjiang, Shaanxi, Gansu, Ningxia, and Shanghai (Zhang et al., 2013a, 2015a; Qi, M. Z., et al., 2015; Cai et al., 2017), although in some other areas *C. parvum* was shown to be common in preweaned dairy calves, especially on large farms or farms experiencing diarrhea outbreaks (Cui et al., 2014; Huang et al., 2014; Qi et al., 2015c; Li F. et al., 2016). Elsewhere in the world, *C. parvum* is the dominant *Cryptosporidium* species in preweaned dairy calves (Xiao, 2010), and the common occurrence of *C. bovis* in this age group has only been reported in a few studies in Sweden and Malaysia (Silverlas et al., 2010; Muhid et al., 2011). In contrast, *C. bovis* is a common species in preweaned beef cattle, which are usually maintained in less extensive animal management systems (Ng et al., 2011; Rieux et al., 2013a,b, 2014; Bjorkman et al., 2015). The short history of concentrated animal feeding operations in

China could be responsible for the low occurrence of *C. parvum* in preweaned dairy calves.

Subtyping of *C. parvum* in bovine studies identified the exclusive occurrence of IId subtypes in dairy calves in China, mostly IIdA15G1 and IIdA19G1 (Table 2). One study identified the exclusive occurrence of a few IId subtypes in yaks in Qinghai (Mi et al., 2013), but this was not supported by data from other studies conducted in the same area (Qi et al., 2015a; Li P. et al., 2016). In contrast, preweaned dairy calves in other countries are mostly infected with *C. parvum* IId subtypes, especially IIdA15G2R1, with IId subtypes common only in some areas in Sweden, Romania, Egypt, and Malaysia (Amer et al., 2010, 2013; Muhid et al., 2011; Silverlas et al., 2013; Bjorkman et al., 2015; Vieira et al., 2015; Ibrahim et al., 2016). The source of *C. parvum* IId subtypes in dairy cattle is not clear (Wang et al., 2014b). The two dominant subtypes in cattle, IIdA15G1 and IIdA19G1, are apparently common in various rodents in China (Table 3). Elsewhere in the world, rodents are seldom infected with *C. parvum* (Feng et al., 2007a), and when infected, mostly with IId subtypes (Danisova et al., 2017). Cross-species transmission of IId subtypes is apparently common in China, as other grazing animals such as goats, horses, donkeys, and takins are also known to be infected with IId subtypes (Mi et al., 2014; Qi et al., 2015a; Zhao G. H. et al., 2015; Jian et al., 2016; Peng et al., 2016). The two dominant IId subtypes have been further identified in humans and nonhuman primates in China, suggesting the potential occurrence of zoonotic transmission of *C. parvum* (Wang et al., 2013b; Du et al., 2015).

TRANSMISSION OF OTHER ZONOTIC *CRYPTOSPORIDIUM* SPP. IN HUMANS IN CHINA

In addition to *C. hominis* and *C. parvum*, several other *Cryptosporidium* species that are traditionally associated with animals, such as *C. meleagridis*, *C. canis*, and *C. felis*, have been found in humans in China (Table 1). This is not surprising

TABLE 2 | Common occurrence of *Cryptosporidium bovis* and dominance of *Cryptosporidium parvum* IId subtypes in preweaned dairy calves in China.

Area	No. positive/No. examined (%)	Species (No.)	<i>C. parvum</i> subtype (No.)	References
Henan	172/801 (21.5)	<i>C. bovis</i> (65), <i>C. parvum</i> (54), <i>C. ryanae</i> (19), <i>C. andersoni</i> (12)	IIdA19G1 (67)	Wang et al., 2011b
Heilongjiang	72/151 (47.7)	<i>C. bovis</i> (34), <i>C. andersoni</i> (26), <i>C. ryanae</i> (5), <i>C. meleagridis</i> (5), <i>C. parvum</i> (2)	IIdA19G1 (1)	Zhang et al., 2013a
Ningxia	49/158 (31)	<i>C. parvum</i> (48), <i>C. bovis</i> (1)	IIdA15G1 (51)	Cui et al., 2014
Ningxia	19/186 (10.2)	<i>C. parvum</i> (15), <i>C. bovis</i> (4)	IIdA15G1 (15)	Huang et al., 2014
Ningxia and Gansu	122/877 (14.0)	<i>C. bovis</i> (62), <i>C. ryanae</i> (23), <i>C. andersoni</i> (19), <i>C. parvum</i> (18)	IIdA15G1 (18)	Zhang et al., 2015a
Xinjiang	37/237 (15.6)	<i>C. parvum</i> (22), <i>C. bovis</i> (9), <i>C. ryanae</i> (1), <i>C. andersoni</i> (2)	IIdA15G1 (11), IIdA14G1 (4)	Qi et al., 2015c
Shaanxi	46/186 (24.7)	<i>C. bovis</i> (22), <i>C. andersoni</i> (13), <i>C. ryanae</i> (11)	–	Qi, M. Z., et al., 2015
Beijing	14/404 (3.5)*	<i>C. parvum</i> (10), <i>C. andersoni</i> (4)	IIdA15G1 (4), IIdA19G1 (1), IIdA17G1 (1)	Li F. et al., 2016
Shanghai	303/818	<i>C. bovis</i> (199), <i>C. parvum</i> (72), <i>C. ryanae</i> (38)	IIdA19G1 (66)	Cai et al., 2017

*Animals <1 year.

TABLE 3 | Rodents and other animals as possible sources of *Cryptosporidium parvum* IId subtype family in dairy cattle in China.

Animal	Location	Sample size	No. <i>Cryptosporidium</i> positive (%)	No. of <i>C. parvum</i>	Subtype	References
Golden hamster	Henan	50	16 (32.0)	4	IIdA15G1 (4)	Lv et al., 2009
Siberian hamster	Henan	51	4 (7.8)	2	IIdA15G1 (2)	
Campbell hamster	Henan	30	3 (10.0)	2	IIdA15G1 (2)	
Siberian chipmunk	Henan	20	6 (30.0)	2	IIdA15G1 (2)	
Brown rat	Henan	168	11 (6.6)	9	IIdA15G1 (9)	Zhao Z. et al., 2015
Golden takin	Shaanxi	191	15 (7.9)	2	IIdA19G1 (2)	Zhao G. H. et al., 2015
Yak	Qinghai, Gansu, Sichuan, Tibet	545	22 (4.0)	12	IIdA15G1 (3), IIdA19G1 (1), IIdA18G1 (1)	Qi et al., 2015a
Goat	Shanghai	302	33 (10.9)	11	IIdA19G1 (8), IIdA17G2R1 (1), IIdA15G2R1 (1)	Mi et al., 2014
Horse	Sichuan, Gansu, Inner Mongolia	–	5	4	IIdA19G1 (3)	Jian et al., 2016
Donkey	Henan, Shandong	–	82	18	IIdA19G1 (18)	
Rhesus monkey	Shaanxi	86	6 (7.0)	1	IIdA15G1 (1)	Du et al., 2015

considering the frequent reports of these pathogens in humans in other developing countries (Xiao, 2010). As these pathogens were identified in children in urban areas in the absence of *C. parvum* (Feng et al., 2012; Wang et al., 2017), anthroponotic transmission of these *Cryptosporidium* species cannot be ruled out. In contrast, *C. andersoni* has been identified at high frequency in humans in two urban studies in China (Table 1). The unusual high *Cryptosporidium* infection rates, especially in adult patients (Jiang et al., 2014), and the identification of the canine-specific assemblage C as the dominant *G. duodenalis* genotype in one of the studies (Liu H. et al., 2014) are some other unusual observations in these studies.

Zoonotic infection appears to be important in cryptosporidiosis epidemiology in rural China, as indicated by the high ratio of zoonotic *Cryptosporidium* spp. (*C. parvum*, *C. meleagridis*, and *C. suis*) in HIV+ patients in a case-control study in Henan, which identified animal contact as a risk factor for cryptosporidiosis occurrence in the study population (Wang et al., 2013b). Subtype analysis identified the *C. parvum* as IIdA19G1, which is one of the two major *C. parvum* subtypes in calves, rodents, and other animals in China (see below).

TRANSMISSION OF OTHER *CRYPTOSPORIDIUM* SPP. IN ANIMALS IN CHINA

The transmission of *Cryptosporidium* spp. appears to be unique in sheep and goats. Although, initial studies in Henan and Sichuan indicated a frequent occurrence of the zoonotic pathogen *C. ubiquitum* in sheep (Wang Y. et al., 2010; Shen et al., 2011), more recent studies have shown a common occurrence of the non-pathogenic *C. xiaoi* in sheep in Inner Mongolia and Qinghai (Ye et al., 2013; Li P. et al., 2016). Goats in China are also mostly infected with these two *Cryptosporidium* species, although in some areas a few *C. parvum* and *C. andersoni* infections were detected (Mi et al., 2014; Wang et al., 2014a; Peng et al., 2016).

Elsewhere in the world, sheep and goats in European countries are frequently infected with the pathogenic and zoonotic species *C. parvum*, whereas those in Americas are mainly infected with *C. ubiquitum*. All three *Cryptosporidium* species appear to be common in sheep in Australia (Ye et al., 2013; Ryan et al., 2014). *Cryptosporidium xiaoi* appears to be common in sheep and goats in Africa, where like in China animal management is less intensive (Soltane et al., 2007; Mahfouz et al., 2014; Parsons et al., 2015; Hijjawi et al., 2016).

There are quite a few molecular epidemiologic studies of *Cryptosporidium* spp. in pigs, but results obtained thus far are in agreement with observations in industrialized nations (Wang R. et al., 2010; Chen et al., 2011; Yin et al., 2011, 2013; Zhang et al., 2013b; Lin et al., 2015; Zou et al., 2017). As in other countries, pigs in China are mostly infected with *C. suis* and *C. scrofarum*, with different distribution of these two between piglets and adults. The former is preferentially found in preweaned piglets whereas the latter is mostly found in postweaned pigs (Wang R. et al., 2010; Yin et al., 2013; Zhang et al., 2013b), although in one study *C. suis* was also found in postweaned and adult pigs at high frequency (Lin et al., 2015). *Cryptosporidium scrofarum* has been identified in Eurasian wild boars in China (Li W. et al., 2017). In contrast, both *C. suis* and *C. scrofarum* are common in these animals in Europe (Garcia-Preseido et al., 2013; Nemejc et al., 2013).

Molecular characterizations of *Cryptosporidium* spp. in other animals are less systematic. There are a few studies on the distribution of *Cryptosporidium* spp. in dogs and cats in China, but likely in most other areas, only *C. canis* and *C. felis* were identified in these animals, respectively (Jian et al., 2014; Li W. et al., 2015; Xu et al., 2016). As expected, these two species have been also found in wild or captive canine and feline animals such as foxes, raccoon dogs, and mauls (*Felis manul*) (Li J. et al., 2015; Zhang S. et al., 2016; Zhang X. X. et al., 2016). As expected, host-adapted *Cryptosporidium* genotypes have been identified in the a few wild mammals examined, such as minks, bats, and other rodents (Wang et al., 2008b; Lv et al., 2009; Wang W. et al., 2013; Zhao Z. et al., 2015; Li Q. et al., 2016; Zhang S. et al., 2016).

Similarly, a new *Cryptosporidium* genotype related to the bear genotype has been identified in giant panda (Liu et al., 2013), although *C. andersoni* appears to be common in both giant and less panda (Wang et al., 2015a,b). Interestingly, *C. ubiquitum* has been identified in pet chinchillas and hedgehogs in China (Qi et al., 2015b; Li Q. et al., 2016). All 11 *C. ubiquitum* isolates from chinchillas belonged to the XIId subtype family previously identified in the United States. Two novel subtype families of *C. ubiquitum* related to XIId have been found in wastewater and storm water in Shanghai, indicating *C. ubiquitum* could be a human pathogen in China (Huang et al., 2017).

SOURCES OF CRYPTOSPORIDIUM CONTAMINATION IN WATER

Because water exposure is frequently associated with human cryptosporidiosis in industrialized nations, extensive efforts have been made to understand the transport of *Cryptosporidium* oocysts in environment. The host-adapted nature of most *Cryptosporidium* spp. has led to the use of genotyping tools to assess the source and human-infective potential of *Cryptosporidium* oocysts in water (Xiao et al., 2000). Due to cost issues, these types of studies are largely restricted to industrialized nations. With the implementation of new Standards for Drinking Water Quality (GB 5749-2006) (http://www.iwa-network.org/filemanager-uploads/WQ_Compendium/Database/Selected_guidelines/016.pdf) in China in July 2012, *Cryptosporidium* spp. and *G. duodenalis* are now among six microbial parameters in the national drinking water quality standard, which has generated increased interest in monitoring for *Cryptosporidium* oocysts in drinking water and source water, as China is the major source for point and nonpoint *Cryptosporidium* emission to surface water (Hofstra et al., 2012; Hofstra and Vermeulen, 2016). Currently, the cost of *Cryptosporidium* detection per water sample in China is >10,000 RMB (>\$1,500). As a result, regulatory testing of water samples for *Cryptosporidium* spp. is, in essence, restricted to large utilities and special events, and genotyping of *Cryptosporidium* spp. in water remains a research effort only in a few elite academic laboratories.

Procedures have been developed in China to genotype *Cryptosporidium* spp. in source water using oocyst concentration by flocculation instead of by expensive cartridge filtration and

immunomagnetic separation, and detection of *Cryptosporidium* spp. by PCR instead of immunofluorescence microscopy (Feng et al., 2011b). Compared with the USEPA Method 1623 (USEPA, 2012), the new test cannot provide accurate quantitative data on the contamination level, but is much less expensive and can assess the source and human-infective potential of *Cryptosporidium* oocysts. This technique has also been used effectively in assessing the human-infective potential of *Cryptosporidium* spp. in treated urban wastewater (Ma et al., 2016). The recent development of three real-time PCR assays for genotyping and source tracking *Cryptosporidium* spp. in water could further promote research on *Cryptosporidium* contamination in drinking source watershed (Li N. et al., 2015).

The use of genotyping tools in the analysis of source water has led to the identification of *C. andersoni* and *C. suis* as the dominant *Cryptosporidium* species in Huangpu River, suggesting that cattle and pigs are major contributors to *Cryptosporidium* contamination for this drinking water source (Feng et al., 2011b). Similar observations have also been made in other areas in China using other approaches. In these studies, two poultry *Cryptosporidium* species, *C. baileyi* and *C. meleagridis*, also appear in source water in China at higher frequencies than in other countries (Table 4). In other countries, *C. suis*, *C. baileyi*, and *C. meleagridis* are rarely identified in drinking water sources.

The importance of pigs in *Cryptosporidium* contamination in source water in China was supported by results of the investigation of a pig carcass incident in March 2013, in which more than 16,000 pig carcasses that had been dumped in Jiaxing, Zhejiang Province reached Shanghai via the upper Huangpu River. Much higher *Cryptosporidium* detection rates were obtained in Huangpu River samples collected upstream of Shanghai during and shortly after the carcass incident, especially the occurrence of *C. suis* and *C. scrofarum*, another *Cryptosporidium* species in pigs (Hu et al., 2014). This was further supported by data on the occurrence of pig-adapted *E. bieneusi* genotypes in the river water samples.

CHALLENGES AND OPPORTUNITIES OF CRYPTOSPORIDIUM RESEARCH IN CHINA

Despite recent progress in *Cryptosporidium* research in China, there remain some challenges. Most studies in this area have

TABLE 4 | *Cryptosporidium* species in drinking source water in China.

Area	Sample size	No. positive (%)	<i>Cryptosporidium</i> species	References
Shanghai	50	17 (34.0)*	<i>C. andersoni</i> (14), <i>C. suis</i> (7), <i>C. baileyi</i> (2), <i>C. meleagridis</i> (1), <i>C. hominis</i> (1)	Feng et al., 2011b
Shanghai	178	67 (37.6)**	<i>C. andersoni</i> (38), <i>C. suis</i> (27), <i>C. baileyi</i> (16), <i>C. scrofarum</i> (8), <i>C. meleagridis</i> (4), <i>C. parvum</i> (3), <i>C. hominis</i> (2), <i>C. ryanae</i> (1), <i>C. cuniculus</i> (1), <i>C. fragile</i> (1), rat genotype IV (1), avian genotype II (1), avian genotype III (1)	Hu et al., 2014
Chongqing	66	19 (28.8)	<i>C. andersoni</i> (9), <i>C. hominis</i> (6), <i>C. suis</i> (5), <i>C. bovis</i> (3), <i>C. meleagridis</i> (1), <i>C. baileyi</i> (1)	Xiao et al., 2013
Zhejiang	47	37 (78.7)	<i>C. suis</i> (2), avian genotype III (2), <i>C. scrofarum</i> (1), <i>C. ubiquitum</i> (1), <i>C. fragile</i> (1)	Xiao et al., 2012

*Seven samples (41.2%) contained more than one *Cryptosporidium* species, including six samples containing two species and one containing three species. **Including unspecified number of samples with mixed *Cryptosporidium* species/genotypes.

used established molecular diagnostic tools (SSU rRNA-based PCR-RFLP tool for species identification and gp60-based PCR-sequencing for subtyping) in prevalence studies. There is a lack of integrated usage of advanced molecular diagnostic tools and sophisticated epidemiological design in field studies of cryptosporidiosis. Studies are urgently needed to address public health issues related to the rapid economic development in China, such as changes in incidence and epidemiology of cryptosporidiosis in humans, impacts of concentrated animal feeding operations on *Cryptosporidium* transmission and environmental contamination, and potential introduction and dispersal of virulent and zoonotic *Cryptosporidium* species and subtypes from the Belt and Road countries. Efforts also could be made to substantiate some unusual molecular epidemiologic observations of cryptosporidiosis in China by research teams, such as the finding of *C. tyzzeri* and *C. serpentis* in several species of farm animals (Chen and Huang, 2007, 2012; Chen and Qiu, 2012) and the unexpectedly high occurrence of *C. andersoni* in urban human populations (Jiang et al., 2014; Liu H. et al., 2014). These observations are in stark contrast to other reports, and have important public health and regulatory implications.

For young scientists new to research, now may be the best time to conduct research on *Cryptosporidium* spp. In the past few years, the identification of cryptosporidiosis as a major cause for moderate-to-severe diarrhea in young infants in developing countries, the increasing occurrence of waterborne cryptosporidiosis in industrialized nations because

of the chlorine-resistant nature of the parasite, and the lack of effective treatment and vaccines have attracted the attention of funding agencies. With new research investments and involvements of scientists from other research areas, major advances have been made in basic research on *Cryptosporidium* spp., including *in vitro* cultivation, cryopreservation, WGS, genetic manipulation, and drug development (Guo et al., 2015b; Vinayak et al., 2015; Morada et al., 2016; Hulverson et al., 2017; Manjunatha et al., 2017). They likely will promote research in pathogen biology of *Cryptosporidium* spp. and improve the depth of molecular epidemiological studies of *Cryptosporidium* spp. With these developments, Chinese *Cryptosporidium* research may be able to broaden its scope and increase its impact.

AUTHOR CONTRIBUTIONS

YF and LX conducted data collection and analysis and prepared the report.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (31425025 and 31630078). The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the U.S. Centers for Disease Control and Prevention.

REFERENCES

- Amer, S., Honma, H., Ikarashi, M., Tada, C., Fukuda, Y., Suyama, Y., et al. (2010). *Cryptosporidium* genotypes and subtypes in dairy calves in Egypt. *Vet. Parasitol.* 169, 382–386. doi: 10.1016/j.vetpar.2010.01.017
- Amer, S., Zidan, S., Adamu, H., Ye, J., Roellig, D., Xiao, L., et al. (2013). Prevalence and characterization of *Cryptosporidium* spp. in dairy cattle in Nile River delta provinces, Egypt. *Exp. Parasitol.* 135, 518–523. doi: 10.1016/j.exppara.2013.09.002
- Bjorkman, C., Lindstrom, L., Oweson, C., Ahola, H., Troell, K., and Axen, C. (2015). *Cryptosporidium* infections in suckler herd beef calves. *Parasitology* 142, 1108–1114. doi: 10.1017/S0031182015000426
- Blanchard, P. C. (2012). Diagnostics of dairy and beef cattle diarrhea. *Vet. Clin. North Am.* 28, 443–464. doi: 10.1016/j.cvfa.2012.07.002
- Cai, M., Guo, Y., Pan, B., Li, N., Wang, X., Tang, C., et al. (2017). Longitudinal monitoring of *Cryptosporidium* species in pre-weaned dairy calves on five farms in Shanghai, China. *Vet. Parasitol.* 241, 14–19. doi: 10.1016/j.vetpar.2017.05.005
- Chen, F., and Huang, K. (2007). Prevalence and phylogenetic analysis of *Cryptosporidium* in pigs in eastern China. *Zoonoses Public Health* 54, 393–400. doi: 10.1111/j.1863-2378.2007.01078.x
- Chen, F., and Huang, K. (2012). Prevalence and molecular characterization of *Cryptosporidium* spp. in dairy cattle from farms in China. *J. Vet. Sci.* 13, 15–22. doi: 10.4142/jvs.2012.13.1.15
- Chen, F., and Qiu, H. (2012). Identification and characterization of a Chinese isolate of *Cryptosporidium serpentis* from dairy cattle. *Parasitol. Res.* 111, 1785–1791. doi: 10.1007/s00436-012-3024-5
- Chen, Z., Mi, R., Yu, H., Shi, Y., Huang, Y., Chen, Y., et al. (2011). Prevalence of *Cryptosporidium* spp. in pigs in Shanghai, China. *Vet. Parasitol.* 181, 113–119. doi: 10.1016/j.vetpar.2011.04.037
- Cho, Y. I., Han, J. I., Wang, C., Cooper, V., Schwartz, K., Engelken, T., et al. (2013). Case-control study of microbiological etiology associated with calf diarrhea. *Vet. Microbiol.* 166, 375–385. doi: 10.1016/j.vetmic.2013.07.001
- Cui, Z., Wang, R., Huang, J., Wang, H., Zhao, J., Luo, N., et al. (2014). Cryptosporidiosis caused by *Cryptosporidium parvum* subtype IIdA15G1 at a dairy farm in Northwestern China. *Parasit. Vectors* 7:529. doi: 10.1186/s13071-014-0529-z
- Danisova, O., Valencakova, A., Stanko, M., Luptakova, L., Hatalova, E., and Canady, A. (2017). Rodents as a reservoir of infection caused by multiple zoonotic species/genotypes of *C. parvum*, *C. hominis*, *C. suis*, *C. scrofarum*, and the first evidence of *C. muskrat* genotypes I and II of rodents in Europe. *Acta Trop.* 172, 29–35. doi: 10.1016/j.actatropica.2017.04.013
- Deng, L., Li, W., Zhong, Z., Gong, C., Cao, X., Song, Y., et al. (2017). Occurrence and genetic characteristics of *Cryptosporidium hominis* and *Cryptosporidium andersoni* in horses from Southwestern China. *J. Eukaryot. Microbiol.* doi: 10.1111/jeu.12399. [Epub ahead of print].
- Du, S. Z., Zhao, G. H., Shao, J. F., Fang, Y. Q., Tian, G. R., Zhang, L. X., et al. (2015). *Cryptosporidium* spp., *Giardia intestinalis*, and *Enterocytozoon bienersi* in captive non-human primates in Qinling Mountains. *Korean J. Parasitol.* 53, 395–402. doi: 10.3347/kjp.2015.53.4.395
- Feng, Y., Alderisio, K. A., Yang, W., Blancero, L. A., Kuhne, W. G., Nadeski, C. A., et al. (2007a). *Cryptosporidium* genotypes in wildlife from a New York watershed. *Appl. Environ. Microbiol.* 73, 6475–6483. doi: 10.1128/AEM.01034-07
- Feng, Y., Li, N., Duan, L., and Xiao, L. (2009). *Cryptosporidium* genotype and subtype distribution in raw wastewater in Shanghai, China: evidence for possible unique *Cryptosporidium hominis* transmission. *J. Clin. Microbiol.* 47, 153–157. doi: 10.1128/JCM.01777-08
- Feng, Y., Li, N., Roellig, D. M., Kelley, A., Liu, G., Amer, S., et al. (2017). Comparative genomic analysis of the IId subtype family of *Cryptosporidium parvum*. *Int. J. Parasitol.* 47, 281–290. doi: 10.1016/j.ijpara.2016.12.002
- Feng, Y., Ortega, Y., He, G., Das, P., Xu, M., Zhang, X., et al. (2007b). Wide geographic distribution of *Cryptosporidium bovis* and the deer-like genotype in bovines. *Vet. Parasitol.* 144, 1–9. doi: 10.1016/j.vetpar.2006.10.001

- Feng, Y., Tiao, N., Li, N., Hlavsa, M., and Xiao, L. (2014). Multilocus sequence typing of an emerging *Cryptosporidium hominis* subtype in the United States. *J. Clin. Microbiol.* 52, 524–530. doi: 10.1128/JCM.02973-13
- Feng, Y., Torres, E., Li, N., Wang, L., Bowman, D., and Xiao, L. (2013). Population genetic characterization of dominant *Cryptosporidium parvum* subtype IIAA15G2R1. *Int. J. Parasitol.* 43, 1141–1147. doi: 10.1016/j.ijpara.2013.09.002
- Feng, Y., Wang, L., Duan, L., Gomez-Puerta, L. A., Zhang, L., Zhao, X., et al. (2012). Extended outbreak of cryptosporidiosis in a pediatric hospital, China. *Emerg. Infect. Dis.* 18, 312–314. doi: 10.3201/eid1802.110666
- Feng, Y., Yang, W., Ryan, U., Zhang, L., Kvac, M., Koudela, B., et al. (2011a). Development of a multilocus sequence tool for typing *Cryptosporidium muris* and *Cryptosporidium andersoni*. *J. Clin. Microbiol.* 49, 34–41. doi: 10.1128/JCM.01329-10
- Feng, Y., Zhao, X., Chen, J., Jin, W., Zhou, X., Li, N., et al. (2011b). Occurrence, source, and human infection potential of *Cryptosporidium* and *Giardia* spp. in source and tap water in Shanghai, China. *Appl. Environ. Microbiol.* 77, 3609–3616. doi: 10.1128/AEM.00146-11
- Garcia-Prsedo, I., Pedraza-Diaz, S., Gonzalez-Warleta, M., Mezo, M., Gomez-Bautista, M., Ortega-Mora, L. M., et al. (2013). Presence of *Cryptosporidium scrofarum*, *C. suis* and *C. parvum* subtypes IIAA16G2R1 and IIAA13G1R1 in Eurasian wild boars (*Sus scrofa*). *Vet. Parasitol.* 196, 497–502. doi: 10.1016/j.vetpar.2013.04.017
- Gong, C., Cao, X. F., Deng, L., Li, W., Huang, X. M., Lan, J. C., et al. (2017). Epidemiology of *Cryptosporidium* infection in cattle in China: a review. *Parasite* 24:1. doi: 10.1051/parasite/2017001
- Guo, Y., Cebelski, E., Matusевич, C., Alderisio, K. A., Lebbad, M., McEvoy, J., et al. (2015a). Subtyping novel zoonotic pathogen *Cryptosporidium* chipmunk genotype I. *J. Clin. Microbiol.* 53, 1648–1654. doi: 10.1128/JCM.03436-14
- Guo, Y., Li, N., Lysen, C., Frace, M., Tang, K., Sammons, S., et al. (2015b). Isolation and enrichment of *Cryptosporidium* DNA and verification of DNA purity for whole-genome sequencing. *J. Clin. Microbiol.* 53, 641–647. doi: 10.1128/JCM.02962-14
- Guo, Y., Tang, K., Rowe, L. A., Li, N., Roellig, D. M., Knipe, K., et al. (2015c). Comparative genomic analysis reveals occurrence of genetic recombination in virulent *Cryptosporidium hominis* subtypes and telomeric gene duplications in *Cryptosporidium parvum*. *BMC Genomics* 16:320. doi: 10.1186/s12864-015-1517-1
- Hijjawi, N., Mukbel, R., Yang, R., and Ryan, U. (2016). Genetic characterization of *Cryptosporidium* in animal and human isolates from Jordan. *Vet. Parasitol.* 228, 116–120. doi: 10.1016/j.vetpar.2016.08.015
- Hofstra, N., Bouwman, A. F., Beusen, A. H., and Medema, G. J. (2012). Exploring global *Cryptosporidium* emissions to surface water. *Sci. Total Environ.* 442C, 10–19. doi: 10.1016/j.scitotenv.2012.10.013
- Hofstra, N., and Vermeulen, L. C. (2016). Impacts of population growth, urbanisation and sanitation changes on global human *Cryptosporidium* emissions to surface water. *Int. J. Hyg. Environ. Health* 219, 599–605. doi: 10.1016/j.ijheh.2016.06.005
- Holland, R. E. (1990). Some infectious causes of diarrhea in young farm animals. *Clin. Microbiol. Rev.* 3, 345–375. doi: 10.1128/CMR.3.4.345
- Hu, Y., Feng, Y., Huang, C., and Xiao, L. (2014). Occurrence, source, and human infection potential of *Cryptosporidium* and *Enterocytozoon bieneusi* in drinking source water in Shanghai, China, during a pig carcass disposal incident. *Environ. Sci. Technol.* 48, 14219–14227. doi: 10.1021/es504464t
- Huang, C., Hu, Y., Wang, L., Wang, Y., Li, N., Guo, Y., et al. (2017). Environmental transport of emerging human-pathogenic *Cryptosporidium* species and subtypes through combined sewer overflow and wastewater. *Appl. Environ. Microbiol.* 83:e00682-17. doi: 10.1128/AEM.00682-17
- Huang, J., Yue, D., Qi, M., Wang, R., Zhao, J., Li, J., et al. (2014). Prevalence and molecular characterization of *Cryptosporidium* spp. and *Giardia duodenalis* in dairy cattle in Ningxia, northwestern China. *BMC Vet. Res.* 10:292. doi: 10.1186/s12917-014-0292-6
- Hulverson, M. A., Vinayak, S., Choi, R., Schaefer, D. A., Castellanos-Gonzalez, A., Vidadala, R. S. R., et al. (2017). Bumped-kinase inhibitors for cryptosporidiosis therapy. *J. Infect. Dis.* 215, 1275–1284. doi: 10.1093/infdis/jix120
- Ibrahim, M. A., Abdel-Ghany, A. E., Abdel-Latef, G. K., Abdel-Aziz, S. A., and Abouelhadid, S. M. (2016). Epidemiology and public health significance of *Cryptosporidium* isolated from cattle, buffaloes, and humans in Egypt. *Parasitol. Res.* 115, 2439–2448. doi: 10.1007/s00436-016-4996-3
- Inacio, S. V., Widmer, G., de Brito, R. L., Zucatto, A. S., de Aquino, M. C., Oliveira, B. C., et al. (2017). First description of *Cryptosporidium hominis* GP60 genotype Ika20G1 and *Cryptosporidium parvum* GP60 genotypes IIAA18G3R1 and IIAA15G2R1 in foals in Brazil. *Vet. Parasitol.* 233, 48–51. doi: 10.1016/j.vetpar.2016.11.021
- Jacobson, C., Williams, A., Yang, R., Ryan, U., Carmichael, I., Campbell, A. J., et al. (2016). Greater intensity and frequency of *Cryptosporidium* and *Giardia* oocyst shedding beyond the neonatal period is associated with reductions in growth, carcass weight and dressing efficiency in sheep. *Vet. Parasitol.* 228, 42–51. doi: 10.1016/j.vetpar.2016.08.003
- Jian, F., Liu, A., Wang, R., Zhang, S., Qi, M., Zhao, W., et al. (2016). Common occurrence of *Cryptosporidium hominis* in horses and donkeys. *Infect. Genet. Evol.* 43, 261–266. doi: 10.1016/j.meegid.2016.06.004
- Jian, F., Qi, M., He, X., Wang, R., Zhang, S., Dong, H., et al. (2014). Occurrence and molecular characterization of *Cryptosporidium* in dogs in Henan Province, China. *BMC Vet. Res.* 10:26. doi: 10.1186/1746-6148-10-26
- Jiang, Y., Ren, J., Yuan, Z., Liu, A., Zhao, H., Liu, H., et al. (2014). *Cryptosporidium andersoni* as a novel predominant *Cryptosporidium* species in outpatients with diarrhea in Jiangsu Province, China. *BMC Infect. Dis.* 14:555. doi: 10.1186/s12879-014-0555-7
- Karim, M. R., Zhang, S., Jian, F., Li, J., Zhou, C., Zhang, L., et al. (2014). Multilocus typing of *Cryptosporidium* spp. and *Giardia duodenalis* from non-human primates in China. *Int. J. Parasitol.* 44, 1039–1047. doi: 10.1016/j.ijpara.2014.07.006
- Kotloff, K. L., Nataro, J. P., Blackwelder, W. C., Nasrin, D., Farag, T. H., Panchalingam, S., et al. (2013). Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet* 382, 209–222. doi: 10.1016/S0140-6736(13)60844-2
- Laatamna, A. E., Wagnerová, P., Sak, B., Květoňová, D., Xiao, L., Rost, M., et al. (2015). Microsporidia and *Cryptosporidium* in horses and donkeys in Algeria: detection of a novel *Cryptosporidium hominis* subtype family (Ik) in a horse. *Vet. Parasitol.* 208, 135–142. doi: 10.1016/j.vetpar.2015.01.007
- Li, F., Wang, H., Zhang, Z., Li, J., Wang, C., Zhao, J., et al. (2016). Prevalence and molecular characterization of *Cryptosporidium* spp. and *Giardia duodenalis* in dairy cattle in Beijing, China. *Vet. Parasitol.* 219, 61–65. doi: 10.1016/j.vetpar.2016.01.023
- Li, J., Dong, H., Wang, R., Yu, F., Wu, Y., Chang, Y., et al. (2017). An investigation of parasitic infections and review of molecular characterization of the intestinal protozoa in nonhuman primates in China from 2009 to 2015. *Int. J. Parasitol. Parasites Wildl.* 6, 8–15. doi: 10.1016/j.ijppaw.2016.12.003
- Li, J., Qi, M., Chang, Y., Wang, R., Li, T., Dong, H., et al. (2015). Molecular Characterization of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* in Captive Wildlife at Zhengzhou Zoo, China. *J. Eukaryot. Microbiol.* 62, 833–839. doi: 10.1111/jeu.12269
- Li, N., Neumann, N. F., Ruecker, N., Alderisio, K. A., Sturbaum, G. D., Villegas, E. N., et al. (2015). Development and evaluation of three real-time PCR assays for genotyping and source tracking *Cryptosporidium* spp. in water. *Appl. Environ. Microbiol.* 81, 5845–5854. doi: 10.1128/AEM.01699-15
- Li, N., Xiao, L., Alderisio, K., Elwin, K., Cebelski, E., Chalmers, R., et al. (2014). Subtyping *Cryptosporidium ubiquitum*, a zoonotic pathogen emerging in humans. *Emerg. Infect. Dis.* 20, 217–224. doi: 10.3201/eid2002.121797
- Li, N., Xiao, L., Cama, V. A., Ortega, Y., Gilman, R. H., Guo, M., et al. (2013). Genetic recombination and *Cryptosporidium hominis* virulent subtype IIAA10G2. *Emerg. Infect. Dis.* 19, 1573–1582. doi: 10.3201/eid1910.121361
- Li, N., Xiao, L., Wang, L., Zhao, S., Zhao, X., Duan, L., et al. (2012). Molecular surveillance of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* by genotyping and subtyping parasites in wastewater. *PLoS Negl. Trop. Dis.* 6:e1809. doi: 10.1371/journal.pntd.0001809
- Li, P., Cai, J., Cai, M., Wu, W., Li, C., Lei, M., et al. (2016). Distribution of *Cryptosporidium* species in Tibetan sheep and yaks in Qinghai, China. *Vet. Parasitol.* 215, 58–62. doi: 10.1016/j.vetpar.2015.11.009
- Li, Q., Li, L., Tao, W., Jiang, Y., Wan, Q., Lin, Y., et al. (2016). Molecular investigation of *Cryptosporidium* in small caged pets in northeast China: host specificity and zoonotic implications. *Parasitol. Res.* 115, 2905–2911. doi: 10.1007/s00436-016-5076-4
- Li, W., Deng, L., Wu, K., Huang, X., Song, Y., Su, H., et al. (2017). Presence of zoonotic *Cryptosporidium scrofarum*, *Giardia duodenalis* assemblage A

- and *Enterocytozoon bieneusi* genotypes in captive Eurasian wild boars (*Sus scrofa*) in China: potential for zoonotic transmission. *Parasit. Vectors* 10:10. doi: 10.1186/s13071-016-1942-2
- Li, W., Li, Y., Song, M., Lu, Y., Yang, J., Tao, W., et al. (2015). Prevalence and genetic characteristics of *Cryptosporidium*, *Enterocytozoon bieneusi* and *Giardia duodenalis* in cats and dogs in Heilongjiang province, China. *Vet. Parasitol.* 208, 125–134. doi: 10.1016/j.vetpar.2015.01.014
- Lin, Q., Wang, X. Y., Chen, J. W., Ding, L., and Zhao, G. H. (2015). *Cryptosporidium* suis infection in post-weaned and adult pigs in Shaanxi province, northwestern China. *Korean J. Parasitol.* 53, 113–117. doi: 10.3347/kjp.2015.53.1.113
- Liu, A., Zhang, J., Zhao, J., Zhao, W., Wang, R., and Zhang, L. (2015). The first report of *Cryptosporidium andersoni* in horses with diarrhea and multilocus subtype analysis. *Parasit. Vectors* 8:483. doi: 10.1186/s13071-015-1102-0
- Liu, H., Shen, Y., Yin, J., Yuan, Z., Jiang, Y., Xu, Y., et al. (2014). Prevalence and genetic characterization of *Cryptosporidium*, *Enterocytozoon*, *Giardia* and *Cyclospora* in diarrheal outpatients in China. *BMC Infect. Dis.* 14:25. doi: 10.1186/1471-2334-14-25
- Liu, S., Roellig, D. M., Guo, Y., Li, N., Frace, M. A., Tang, K., et al. (2016). Evolution of mitosome metabolism and invasion-related proteins in *Cryptosporidium*. *BMC Genomics* 17:1006. doi: 10.1186/s12864-016-3343-5
- Liu, X., He, T., Zhong, Z., Zhang, H., Wang, R., Dong, H., et al. (2013). A new genotype of *Cryptosporidium* from giant panda (*Ailuropoda melanoleuca*) in China. *Parasitol. Int.* 62, 454–458. doi: 10.1016/j.parint.2013.06.004
- Liu, X., Xie, N., Li, W., Zhou, Z., Zhong, Z., Shen, L., et al. (2015a). Emergence of *Cryptosporidium hominis* monkey genotype II and novel subtype family Ik in the squirrel monkey (*Saimiri sciureus*) in China. *PLoS ONE* 10:e0141450. doi: 10.1371/journal.pone.0141450
- Liu, X., Zhou, X., Zhong, Z., Chen, W., Deng, J., Niu, L., et al. (2014a). New subtype of *Cryptosporidium cuniculus* isolated from rabbits by sequencing the Gp60 gene. *J. Parasitol.* 100, 532–536. doi: 10.1645/13-223.1
- Liu, X., Zhou, X., Zhong, Z., Deng, J., Chen, W., Cao, S., et al. (2014b). Multilocus genotype and subtype analysis of *Cryptosporidium andersoni* derived from a Bactrian camel (*Camelus bactrianus*) in China. *Parasitol. Res.* 113, 2129–2136. doi: 10.1007/s00436-014-3863-3
- Liu, X., Zhou, X., Zhong, Z., Zuo, Z., Shi, J., Wang, Y., et al. (2015b). Occurrence of novel and rare subtype families of *Cryptosporidium* in bamboo rats (*Rhizomys sinensis*) in China. *Vet. Parasitol.* 207, 144–148. doi: 10.1016/j.vetpar.2014.11.009
- Lv, C., Zhang, L., Wang, R., Jian, F., Zhang, S., Ning, C., et al. (2009). *Cryptosporidium* spp. in wild, laboratory, and pet rodents in china: prevalence and molecular characterization. *Appl. Environ. Microbiol.* 75, 7692–7699. doi: 10.1128/AEM.01386-09
- Ma, J., Cai, J., Ma, J., Feng, Y., and Xiao, L. (2014). Occurrence and molecular characterization of *Cryptosporidium* spp. in yaks (*Bos grunniens*) in China. *Vet. Parasitol.* 202, 113–118. doi: 10.1016/j.vetpar.2014.03.030
- Ma, J., Feng, Y., Hu, Y., Villegas, E. N., and Xiao, L. (2016). Human infective potential of *Cryptosporidium* spp., *Giardia duodenalis* and *Enterocytozoon bieneusi* in urban wastewater treatment plant effluents. *J. Water Health* 14, 411–423. doi: 10.2166/wh.2016.192
- Ma, J., Li, P., Zhao, X., Xu, H., Wu, W., Wang, Y., et al. (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. doi: 10.1016/j.vetpar.2014.10.011
- Mahfouz, M. E., Mira, N., and Amer, S. (2014). Prevalence and genotyping of *Cryptosporidium* spp. in farm animals in Egypt. *J. Vet. Med. Sci.* 76, 1569–1575. doi: 10.1292/jvms.14-0272
- Manjunatha, U. H., Vinayak, S., Zambriski, J. A., Chao, A. T., Sy, T., Noble, C. G., et al. (2017). A *Cryptosporidium* PI(4)K inhibitor is a drug candidate for cryptosporidiosis. *Nature* 546, 376–380. doi: 10.1038/nature22337
- Meganck, V., Hoflack, G., and Opsomer, G. (2014). Advances in prevention and therapy of neonatal dairy calf diarrhoea: a systematical review with emphasis on colostrum management and fluid therapy. *Acta Vet. Scand.* 56:75. doi: 10.1186/s13028-014-0075-x
- Mi, R., Wang, X., Huang, Y., Zhou, P., Liu, Y., Chen, Y., et al. (2014). Prevalence and molecular characterization of *Cryptosporidium* in goats across four provincial level areas in China. *PLoS ONE* 9:e111164. doi: 10.1371/journal.pone.0111164
- Mi, R., Wang, X., Li, C., Huang, Y., Zhou, P., Li, Z., et al. (2013). Prevalence and genetic characterization of *Cryptosporidium* in yaks in Qinghai Province of China. *PLoS ONE* 8:e74985. doi: 10.1371/journal.pone.0074985
- Morada, M., Lee, S., Gunther-Cummins, L., Weiss, L. M., Widmer, G., Tzipori, S., et al. (2016). Continuous culture of *Cryptosporidium parvum* using hollow fiber technology. *Int. J. Parasitol.* 46, 21–29. doi: 10.1016/j.ijpara.2015.07.006
- Muhid, A., Robertson, I., Ng, J., and Ryan, U. (2011). Prevalence of and management factors contributing to *Cryptosporidium* sp. infection in pre-weaned and post-weaned calves in Johor, Malaysia. *Exp. Parasitol.* 127, 534–538. doi: 10.1016/j.exppara.2010.10.015
- Naciri, M., Lefay, M. P., Mancassola, R., Poirier, P., and Chermette, R. (1999). Role of *Cryptosporidium parvum* as a pathogen in neonatal diarrhoea complex in suckling and dairy calves in France. *Vet. Parasitol.* 85, 245–257. doi: 10.1016/S0304-4017(99)00111-9
- Nemejc, K., Sak, B., Kvetonova, D., Hanzal, V., Janiszewski, P., Forejtek, P., et al. (2013). *Cryptosporidium suis* and *Cryptosporidium scrofarum* in Eurasian wild boars (*Sus scrofa*) in Central Europe. *Vet. Parasitol.* 197, 504–508. doi: 10.1016/j.vetpar.2013.07.003
- Ng, J., Yang, R., McCarthy, S., Gordon, C., Hijawi, N., and Ryan, U. (2011). Molecular characterization of *Cryptosporidium* and *Giardia* in pre-weaned calves in Western Australia and New South Wales. *Vet. Parasitol.* 176, 145–150. doi: 10.1016/j.vetpar.2010.10.056
- Paiva, P. R., Grego, K. F., Lima, V. M., Nakamura, A. A., da Silva, D. C., and Meireles, M. V. (2013). Clinical, serological, and parasitological analysis of snakes naturally infected with *Cryptosporidium serpentis*. *Vet. Parasitol.* 198, 54–61. doi: 10.1016/j.vetpar.2013.08.016
- Parsons, M. B., Travis, D., Lonsdorf, E. V., Lipende, I., Roellig, D. M., Kamenya, S., et al. (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. doi: 10.1371/journal.pntd.0003529
- Peng, M. M., Matos, O., Gatei, W., Das, P., Stantic-Pavlinic, M., Bern, C., et al. (2001). A comparison of *Cryptosporidium* subgenotypes from several geographic regions. *J. Eukaryot. Microbiol.* 48, 28S–31S. doi: 10.1111/j.1550-7408.2001.tb00442.x
- Peng, X. Q., Tian, G. R., Ren, G. J., Yu, Z. Q., Lok, J. B., Zhang, L. X., et al. (2016). Infection rate of *Giardia duodenalis*, *Cryptosporidium* spp. and *Enterocytozoon bieneusi* in cashmere, dairy and meat goats in China. *Infect. Genet. Evol.* 41, 26–31. doi: 10.1016/j.meegid.2016.03.021
- Platts-Mills, J. A., Babji, S., Bodhidatta, L., Gratz, J., Haque, R., Havt, A., et al. (2015). Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). *Lancet Glob. Health* 3, e564–e575. doi: 10.1016/S2214-109X(15)00151-5
- Qi, M., Cai, J., Wang, R., Li, J., Jian, F., Huang, J., et al. (2015a). Molecular characterization of *Cryptosporidium* spp. and *Giardia duodenalis* from yaks in the central western region of China. *BMC Microbiol.* 15:108. doi: 10.1186/s12866-015-0446-0
- Qi, M., Huang, L., Wang, R., Xiao, L., Xu, L., Li, J., et al. (2014). Natural infection of *Cryptosporidium muris* in ostriches (*Struthio camelus*). *Vet. Parasitol.* 205, 518–522. doi: 10.1016/j.vetpar.2014.06.035
- Qi, M., Luo, N., Wang, H., Yu, F., Wang, R., Huang, J., et al. (2015b). Zoonotic *Cryptosporidium* spp. and *Enterocytozoon bieneusi* in pet chinchillas (*Chinchilla lanigera*) in China. *Parasitol. Int.* 64, 339–341. doi: 10.1016/j.parint.2015.05.007
- Qi, M., Wang, H., Jing, B., Wang, D., Wang, R., and Zhang, L. (2015c). Occurrence and molecular identification of *Cryptosporidium* spp. in dairy calves in Xinjiang, Northwestern China. *Vet. Parasitol.* 212, 404–407. doi: 10.1016/j.vetpar.2015.07.002
- Qi, M., Wang, R., Jing, B., Jian, F., Ning, C., and Zhang, L. (2016). Prevalence and multilocus genotyping of *Cryptosporidium andersoni* in dairy cattle and He cattle in Xinjiang, China. *Infect. Genet. Evol.* 44, 313–317. doi: 10.1016/j.meegid.2016.07.022
- Qi, M., Zhou, H., Wang, H., Wang, R., Xiao, L., Arrowood, M. J., et al. (2015d). Molecular identification of *Cryptosporidium* spp. and *Giardia duodenalis* in grazing horses from Xinjiang, China. *Vet. Parasitol.* 209, 169–172. doi: 10.1016/j.vetpar.2015.02.030
- Qi, M. Z., Fang, Y. Q., Wang, X. T., Zhang, L. X., Wang, R. J., Du, S. Z., et al. (2015). Molecular characterization of *Cryptosporidium* spp. in pre-weaned calves

- in Shaanxi Province, north-western China. *J. Med. Microbiol.* 64, 111–116. doi: 10.1099/jmm.0.079327-0
- Rieux, A., Chartier, C., Pors, I., and Paraud, C. (2013a). Dynamics of excretion and molecular characterization of *Cryptosporidium* isolates in pre-weaned French beef calves. *Vet. Parasitol.* 195, 169–172. doi: 10.1016/j.vetpar.2012.12.043
- Rieux, A., Paraud, C., Pors, I., and Chartier, C. (2013b). Molecular characterization of *Cryptosporidium* isolates from pre-weaned calves in western France in relation to age. *Vet. Parasitol.* 197, 7–12. doi: 10.1016/j.vetpar.2013.05.001
- Rieux, A., Paraud, C., Pors, I., and Chartier, C. (2014). Molecular characterization of *Cryptosporidium* isolates from beef calves under one month of age over three successive years in one herd in western France. *Vet. Parasitol.* 202, 171–179. doi: 10.1016/j.vetpar.2014.03.004
- Ryan, U., Fayer, R., and Xiao, L. (2014). *Cryptosporidium* species in humans and animals: current understanding and research needs. *Parasitology* 141, 1667–1685. doi: 10.1017/S0031182014001085
- Santin, M. (2013). Clinical and subclinical infections with *Cryptosporidium* in animals. *N. Z. Vet. J.* 61, 1–10. doi: 10.1080/00480169.2012.731681
- Shen, Y., Yin, J., Yuan, Z., Lu, W., Xu, Y., Xiao, L., et al. (2011). The identification of the *Cryptosporidium ubiquitum* in pre-weaned Ovises from Aba Tibetan and Qiang autonomous prefecture in China. *Biomed. Environ. Sci.* 24, 315–320. doi: 10.3967/0895-3988.2011.03.016
- Sikora, P., Andersson, S., Winecka-Krusnell, J., Hallstrom, B., Alsmark, C., Troell, K., et al. (2016). Genomic variation in IBA10G2 and other patient derived *Cryptosporidium hominis* subtypes. *J. Clin. Microbiol.* 55, 844–858. doi: 10.1128/JCM.01798-16
- Silverlas, C., Bosaeus-Reineck, H., Naslund, K., and Bjorkman, C. (2013). Is there a need for improved *Cryptosporidium* diagnostics in Swedish calves? *Int. J. Parasitol.* 43, 155–161. doi: 10.1016/j.ijpara.2012.10.009
- Silverlas, C., Naslund, K., Bjorkman, C., and Mattsson, J. G. (2010). Molecular characterisation of *Cryptosporidium* isolates from Swedish dairy cattle in relation to age, diarrhoea and region. *Vet. Parasitol.* 169, 289–295. doi: 10.1016/j.vetpar.2010.01.003
- Soltane, R., Guyot, K., Dei-Cas, E., and Ayadi, A. (2007). Prevalence of *Cryptosporidium* spp. (Eucoccidiorida: Cryptosporiidae) in seven species of farm animals in Tunisia. *Parasite* 14, 335–338. doi: 10.1051/parasite/2007144335
- Tang, Y., Li, N., Song, M., Roellig, D. M., Feng, Y., and Xiao, L. (2016). Development of a multilocus sequence typing tool for high-resolution subtyping and genetic structure characterization of *Cryptosporidium ubiquitum*. *Infect. Genet. Evol.* 45, 256–261. doi: 10.1016/j.meegid.2016.09.011
- Taylan-Ozkan, A., Yasa-Duru, S., Usluca, S., Lysen, C., Ye, J., Roellig, D. M., et al. (2016). *Cryptosporidium* species and *Cryptosporidium parvum* subtypes in dairy calves and goat kids reared under traditional farming systems in Turkey. *Exp. Parasitol.* 170, 16–20. doi: 10.1016/j.exppara.2016.06.014
- USEPA (2012). *Method 1623.1: Cryptosporidium and Giardia in Water by Filtration/IMS/FA (Washington, D.C., EPA 816-R-12-001)*. Office of Water, U.S. Environmental Protection Agency, Washington, DC.
- Vieira, P. M., Mederle, N., Lobo, M. L., Imre, K., Mederle, O., Xiao, L., et al. (2015). Molecular characterisation of *Cryptosporidium* (Apicomplexa) in children and cattle in Romania. *Folia Parasitol.* 62:2015.002. doi: 10.14411/fp.2015.002
- Vinayak, S., Pawlowic, M. C., Sateriale, A., Brooks, C. F., Studstill, C. J., Bar-Peled, Y., et al. (2015). Genetic modification of the diarrhoeal pathogen *Cryptosporidium parvum*. *Nature* 523, 477–480. doi: 10.1038/nature14651
- Wang, L., Xiao, L., Duan, L., Ye, J., Guo, Y., Guo, M., et al. (2013a). Concurrent infections of *Giardia duodenalis*, *Enterocytozoon bienersi*, and *Clostridium difficile* in children during a cryptosporidiosis outbreak in a pediatric hospital in China. *PLoS Negl. Trop. Dis.* 7:e2437. doi: 10.1371/journal.pntd.0002437
- Wang, L., Xue, X., Li, J., Zhou, Q., Yu, Y., and Du, A. (2014). Cryptosporidiosis in broiler chickens in Zhejiang Province, China: molecular characterization of oocysts detected in fecal samples. *Parasite* 21:36. doi: 10.1051/parasite/2014035
- Wang, L., Zhang, H., Zhao, X., Zhang, L., Zhang, G., Guo, M., et al. (2013b). Zoonotic *Cryptosporidium* species and *Enterocytozoon bienersi* genotypes in HIV-positive patients on antiretroviral therapy. *J. Clin. Microbiol.* 51, 557–563. doi: 10.1128/JCM.02758-12
- Wang, R., Jian, F., Zhang, L., Ning, C., Liu, A., Zhao, J., et al. (2012). Multilocus sequence subtyping and genetic structure of *Cryptosporidium muris* and *Cryptosporidium andersoni*. *PLoS ONE* 7:e43782. doi: 10.1371/journal.pone.0043782
- Wang, R., Li, G., Cui, B., Huang, J., Cui, Z., Zhang, S., et al. (2014a). Prevalence, molecular characterization and zoonotic potential of *Cryptosporidium* spp. in goats in Henan and Chongqing, China. *Exp. Parasitol.* 142, 11–16. doi: 10.1016/j.exppara.2014.04.001
- Wang, R., Ma, G., Zhao, J., Lu, Q., Wang, H., Zhang, L., et al. (2011a). *Cryptosporidium andersoni* is the predominant species in post-weaned and adult dairy cattle in China. *Parasitol. Int.* 60, 1–4. doi: 10.1016/j.parint.2010.09.002
- Wang, R., Qiu, S., Jian, F., Zhang, S., Shen, Y., Zhang, L., et al. (2010). Prevalence and molecular identification of *Cryptosporidium* spp. in pigs in Henan, China. *Parasitol. Res.* 107, 1489–1494. doi: 10.1007/s00436-010-2024-6
- Wang, R., Wang, H., Sun, Y., Zhang, L., Jian, F., Qi, M., et al. (2011b). Characteristics of *Cryptosporidium* transmission in preweaned dairy cattle in Henan, China. *J. Clin. Microbiol.* 49, 1077–1082. doi: 10.1128/JCM.02194-10
- Wang, R., Wang, J., Sun, M., Dang, H., Feng, Y., Ning, C., et al. (2008a). 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. doi: 10.1007/s00436-008-1069-2
- Wang, R., Zhang, L., Axen, C., Bjorkman, C., Jian, F., Amer, S., et al. (2014b). *Cryptosporidium parvum* IId family: clonal population and dispersal from Western Asia to other geographical regions. *Sci. Rep.* 4:4208. doi: 10.1038/srep04208
- Wang, R., Zhang, L., Feng, Y., Ning, C., Jian, F., Xiao, L., et al. (2008b). Molecular characterization of a new genotype of *Cryptosporidium* from American minks (*Mustela vison*) in China. *Vet. Parasitol.* 154, 162–166. doi: 10.1016/j.vetpar.2007.12.038
- Wang, R., Zhang, L., Ning, C., Feng, Y., Jian, F., Xiao, L., et al. (2008c). Multilocus phylogenetic analysis of *Cryptosporidium andersoni* (Apicomplexa) isolated from a bactrian camel (*Camelus bactrianus*) in China. *Parasitol. Res.* 102, 915–920. doi: 10.1007/s00436-007-0851-x
- Wang, R., Zhang, X., Zhu, H., Zhang, L., Feng, Y., Jian, F., et al. (2011c). Genetic characterizations of *Cryptosporidium* spp. and *Giardia duodenalis* in humans in Henan, China. *Exp. Parasitol.* 127, 42–45. doi: 10.1016/j.exppara.2010.06.034
- Wang, T., Chen, Z., Xie, Y., Hou, R., Wu, Q., Gu, X., et al. (2015a). Prevalence and molecular characterization of *Cryptosporidium* in giant panda (*Ailuropoda melanoleuca*) in Sichuan province, China. *Parasit. Vectors* 8, 344. doi: 10.1186/s13071-015-0953-8
- Wang, T., Chen, Z., Yu, H., Xie, Y., Gu, X., Lai, W., et al. (2015b). Prevalence of *Cryptosporidium* infection in captive lesser panda (*Ailurus fulgens*) in China. *Parasitol. Res.* 114, 773–776. doi: 10.1007/s00436-014-4290-1
- Wang, T., Fan, Y., Koehler, A. V., Ma, G., Li, T., Hu, M., et al. (2017). First survey of *Cryptosporidium*, *Giardia* and *Enterocytozoon* in diarrhoeic children from Wuhan, China. *Infect. Genet. Evol.* 51, 127–131. doi: 10.1016/j.meegid.2017.03.006
- Wang, W., Cao, L., He, B., Li, J., Hu, T., Zhang, F., et al. (2013). Molecular characterization of *Cryptosporidium* in bats from Yunnan province, southwestern China. *J. Parasitol.* 99, 1148–1150. doi: 10.1645/13-322.1
- Wang, Y., Feng, Y., Cui, B., Jian, F., Ning, C., Wang, R., et al. (2010). Cervine genotype is the major *Cryptosporidium* genotype in sheep in China. *Parasitol. Res.* 106, 341–347. doi: 10.1007/s00436-009-1664-x
- Xiao, G., Qiu, Z., Qi, J., Chen, J. A., Liu, F., Liu, W., et al. (2013). Occurrence and potential health risk of *Cryptosporidium* and *Giardia* in the Three Gorges Reservoir, China. *Water Res.* 47, 2431–2445. doi: 10.1016/j.watres.2013.02.019
- Xiao, L. (2010). Molecular epidemiology of cryptosporidiosis: an update. *Exp. Parasitol.* 124, 80–89. doi: 10.1016/j.exppara.2009.03.018
- Xiao, L., Alderisio, K., Limor, J., Royer, M., and Lal, A. A. (2000). Identification of species and sources of *Cryptosporidium* oocysts in storm waters with a small-subunit rRNA-based diagnostic and genotyping tool. *Appl. Environ. Microbiol.* 66, 5492–5498. doi: 10.1128/AEM.66.12.5492-5498.2000
- Xiao, S., An, W., Chen, Z., Zhang, D., Yu, J., and Yang, M. (2012). Occurrences and genotypes of *Cryptosporidium* oocysts in river network of southern-eastern China. *Parasitol. Res.* 110, 1701–1709. doi: 10.1007/s00436-011-2688-6
- Xu, H., Jin, Y., Wu, W., Li, P., Wang, L., Li, N., et al. (2016). Genotypes of *Cryptosporidium* spp., *Enterocytozoon bienersi* and *Giardia duodenalis* in dogs and cats in Shanghai, China. *Parasit. Vectors* 9, 121. doi: 10.1186/s13071-016-1409-5
- Yang, Z., Zhao, W., Shen, Y., Zhang, W., Shi, Y., Ren, G., et al. (2016). Subtyping of *Cryptosporidium cuniculus* and genotyping of *Enterocytozoon*

- bieneusi* in rabbits in two farms in Heilongjiang Province, China. *Parasite* 23:52. doi: 10.1051/parasite/2016063
- Ye, J., Xiao, L., Li, J., Huang, W., Amer, S. E., Guo, Y., et al. (2014). Occurrence of human-pathogenic *Enterocytozoon bieneusi*, *Giardia duodenalis* and *Cryptosporidium* genotypes in laboratory macaques in Guangxi, China. *Parasitol. Int.* 63, 132–137. doi: 10.1016/j.parint.2013.10.007
- Ye, J., Xiao, L., Ma, J., Guo, M., Liu, L., and Feng, Y. (2012). Anthroponotic enteric parasites in monkeys in public park, China. *Emerg. Infect. Dis.* 18, 1640–1643. doi: 10.3201/eid1810.120653
- Ye, J., Xiao, L., Wang, Y., Wang, L., Amer, S., Roellig, D. M., et al. (2013). Periparturient transmission of *Cryptosporidium xiaoi* from ewes to lambs. *Vet. Parasitol.* 197, 627–633. doi: 10.1016/j.vetpar.2013.07.021
- Yin, J. H., Yuan, Z. Y., Cai, H. X., Shen, Y. J., Jiang, Y. Y., Zhang, J., et al. (2013). Age-related infection with *Cryptosporidium* species and genotype in pigs in China. *Biomed. Environ. Sci.* 26, 492–495. doi: 10.3967/0895-3988.2013.06.010
- Yin, J., Shen, Y., Yuan, Z., Lu, W., Xu, Y., and Cao, J. (2011). Prevalence of the *Cryptosporidium* pig genotype II in pigs from the Yangtze River Delta, China. *PLoS ONE* 6:e20738. doi: 10.1371/journal.pone.0020738
- Zhang, S., Tao, W., Liu, C., Jiang, Y., Wan, Q., Li, Q., et al. (2016). First report of *Cryptosporidium canis* in foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) and identification of several novel subtype families for *Cryptosporidium* mink genotype in minks (*Mustela vison*) in China. *Infect. Genet. Evol.* 41, 21–25. doi: 10.1016/j.meegid.2016.03.016
- Zhang, W., Wang, R., Yang, F., Zhang, L., Cao, J., Zhang, X., et al. (2013a). Distribution and genetic characterizations of *Cryptosporidium* spp. in pre-weaned dairy calves in Northeastern China's Heilongjiang Province. *PLoS ONE* 8:e54857. doi: 10.1371/journal.pone.0054857
- Zhang, W., Yang, F., Liu, A., Wang, R., Zhang, L., Shen, Y., et al. (2013b). Prevalence and genetic characterizations of *Cryptosporidium* spp. in pre-weaned and post-weaned piglets in Heilongjiang Province, China. *PLoS ONE* 8:e67564. doi: 10.1371/journal.pone.0067564
- Zhang, X. X., Cong, W., Ma, J. G., Lou, Z. L., Zheng, W. B., Zhao, Q., et al. (2016). First report of *Cryptosporidium canis* in farmed Arctic foxes (*Vulpes lagopus*) in China. *Parasit. Vectors* 9:126. doi: 10.1186/s13071-016-1396-6
- Zhang, X. X., Tan, Q. D., Zhou, D. H., Ni, X. T., Liu, G. X., Yang, Y. C., et al. (2015a). Prevalence and molecular characterization of *Cryptosporidium* spp. in dairy cattle, northwest China. *Parasitol. Res.* 114, 2781–2787. doi: 10.1007/s00436-015-4537-5
- Zhang, X. X., Zhang, N. Z., Zhao, G. H., Zhao, Q., and Zhu, X. Q. (2015b). Prevalence and genotyping of *Cryptosporidium* infection in pet parrots in North China. *Biomed. Res. Int.* 2015:549798. doi: 10.1155/2015/549798
- Zhao, G. H., Du, S. Z., Wang, H. B., Hu, X. F., Deng, M. J., Yu, S. K., et al. (2015). First report of zoonotic *Cryptosporidium* spp., *Giardia intestinalis* and *Enterocytozoon bieneusi* in golden takins (*Budorcas taxicolor bedfordi*). *Infect. Genet. Evol.* 34, 394–401. doi: 10.1016/j.meegid.2015.07.016
- Zhao, G. H., Ren, W. X., Gao, M., Bian, Q. Q., Hu, B., Cong, M. M., et al. (2013). Genotyping *Cryptosporidium andersoni* in cattle in Shaanxi Province, Northwestern China. *PLoS ONE* 8:e60112. doi: 10.1371/journal.pone.0060112
- Zhao, W., Wang, R., Zhang, W., Liu, A., Cao, J., Shen, Y., et al. (2014). 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. doi: 10.1371/journal.pone.0102006
- Zhao, Z., Wang, R., Zhao, W., Qi, M., Zhao, J., Zhang, L., et al. (2015). Genotyping and subtyping of *Giardia* and *Cryptosporidium* isolates from commensal rodents in China. *Parasitology* 142, 800–806. doi: 10.1017/S0031182014001929
- Zhu, H., Zhao, J., Wang, R., and Zhang, L. (2012). Molecular identification of a rare subtype of *Cryptosporidium hominis* in infants in China. *PLoS ONE* 7:e43682. doi: 10.1371/journal.pone.0043682
- Zou, Y., Ma, J. G., Yue, D. M., Zheng, W. B., Zhang, X. X., Zhao, Q., et al. (2017). Prevalence and risk factors of *Cryptosporidium* infection in farmed pigs in Zhejiang, Guangdong, and Yunnan provinces, China. *Trop. Anim. Health Prod.* 49, 653–657. doi: 10.1007/s11250-017-1230-y

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer MY and handling Editor declared their shared affiliation.

Copyright © 2017 Feng and Xiao. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.