Cryptosporidium in asymptomatic children in Southern Xinjiang, China and the potential of zoonotic transmission

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Abstract

Background: *Cryptosporidium* is a primary cause of diarrhea in children globally. However, there is limited information on the prevalence and genetic characterization of *Cryptosporidium* in children in Xinjiang, China. This study aimed to assess the genetic characteristics and epidemiological status of *Cryptosporidium* in asymptomatic children in Southern Xinjiang, China.

Methods: A total of 609 fecal samples were collected from kindergartners aged 2-6 y from 11 counties of Southern Xinjiang, China. We used nested PCR amplification of partial SSU rDNA gene to screen the samples for *Cryptosporidium* spp. The isolates containing *C. parvum* and *C. hominis* were further subtyped by a 60-kDa glycoprotein (*gp60*). We used MEGA7 to construct a phylogenetic tree to study the genetic relationship between the *gp60* subtypes of these two species via the Maximum Likelihood method based on the Tamura-Nei model.

Results: Only 1.3% (8/609) of asymptomatic children were confirmed as infected with *Cryptosporidium* with 2.0% (6/299) infection rate in boys and 0.6% (2/310) infection rate in girls. Three *Cryptosporidium* species were identified including *C. felis* (37.5%; 3/8), *C. hominis* (37.5%; 3/8) and *C. parvum* (25.0%; 2/8). Three *C. hominis* subtypes (IbA9G3, IdA14 and IfA12G1) and two *C. parvum* subtypes (IIdA14G1 and IIdA15G1) were also found.

Conclusions: This study was the first to identify the presence of *cryptosporidium* in asymptomatic children in Southern Xinjiang, China. The presence of zoonotic *C. parvum* subtypes IIdA14G1 and IIdA15G1 indicates possible crossspecies transmission of *Cryptosporidium* between children and animals.

Background

Cryptosporidium spp. are obligate intracellular protozoan parasites, which can be found in all classes of vertebrates [1]. *Cryptosporidium* infects the gastric and intestinal epithelial cells leading to self-limiting diarrhea or asymptomatic symptoms in healthy people. However, infection can be fatal amongst immunocompromised individuals [2]. *Cryptosporidium* infection leads to stunted growth and weight loss, among other features. *Cryptosporidiosis* accounts for approximately 30.0%-50.0% global deaths per-year in children < 5 y of age and is the second leading cause of diarrhea-related fatalities in children after rotavirus [3]. *Cryptosporidium* can be transmitted via various routes, including direct contact with infected individuals/animals, or uptake of contaminated food/water [4].

Cryptosporidiosis is a complex genus in which a total of more than 40 species and over 40 genotypes (yet to be assigned a species) have been formally described, all with high genetic diversity [5, 6]. Molecular studies have demonstrated that human cases of cryptosporidiosis are caused by minimum 20 different species. The dominant subtypes include *C. hominis* and *C. parvum* followed by *Cryptosporidium meleagridis*, *C. ubiquitum* and *C. viatorum*. Meanwhile, *C. canis*, *C. felis*, *C. cuniculus*, and *C. andersoni*, in addition to three *Cryptosporidium* genotypes (horse, chipmunk I and skunk) cause disease in humans

and are zoonotic [7]. Various gp60-targeted subtyping tools have been designed to assess the importance of zoonotic transmission, using comparative analyses of human and animal samples [7, 8].

In China, recent studies have focused on *Cryptosporidium* in children in Central and Southern China but molecular detection methods have been used in no more than three reported studies [9]. Four species of *Cryptosporidium*, including *C. meleagridis*, *C. hominis*, *C. canis*, and *C. felis* have been identified in children from China. Amongst them, *C. hominis* is the predominant species and four subtype families including Ia, Ib, Id and Ig, are present within the *C. hominis* isolates [9]. Nevertheless, the species and subtypes of *Cryptosporidium* in children in China remain poorly defined, particularly in specific provinces or regions such as in Xinjiang Uygur Autonomous Region (XUAR), where a number of zoonotic species of *Cryptosporidium* are distributed in local animals. We therefore propose that the scale of infection by *Cryptosporidium* in children has been underestimated in China. This population poses a significant threat if the infections continue to be ignored.

In this study, we investigated the epidemiology of *Cryptosporidium* infection in children aged 1–6 years in XUAR, North-West China, and tracked possible sources of infection and assessed the crossspecies spread of *Cryptosporidium* spp. between children and animals using genotyping and subtyping tools. Such knowledge can provide insight into control and prevention strategies against infections for this pathogen.

Methods

Sample Collection

Between August 2017 to January 2019, 609 fecal samples were collected from kindergarteners aged 2–6 y from 11 counties of Southern Xinjiang, China (Table 1). The parents/guardians who provided consent on behalf of their children were trained with relevant guidelines by the staff and were provided a labeled plastic fecal collector marked with the date of collection and patient identity information (age and sex). None of the kids experienced diarrhea during the sampling period. After collection, samples were stored at 4°C.

Table 1
Prevalence and species distribution of *Cryptosporidium* among children from southern Xinjiang by counties and gender

Collection site	Boy		Girl	
	No. Positive/No. Samples (%)	Species and subtype (n)	No. Positive/No. Samples (%)	Species and subtype (n)
Baicheng	0/11	/	0/12	/
Hotan	0/40	/	0/40	/
Kuqa	1/19 (5.3)	<i>C. parvum</i> (1), lldA15G1 (1)	1/19 (5.3)	<i>C. parvum</i> (1), lldA14G1 (1)
Lop	0/43	/	0/66	/
Payzawat	1/14 (7.1)	<i>C. hominis</i> (1), lfA12G1 (1)	0/11	/
Pishan	0/16	/	0/21	/
Poskam	0/17	/	1/18 (5.6)	<i>C. hominis</i> (1), lbA9G3 (1)
Shufu	0/26	/	0/22	/
Tumushuke	0/33	/	0/29	/
Yecheng	2/46 (4.3)	<i>C. felis</i> (2)	0/43	/
Yopurga	2/34 (5.9)	<i>C. hominis</i> (1), ldA14 (1); <i>C. felis</i> (1)	0/29	/
Subtotal	6/299 (2.0)	<i>C. felis</i> (3); <i>C. hominis</i> (2), ldA14 (1), lfA12G1 (1); <i>C. parvum</i> (1), lldA15G1 (1)	2/310 (0.6)	<i>C. hominis</i> (1), lbA9G3 (1); <i>C. parvum</i> (1), lldA14G1 (1)
Total	8/609 (1.3)	<i>C. felis</i> (3); <i>C. hominis</i> (3), lbA9G3 (1), ldA14 (1), lfA12G1 (1); <i>C. parvum</i> (2), lldA14G1 (1), lldA15G1 (1)		

DNA Extraction and PCR Amplification

Approximately 200 mg of each fecal sample was extract the genomic DNA used the *E.Z.N.A*® stool DNA kit, and stored the extracted DNA at -20°C before PCR analysis.

We used nested PCR amplification of an approximately 830 bp partial SSU rDNA gene fragment to screen the samples for the presence of *Cryptosporidium* spp. We used primers described previously in a study by Xiao et al. [10]. Additionally, we used nested PCR to amplify an 850 bp gene fragment of a 60-kDa glycoprotein (*gp60*) to subtype *Cryptosporidium* positive isolates. This study was done using primers

described previously in a study by Alves et al. [11]. Also, dH₂O was included as a negative control and DNA from chicken-derived *C. bailey* was used as the positive control.

Sequence and Phylogenetic Analyses

We used GENEWIZ to bidirectionally sequence positive secondary PCR products. Sequencing of PCR products was performed to confirm sequence accuracy during DNA preparation. We used DNASTAR Lasergene EditSeq v7.1.0 (<http://www.dnastar.com/>) to edit the derived sequences and used Clustal X v2.1 (<http://www.clustal.org/>) to align them with reference sequences downloaded from GenBank.

A phylogenetic tree was constructed using MEGA7 using the Maximum Likelihood method based on the Tamura-Nei model to evaluate the genetic relationship between the *gp60* subtypes of *C. hominis* and *C. parvum*.

Results

Prevalence of Cryptosporidium

Cryptosporidium spp. was identified in a few asymptomatic children, with only eight *Cryptosporidium* positive (1.3%, 8/609) based on the SSU rDNA gene. The infection rate of *Cryptosporidium* in children in 5 of the 11 collection sites of the Xinjiang Province was: Payzawat (4.0%; 1/25), Yopurga (3.2%; 2/63), Yecheng (2.2%; 2/89), Poskam (2.9%; 1/35), and Kuqa (5.3%; 2/38). No substantial difference in the infection rate of *Cryptosporidium* in children from the five sites ($P > 0.05$) (Table 1). The infection rate of *Cryptosporidium* was 2.0% (6/299) in boys and 0.6% (2/310) in girls. There was no insignificant difference in the infection rate of *Cryptosporidium* in boys and in girls ($P > 0.05$).

Genetic characterizations at the SSU rRNA gene

Three *Cryptosporidium* species were identified, including *C. felis* (37.5%; 3/8), *C. hominis* (37.5%; 3/8), and *C. parvum* (25.0%; 2/8). The three, three and two SSU rDNA sequences of *C. felis*, *C. hominis* and *C. parvum* were identical, respectively and showed 100% similarity with the sequences in Genbank: AF159113 for *C. felis*, MK990042 for *C. hominis* and MK982463 for *C. parvum*. Amongst them, *C. hominis* was identified at three collection sites (Payzawat, Poskam and Yopurga), *C. felis* in Yopurga and Yecheng, and *C. parvum* only in Kuqa. *C. felis* was only identified in boys, whilst *C. hominis* and *C. parvum* were both identified in both boys and girls.

Subtyping of *C. hominis* and *C. parvum* of the *gp60* gene

All six *C. hominis* and *C. parvum* isolates were successfully amplified at the *gp60* gene. Sequence analysis of three *C. hominis* isolates showed three subtypes, termed IbA9G3 (1), IdA14 (1) and IfA12G1 (1). The sequences of two *C. parvum* isolates identified in the study showed 100% similarity to those of IIdA14G1 and IIdA15G1, respectively.

Discussion

Almost all countries in the world except Antarctica has been recorded to have a high incidence of cryptosporidiosis in humans [12]. The African nations, central and south American countries, Asian nations, European countries, and North American countries have been reported to have 2.6%-21.3%, 3.2%-31.5%, 1.3%-13.1%, 0.1%-14.1%, 0.3%-4.3% cases of *Cryptosporidium* infection, respectively, resulting in a pooled prevalence of 7.6% [9, 13]. Statistics have shown that up to 2018 in China, approximately 200,054 individuals participated in *Cryptosporidium* related studies, of which 5,933 (3.0%) were diagnosed with *Cryptosporidium* infection/cryptosporidiosis. The disease was found to be less prevalent in adults (1.9%, 402/21316) than in children < 5 years old (2.6%, 269/10491) in China ($P < 0.01$) [9].

Other than age, sensitivity and specificity of the detection techniques, host health, and living criteria might also affect disease prevalence [14]. The estimated prevalence was high in those from low-income countries, individuals with gastrointestinal symptoms and residents not living in urban areas [12]. In developing countries, the prevalence of *Cryptosporidium* in children under five years with diarrhea was 27.4% in India (ELISA) [15], 27.8% and 32.0% in Ghana and Guatemala (microscopic analysis), respectively [16, 17], and 25.0% and 10.4% in Uganda and Tanzania (PCR), respectively [18, 19]. In China, significantly higher prevalence of *Cryptosporidium* was observed in rural population (1.8%-12.9%) than in urban population (0-3.7%) [9]. In the USA, cryptosporidiosis was mainly observed in children between 1–9 y of age; its high incidence was associated with recreational water use and communal swimming venues, resulting in peak infection in the summer [20].

There was a limited of information on the prevalence of asymptomatic infection. *Cryptosporidium* was detected in 20 (7.2%) of 276 asymptomatic aboriginal children living in villages in Malaysia [21]. In Jeddah, South Africa, almost 4.7% *Cryptosporidium*-positive cases were asymptomatic compared with 32.0% cases with diarrhea from pediatric clinics [22]. This study is the first study to explore the infection of *Cryptosporidium* in asymptomatic children in China with low infection rates (1.3%; 8/609).

Three *Cryptosporidium* species were identified, including *C. felis* (37.5%; 3/8), *C. hominis* (37.5%; 3/8) and *C. parvum* (25.0%; 2/8). *C. felis* oocysts were first identified in cat feces and were therefore considered a host-adapted species [23]. Human *C. felis* infections have shown more recent prevalence in developing countries including China, where it was found to cause *Cryptosporidium* infection in minimum eight human cases [7]. In addition to cats and humans, *C. felis* has also been found in other animals including non-human primates, calves, horses, and foxes, suggestive of a possible risk of zoonotic transmission [24]. *C. hominis* is a pathogenic species commonly found in humans and natural infections have been reported in nonhuman primates, cattle, dugong, marsupials, and goats [25]. The major *Cryptosporidium* species in Chinese population is *C. hominis*, which has been shown to cause approximately 48.3% (127/263) of human cases [9]. Recent studies have identified *C. hominis* as the dominant *Cryptosporidium* species in other species in China, such as horses and donkeys [26–28]. This supports the theory that these animals have been infected by human feces. *C. parvum* has been shown to have the

highest zoonotic potential among the *Cryptosporidium* species. In China, 16.7% (44/263) of human cases are caused by *C. parvum* [9]. In this study, we identified *C. parvum* in yaks, sheep, goats, golden takins, horses, cattle, and donkeys, indicating zoonotic potential.

Subtyping tools based on the analysis of the *gp60* gene have been developed for human-pathogenic *Cryptosporidium* spp. to track potential sources of infection [7]. In this study, three subtype families (Ib, Id, and If) were identified for *C. hominis*, composed of IdA14, IfA12G1 and IbA9G3. Elevated frequency of all three subtype families (Ib, Id, and If) were identified in rhesus monkeys in Guizhou, where humans and animals are known to closely interact [29]. This study is the first work that is performed on humans with the discovery that those subtypes can spread between humans and monkeys.

There is a limited availability of data on the subtypes of *C. parvum* in humans in China compared to *C. hominis*. Only two subtypes (IIa and IId) have been identified and only IIdA19G1 was found [30]. In this study, subtyping successfully identified two *C. parvum* isolates of the IId family; IIdA15G1 and IIdA14G1. These were the dominant groups in animals in China indicating that these animals may represent an important source of zoonotic *Cryptosporidium* in China [7].

Conclusions

This study was the first to identify the presence of asymptomatic *cryptosporidium* infections in children in Southern Xinjiang China. Three species of *Cryptosporidium* including *C. felis*, *C. hominis* and *C. parvum* were found. Meanwhile, three *C. hominis* subtypes (IdA14, IfA12G1 and IbA9G3) and two *C. parvum* subtypes (IIdA14G1 and IIdA15G1) were identified. The presence of common zoonotic *C. parvum* subtypes highlights the possible cross-species transmission of *Cryptosporidium* between children and animals.

Abbreviations

gp60: 60-kDa glycoprotein; SSU: small subunit

Declarations

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Ethics approval and consent to participate

The Research Ethics Committee and the Ethics Committee of Tarim University approved the study protocol. During the entire procedure, no children were injured.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MQ and WZTW, ZW, YZ and QZTW and ZWTW, TW and MQ and LZ

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Figures

Figure 1

Molecular Phylogenetic analysis. The Tamura-Nei model-based Maximum Likelihood method was used to determine the evolutionary history. Here is the tree with the highest log likelihood with branches showing the % of trees with clusters of relevant taxa. The NJ and BioNJ algorithms were used to obtain the initial tree(s) for heuristic searches using the MCL method. We selected the topology with a superior log likelihood value. The tree has been drawn to scale, and the number of substitutions per-site indicate

branch lengths. There were 32 nucleotide sequences used for analysis. Codon positions included 1st + 2nd + 3rd + non-coding. All positions with missing data and gaps were excluded, resulting in 292 positions. MEGA7 was used to perform the evolutionary analyses.

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