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ORIGINAL PAPER

Detection of *Cryptosporidium* and *Giardia* in agricultural and water environments in the Qinghai area of China by IFT and PCR

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Abstract Qinghai Province in northwest China is strongly influenced by agricultural activities and is an important source of food and drinking water. Here, we present findings regarding the occurrence and molecular epidemiology of *Cryptosporidium* and *Giardia* species based on a large-scale investigation of areas of Qinghai Province. The diagnosis and molecular detection of *Cryptosporidium* oocysts and *Giardia* cysts was carried out using immunofluorescence microscopy (IFT), whereas nested polymerase chain reaction (PCR) in fecal smears and water samples was used for the detection and molecular characterization of the species. In total, 561 samples (260 water samples and 301 fecal samples from animals) were collected and analyzed. Of the 260 water samples, 66 samples were *Cryptosporidium*-positive by IFT and 71 samples were positive by nested PCR; in addition, 39 samples were *Giardia*-positive by IFT and 40 samples were positive by nested PCR. Of the 301 fecal samples from animals, 98 samples were *Cryptosporidium*-positive by IFT and 61 samples were positive by nested PCR, whereas 52 samples were *Giardia*-positive by IFT and 31 samples were positive by nested PCR. We showed that the water supplies and animals investigated contained *Cryptosporidium* and *Giardia* (oo)cysts. Thus, we recommend that the Chinese Government and Chinese health authorities undertake control measures to protect the food and drinking water sources in Qinghai from these pathogenic protozoa.

Keywords Water · Feces · *Giardia* · *Cryptosporidium* · Nested PCR · Immunofluorescence test

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Introduction

The occurrence of the (oo)cysts of *Cryptosporidium* and *Giardia* in agricultural water and other aquatic environments is a globally acknowledged public health problem. There are numerous documented reports of water-borne outbreaks of giardiasis and cryptosporidiosis throughout the world (Karanis et al. 2007a). At least 199 outbreaks resulting from the waterborne transmission of parasitic protozoa were reported worldwide during 2004–2010 (Baldursson and Karanis 2011). A widely used method for the detection of the (oo)cysts in environmental samples is the immunofluorescence assay (IFA), which is time-consuming and subject to variations in sensitivity. However, identifying the species infecting humans and animals is important in determining the epidemiology of the disease and the transmission routes of *Cryptosporidium* (Plutzer et al. 2007; 2008; Plutzer and Karanis 2009).

In many countries, including China, there are no requirements for testing surface water for the presence of these parasites. The numbers of clinical giardiasis and cryptosporidiosis cases are likely to be underestimated in China because of the lack of systematic methods for the diagnosis of these parasites. Molecular tools for the detection and characterization of *Giardia* and *Cryptosporidium* species have been widely applied in recent years, particularly for the detection and characterization of species from water supplies.

In the present study, we used IFT, polymerase chain reaction (PCR) assays for comparative detection of *Giardia* and *Cryptosporidium* (oo)cysts in environmental water samples from fecal samples and drinking water supplies in the Qinghai province of northwest China.

Materials and methods

General information on geography

Qinghai is located on the north-eastern part of the Tibetan Plateau. The Yellow River originates in the south of the province, whereas the Yangtze River and the Mekong have their sources in the south west. Qinghai is separated by the Riyue Mountain into pastoral and agriculture zones in the west and east. Located mostly on the Qinghai-Tibet plateau, the province has long been a melting pot for several ethnic groups, including the Han, Tibetans, Hui, Tu, Mongols, and Salars. Qinghai borders Gansu in the northeast, Xinjiang in the northwest, Sichuan in the southeast, and Tibet Autonomous Region in the southwest.

During the hot summer months, many tourists from the hot southern and eastern parts of China travel to Xining because its climate in July and August is relatively mild and comfortable, making the city an ideal summer retreat. Thousands of tourists use the seashore and rivers for swimming and camping activities. Because it is a popular tourist destination and the use of rivers, etc., is very popular, there is a need to understand the protozoan burden of the area.

Sample sizes and sites

During 2011 and 2012, a total of 561 samples were collected, including 260 water samples and 301 fecal samples. For each sample, information was collected about its location, river name, and size (Fig. 1; Table 1). There are no seasonal differences in sampling. Fecal samples were collected from domesticated animals (including yak, sheep, goat, cattle, horse, donkey, and dog) on farms (including national and private) and some wild animals, such as the Tibetan antelope, pika, and sokhor. The location, animal species, and size of each sample are shown in Fig. 1, and Tables 2 and 3. All the samples were collected from the south and east parts of Qinghai Province where the land is used for farming. The interaction of humans, animals, and the environment with *Cryptosporidium* and *Giardia* enable these organisms to complete their life circle. Therefore, it is crucial to understand the prevalence of *Cryptosporidium* and *Giardia* in this region.

Sample collection method

Water samples

At each sampling point, a 20-L water sample was collected and divided in two equal portions; 10 L was used for IFT, and 10 L was analyzed using molecular methods. The water was collected in sterile plastic tanks by the sample containers dropped into the water and transferred to the laboratory for parasitological analysis. The samples were analyzed in the laboratory of the Academy of Animal Sciences in Xining in the Department of Infectious Diseases.

Fecal samples

Fecal material from each animal was collected in labeled Eppendorf cups (2×1.5 mL); duplicate samples were placed in 1.5 mL cups, capped, and then immediately placed on ice and transported to the laboratory. In the laboratory, the fecal samples were preserved in 2.5 % potassium dichromate and kept at 4 °C until use. In total, 301 fresh fecal samples were collected during 2011 and 2012 from different animal species (Tables 2 and 3).

(Oo)cyst concentration

Water samples

The (oo)cyst concentration in the investigated environmental water samples was carried out following a modified version of the methodology of Karanis et al. (2006). Briefly, 10 L of river water was filtered through membrane filters (diameter 142 mm) with a pore size of 1.2 μm by means of a vacuum device. After filtration, the filters were washed at least three times with 15 mL 0.1 % Tween 80. The eluate and the wash solutions were collected in sterile 50-mL conical tubes and centrifuged at 1,500×*g* for 15 min. The supernatant was removed, and the pellet (1–2 mL, depending on the water turbidity) was subjected to sucrose flotation (Karanis et al. 2006, 2007a).

Microscopic examination

Microscopic examinations using immunofluorescence (CRYPTO CEL, Cellabs Pty, Brookvale, Australia) were performed on all samples after discontinuous sucrose gradient purification and diethyl-ether/phosphate-buffered saline (2:1 *v/v*) biphasic concentration as described previously (Karanis et al. 2006, 2007a).

Fig. 1 Distribution of the sites of sample collection in Qinghai Province. All samples were collected from the south and east parts of Qinghai Province, which are the main farming regions. In addition, the Three River Source area is important to downstream areas for farming and drinking water supplies. *Black triangles* indicate the main rivers sampled

Detection and enumeration of (oo)cysts by IFT

A 25-μl aliquot of each suspension was placed on a glass-well microscope slide for the enumeration of *Cryptosporidium* oocysts and *Giardia* cysts. The samples were mounted onto slides, fixed with methanol, and then stained with fluorescing iso-thiocyanate-conjugated anti-*Cryptosporidium* spp. and anti-*Giardia* spp. monoclonal antibodies obtained from Cellabs Biotechnology (Australia). Each slide was scanned completely for cysts and oocysts by using an immunofluorescence microscope (Olympus BHF-T). Organisms that met the criteria for *Giardia* cysts and *Cryptosporidium* oocysts (based on size, shape, and fluorescence under a total magnification of×200 to×400) were labeled as (oo)cysts.

Detection of (oo)cysts by PCR

DNA extraction

DNA extraction was performed for all water and fecal samples. DNA from the water samples was extracted from seeded water and concentrations of river water samples using a QIAamp water Mini Kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions, with the addition of ten freeze–thaw cycles, as described previously (Plutzer et al. 2008, 2009). DNA from the fecal samples was extracted using a QIAamp DNA Stool Mini Kit (Qiagen GmbH) according to the manufacturer's instructions.

PCR for Cryptosporidium and Giardia

Cryptosporidium A nested PCR was performed to detect *Cryptosporidium* oocysts. PCR primers that had been previously described were used to amplify the 825-bp fragment of *C. parvum* 18S RNA (Karanis et al. 2007a, c). Both PCRs were performed in standard mixtures of 50 μL containing 1.25 μL primer mixtures (200 nmol of each primer), 8 μL of 200 mM dNTP mix (200 mM of each dNTP) (Finnzymes, Espoo, Finland), 1× PCR buffer containing 5 μL 1.5 mM $MgCl_2$ (Qiagen GmbH), 3 μL 3 mM $MgCl_2$ (Qiagen GmbH), 0.5 μL 2.5 U HotstarTaq DNA polymerase (Qiagen GmbH), 2 μL bovine serum albumin (BSA; acetylated, 10 mg/mL) (Promega, Madison, WI, USA), 3 μL DNA, and 27.25 μL distilled water.

For primary PCR, the amplification reactions were run according to the following PCR program: An initial step at 94 °C for 3 min; 35 cycles of 94 °C for 45 s, 55 °C for 45 s,

Table 1 All water samples' information about their location, river, sample size per location, and detection results by IFT and nPCR

Location	River	Sample Size	*Cryptosporidium*				*Giardia*			
			IFT	Pos-rate (%)	nPCR	Pos-rate (%)	IFT	Pos-rate (%)	nPCR	Pos-rate (%)
Huangzhong county	Mayigou reserve	8	3	37.50	1	12.50	2	25.00	2	25.00
	Nanchuan River	5	1	20.00	2	40.00	0	0.00	0	0.00
	Pandao reserve	9	2	22.22	1	11.11	3	33.33	2	22.22
Xining	Huangshui River	10	1	10.00	3	30.00	3	30.00	1	10.00
	Beichuan River	11	2	18.18	2	18.18	4	36.36	2	18.18
	Nanchuan River	8	1	12.50	1	12.50	0	0.00	1	12.50
Guide county	Yellow River	5	2	40.00	2	40.00	1	20.00	1	20.00
	Nongna Power station	9	3	33.33	3	33.33	1	11.11	1	11.11
Menyuan county	Menyuan River	6	2	33.33	4	66.67	1	16.67	2	33.33
	Huangcheng River	8	1	12.50	1	12.50	2	25.00	3	37.50
Gangcha county	Haergai River	3	0	0.00	1	33.33	0	0.00	0	0.00
	Shaliu River	5	1	20.00	3	60.00	2	40.00	0	0.00
	Wetland	7	1	14.29	1	14.29	3	42.86	1	14.29
	Huangshui River tributary	9	2	22.22	4	44.44	1	11.11	0	0.00
Haiyan county	Reshui River	6	2	33.33	1	16.67	0	0.00	1	16.67
	Huangshui River tributary	8	2	25.00	3	37.50	0	0.00	0	0.00
	Wetland	7	1	14.29	2	28.57	0	0.00	0	0.00
	Dongdatan reserve	8	4	50.00	1	12.50	2	25.00	2	25.00
Datong county	Heiquan reserve	9	5	55.56	1	11.11	1	11.11	1	11.11
	Xiazhi Spring	9	4	44.44	1	11.11	0	0.00	1	11.11
	Dongxia River	6	1	16.67	3	50.00	1	16.67	1	16.67
Huzhu county	Ximen River	9	3	33.33	1	11.11	1	11.11	2	22.22
	Huzhu Lake	8	2	25.00	3	37.50	0	0.00	1	12.50
	Nanmenxia reserve	10	1	10.00	1	10.00	1	10.00	2	20.00
	Nanmenxia Vetland	5	1	20.00	3	60.00	1	20.00	2	40.00
Qilian county	Ebo River	7	2	28.57	1	14.29	0	0.00	1	14.29
	Binggou River	7	1	14.29	2	28.57	2	28.57	1	14.29
	Babao River	9	2	22.22	4	44.44	1	11.11	2	22.22
Jianzha county	Yellow River	9	3	33.33	1	11.11	0	0.00	0	0.00
	Longyangxia reserve	5	1	20.00	2	40.00	1	20.00	1	20.00
Gonghe county	Quantou wetland	8	1	12.50	1	12.50	2	25.00	2	25.00
Zeku county	Yellow River	7	2	28.57	3	42.86	1	14.29	1	14.29
Qinghai Lake tributary	Heima river	4	1	25.00	1	25.00	1	25.00	1	25.00
	Daotang river	4	2	50.00	3	75.00	1	25.00	0	0.00
	Buhahe river	4	1	25.00	2	50.00	0	0.00	0	0.00
	Quanji river	4	1	25.00	1	25.00	0	0.00	1	25.00
	Shaliu river	4	1	25.00	1	25.00	0	0.00	1	25.00
Total	37	260	66	25.38	71	27.31	39	15.00	40	15.38

Sample size provides the number of the samples taken and investigated

IFT, nPCR provide the number of the positives samples detected by IFT and nPCR, *pos-rate* positivity rate in percent

and 72 °C for 1 min; then 72 °C for 7 min and a final hold at 4 °C. For secondary PCR, each reaction was prepared as for primary PCR, but F2 and R2 primers were used and the following PCR program was run: 94 °C, 3 min; 35 cycles of 94 °C for 45 s, 58 °C for 45 s, and 72 °C for 1 min; then 72 °C for 7 min and a final hold at 4 °C.

Giardia Semi-nested PCR was performed using the primers published by Read et al. (2004) to amplify a 432-bp fragment of the *Giardia* glutamate dehydrogenase gene. A nested PCR was performed in a standard mixture of 25 μL containing 200 nmol of each primer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 2.5 U Taq DNA polymerase

Table 2 All fecal samples' information about their location, size, and detection results by IFT and nPCR

Location	Sample size	*Cryptosporidium*				*Giardia*			
		IFT	Pos-rate (%)	nPCR	Pos-rate (%)	IFT	Pos-rate (%)	nPCR	Pos-rate (%)
Huangzhong County	28	11	39.29	5	17.86	7	25.00	4	14.29
Xining City	22	7	31.82	4	18.18	6	27.27	1	4.55
Guide County	16	7	43.75	5	31.25	6	37.50	2	12.50
Menyuan County	13	6	46.15	6	46.15	5	38.46	2	15.38
Gangcha County	18	5	27.78	4	22.22	2	11.11	0	0.00
Haiyan County	19	4	21.05	3	15.79	3	15.79	1	5.26
Datong County	25	8	32.00	5	20.00	8	32.00	3	12.00
Huzhu County	25	11	44.00	5	20.00	0	0.00	2	8.00
Qilian County	28	9	32.14	3	10.71	3	10.71	5	17.86
Jianzha County	29	5	17.24	6	20.69	5	17.24	3	10.34
Gonghe County	31	11	35.48	5	16.13	3	9.68	2	6.45
Zeku County	23	6	26.09	3	13.04	0	0.00	4	17.39
Qinghai Lake tributary	24	8	33.33	7	29.17	4	16.67	2	8.33
Total	301	98	32.56	61	20.27	52	17.28	31	10.30

Sample size number of samples investigated, *IFT* immunofluorescence test, *nPCR* nested PCR, *Pos-rate* positivity rate

(Qiagen), and 2 μL BSA (10 mg/mL) plus 10× Perkin-Elmer PCR buffer. The templates were subjected to an initial denaturation at 94 °C for 2 min followed by 35 cycles of 94 °C for 2 min, 55 °C for 10 s, 72 °C for 30 s and a final extension at 72 °C for 5 min. A secondary PCR was run using the following conditions: An initial step at 96 °C for 4 min, followed by 35 amplification cycles of 94 °C for 20 s, 53 °C for 20 s, 72 °C for 40 s and a final extension at 72 °C for 5 min. PCR products were electrophoresed on a 1.5 % agarose gel containing ethidium bromide (0.6 mg/mL) and visualized using a Gel Doc device.

Results

Water samples

The study determined that most of the water samples contained high concentrations of *Cryptosporidium* oocysts

Table 3 The fecal samples' detection result assigned by animal species

Species	Sample size	*Cryptosporidium*				*Giardia*			
		IFT	Pos-rate (%)	nPCR	Pos-rate (%)	IFT	Pos-rate (%)	nPCR	Pos-rate (%)
Yak	57	19	33.33	13	22.81	10	17.54	7	12.28
Sheep	61	21	34.43	12	19.67	13	21.31	8	13.11
Goat	51	14	27.45	8	15.69	11	21.57	2	3.92
Cattle	47	18	38.30	10	21.28	5	10.64	3	6.38
Horse	15	5	33.33	4	26.67	2	13.33	0	0.00
Donkey	13	4	30.77	3	23.08	2	15.38	1	7.69
Dog	31	8	25.81	5	16.13	4	12.90	2	6.45
Tibet antelope	7	2	28.57	1	14.29	0	0.00	0	0.00
Pika	11	4	36.36	3	27.27	3	27.27	1	9.09
Zokor sokhor	8	3	37.50	2	25.00	2	25.00	1	12.50
Total	301	98	32.56	61	20.27	52	17.28	25	8.31

Sample size number of samples investigated, *IFT* immunofluorescence test, *nPCR* nested PCR, *Pos-rate* positivity rate

and low concentrations of *Giardia* cysts (Tables 1). *Cryptosporidium* oocysts were more prevalent (66 out of 260 water samples; 98 out of 301 fecal samples) compared with *Giardia* cysts (39 out of 260 water samples; 52 out of 301 fecal samples) based on the IFA method. The nested PCR results showed that 71 out of 260 water samples and 61 out of 301 fecal samples were *Cryptosporidium* positive, whereas 40 out of 260 water samples and 31 out of 301 fecal samples were *Giardia*-positive.

The highest *Cryptosporidium* positive rate was Daotangh River with a rate of 75 % (Table 1). The lowest *Cryptosporidium* rate was the Nanmenxia reservoir (10.0 %). In terms of *Giardia*, the highest positive rate was the Nanmenxia wetland (40.0 %) (Table 1). No *Giardia* cysts have been detected in any of the samples from the nine rivers in the present study (Table 1).

Fecal samples

Table 2 summarizes the information on the fecal samples relating to location, sample size, and detection results. Of the 301 fecal samples, 98 were *Cryptosporidium*-positive by IFA and 61 were positive by nested PCR, whereas 52 samples were *Giardia*-positive by IFA and 31 samples were positive by nested PCR. All the fecal samples were taken from areas that are known for their farming of cattle, yak, sheep, goat, and other domestic animals. The highest *Cryptosporidium* positive rate was from Menyuan county (46.15 %) by both IFA and PCR, whereas the lowest rate was 10.71 %, from Qilian county. Menyuan county also had the highest rate of detection of *Giardia* (38.46 %). The lowest positive rate of *Giardia* was found in Gangcha and Zeku counties, with the IFT and nested PCR detection methods, respectively. Two out of the 18 samples from Gangcha county were *Giardia*-positive by IFT, but not by nested PCR. From the 23 samples collected from Zeku county, IFT detected no *Giardia*, whereas four samples were *Giardia*-positive using nested PCR.

The fecal samples varied in composition, with those animals harboring the parasites usually being watery and containing mucus. In addition, blood or leukocytes in such samples were occasionally observed.

Table 3 summarizes the results of fecal sample by animal species. The 301 fecal samples were collected from ten animal species, including both domestic and wild animals, although the latter were hard to sample. Although the sample sizes differed among species, the *Cryptosporidium*-positive rates were almost the same, at approximately 30 % by IFT, whereas the results detected by nested PCR varied from 14.29 % to 27.27 % (Table 3). No *Giardia* infections were found in the seven Tibetan antelope samples with either IFT or nested PCR (Table 3).

Discussion

Waterborne diseases occur worldwide, and outbreaks caused by the contamination of community water systems have the potential to cause disease in large numbers of consumers. In addition to outbreaks caused by contaminated potable water, there are outbreaks caused following the accidental ingestion of recreational waters. Rapid and effective monitoring methods are needed at drinking-water facilities to determine the occurrence of (oo)cysts in source and treated water. The development of such methods would lead to better decisions concerning the public health risks and treatment associated with water sources (Karanis et al. 2007a, b, c; Baldursson and Karanis 2011).

In our study, the microscopic identification of (oo)cysts was based on IFA analysis, which showed that *Cryptosporidium* was more prevalent than *Giardia*. The number of positive results may have differed because the samples were collected at different times, seasons, or locations between 2011 and 2012.

However, oocysts of *Cryptosporidium* are smaller (approx. 4–6 μm) and are able to change shape, which enables them to squeeze through the pores of membrane filters during vacuum pump filtration (Mayer and Palmer 1996). Therefore, the filtration techniques might be more efficient for the detection of *Giardia* cysts (approx. 8–12 μm) than of *Cryptosporidium* oocysts.

More frequent detection of *Giardia* cysts in untreated waters relies on the assumption that giardiasis is more widespread and occurs with greater intensity than cryptosporidiosis. Other studies reported this trend in surface waters in other countries (Plutzer et al. 2007; Carmena et al. 2007) too.

The concentration of (oo)cysts in surface water is usually low, being reported from different countries as ranging from 1 to 18 (oo)cysts/10 L (Robertson and Gjerde 2001; Hanninen et al. 2005). In The Netherlands, a higher concentration of (oo)cysts in surface water has been detected. The average concentration, corrected with the average recovery efficiency, was 4.5 and 5.4 oocysts/L and 22 and 95 cysts/L in the rivers Rhine and Meuse, respectively (Medema and Schijven 2001). Natural surface water from rivers and reservoirs in northern Spain have been found with concentrations that reached 1,767 *Cryptosporidium* oocysts and >25,000 *Giardia* cysts per 100 L (Carmena et al. 2007). Surface water in Italy was contaminated with 0–5 *Cryptosporidium* oocysts and 8×10^1 to 6×10^3 *Giardia* cysts per L (Briancesco and Bonadonna 2005). *Cryptosporidium* oocysts in surface waters draining a livestock farm on a Warwickshire (UK) estate showed a median concentration of 0.48 oocysts/L (Bodley-Tickell et al. 2002). In a study of water supplies in southern Russia and Bulgaria, 16 out of 166 samples (9.6 %) were positive for *Giardia* and 30 (18.1 %) were positive for *Cryptosporidium* (Karanis et al. 2006). Despite that the detection methodologies

are different, it is difficult to make comparisons in the results of the various studies. However, in our study, we do not provide oocyst concentrations (number of oocysts per liter); we provide only the positivity rate, and this was very high according to our results.

Several studies have shown that nested PCR appears to be more sensitive than microscopy for the detection of *Cryptosporidium* and *Giardia* in water samples (Jiang et al. 2005; Nichols et al. 2003). In our study, only five samples out of 20 that were *Cryptosporidium*-positive by IFA and one out of 20 samples that were *Giardia*-positive by IFA were also positive by PCR. This could be because of the high concentration of PCR inhibitors or the presence of empty oocysts in the investigated water samples, which would prevent their detection by molecular-based methods (Karanis et al. 2006, 2007c; Jiang et al. 2005). Additionally, these results might be the result of a low concentration of DNA and the uneven distribution of template DNA, especially in samples containing low numbers (1–3) of oocysts (Smith and Nichols 2010).

Although, in our study, IFA was a more sensitive technique than the nested PCR, PCR can be used to determine the species and genotype of an isolate. The discrepancies caused by IFA-positive–PCR-negative results could also result from the tendency of the IFA reagents to cross-react with non-target organisms, such as algae, or from the inhibitory effects on PCR enzymes caused by interfering substances, such as humic acids. It is important to note that, because of the specificity of the targeting to actual genetic sequences that are unique to a given organism, the PCR method is less likely to show cross-reactivity. This provides it with a distinct advantage over the IFA method. At present, empty oocysts cannot be detected by molecular-based methods, which leads to underestimation of (oo)cyst contamination (Karanis et al. 2006; Smith and Nichols 2010).

As mentioned above, at present, each method has its own advantages and disadvantages; therefore, depending on the aim and the design of the study, a combination of more detection methods is likely to be best.

In our study, only new information on the contamination of rivers by the pathogenic protozoa *Cryptosporidium* and *Giardia* in Qinghai province and Xining City is provided. Furthermore, the study reveals for the first time that Guoluo and Yushu prefectures, which provide water for irrigation, are heavily contaminated by *Cryptosporidium* and *Giardia*. Among the samples collected from the Three River source region (i.e., source of the Yangtze River, the Yellow River, and Lancang River), both parasites were detected by at least one of the IFA or PCR methods.

The presence of *Giardia* and *Cryptosporidium* in aquatic ecosystems in Qinghai makes it imperative to develop prevention strategies for water and food safety. Human incidences of giardiasis and cryptosporidiosis and prevalence-based studies provide baseline data so that risk factors associated with waterborne and foodborne transmission can be identified. However, this type of information is not yet available in China. Standardized methods are required to maximize public health surveillance, and documentation of outbreaks will provide better insight into the public health impact of waterborne pathogenic protozoa such as *Giardia* and *Cryptosporidium*. National statistics on outbreaks linked to contaminated water are not available for China. We and others have shown that these pathogens are in recreational and drinking water in China (Karanis et al. 2007a; Baldursson and Karanis 2011). However, in China, there are no requirements for testing surface waters for the presence of these parasites, and the detection of *Giardia* and *Cryptosporidium* and information on the prevalence of these organisms in water is scarce. The present work will contribute to filling this gap and functioning as a platform for future research. More systematic studies and further monitoring of these pathogens in China are required.

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