Letter to the Editor

Quantitative Microbial Risk Assessment of *Cryptosporidium* and *Giardia* in Public Drinking Water in China*

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We aimed to assess the risks of *Cryptosporidium* and *Giardia* infections associated with drinking water for local residents, based on a quantitative microbial risk assessment, in three densely populated regions of China. In total, 45 source water samples and 45 treated water samples were collected from June to December 2014. Five *Cryptosporidium*-positive samples and 5 *Giardia*-positive samples were found. The annual probability of infection for individuals in Jintan (6.27×10^{-4}–2.05×10^{-3} for *Cryptosporidium* and 7.18×10^{-4}–2.32×10^{-3} for *Giardia*), Ezhou (6.27×10^{-4}–1.10×10^{-2} for *Cryptosporidium* and 3.65×10^{-4}–1.20×10^{-3} for *Giardia*), and Binyang (3.79×10^{-4}–1.25×10^{-3} for *Cryptosporidium*) exceeded the tolerable risk of infection of 10^{-4} set by the United States Environmental Protection Agency. Moreover, the corresponding disease burdens of cryptosporidiosis and giardiasis, due to direct drinking and residual water in these regions, exceeded the threshold of 10^{-6} disability-adjusted life years per person per year set by the World Health Organization. These results provide insights into strategies to improve the safety of drinking water.

Key words: *Cryptosporidium*; *Giardia*; Quantitative microbial risk assessment

Cryptosporidium spp. and *Giardia* spp. are important intestinal protozoa of humans and animals, causing severe diarrhea. To date, many outbreaks have been caused by both pathogens[1], most notably the massive waterborne *Cryptosporidium* outbreak in Milwaukee, Wisconsin in 1993, which caused 400,000 infections and approximately 100 deaths. Fecal-oral transmission of *Cryptosporidium* oocysts and *Giardia* cysts occurs through the consumption of contaminated drinking or recreational water or food or contact with infected people or animals. Risk assessments for *Cryptosporidium* and *Giardia* in bodies of water have become crucial tools in many countries to help public health practitioners make appropriate environmental decisions for the maintenance of public health. For example, the daily consumption of drinking water was identified as an important contributing factor for the high risk of *Cryptosporidium* and *Giardia* infections in Mexico[2]. As recommended by the World Health Organization (WHO), quantitative microbial risk assessment (QMRA) permits the evaluation of the risk of infection by biological agents through data monitoring in the laboratory. The process includes four steps: hazard identification, exposure assessment, dose-response modeling, and risk characterization. For *Cryptosporidium* and *Giardia*, the risks for individuals should be less than 10^{-4} infections per year, according to the United States Environmental Protection Agency (USEPA). Furthermore, the acceptable threshold for disease burden from waterborne exposure has been set at 10^{-6} disability-adjusted life years (DALYs) per person per year (pppy) by WHO, which is approximately equivalent to a 10^{-5} excess lifetime risk of cancer (i.e., 1 excess case of cancer per 100,000 people ingesting drinking water at the water quality target, daily over a 70-year period).

Epidemiological data on human cryptosporidiosis and giardiasis have confirmed that these organisms are ubiquitous in China. For example, the occurrence

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rates were found to be 2.0% for *Cryptosporidium* and 1.4% for *Giardia* in children with a history of diarrhea in Wuhan, Hubei Province[3]. However, the routes of *Cryptosporidium* and *Giardia* infections have received little attention. A series of outbreaks have been associated with the presence of both protozoa in surface water, and most of these have occurred in developed countries[1]. Water is a significant route of transmission for both protozoa, which should be of great concern, especially in bodies of water used for the production of domestic drinking water and recreational activities.

The objective of this study was to determine the prevalence of both protozoa in source water and treated drinking water of waterworks in three densely populated countryside regions: Jintan, Jiangsu Province (eastern China); Ezhou, Hubei Province (central China); and Binyang, Guangxi Zhuang Autonomous Region (southern China). Based on the survey data, the risks of protozoan infection and disease burdens were assessed for the populations supplied by these drinking water samples, taking into account the water consumption habits of the Chinese population and two subpopulations with different immune statuses. Our study may be the first QMRA of *Cryptosporidium* and *Giardia* in these regions, which will be helpful in prompting actions to reduce the disease burden of protozoan infections in China.

Cryptosporidium oocysts and *Giardia* cysts are highly resistant to chemical disinfection and environmental stressors, and may survive for a long period of time in water. They cannot be completely inactivated by conventional waterwork treatment methods (comprising coagulation, flocculation, sedimentation, sand filtration, chlorination, and fluoridation), thus affecting the health of surrounding residents. In the regions we investigated, the waterworks were the sole source of drinking water for surrounding residents and they used conventional water treatment practices. Consequently, *Cryptosporidium* and *Giardia* were considered to pose a threat to local residents.

Samples were collected from Jintan District, Ezhou City, and Binyang County in China from June to December 2014, which included both the rainy and dry seasons. Five different waterwork facilities were sampled in each region, and at each sampling site, three parallel water samples were collected at different times. The volume of each sample was 10 and 100 L for source and treated water, respectively. All samples were flocculated and filtered within 24 hours of sampling. Clean polypropylene containers (15 L) were used for the collection of source water, which was collected from approximately 10–20 cm below the surface of the surface water sites, e.g., a river or lake. Treated water samples were collected directly through taps in the waterwork facilities.

The detection of (oo)cysts has been described elsewhere. Each source water sample was processed using the calcium carbonate flocculation (CCF) modified Method 1623[4]. The number of (oo)cysts was recorded as the detected number in 10 L of source water. Each treated water sample was concentrated on site using a 3.0-μm pore-size cellulose acetate membrane modified Method 1623[5]. The number of (oo)cysts was recorded as the detected number in 100 L of treated water. Simultaneously, the recovery efficiencies (R) for *Cryptosporidium* and *Giardia* were evaluated using seeded water containing 99 ± 1.1 oocysts and 99 ± 1.5 cysts (EasySeed, ESCG100-5; BioPoint, Sydney, Australia) in purified water samples. The R value was expressed as a percentage and was calculated as follows:

$$R = C/C_i \times 100\% \quad (1)$$

where C_i is the initial known concentration of (oo)cysts in the seeded water, and C is the detected concentration of (oo)cysts. The concentrations and the recovery efficiencies of *Cryptosporidium* and *Giardia* are presented as the arithmetic mean ± standard deviation (s).

In consideration of the habits and customs of local residents and the coverage of sampling sites, the exposure was evaluated based the entire local resident population. Exposure to source and treated water was estimated using two different approaches.

For source water, exposure (N_s) was calculated using the following Equation[6]:

$$N_s = C \times R^{-1} \times I \times V \times TR \quad (2)$$

where N_s is the exposure of individuals to (oo)cysts; C is the mean detected concentration of (oo)cysts [(oo)cysts/L]; R is the recovery efficiency of the CCF-modified Method 1623; I is the fraction of infective protozoa [assuming that all (oo)cysts were viable and infectious]; V is the daily consumption of unboiled water by individuals, which was considered to include two main exposure routes: direct consumption of tap water and the intake of residual water during tooth-brushing or washing. The mean ingestion volume of unboiled tap water was approximately 21 mL/day and the residual water remaining in the mouth after tooth-brushing or washing has been estimated to be approximately 7–71 mL/day for Chinese individuals[5]. *TR* is the removal efficiency of (oo)cysts by the waterwork facility, which was inferred to approximately 2 $\log_{10}$ and 3 $\log_{10}$ units for oocysts and cysts, respectively, when using conventional treatment methods.

For treated water, the exposure (N_t) was calculated using the following Equation[2]:

$$N_t = C \times R^{-1} \times I \times V \quad (3)$$

where N_t, C, R, I, and V are the same as those used for the source water calculation and R is the recovery efficiency of membrane-modified Method 1623.

The probability of infection by (oo)cyst ingestion was determined using the exponential dose-response model.

The daily risk of infection (P_d) for individuals was estimated using the following Equation:

$$P_d = 1 - e^{-rN} \quad (4)$$

where r represents infectivity constants of 0.09 for *Cryptosporidium*[7] and 0.0199 for *Giardia*[2].

The annual risk of infection (P_y) for individuals was estimated using the following Equation:

$$P_y = 1 - (1 - P_d)^n \quad (5)$$

where n is the number of days for an individual exposed to the pathogens per year. In these regions, the investigated waterwork facilities were the sole local water sources, and thus, n was assumed to be 365 days.

The risk was characterized by calculating DALYs, which represents disease burden and includes the years of life lost due to mortality and the loss of healthy years due to disability. In consideration of different disease burdens in different individuals, two subgroups were evaluated: immunocompetent (99.945%) and immunodeficient individuals (0.055%)[8]. Disease burdens associated with *Cryptosporidium* and *Giardia* for the entire population were calculated as follows[8]:

$$P_{illyear} = P_y \times P_{ill/Inf} \times f \quad (6)$$

$$B = \sum_{i}^{n} P_{illyear_i} \times DBPC_i \times P_i \quad (7)$$

where $P_{illyear}$ is the annual probability of illness for the population; $P_{ill/Inf}$ is the probability of developing an illness after being infected, i.e., morbidity, which is 0.39 for the immunocompetent population and 1 for the immunodeficient population for *Cryptosporidium* infection[9] and 0.5 for *Giardia* infection[8]; f is the susceptibility fraction of the total population, which is considered to be 100% here because tap water and residual water from tooth-brushing or washing affect all individuals; B is the disease burden; i is the population subgroup number; $DBPC_i$ is the disease burden per case in the ith subgroup, which was estimated to be 0.32 DALYs and 1.91 × 10^{-3} DALYs per case for the immunodeficient and immunocompetent population, respectively, due to cryptosporidiosis, and 1.7 × 10^{-3} DALYs per case due to giardiasis in China[8]; and P_i is the proportion of the ith subgroup in the total population, as previously mentioned.

In total, 45 source water samples and 45 treated water samples from 15 different waterworks were collected and examined for *Cryptosporidium* and *Giardia* using CCF-modified Method 1623 and membrane-modified Method 1623, respectively. The mean concentrations in each region are shown in Table 1. For source water, 4 *Cryptosporidium*-positive samples (mean concentration: 0.020–0.033 oocysts/L) and 3 *Giardia*-positive samples (mean concentration: 0–0.033 cysts/L) were found. For treated water, 1 *Cryptosporidium*-positive sample (mean concentration: 0–0.013 oocysts/L) and 2 *Giardia*-positive samples (mean concentration: 0–0.0013 cysts/L) were found. In a real waterwork treatment system, the water samples taken before and after the treatment zone are almost unpaired because of a time delay during the water treatment process, i.e., they are not exactly the same water sample. Meanwhile, the occurrence of both protozoa in environmental water samples is influenced by many factors, including the timing and frequency of sampling and the sampling site characteristics (e.g., rainfall or distance from pathogen sources). This resulted in inconsistencies in concentrations between source water and treated water samples collected from the same region. Furthermore, a large number of samples are needed to achieve consistent results due to the low density, motility, and variability of both protozoa.

The recovery efficiencies for *Cryptosporidium* and *Giardia* from source water samples were 48.5%

± 0.5% and 52.5% ± 0.8% using CCF-modified Method 1623, respectively, and from treated water samples, the recovery efficiencies were 35.4% ± 0.4% and 37.4% ± 0.6% using membrane-modified Method 1623, respectively. All of these recovery efficiencies met the acceptable level described in Method 1623 (24%–100%).

People living in Jintan, Ezhou, and Binyang were exposed to 0–6.26 × 10^{-5}, 1.91 × 10^{-5}–3.38 × 10^{-4}, and 0–3.79 × 10^{-5} oocysts and 0–3.20 × 10^{-4}, 1.76 × 10^{-6}–1.65 × 10^{-4}, and 0–3.50 × 10^{-6} cysts per day, in the best and worst exposure scenarios based on water consumption, respectively. These data were obtained from the integration of calculated results, including source and treated water samples. The annual probability of infection and DALYs per person per year caused by (oo)cysts are shown in Table 2. In the three regions studied, the estimated risks of *Cryptosporidium* or *Giardia* infection for individuals per year ranged from 0–1.10 × 10^{-2} and from 0–2.32 × 10^{-3}, respectively, and the corresponding disease burdens ranged from 0–1.01 × 10^{-5} DALYs pppy and from 0–1.97 × 10^{-6} DALYs pppy, respectively.

Many previous studies have performed health risk assessments of *Cryptosporidium* and *Giardia* using the annual probability of infection for individuals (QMRA); however, except for several publications by Xiao et al. appraising DALYs[5,6], there is a sparsity of data with respect to drinking water in China. QMRA is the most commonly used method for microbiological risk assessment. The annual risk threshold of 10^{-4} suggested by the USEPA and the acceptable disease burden threshold of 10^{-6} DALYs pppy from waterborne exposure set by the WHO have been adopted for the evaluation of *Cryptosporidium* and *Giardia* infections. In this study, as shown in Table 2, the annual probability of infection for individuals in Jintan (*Cryptosporidium* and *Giardia*), Ezhou (*Cryptosporidium* and *Giardia*), and Binyang (*Cryptosporidium*) exceeded the USEPA threshold. The cryptosporidiosis burden per person per year due to the direct consumption of drinking and residual water in these three regions and the giardiasis burden in Jintan and Ezhou exceeded the tolerable value. A recent study showed that the occurrence rates of *Giardia* in humans were 0.15%,

Table 1. Occurrence of *Cryptosporidium* and *Giardia* in source and treated water samples from three regions in China

Type of water	Location	Number of waterworks	Samples	Positive samples*		Mean concentrations (± *s*)	
				Cryptosporidium	*Giardia*	*Cryptosporidium* (oocysts/L)	*Giardia* (cysts/L)
Source water (10 L)	Jintan, Jiangsu	5	15	2 (total 5 oocysts)	0	0.033 ± 0.089	0
	Ezhou, Hubei	5	15	1 (total 5 oocysts)	2 (total 5 cysts)	0.033 ± 0.12	0.033 ± 0.089
	Binyang, Guangxi	5	15	1 (total 3 oocysts)	1 (total 3 cysts)	0.020 ± 0.077	0.020 ± 0.077
Treated water (100 L)	Jintan, Jiangsu	5	15	0	1 (total 2 cysts)	0	0.013 × 10^{-1} ± 0.051 × 10^{-1}
	Ezhou, Hubei	5	15	1 (total 2 oocysts)	1 (total 1 cyst)	0.013 × 10^{-1} ± 0.051 × 10^{-1}	0.067 × 10^{-2} ± 0.025 × 10^{-2}
	Binyang, Guangxi	5	15	0	0	0	0

Note. *Including detected number of *Cryptosporidium* or *Giardia* (oo)cysts in samples analyzed. The total (oo)cysts denote number in 10 L source water or 100 L treated water, respectively.

Table 2. Annual probability of individual infection and disease burdens caused by *Cryptosporidium* and *Giardia*

Location	P_y from source water (×10^{-4})		DALYs pppy from source water (×10^{-6})		P_y from treated water (×10^{-4})		DALYs pppy from treated water (×10^{-6})	
	$P_{y\text{-}cry}$ [a]	$P_{y\text{-}gd}$ [b]	B_{cry} [c]	B_{gd} [d]	$P_{y\text{-}cry}$ [a]	$P_{y\text{-}gd}$ [b]	B_{cry} [c]	B_{gd} [d]
Jintan, Jiangsu	6.27–20.50	0	0.577–1.890	0	0	7.18–23.20	0	0.610–1.970
Ezhou, Hubei	6.27–20.50	0.11–0.40	0.577–1.890	0.009–0.034	33.70–110.00	3.65–12.00	3.100–10.100	0.310–1.020
Binyang, Guangxi	3.79–12.50	0.07–0.26	0.349–1.150	0.006–0.022	0	0	0	0

Note. [a]$P_{y\text{-}cry}$: annual probability of infection by *Cryptosporidium*; [b]$P_{y\text{-}gd}$: annual probability of infection by *Giardia*; [c]B_{cry}: disease burdens caused by *Cryptosporidium*; [d]B_{gd}: disease burdens caused by *Giardia*.

2.08%, and 1.35% in Jiangsu Province, Hubei Province, and the Guangxi Zhuang Autonomous Region, respectively[10]. However, the annual probability of developing giardiasis for individuals ($P_{illyear}$) in our study was 0–1.16 × 10^{-3}, 5.45 × 10^{-6}–6.00 × 10^{-4}, and 0–1.27 × 10^{-5} in Jintan, Ezhou, and Binyang, respectively. The main reason for the differences in Ezhou and Binyang may be that transmission through the daily consumption of water occupies only a small part of the various waterborne routes of transmission of *Giardia* in both studied regions, eg., swimming may also be an important route of transmission in these regions. The estimated disease burdens caused by *Cryptosporidium* and *Giardia* through the direct consumption of drinking and residual water in our study were lower than previous estimates in Zhejiang Province, China (6.51 × 10^{-5} DALYs pppy for *Cryptosporidium* and 6.25 × 10^{-6} DALYs pppy for *Giardia*), where swimming is considered to be the major exposure route[5,6]. In Ezhou, the risk estimates based on treated water samples tended to be higher than those based on source water samples, which may imply ineffective treatment of source water in these waterwork facilities. Improved management and regular disinfection may be needed in these facilities.

The probability of infection from source water listed in Table 2 may be underestimated, since there are other exposure routes associated with infection, e.g., irrigation and swimming. These have not been considered here when assessing the risks of infection by *Cryptosporidium* and *Giardia* in water. On the contrary, the risks may be overestimated because all the detected (oo)cysts were assumed to be viable and different species were not be discriminated. Not all *Cryptosporidium* and *Giardia* species are pathogenic to humans. These non-pathogenic species may have been included in positive samples, but they do not result in clinical infection. In addition, chlorine dioxide used by Hubei province and the Guangxi Zhuang Autonomous Region, may have improved the removal efficiency for both protozoa, but this was not factored into the analysis due to its low dose and irregular use. Despite the potential under- and over-estimation of the risks, the information generated in this study is useful in drawing the attention of health administration departments endeavoring to develop strategies to reduce the exposure of local residents to (oo)cysts.

In conclusion, the QMRA conducted in these three densely populated regions of China demonstrated that the infection risks and disease burdens caused by *Cryptosporidium* and *Giardia* are potentially significant. The protection of water sources should be implemented by the government. For local residents, drinking boiled water is recommended, and residual water consumption should be reduced to the greatest extent.

Authors' Contributions SHEN Yu Juan, CAO Jian Ping, and CAO Sheng Kui conceived and designed the experiments; CAO Sheng Kui, JIANG Yan Yan, YUAN Zhong Ying, and YIN Jian Hai performed the experiments; XU Meng and XUE Jing Bo analyzed the data; CAO Sheng Kui wrote the manuscript; CAO Jian Ping, SHEN Yu Juan, and TANG Lin Hua revised the manuscript. All authors read and approved the final version of the manuscript.

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Conflict of Interest The authors declare that no competing interests exist.

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