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Influence of sewage treatment plant effluent discharge into multipurpose river on its water quality: A quantitative health risk assessment of *Cryptosporidium* and *Giardia*☆

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ABSTRACT

Sewage treatment plants (STPs) are one of the sources of pathogens discharged into surface water. An investigation was carried out over the duration of 12 months in Henan Province, China, to evaluate the health influence of municipal wastewater effluent discharge on water quality of the receiving water. A discharge-based quantitative microbial risk assessment (QMRA) was employed, taking into account the vegetables consumption habits of the Chinese, population subgroups with different immune statuses and ages, to evaluate the incremental disease burden from agricultural irrigation and swimming exposure scenarios associated with increased concentration of the protozoan *Cryptosporidium* and/or *Giardia* in the receiving river. The results shown that all the STP influent samples contained *Cryptosporidium* and *Giardia* with average density of 142.31 oocysts/L and 1187.06 cysts/L, respectively. The QMRA results demonstrated that the estimated additional health burdens due to discharged effluent for both parasites were slightly violated the threshold of 10^{-6} DALYs per person per year set by WHO. Mitigation measures should be planned and executed by season since more disease burdens were borne during hot season than other seasons. The sensitivity analysis highlighted the great importance of stability of STP treatment process. This study provides useful information to improve the safety of surface water and deduce the disease burden of the protozoa in Henan Province and other region inside and outside China.

1. Introduction

Sewage discharge is one of the problems in both developed and developing countries. It contributes to a compounded unstable aquatic ecosystem, including but not limited to oxygen demand and nutrient loading of the receiving watershed, promoting toxic, and algal blooms (Gonzalo and Camargo, 2013; Li et al., 2016; Aubertheau et al., 2017; Hilario Garcia et al., 2017). In addition, sewage effluent may be of public health significance, especially if effluent is discharged into water that is subsequently used for drinking, recreation, or agricultural purpose (Robertson et al., 2006; Zafar et al., 2017). Effluents from industrial sources, generally because of their chemical pollution, are associated with non-communicable disease (Zafar et al., 2017), while those from municipal wastewater would contribute to infectious disease (King et al., 2017; Mok et al., 2014). The infectious agents associated with municipal wastewater are those found in the domestic sanitary waste of the population.

Although a variety of pathogens have been identified in raw sewage, relatively few types of pathogens seem to be responsible for most of the waterborne diseases caused by sewage-original pathogens (Seto et al., 2016; Mead et al., 1999). Among them, protozoan parasites such as *Cryptosporidium* and *Giardia* are the key types of pathogens due to their high infectivity and high resistance to water treatment and disinfection (WHO, 2009). Consequently any additional sources of *Cryptosporidium* oocyst and *Giardia* cyst in receiving water are highly undesirable.

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In fact, *Cryptosporidium* and *Giardia* were responsible for the most prevalent worldwide waterborne infection producing diarrhea. In immunodeficiency person such as human immunodeficiency virus (HIV)-infected patients, this infection is more common and can be life-threatening (Hunter and Nichols, 2002). During the time period from 2004 to 2010, at least 60.5% (120) and 35.1% (70) of worldwide waterborne outbreaks of human diseases caused by *Cryptosporidium* and *Giardia*, respectively, and most of the outbreaks occurred in developed countries (Baldursson and Karanis, 2011). This may be a distorted picture of the global conditions which was attributed to the improved reporting from regions of high economic and sanitation status (WHO and UNICEF, 2017). The infection prevalence and outbreak incidence in developing countries probably most affected by both of the parasites are not able to identify or provide any reports of outbreak events due to the lack of surveillance systems (Efstratiou et al., 2017a). In Thailand, which belongs to developing countries like China, the reported data of diarrheal diseases incidence were suspected to be under-reported and the causal agents of diarrheal diseases were undiscovered (Yotthanooi and Choonpradub, 2010). It has been estimated that nearly 2% of adults and 6%–8% of children in developed countries worldwide, while nearly 33% of the people in the developing countries have had giardiasis (www.cdc.gov).

Many factors including the multiple exposure routes reflecting living and sanitation conditions, and contamination of the aquatic ecosystem by the high environmental burden, have been contributed to the high infection caused by *Cryptosporidium* and *Giardia* (Squire and Ryan, 2017). Between January 2007 and December 2008, 96.7% of waterborne cryptosporidiosis outbreaks in the United States of America were associated with recreational water other than drinking water (Brunkard et al., 2011). On the other hand, the health risks of *Cryptosporidium* and *Giardia* infection associated with agricultural irrigation have become one of major concerns of the world, particularly in developing countries (Mok and Hamilton, 2014; Aiello et al., 2013).

Quantitative microbial risk assessment (QMRA) has been widely employed to predict the human health risks associated with exposure to infectious pathogens in drinking water (Xiao et al., 2012a), surface water (An et al., 2011; Adell et al., 2016), and reclaimed water (Zhang et al., 2015; Ryu et al., 2007). Given the concentrations of pathogen in receiving water are often 'non-detect' with limits of detection, the lack of appropriate input data for pathogen concentrations at the point of exposure limits the application of QMRA. To meet this challenge, McBride et al. (2013) developed a discharge-based QMRA that uses pathogens detected in discharges into flowing inland waters rather than relying on measurements in receiving waters themselves to assess the risk of seven pathogens in recreational waters. Later, the discharge-based QMRA was employed in drinking water management (Sokolova et al., 2015).

Effluents of sewage treatment plant (STP) have been increasingly used for irrigation, recreational impoundments, and wetland reconstruction in many arid regions of the world (Ma et al., 2016). In China, there are about 1.33 million hectares of land irrigated directly and indirectly with wastewater (Jiménez, 2006) and 10.5–21.6% of people swim in river (An et al., 2011). Considering that China is the world's most populated country, with 74,000 HIV-positive persons (UNAIDS, 2011), there may be millions of people exposed to health risks from water. As far as we know, to date there have not been any studies on the microbial risks attendant with effluent discharging into river in China. The aim of this research was to investigate the health influence of municipal wastewater effluent discharges into river on its water quality. For this purpose, the concentrations of *Cryptosporidium* oocysts and *Giardia* cysts in influent of STP were surveyed for a year, and a discharge-based QMRA combined with hydrologic modeling was employed, taking into account the vegetables consumption habits of the Chinese, population subgroups with different immune statuses and ages, to evaluate the incremental disease burden from agricultural irrigation and recreational exposure associated with increased concentration of these protozoa in river. The uncertainties in these attributable risk estimates are discussed as well as their implications. The methodology and results of this study will be useful in better evaluating and reducing the burden of protozoan infection associated with wastewater in Henan Province and other region inside and outside China.

2. Material and methods

2.1. Study site description

The STP is located in the county of Wuzhi, Henan Province, China (Fig. 1). The treatment system at the STP consists of a preliminary treatment (rotating screens and aerated grit chamber), oxidation ditch, and disinfection with chlorine. The population served by this STP is approximately 150,000 inhabitants. This plant treats around 24,000 m^3 of domestic sewage per day, which is discharged into a small receiving water body, and finally about 10% of the effluent reaches the nearby *Qinhe* River.

The *Qinhe* River is the main source of agricultural irrigation in the local farmland, and is recreational water for some people in the hot season. In 2015, the water flow of the river varied between 3.14 and 10.82 m^3 per second, with an average of 6.98 m^3 per second. The lowest and highest water level of the *Qinhe* Rive usually occurred in the dry season (November–March) and rainy season (June–August), respectively.

2.2. Hazard identification

Bacteria, viruses, and protozoans are the major groups of sewage pathogens. Most of these pathogenic microorganisms could be controlled by the application of disinfection with chlorine. However, *Giardia* cyst and *Cryptosporidium* oocyst are moderately even highly resistant to both environmental stress and chemical disinfection (WHO, 2009). Furthermore, these protozoa cannot be completely removed by the conventional wastewater treatment. Consequently, *Cryptosporidium* and *Giardia* in the discharged effluent were considered to be the pathogens most likely to pose a threat, and were selected as indicators, for this study, to assess the public health influence of effluent on river water quality.

2.3. Exposure assessment

2.3.1. Concentration of parasitic pathogens

Compared to the much more removed and dilute concentration expected in effluent and receiving water, pathogens were generally present at higher concentration in the influent, increasing the likelihood of their detection without the need for large-volume sampling (McBride et al., 2013). The concentrations of *Cryptosporidium* oocyst and *Giardia* cyst in sewage influent of the STP were monitored every two weeks from April 2015 to March 2016 ($n = 24$). Each of monitoring days, 50 mL of sample was collected and concentrated by centrifugation at 1500 $\times$ g for 10 min. Then, oocysts and/or cysts in the pellet were separated from debris by flotation on Percoll-sucrose gradients, labeled with fluorescein isothiocyanate conjugated anti-*Cryptosporidium* and anti-*Giardia* monoclonal antibodies (Waterborne, Inc. New Orleans, LA) as well as 4′6 diamidino-2-phenyl indole (DAPI), and were examined microscopically, as previously described by Xiao et al. (2012a). This analytical method based on microscopic examination can detect as

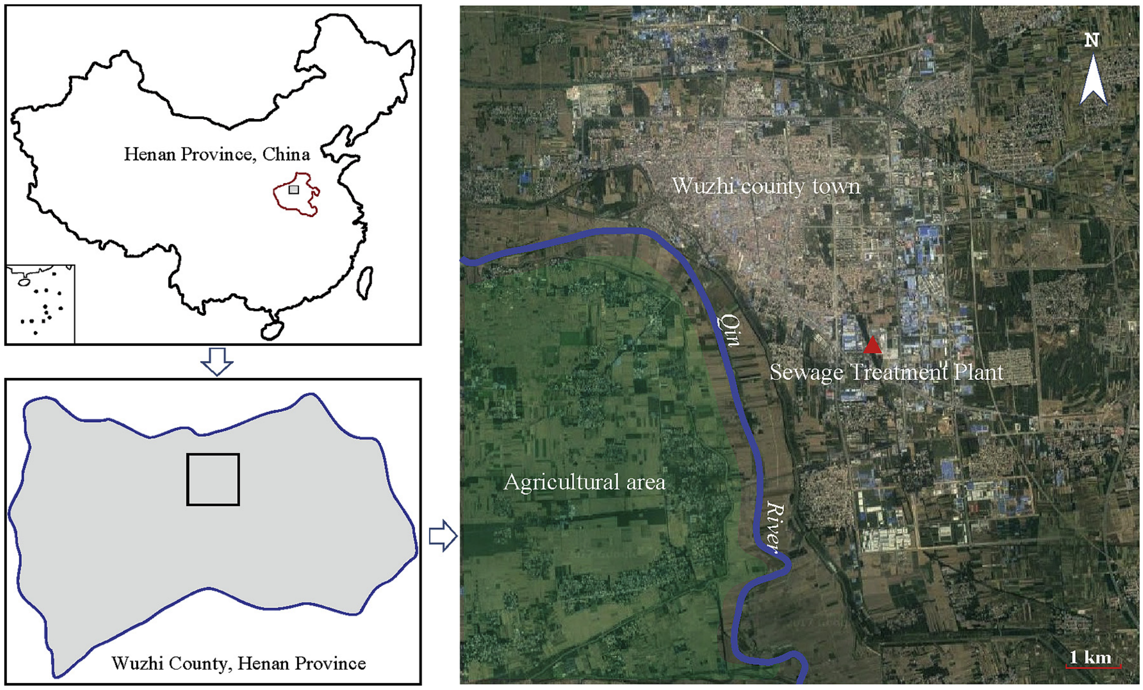

Fig. 1. Map of the study area.

low as one *Cryptosporidium* oocyst or *Giardia* cyst (Xiao et al., 2012b).

In the McBride et al. (2013) study, concentration data of seven pathogens, including the parasites, bacteria and viruses, were collected from different types of stormwater discharge and were used to calculate the concentrations of these pathogens in the receiving recreational waters. Similarly, in the present study, the parasitic concentrations in the effluent of STP (C_{eff} in equation (1)) and *Qinhe* River (C_{river} in equation (2)) were estimated by the pathogen concentration in influent (C_{inf} in equation (1)), which is modeled using a continuous distribution similar to the previous studies (Symonds et al., 2014; Verbyla et al., 2016), and removal/dilution modeling. The average removal efficiency (R in equation (1)) of these protozoan parasites by oxidation ditch treatment system was assumed to be 99% (i.e. 2.0 $\log_{10}$) (Nasser, 2016; Nasser et al., 2012). Stream flow was modeled as the average flow in the river, which was around 6.98 m^3 per second. Since the study was aimed at assessing the additional impact due to the effluent discharge, the concentration of *Cryptosporidium* oocyst and *Giardia* cyst in the river upstream was assumed to be zero. So, the estimated concentration of parasite in the river downstream is given by the total oocyst/cyst load divided by the total discharge (stream flow (Q_{river}) plus effluent (W_{river}), equation (3)). The concentrations were calculated as

$$C_{eff} = C_{inf} \times (1 - R) \tag{1}$$

$$C_{river} = \frac{C_{eff} \times W_{eff}}{W_{eff} + W_{river}} \tag{2}$$

$$W_{rive} = Q_{river} \times 60 \times 60 \times 24 \tag{3}$$

Where C_{eff} is the parasite concentration in effluent (oocyst/L or cyst/L); C_{inf} is the parasite concentration in sewage influent (oocyst/L or cyst/L); R is the removal efficiency of *Cryptosporidium* oocyst or *Giardia* cyst by STP (%); C_{river} is the estimated additional parasite concentration in river water (oocyst/L or cyst/L); W_{eff} is daily effluent volume reaching *Qinhe* River (m^3/day); W_{river} is the daily stream flow of *Qinhe* River (m^3/day); and Q_{river} is the average river flow (m^3/second).

2.3.2. Water ingestion

When assessing the risk of *Cryptosporidium* and *Giardia* in water, the unboiled water consumption by all exposure routes should be considered. Nevertheless, considering the use of *Qinhe* River, only two exposure scenarios including agricultural irrigation and swimming were assessed in the study. Recently, Mok and Hamilton (2014) evaluated the volume of water left on Asian vegetables and lettuce after irrigation, consumption of vegetables from wastewater irrigation in China, and estimated about 4.80 mL water was ingested per person per day for all population by this unrestricted irrigation route A (i.e. f in equation (6) = 100%). Another irrigation route B involved an involuntary ingestion of 1–10 mg soil per single exposure (approximately equivalent to 1–10 mL river water) by farmers working three days per week (150 days per year) or children playing in the restrictedly river-irrigated fields (Diallo et al., 2008; Mara et al., 2007). According to the 2010 population census of the People's Republic of China (www.stats.gov.cn), 45.6% of population is engaged in agricultural work and we assumed all these people involved in the irrigation route B (i.e. f in equation (6) = 45.6%). The volumes of water by swimmers ingested were estimated to be 7.56 and 45.98 mL/visit for adults (>14 years old) and children, respectively (Suppes et al., 2014). An et al. (2011) assumed that 10.5–21.6% of Chinese (f in equation (6)) swim in rivers twice-weekly in the hot season (generally between May and September).

2.4. Dose–response assessment

Probability of infection was estimated using the exponential dose–response models (P_{Infday} in equation (4)) with infectivity constants (r) of 0.09 for *Cryptosporidium* (Soller et al., 2010) and

0.02 for *Giardia* (McBride et al., 2013). To account for the variability, a lognormal distribution was used to describe the infectivity constant of each parasite in this study (Brouwer et al., 2017). The annual individual-level risk of infection ($P_{Infyear}$ in equation (5)) is calculated from the daily risk. The probability of illness ($P_{Ill|Inf}$) due to *Cryptosporidium* infection was modeled using a beta distribution ($\alpha = 20$, $\beta = 8$) with a mean of 0.71 (Xiao et al., 2012a), whereas a distribution varies uniformly from 0.2 to 0.7 was used to model the morbidity of *Giardia* infection (Chhipi-Shrestha et al., 2017). So, the risk of illness ($P_{Illyear}$ in equation (6)) due to *Cryptosporidium* or *Giardia* infection can then be calculated by multiplying the risk of infection and the probability of illness. These models were calculated by using the following equations.

$$P_{Infday} = 1 - e^{-r \times C_{river} \times V} \tag{4}$$

$$P_{Infyear} = 1 - \left(1 - P_{Infday}\right)^{n} \tag{5}$$

$$P_{Illyear} = P_{Infyear} \times P_{\mathrm{Ill|Inf}} \times f \tag{6}$$

Where P_{Infday} is the daily probability of infection; r is the infectivity constants of the exponential dose–response function; V is the exposure volume; $P_{Infyear}$ is the estimated annual probability of infection; n is the number of exposures per year; $P_{Illyear}$ is the estimated annual probability of illness for total population; $P_{\mathrm{Ill|Inf}}$ is the probability of developing illness given infection, i.e., morbidity; and f is the susceptibility fraction of total population.

2.5. Risk characterization

The risk was characterized by disease burden, which was expressed in disability adjusted life year (DALY). The DALY calculation included both the years of life lost due to mortality and loss of healthy years with disability. Water diarrhea was taken as the most common disease outcome from *Cryptosporidium* infection with duration of 7.2 days (Havelaar and Melse, 2003) in immunocompetent population and mortality would occur especially in immunodeficient population such as HIV/AIDS (Dietz et al., 2000). The disease burden of cryptosporidiosis in China was estimated to be 0.32 DALYs and 1.91×10^{-3} DALYs per case in the immunodeficient population and immunocompetent population, respectively (Xiao et al., 2012a). Considering the illness duration of *Giardia* infection varied between 5 and 33 days (for mild and hospitalized patient), and death rarely occur, Gibney et al. (2014) estimated the health burden due to giardiasis was 1.7×10^{-3} DALYs per case. As mentioned earlier, the volume of water ingested and the cryptosporidiosis burden in different people is different. So, in the calculation of disease burden (B in equation (7)), four population subgroups, i.e. (1) immunocompetent adults, (2) immunocompetent children, (3) immunodeficient adults, and (4) immunodeficient children, were calculated separately taking into account their proportions of all population (p in equation (7); 82.629%, 17.316%, 0.054% and 0.002%, respectively)(Xiao et al., 2012a). Consequently, each of the parasitic disease burdens in all population was calculated as follows (equation (7)) (Chhipi-Shrestha et al., 2017).

$$B = \sum_{i}^{n} PIllyear_i \times DBPC_i \times p_i \tag{7}$$

Where i is the subgroup number of population; $P_{Illyear\ i}$ is the estimated annual probability of illness in ith population subgroup; $DBPC_i$ is disease burden per case in ith group; and p_i is the proportion of ith subgroup of all population.

2.6. Model implementation

All the model parameters (summarized in Table 1) were constructed in Microsoft Excel running the @Risk 5.5 add-on package (Palisade Corporation, USA). To account for uncertainty and variability in the parameters, Monte Carlo simulation of 10,000 iterations was performed to calculate probabilities. Each of iterations involved drawing a set of values from the input parameter probability distributions. Spearman rank order correlation was also conducted comparing the uncertainty relationships between the input variables and annual disease burden.

3. Results and discussion

3.1. Concentration of Cryptosporidium oocyst and Giardia cyst

Monitoring data from April 2015 to March 2016 indicated that all the STP influent samples contained *Cryptosporidium* oocyst and *Giardia* cyst with average/annualized concentrations of 142.31 (min–max: 40–420) oocysts/L and 1187.06 (590–2120) cysts/L, respectively (Table 2). Both protozoan concentrations in the hot season (May to September) were higher than those in other seasons. Using equations (1) and (2), the concentrations of *Cryptosporidium* and *Giardia* in STP effluent and *Qinhe* River were estimated to be 1.43 (95% confidence intervals (CI): 0.05–12.42) oocysts/L and 11.87 (95% CI: 0.60–106.72) cysts/L, on average, and 0.006 (95% CI: 0.000–0.052) oocysts/L and 0.050 (95% CI: 0.002–0.454) cysts/L, respectively. Their seasonal probability density functions are shown in Fig. 2. The figure shows a wide variation in the concentration estimate.

Cryptosporidium oocyst and *Giardia* cyst have been found commonly in sewage waters worldwide. The protozoan concentration in sewage influent were 33–600 oocysts/L and 130–3600 cysts/L in Northern China (Zhang et al., 2015; Fu et al., 2010), and 69–1210 oocysts/L and 7200–18300 cysts/L in South China (Zong et al., 2005). An survey of 40 sewage treatment works throughout Norway shown that parasites were detected in influent of 100–1100 oocysts/L for *Cryptosporidium* and 100–13600 cysts/L for *Giardia* (Robertson et al., 2006). Monitoring in Spain revealed that the *Cryptosporidium* concentration in 29 STPs influents was 1–80 oocysts/L and the *Giardia* concentration in 49 STPs influents was 2–14400 cysts/L (Castro-Hermida et al., 2010). Similarly, a count that ranged between 50 and 1280 oocysts/L of *Cryptosporidium* was obtained in swage influent from North Germany (Ajonina et al., 2012), and 3–21335 oocysts/L in 5 STPs from Australia (King et al., 2017). Therefore, the concentration levels (40–420 oocysts/L and 590–2120 cysts/L) of the parasites in this study were in accordance with those reported elsewhere, showing that *Cryptosporidium* and *Giardia* are highly prevalent in wastewater and requires effective control to ensure biosecurity.

To prevent the environmental transmission by pathogenic microorganism through sewage, treatment should be applied to remove or inactivate these pathogens in effluent before discharge. In the present study, an average removal of 99% (i.e. 2.0 $\log_{10}$) was used to estimate the protozoan concentration in effluent. Actually, secondary treatment processes have been reported to be highly variable and by some accounts ineffective in removing *Cryptosporidium* oocyst and *Giardia* cyst (Nasser, 2016; Nasser et al., 2012). Fu et al. (2010) reported that the removal of protozoan by conventional activated sludge, anaerobic-anoxic–oxic treatment and oxidation ditch process were 1.52, 1.79 and 2.17 $\log_{10}$, respectively, for *Cryptosporidium*, and 1.68, 2.04 and 2.60 $\log_{10}$, respectively, for *Giardia*. Recently, King et al. (2017) reported that oocyst removals for secondary treatment processes were highly variable for 5 STPs in Australia, ranging from 0.21 to 3.27 $\log_{10}$. Besides their

Table 1
Quantitative microbial risk assessment parameters and assumptions.

Parameter (units)	Symbol	Mean values	Assumptions [a]	References
Concentration in sewage influent	C_{inf}			Raw data collected in this study
Cryptosporidium in hot season (oocyst/L)		197.50	Lognorm(197.50, 85.09)	
Cryptosporidium in other season (oocyst/L)		102.88	Lognorm(102.88, 45.65)	
Giardia in hot season (cyst/L)		1597.50	Lognorm(1597.50, 254.81)	
Giardia in other season (cyst/L)		893.88	Lognorm(893.88, 282.75)	
Removal efficiency of parasite by STP (%)	R	99	BetaGeneral(9.95, 0.0912, 0.01, 0.999)	(Nasser, 2016).
Daily effluent volume reaching *Qinhe* River (m^3)	W_{eff}	2400	Uniform(22000,26000) × 10%	This study
Daily stream flow of *Qinhe* River (m^3)	W_{river}	603072	BetaGeneral(2, 2, 3.14, 10.82) × 60 × 60 × 24	This study
Water consumption	V			
via irrigation route A (mL/day)		4.8	Gamma(53.4, 0.09)	(Mok and Hamilton, 2014)
via irrigation route B (mL/day)		5.5	Uniform(1, 10)	(Mara et al., 2007)
via swimming route for adults (mL/visit)		7.56	Gamma(30.2, 0.25)	(Suppes et al., 2014)
via swimming route for children (mL/visit)		45.98	Gamma(183.9, 0.25)	(Suppes et al., 2014)
Susceptibility fraction of exposure population	f			
via irrigation route A (%)		100	Fixed value	(Mok and Hamilton, 2014)
via irrigation route B (%)		45.6	Fixed value	(www.stats.gov.cn)
via swimming route (%)		16.05	Uniform(10.5, 21.6)	(An et al., 2011)
Frequency of exposures per year	n			
via irrigation route A (day)		365	Fixed value	(Mok and Hamilton, 2014)
via irrigation route B (day)		150	Fixed value	(Diallo et al., 2008)
via swimming route (visit)		40	Fixed value	(An et al., 2011)
Infectivity constants of the exponential dose response model	r			
Cryptosporidium		0.09	Lognorm(0.09, 0.01)	(Soller et al., 2010)
Giardia		0.02	Lognorm(0.02, 0.001)	(McBride et al., 2013)
Probability of illness given infection	$P_{Ill\|Inf}$			
Cryptosporidium		0.71	Beta(20, 8)	(Xiao et al., 2012a)
Giardia		0.5	Uniform(0.2, 0.7)	(Chhipi-Shrestha et al., 2017)
Disease burden per case	*DBPC*			
cryptosporidiosis in immunodeficient population (DALY)		0.32	Uniform(0.1, 0.54)	(Xiao et al., 2012a)
cryptosporidiosis in immunocompetent population (DALY)		1.91×10^{-3}	Uniform(0.0015, 0.003)	(Xiao et al., 2012a)
giardiasis (DALY)		1.7×10^{-3}	Uniform(0.0011,0.0028)	(Gibney et al., 2014)

[a] Lognorm(mean, standard deviation) specifies a lognormal distribution with the entered mean and standard deviation. BetaGeneral(α1, α2, minimum, maximum) specifies a beta distribution with the defined minimum and maximum using the shape parameters α1 and α2. Uniform(minimum, maximum) specifies a uniform probability distribution with the entered minimum and maximum values. Gamma(α, β) specifies a gamma distribution using the shape parameter α and the scale parameter β.

Table 2
– Concentration of *Cryptosporidium* oocysts and *Giardia* cysts in sewage influent.

Pathogens	Seasons	No. of samples	Mean no. of (oo)cysts/L (Min–Max)	95% CI [a]
Cryptosporidium	Hot season	10	197.50 (120–420)	80.78–407.14
	Other seasons	14	102.88 (40–180)	40.81–215.56
	Total	24	142.31 (40–420)	44.79–344.65
Giardia	Hot season	10	1597.50 (1260–2120)	115.12–2151.74
	Other seasons	14	893.88 (590–1740)	465.21–1560.69
	Total	24	1187.06 (590–2120)	548.37–2254.43

[a] 95% CI: 95% confidence intervals were based on Monte Carlo simulation of 10,000 iterations using the statistical distribution in Table 1.

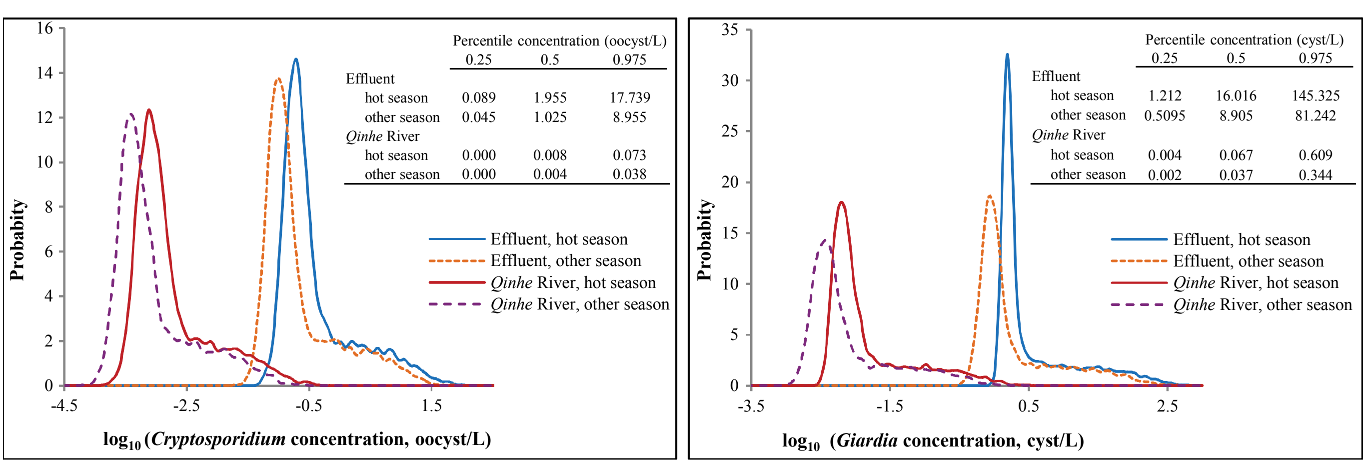

Fig. 2. Probability density functions of estimated concentrations of *Cryptosporidium* and *Giardia* in STP effluent and in *Qinhe* River by season.

concentration in the influent, the removal efficiency of *Cryptosporidium* oocyst and *Giardia* cyst by STP depends upon the processes applied (Cheng et al., 2009). A failure in the processes can result in high protozoan concentration in the effluent (Zhang et al., 2015). It is important consequently to describe average removal of pathogen by using probability density distribution to account for the variability and uncertainty of treatment efficiency. In this study, the computed average concentrations of *Cryptosporidium* and *Giardia* in STP effluent (1 oocyst/L and 12 cysts/L, respectively) were similar to those in STPs effluents from Shanghai, where the concentration were 0–1 oocyst/L and 0–49 cysts/L, respectively (Ma et al., 2016).

The additional concentration of protozoa in *Qinhe* River were estimated to be 0.006 oocysts/L and 0.053 cysts/L, respectively. The concentration was found to be lower than that in Yangtze River, where 0.19 oocysts/L and 0.078 cysts/L were detected (Xiao et al., 2013), and the main reason may be that the assumption of their concentration in the river upstream was zero in this study.

As discussed above, the monitored or computed concentrations of protozoa were consistent with those reported elsewhere. Therefore, the concentration data of pathogens collected from STP influent could use in assessing the risk of pathogens in the receiving water. In addition, collecting pathogens data from influent is more economical than that from the removed STP effluent or diluted receiving water, since the former does not need to collect and process large volumes of water samples.

3.2. Health impact assessment

The increased health impact of STP effluent discharge into *Qinhe* River was estimated using both probability of infection and disease burden by exposure route and by season (Table 3). For swimming in the river, the incremental risk of infection by *Cryptosporidium* and *Giardia* were 7.0 (95% CI: 0.2–61.1) and 12.6 (95% CI: 0.6–116.3) per 100,000 population per year, respectively. It should be noted that the estimates are based on 40 swimming exposure events in hot season and 10.5–21.6% of the population swimming in the river. Whereas for the exposure route by river agricultural irrigation, the additional protozoan infection was estimated separately since the protozoan concentration was seasonal (Fig. 2). The *Cryptosporidium* and *Giardia* infection risk via river irrigation in hot season were 158.2 (95% CI: 5.9–1401.9) and 286.5 (95% CI: 15.7–2676.5) per 100,000 population per year, respectively, both of which are higher than those in other season (84.3 and 161.1, respectively). From the perspective of disease burden, the total incremental health burden due to effluent discharge was calculated as 2.85×10^{-6} (95% CI: 0.09×10^{-6}–25.02×10^{-6}) DALYs per person per year (pppy) for *Cryptosporidium* and 3.17×10^{-6} (95% CI: 0.11×10^{-6}–29.58×10^{-6}) DALYs pppy for *Giardia* in hot season, and 1.45×10^{-6} (95% CI: 0.04×10^{-6}–13.26×10^{-6}) DALYs pppy for *Cryptosporidium* and 1.56×10^{-6} (95% CI: 0.05×10^{-6}–13.99×10^{-6}) DALYs pppy for *Giardia* in other seasons (Table 3). Generally, the increased combined disease burden from *Cryptosporidium* and/or *Giardia* infection due to effluent discharge was much higher in hot season than in other seasons (Fig. 3).

In addition to direct drinking, exposure routes by irrigation and recreational activities have been the most frequently considered in assessing the risk associated with *Cryptosporidium* and/or *Giardia* in water (Ryu et al., 2007; Chhipi-Shrestha et al., 2017; Jolis et al., 1999). The risk of infection for *Cryptosporidium* and/or *Giardia* in reclaimed wastewater via landscape irrigation for recreation (golf courses or playgrounds) were variable for 7 STPs in United States of America, ranging from as low as 6.35×10^{-7} to as high as 1.58×10^{-1}, but each of them was much lower than that via recreational impoundments for swimming (Ryu et al., 2007). Whereas the increased risks of protozoan infection, in this study, from agricultural irrigation exposure route were higher than from swimming route. The main reason may be that the different frequency of exposure between agriculture and landscape irrigation exposure scenarios.

Although both *Cryptosporidium* and *Giardia* are parasitic protozoa that constitute the leading causes of waterborne enteric disease outbreaks worldwide (Efstratiou et al., 2017a), epidemiological statistics indicates that incidence of giardiasis is typically five times that of cryptosporidiosis (Efstratiou et al., 2017b). This is in agreement with the result reported herein that the risks of infection for *Giardia* were much higher than those for *Cryptosporidium*.

The level of acceptable risk at 10^{-6} DALYs pppy from waterborne exposure was set by WHO (2011). In the present study, in the case of exposure route with agricultural irrigation, the incremental cryptosporidiosis and giardiasis burdens due to effluent discharge are both exceed the acceptable value, though those from exposure route of swimming was below the 10^{-6} DALYs pppy threshold.

The health impact of discharged effluent in receiving *Qinhe* River was seasonal. More disease burdens from both parasites were borne during hot season than other seasons in the present study. Similar study also found that temperature and humidity in different season may relate to variable infection rate of *Cryptosporidium* and *Giardia* (Alum et al., 2014). As is well-known, in the high temperature and humidity season, people are more inclined to swim to be cool and refreshing, which make more opportunities of infection by protozoa in water. High infection rate of *Cryptosporidium* and *Giardia* in population in hot season which in turn increase concentration of oocysts and/or cysts in sewage since infected hosts can shed large numbers of oocysts and/or cysts in their diarrheic feces (Xiao and Fayer, 2008). In fact, a recent study in Australia confirmed that oocyst density in raw sewage was seasonal and commensurate with community disease burden, showing that the cryptosporidiosis notification rate is a robust predictor of oocyst concentration in sewage (King et al., 2017). Therefore, data from

Table 3
– Additional risk of infection and disease burden caused by *Cryptosporidium* and *Giardia* in river posed by the STP effluent discharge by exposure route and by season.

Pathogens	Seasons	Probability of infection pppy (95% CI [a])		DALYs pppy (95% CI [a])		
		Swimming	Irrigation	Swimming	Irrigation	2-route
Cryptosporidium	Hot season	6.95×10^{-5} (0.23×10^{-5} -61.11×10^{-5})	1.58×10^{-3} (0.06×10^{-3} -14.02×10^{-3})	1.17×10^{-7} (0.03×10^{-7} -10.28×10^{-7})	2.74×10^{-6} (0.09×10^{-6} -24.04×10^{-6})	2.85×10^{-6} (0.09×10^{-6} -25.02×10^{-6})
	Other seasons	–	8.43×10^{-4} (0.29×10^{-4} -77.67×10^{-4})	–	1.45×10^{-6} (0.04×10^{-6} -13.26×10^{-6})	1.45×10^{-6} (0.04×10^{-6} -13.26×10^{-6})
Giardia	Hot season	1.26×10^{-4} (0.06×10^{-4} -11.63×10^{-4})	2.87×10^{-3} (0.16×10^{-3} -26.77×10^{-3})	4.11×10^{-7} (0.12×10^{-7} -37.28×10^{-7})	2.76×10^{-6} (0.10×10^{-6} -25.48×10^{-6})	3.17×10^{-6} (0.11×10^{-6} -29.58×10^{-6})
	Other seasons	–	1.61×10^{-3} (0.07×10^{-3} -14.83×10^{-3})	–	1.56×10^{-6} (0.05×10^{-6} -13.99×10^{-6})	1.56×10^{-6} (0.05×10^{-6} -13.99×10^{-6})

[a] 95% CI: 95% confidence intervals were based on Monte Carlo simulation of 10,000 iterations.

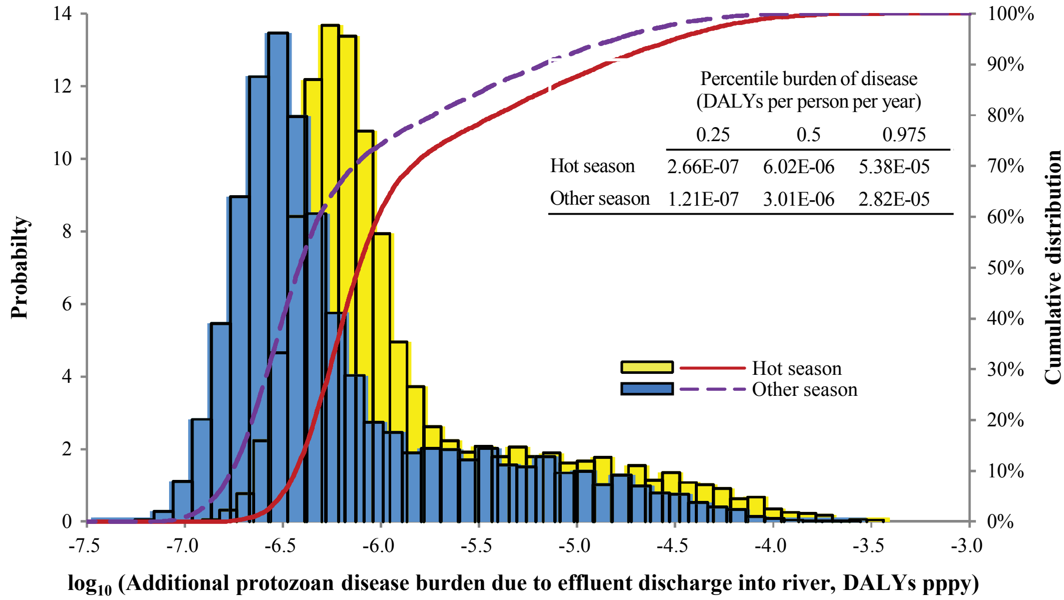

Fig. 3. Density functions of the estimated additional health burden from *Cryptosporidium* and *Giardia* infection due to effluent discharge into receiving *Qinhe* River by season.

disease surveillance could provide a valuable resource for utilities to operate STPs effectively to copy with the impact of seasonality on the oocyst and/or cyst density. Conversely, the monitoring data of oocyst and/or cyst density in sewage could reflect the community disease burden.

3.3. Sensitivity analysis

Sensitivity analysis was performed to evaluate the effects of the variation of input parameters on the final output of disease burden. It was revealed that uncertainty in the protozoan removal efficiency by STP, R, was the most influential factor of all the variables affecting variability in disease burden of cryptosporidiosis and giardiasis (Fig. 4). Similar results were also found that uncertainty in the treatment processes had the most influence on the probability of infection for norovirus from wastewater irrigation of vegetables in Australia (Mok et al., 2014). Consequently, improving the management of existing STP water systems could reduce the risk of human exposure to *Cryptosporidium* and *Giardia*. On the other hand, adopting advanced treatment, such as microfiltration and ozonation, in STP treatment process is also one of the ways to solve the potentially predicted health problems since advanced treatment can significantly reduce the risk of infection of *Cryptosporidium* in water compared with conventional chlorine treatment

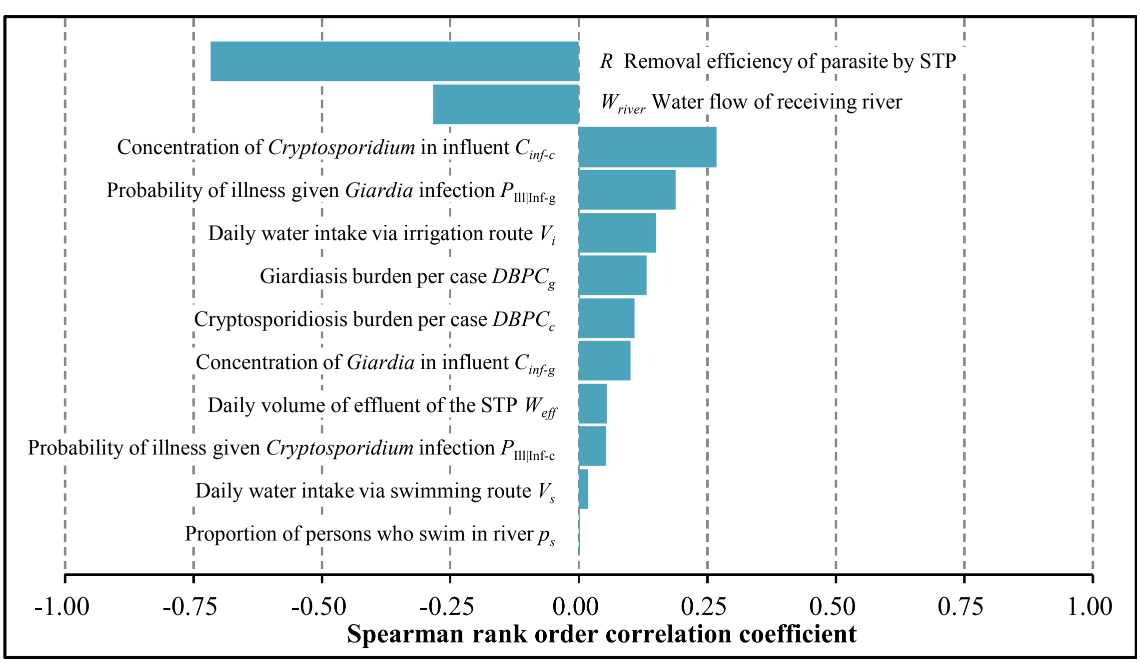

Fig. 4. Tornado chart showing Spearman rank order correlation coefficients between QMRA model parameters and the predicted health burden caused by protozoan parasites in receiving river posed by the STP effluent discharge.

(Xiao et al., 2012a).

Another important consideration is the uncertainty in the water flow of receiving river. The *Cryptosporidium* oocyst concentration in the Three Gorges Reservoir watershed in flood period were higher than those in impounding period, while concentration of *Giardia* cyst in the Yangtze River in flood period were lower than in impounding period (Xiao et al., 2013). This also indicated that health burden from *Cryptosporidium* and/or *Giardia* infection in water was seasonal. The concentrations of parasites in sewage influent, the conditional probability of illness given infection, the disease burden per case, and parameters used to estimate the exposure volume of the population were also significant variables contributing to the uncertainty of the final health impact of effluent on receiving water quality (Fig. 3).

4. Conclusions

According to the above risk assessment, we reached the following conclusions: 1) using concentrations data of pathogens from the influent of STP can be applied to risk assessment of pathogen in the receiving water. It can not only reduce the test costs by reducing the volume of sampled water, but also can reflect the disease burden in communities served by STPs; 2) the increased disease burden of cryptosporidiosis and giardiasis attributable to the influence of effluent discharging into receiving river were both slightly exceed the 10^{-6} DALYs pppy threshold set by WHO, and this should be paid attention by the relevant authorities for China and other developing countries because health effect of treated effluent from the STP in Henan Province may not be very different from other STPs in developing countries; 3) the health impact of effluent on receiving water was seasonal which was higher in hot season than in others, suggesting mitigation measures should be planned and executed by season; 4) improving the treatment efficiency of STP systems is the most effective to reduce the impact of discharged effluents on the receiving water quality.

Conflicts of interest

The authors declare no conflict of interest.

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