Assessment of the risk of infection by *Cryptosporidium* and *Giardia* in non-potable reclaimed water

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Abstract Quantitative risk assessment for *Cryptosporidium* oocysts and *Giardia* cysts was performed to determine the public health significance of non-potable use of tertiary treated reclaimed water. Seven reclaimed water treatment plants in the southwestern United States participated in this study. The average public exposure to oocysts and cysts was estimated, based on concentrations, recovery efficiency, viability and three exposure scenarios. The exponential dose-response model was chosen to determine the probability of infection from ingestion of various numbers of oocysts and cysts. The risks of infection for *Giardia* were approximately one or two orders of magnitude higher than those for *Cryptosporidium*. The combined risks of infection from oocysts and cysts at sites using a combination of chlorination and UV disinfection would meet the annual acceptable risk of 1.00E-04, whereas those at the other utilities using only chlorination indicated higher probability of infection than the 1.00E-04 resulting from accidental consumption of a small amount of non-potable reclaimed water.

Keywords *Cryptosporidium*; *Giardia*; risk assessment; water reuse

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Introduction

The arrival of the 21st century brings with it significant global concern regarding the diminishing supply of fresh water in arid areas. New resources of water are being actively explored to ensure the availability of a sufficient amount of fresh water. Reclaimed water, which is treated municipal wastewater, plays a significant role in meeting escalating municipal water demands in highly populated areas. Historically, reclaimed water was used for agricultural applications such as pasture irrigation or nonfood crop irrigation, and was often perceived as a method of wastewater disposal (WHO, 2003). The trend has now shifted toward unconventional uses such as urban irrigation, toilet and urinal flushing, commercial and industrial uses, and indirect potable reuse (USEPA, 2004). However, concern about the microbial quality of reclaimed water limits its widespread use.

Risk assessment may be defined as the qualitative or quantitative characterisation and estimation of potentially adverse health effects associated with exposure to environmental hazards (Haas *et al.*, 1999). A standard methodology for risk assessment is to identify hazards, to assess the dose–response relationship, to evaluate exposure and to characterise risk. To perform risk assessment, it is necessary to know the concentration of environmental hazards. The occurrence of enteric microbial pathogens including viruses, bacteria and protozoan parasites in water has been examined, and the related quantitative microbial risk assessments have been performed over the past several decades (Rose *et al.*, 1991; Asano *et al.*, 1992; Yanko, 1993; Rusin *et al.*, 1997; Tanaka *et al.*, 1998). However, the occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in reclaimed water and assessment of the risks associated with these protozoan parasites have not been well studied (Jolis *et al.*, 1999; Gennaccaro *et al.*, 2003; Quintero-Betancourt *et al.*, 2003).

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Risk assessment is closely tied to governmental policies focusing on the control of contaminants. The United States Environmental Protection Agency (US-EPA, 2004) has published guidelines for water reuse, yet no federal regulations for water reuse exist in the US; individual states are responsible for the development and implementation of water reuse criteria (State of California, 2000). The objective of this study is to perform quantitative risk assessments for *Cryptosporidium* oocysts and *Giardia* cysts in non-potable reclaimed water. The results will aid in revising the state criteria and implementing federal reclaimed water guidelines.

Materials and methods

Study design and sampling

Based on the size of the utilities, treatment processes and water reuse applications, seven reclaimed water treatment plants in the southwestern US including metropolitan areas in Arizona (AZ), California (CA1, CA2, and CA3), Nevada (NV) and Texas (TX1 and TX2) were selected (Ryu, 2003). Tertiary treated effluents at each utility were collected on a bimonthly basis between June 2002 and June 2003. The water samples were dechlorinated by adding 10% sodium thiosulfate and were filtered through an Envirochek-HV sampling capsule (Gelman Sciences, Ann Arbor, MI) at flow rates of no more than 2 L/min. Sample volumes ranged from 20 to 170 L due to differences in water turbidity at each utility.

Recovery of *Cryptosporidium* and *Giardia*

A total of 77 samples were assayed for *Cryptosporidium* oocysts and *Giardia* cysts using immunomagnetic separation (IMS) (Dynabeads GC-Combo; Dynal A.S., Oslo, Norway) followed by immunofluorescence assay (IFA) (Hydroflour Combo; Strategic Diagnostics Inc., Newark, DE) as described in the US-EPA method 1623 (2001). An integrated cell culture-polymerase chain reaction assay (ICC-PCR) was used to detect infectious *Cryptosporidium* oocysts as described by Di Giovanni *et al*. (1999).

Statistical analysis

All of the data were entered into a Microsoft Excel spreadsheet, and calculations for risk assessment were performed using Microsoft Excel 2002.

Exposure assessment

Exposure assessment is the estimation of how likely it is that an individual or a population will be exposed to the identified hazard and what quantity is likely to be ingested. *Cryptosporidium* oocysts and *Giardia* cysts are transmitted via the faecal-oral route. Based on concentrations, recovery efficiency, viability, disinfection efficiency and exposure scenarios, the average exposure (N) to oocysts and cysts was estimated using the following equation:

$$N = C \times R^{-1} \times I \times 10^{-DR} \times V$$

where N is the number of oocysts and cysts daily ingested by a person through reclaimed water, C is the concentration of pathogens ((oo)cysts/100 L), R is the recovery efficiency of the detection method, I is the fraction of detected pathogens capable of infection, DR is the disinfection efficiency, and V is accidental consumption of a small amount of non-potable reclaimed water (L).

Concentrations of oocysts and cysts (C)

The best estimation of risk is obtained using an unbiased estimate of the true mean concentration. However, some factors make it difficult to obtain the unbiased estimate of the mean concentrations of microbial pathogens in the environment. Firstly, many of the environmental water samples analysed resulted in non-detection (ND) of *Cryptosporidium* oocysts and *Giardia* cysts. Secondly, the sample volumes investigated varied, possibly due to filter clogging and limits of a sample purification technique. Lastly, the microbial concentrations may vary over time (e.g. day to day). Compensating for the low precision of single concentration estimates requires many samples and appropriate averaging. In this study, the effective volume (EV)-weighted average (i.e. total number of oocysts and cysts observed divided by total effective volume of water investigated) was used to calculate the mean concentration and its upper 95% confidence limits (CLs) to prevent underestimation (Parkhurst and Stern, 1998).

Table 1 summarises the results of the concentrations of oocysts and cysts in reclaimed water. *Cryptosporidium* oocysts and *Giardia* cysts were detected in 16% (12/77) and 43% (33/77) of samples using IMS-IFA, respectively. No infectious *Cryptosporidium* oocysts using ICC-PCR were detected in any sample. The highest mean concentration of oocysts was 15.9 oocysts/100 L at site CA1, whereas no oocysts were detected in any sample from sites NV, TX1 and TX2. *Giardia* cysts were detected in all sites, and their mean concentrations ranged from 0.46 to 211 cysts/100 L.

Recovery efficiency (R)

Mean recoveries of *Cryptosporidium* oocysts and *Giardia* cysts from seeded reclaimed water ($n = 4$) were measured to determine the overall recovery efficiency of the detection method, IMS-IFA. The percent recovery efficiencies of oocysts and cysts averaged 48 ± 53 (mean ± relative standard deviation) and 26 ± 33, respectively (Ryu, 2003).

Viability (I)

To date, there are limited data of viability of *Cryptosporidium* oocysts in reclaimed water (Gennaccaro *et al.*, 2003; Quintero-Betancourt *et al.*, 2003; Ryu, 2003), but no data for *Giardia* cysts in reclaimed water are available. In this study, previously published data were used for the estimation of the fraction of viable oocysts and cysts, the best fit probabilities of which are 25% and 13%, respectively (LeChevallier *et al.*, 1991; Gennaccaro *et al.*, 2003). These viable fractions of oocysts and cysts were determined

Table 1 Descriptive statistics of *Cryptosporidium* and *Giardia* in reclaimed water

Sampling Sites	N	*Cryptosporidium* (oocysts/100 L)				*Giardia* (cysts/100 L)			
		% ND[a]	EV-Mean[b]	Upper 95% CLs	Highest concentration	% ND[a]	EV- Mean[b]	Upper 95% CLs	Highest concentration
AZ	12	83	0.53	1.35	3.70	75	1.58	2.75	9.88
NV	12	100	0.00	0.71	0.00	42	31.2	36.0	990
CA1	12	58	15.9	21.4	159	33	36.5	44.5	174
CA2	6	67	0.76	2.73	2.94	0	211	229	1070
CA3	11	73	0.46	1.35	2.33	91	0.46	1.35	2.68
TX1	12	100	0.00	0.66	0.00	42	6.64	9.16	17.6
TX2	12	100	0.00	0.38	0.00	92	2.29	3.46	31.1
Total	77	84	–	–	–	57	–	–	–

[a]Ratio of nondetect (ND) to total samples
[b]Effective volume (EV)-weighted mean concentrations (total number of oocysts and cysts divided by total effective volume of water)

from utilities using only chlorination and from surface water prior to disinfection practice, respectively.

Disinfection efficiency (DR)

The disinfection process at four utilities (CA1, CA2, CA3 and TX1) included only chlorination and, at sites TX2, NV and AZ, chlorination was supplemented with ultraviolet (UV) irradiation (Ryu, 2003). Since both oocysts and cysts are very resistant to chlorination, no inactivation of oocysts and 0.5 log-units reduction of cysts at traditional chlorination conditions used in water plants were considered (USEPA, 1989; Clark and Regli, 1993; Finch *et al.*, 1997). It is well known that UV irradiation is much more effective at killing oocysts and cysts than chlorination (Clancy *et al.*, 2000; Morita *et al.*, 2002; Zimmer *et al.*, 2003). Qian *et al*. (2004) have performed a statistical assessment of the UV dose–response for *Cryptosporidium* oocysts and *Giardia* cysts using several published data. The results demonstrated that UV doses commonly used in water treatment (16 to $\geqslant$ 40 mJ/cm^2) were high enough to achieve 3.0 log-units reduction of oocysts and cysts. To acquire viability of oocysts and cysts in post disinfected reclaimed water using a combination of chlorination and UV irradiation, in this study, a 3.0 log-unit removal credit was added.

Exposure routes (V)

To assess the potential risks of *Cryptosporidium* and *Giardia* associated with the use of reclaimed wastewater, three exposure scenarios were considered:

1. Scenario 1 (Landscape Irrigation for Golf Courses). It is assumed that golfers are exposed to 0.001 L (1 mL) of reclaimed water in a day by handling and cleaning golf balls (Asano *et al.*, 1992). For the sake of an annual risk assessment, it is assumed that an avid person golfs twice a week, which equates to 104 days a year. Night time irrigation and golfing on a dry field are assumed. Additionally, no die-off of oocysts and cysts by desiccation, sunlight, predation, etc. is assumed for the worst possible case.
2. Scenario 2 (Landscape Irrigation for Playgrounds). It is assumed that a person playing football is exposed to 0.005 L (5 mL) of reclaimed water in a day by handling a ball (Ryu, 2003). The person is assumed to play once a week (52 days in a year), with all other assumptions the same as the golf course scenario.
3. Scenario 3 (Recreational Impoundments). A person swimming in a recreational impoundment is assumed to ingest accidentally 0.1 L (100 mL) of reclaimed water in a day (Haas, 1983). The person is assumed to swim for 2 hours a day on the weekends over a five month period (40 days in a year). No pathogen die-off in water is assumed for the worst possible case.

Dose-response modelling

The exponential dose–response model developed by Haas (1983) was chosen to determine the probability of infection from ingestion of various numbers of *Cryptosporidium* and *Giardia*. The exponential model is:

$$P_d = 1 - \exp\left(-\frac{N}{K}\right)$$

where P_d is the probability of infection resulting from daily ingestion of the number of pathogens (N). K is the average number of organisms that must be ingested to initiate an infection. The best-fit K values for *Cryptosporidium* and *Giardia* are 238.6 (95% confidence limits, 132.0–465.4) and 50.5 (95% confidence limits, 27.9–102.1), respectively (Rose *et al.*, 1991; Haas *et al.*, 1996; DuPont *et al.*, 1995).

Estimates of daily risk may be extrapolated to the risk of infection over extended periods of time using the following Default (Haas, 1983):

$$P_t = 1 - (1 - P_d)^t$$

where P_t and P_d are the probability of infection after t days and one day of exposure, respectively.

Assuming that the risks of infection from both parasites are independent, combined risks (P_{comb}) can be estimated using the following Default (Teunis *et al.*, 1997):

$$P_{comb} = 1 - (1 - P_c)(1 - P_g) = P_c + P_g - (P_c \times P_g)$$

where P_c and P_g are the risks of infection from *Cryptosporidium* and *Giardia*, respectively.

The risk of infection

Although reclaimed water provided from the participating utilities for this study is mainly used for non-potable applications, it is possible to be ingested accidentally. Three exposure scenarios were considered, and then daily and annual risks of infection associated with the oocysts and cysts were calculated using the exponential dose-response model. An annual acceptable microbial risk of infection of 1.00E-04 (10^{-4}) from waterborne exposure through potable water was applied for performing risk characterizations (Regli *et al.*, 1991).

The risks of infection from *Cryptosporidium* at sites NV, TX1 and TX2 were not determined due to the lack of positive samples. With multiple days of exposure, the annual risks of infection for *Cryptosporidium* for golf courses and playgrounds would not meet the annual acceptable risk at three California sites (Table 2). For recreational impoundments, even the daily risks of infection for *Cryptosporidium* exceeded the annual acceptable risk of 1.00E-04 at the California sites (data not shown). The annual risks of infection for *Giardia* for all scenarios (except for site NV for the worst scenario) would meet the annual acceptable risk at sites AZ, NV and TX2 where a combination of chlorination and UV disinfection was used, whereas those at the other sites using only chlorination exceeded the annual acceptable risk, resulting in the range of 9.29E-01 to 1.50E-04. Since the risks of infection for *Giardia* were much higher than those for *Cryptosporidium*, spatial trend of the combined risks from both parasites among the utilities are almost identical to that from *Giardia*. In terms of risk characterisation, the risks of infection calculated using upper 95% CLs used to ensure against underestimation showed similar results.

The probability of infection may be overestimated since human pathogenic-species of *Cryptosporidium* and *Giardia* were not considered for risk calculation. It is unlikely that all *Cryptosporidium* and *Giardia* detected in water samples are human pathogenic-species. Two species of *Cryptosporidium* (*hominis* and *parvum*) and *Giardia lamblia* are responsible for most human infections. However, USEPA method 1623 used for this study does not differentiate among species of *Cryptosporidium* or *Giardia*.

Summary and suggestions

In conclusion, the combined risks of infection from *Cryptosporidium* and *Giardia* at utilities using a combination of chlorination and UV disinfection met the annual acceptable risk of 1.00×10^{-4}, whereas utilities using only chlorination indicated high probability of infection resulting from accidental consumption of a small amount of non-potable reclaimed water. A general strategy to resolve health-related concerns and issues

Table 2 Annual risks of infection for oocysts and cysts from three exposure scenarios to non-potable reclaimed water

Sites	Treatment processes	Golf Courses (scenario 1)					
		Oocysts		Cysts		Oocysts + Cysts	
		EV-Mean	Upper 95% CLs	EV-Mean	Upper 95% CLs	EV-Mean	Upper 95% CLs
AZ	Tertiary/UV, Chlorine	1.20×10^{-7}	3.06×10^{-7}	5.14×10^{-7}	8.95×10^{-7}	6.35×10^{-7}	1.20×10^{-6}
NV		N/A	1.61×10^{-7}	1.02×10^{-5}	1.17×10^{-5}	1.02×10^{-5}	1.19×10^{-5}
TX2		N/A	8.63×10^{-8}	7.46×10^{-7}	1.13×10^{-6}	7.46×10^{-7}	1.21×10^{-6}
CA1	Tertiary/Chlorine	3.60×10^{-3}	4.85×10^{-3}	1.18×10^{-2}	1.44×10^{-2}	1.54×10^{-2}	1.92×10^{-2}
CA2		1.73×10^{-4}	6.20×10^{-4}	6.64×10^{-2}	7.19×10^{-2}	6.66×10^{-2}	7.24×10^{-2}
CA3		1.04×10^{-4}	3.06×10^{-4}	1.50×10^{-4}	4.39×10^{-4}	2.54×10^{-4}	7.46×10^{-4}
TX1		N/A	1.50×10^{-4}	2.16×10^{-3}	2.98×10^{-3}	2.16×10^{-3}	3.13×10^{-3}
Sites	Treatment processes	Playgrounds (scenario 2)					
		Oocysts		Cysts		Oocysts + Cysts	
		EV-Mean	Upper 95% CLs	EV-Mean	Upper 95% CLs	EV-Mean	Upper 95% CLs
AZ	Tertiary/UV, Chlorine	3.01×10^{-7}	7.66×10^{-7}	1.29×10^{-6}	2.24×10^{-6}	1.59×10^{-6}	3.00×10^{-6}
NV		N/A	4.03×10^{-7}	2.54×10^{-5}	2.93×10^{-5}	2.54×10^{-5}	2.97×10^{-5}
TX2		N/A	2.16×10^{-7}	1.86×10^{-6}	2.82×10^{-6}	1.86×10^{-6}	3.03×10^{-6}
CA1	Tertiary/Chlorine	8.98×10^{-3}	1.21×10^{-2}	2.93×10^{-2}	3.56×10^{-2}	3.80×10^{-2}	4.72×10^{-2}
CA2		4.31×10^{-4}	1.55×10^{-3}	1.58×10^{-1}	1.70×10^{-1}	1.58×10^{-1}	1.71×10^{-1}
CA3		2.61×10^{-4}	7.66×10^{-4}	3.74×10^{-4}	1.10×10^{-3}	6.35×10^{-4}	1.86×10^{-3}
TX1		N/A	3.75×10^{-4}	5.39×10^{-3}	7.43×10^{-3}	5.39×10^{-3}	7.80×10^{-3}
Sites	Treatment processes	Recreational impoundments (scenario 3)					
		Oocysts		Cysts		Oocysts + Cysts	
		EV-Mean	Upper 95% CLs	EV-Mean	Upper 95% CLs	EV-Mean	Upper 95% CLs
AZ	Tertiary/UV, Chlorine	4.63×10^{-6}	1.18×10^{-5}	1.98×10^{-5}	3.44×10^{-5}	2.44×10^{-5}	4.62×10^{-5}
NV		N/A	6.20×10^{-6}	3.91×10^{-4}	4.51×10^{-4}	3.91×10^{-4}	4.57×10^{-4}
TX2		N/A	3.32×10^{-6}	2.87×10^{-5}	4.33×10^{-5}	2.87×10^{-5}	4.66×10^{-5}
CA1	Tertiary/Chlorine	1.30×10^{-1}	1.70×10^{-1}	3.67×10^{-1}	4.27×10^{-1}	4.49×10^{-1}	5.25×10^{-1}
CA2		6.61×10^{-3}	2.36×10^{-2}	9.29×10^{-1}	9.43×10^{-1}	9.29×10^{-1}	9.45×10^{-1}
CA3		4.01×10^{-3}	1.17×10^{-2}	5.74×10^{-3}	1.68×10^{-2}	9.73×10^{-3}	2.83×10^{-2}
TX1		N/A	5.75×10^{-3}	7.98×10^{-2}	1.08×10^{-1}	7.98×10^{-2}	1.14×10^{-1}

N/A: Not available due to non-positive samples pertaining to treated waters such as reclaimed water and drinking water includes source control and application of advanced treatment processes. Conventional reclaimed water treatment processes include primary treatment (bar screens, grit chamber, primary clarification), secondary treatment (aeration basin, secondary clarification), tertiary treatment (filtration) and disinfection (chlorination/chloramination). The conventional treatment is known to reduce the numbers of *Cryptosporidium* oocysts and *Giardia* cysts by an average of 99.950% (3.17 log reduction) and 99.993% (4.14 log reduction), respectively (Rose *et al.*, 1996). Nevertheless, these protozoan parasites were often detected in tertiary treated effluents (Gennaccaro *et al.*, 2003; Quintero-Betancourt *et al.*, 2003; Ryu, 2003). High chlorination could be considered for achieving sufficient inactivation of microorganisms. However, high levels of dissolved organic matter in reclaimed water make it undesirable to use higher chlorine doses, since they may result in greater formation of disinfection-by-products. Current trends in advanced reclaimed water treatment processes include UV disinfection and membranes. In this study, relatively low risks of infection for *Cryptosporidium* and *Giardia* were estimated at the utilities using a combination of chlorination and UV disinfection, indicating better treatability of these parasites using dual disinfection practices. The authors suggest that reclaimed water utilities should apply advanced treatment processes such as UV disinfection to achieve additional inactivation of *Cryptosporidium* and *Giardia* and then to acquire safe water.

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