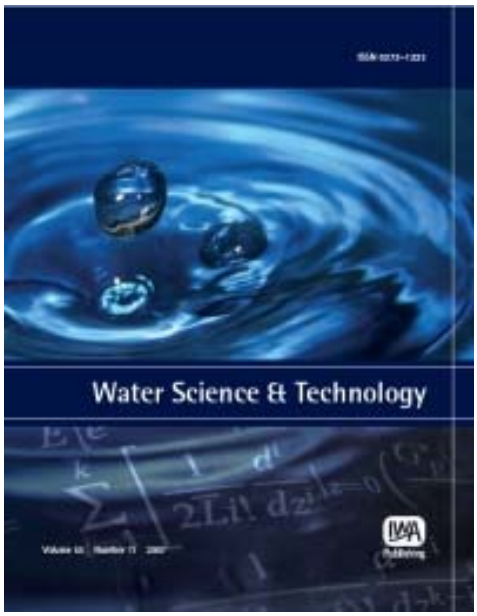

Pathogenic parasites and enteroviruses in wastewater: support for a regulation on water reuse

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ABSTRACT

Brazilian regulations for nonpotable reuse are being established using World Health Organization guidelines, however, they should be developed based on local monitoring studies. This study intended to analyze enteroviruses, protozoa and viable *Ascaris* sp. eggs in raw (24) and treated (24) effluents from four Wastewater Treatment Plants of São Paulo State, Brazil. The protozoa were detected with the US Environmental Protection Agency (USEPA) Method 1623 in the treated effluents and by centrifugation/Immunomagnetic Separation in the raw influent samples. Viable *Ascaris* sp. eggs were analyzed according to a modified USEPA method. Enteroviruses were quantified by using human rhabdomyosarcoma cells after adequate concentration procedures. All wastewater influents were positive for *Giardia* sp. whereas *Cryptosporidium* sp. was detected in 58.3% of the samples. *Giardia* sp. and *Cryptosporidium* sp. were present in 79.2 and 25.0% respectively, of the treated wastewater samples. Viable *Ascaris* sp. eggs were detected in 50.0 and 12.5% of influent and treated wastewater samples. Enteroviruses were isolated in the 24 raw influent samples and in 46% of the treated samples. Taking into account the densities of *Giardia* sp. in some treated wastewaters intended to be used as reclaimed water, Quantitative Microbial Risk Assessment studies should be conducted to establish pathogen quantitative criteria for a future Brazilian regulation for water reuse.

Key words | *Ascaris* sp., *Cryptosporidium* sp., enterovirus, *Giardia* sp., reclaimed water, wastewater

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INTRODUCTION

Wastewater reuse has been increasing in recent years due to the scarcity of water resources, industrial development and the population increase in large cities. Besides agricultural use, urban non-potable reuse includes, among other things, the irrigation of public parks, recreation centers, athletic fields, gardens of public buildings and enterprises, highway medians and shoulders, and the washing of streets (USEPA 2004). Regardless of use, it should be taken into account that wastewater polluted by human and animal excreta contains many pathogenic microorganisms including viruses, bacteria, protozoa and helminths, which may represent a risk to human health. Therefore, wastewater treatment processes must reduce the densities of these pathogens to acceptable levels, and quality criteria, ideally based on microbiological risk assessment, should be established in order to minimize such risks (National Research Council 2012). Aiming to provide such data, a study was undertaken to evaluate the densities of pathogenic microorganisms (protozoa, helminths and enteroviruses) in raw and treated wastewater (reclaimed water) of four Wastewater Treatment Plants (WTPs) from São Paulo State, Brazil. The results of these analyses will be used to estimate the microbiological risk associated with water reuse and support future Brazilian regulation.

METHODS

Sampling

The sampling was performed from February to December 2009 in four WTPs located in the Metropolitan Region of

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São Paulo (WTP 1, 2 and 3) and at a country town 350 km from the capital of São Paulo State (WTP 4). WTP 1 and 3 also produce reclaimed water. The treatment procedures carried out in each plant are summarized in Table 1. Six raw and six treated wastewater samples were collected and analyzed from each WTP. The samples were collected according to the procedures established by APHA (2006).

Pathogen analysis

Protozoa

Giardia sp. and *Cryptosporidium* sp. were detected and quantified by the IMS/IFA assay (Immunomagnetic Separation and Immunofluorescence Microscopy). For the treated samples, Method 1623 (filtration/IMS/IFA) developed by the US Environmental Protection Agency (USEPA 2005) was employed. Briefly, sample volumes of 20 L were concentrated by filtration (FiltaMax, Idexx) and the filters were eluted on a stomacher. The eluate was centrifuged at 1,500 *g* for 15 min and samples were processed by immunomagnetic separation (Dynal, Inc.) using a dry bath for the (oo)cysts dissociation (Ware *et al.* 2003). For the raw samples, the protozoa were analyzed by performing minor modifications to the method described by McCuin & Clancy (2005) (centrifugation/IMS/IFA). Sample volumes of 50 and 400 mL were used for *Giardia* sp. and *Cryptosporidium* sp., respectively. After the addition of sufficient volumes of Tween 80 (20%) in order to obtain a final concentration of 1%, the samples were centrifuged at 1,500 *g* for 15 min. Kaolin (0.75 g for each concentrated sample) was added to perform the immunomagnetic separation as described above, but two additional washings with buffered phosphate solution were necessary to remove the kaolin and the particulate matter usually found in these samples. The cysts and oocysts were identified and counted by immunofluorescence reaction and confirmed by DAPI fluorescence and DIC (differential interference contrast microscopy). Negative and positive control slides were also prepared. The Initial Precision and Recovery (IPR) as well as Matrix Spike (MS) were determined according to the USEPA Method 1623 (USEPA 2005), using the reference material EasySeed and ColorSeed (BTFbio, Biomérieux).

Table 1 | Description of the wastewater treatment procedures used by the four WTPs

Plant	Daily volume treated (L/s)	Type of treatment	Treatment
WTP 1	1,800	Secondary	Activated sludge
		Tertiary	Filter screen, sand–anthracite filter, membrane filtration (1 μm cartridge) and chlorine disinfection
WTP 2	1.7	Secondary	UASB, MBR
		Tertiary	Ferric chloride coagulation, sedimentation and chlorine disinfection
WTP 3	9,951	Secondary	Activated sludge
		Tertiary	Sand–anthracite filter and chlorine disinfection
WTP 4	40.4	Secondary	Anaerobic and facultative pond
		Tertiary	Maturation pond and trickling filter

UASB: Upflow anaerobic sludge blanket; MBR: Membrane bioreactor.

Viable *Ascaris* sp. eggs

A modified USEPA method established to assay viable *Ascaris* sp. eggs in wastewater, sludge and compost (USEPA 2003) was employed for the enumeration of these organisms. For treated effluents, volumes of 10 L were left to settle overnight, whereas for raw wastewater samples, volumes of 5 L were centrifuged at 1,000 *g* for 10 min. The supernatant of raw wastewater samples was aspirated and an anionic detergent (7X, MP Biomedicals) was added. After two periods of overnight incubation at 4–10 °C, the homogenized sediment was strained through a 50 mesh sieve and submitted to a new overnight incubation at 4–10 °C. The sediments of both, treated and raw samples, was floated with 1.20 (sg) magnesium sulfate. The supernatant was strained through a 400 mesh sieve and the material retained by the sieve was centrifuged at 1,000 *g* for 10 min. The sediment was centrifuged at 1,000 *g* for 10 min, floated with 1.20 (sg) magnesium sulfate, centrifuged at 1,000 *g* for 5 min and strained through a 400 mesh sieve. The material retained by the sieve was centrifuged at 1,000 *g* for 10 min. The final sediment from the raw and treated samples was resuspended in 4.0 mL of 0.05 mol/L sulfuric acid. Positive and negative controls with *Ascaris summ* eggs (Excelsior Sentinel Inc.) were also prepared. Incubation was carried out at 26 °C for 3–4 weeks to allow the embryonation of the eggs. Slides of the positive control were prepared and examined periodically. When at least 90% of the positive control eggs were fully embryonated,

samples were ready to be examined. The performance of the method was evaluated by spiking raw and treated wastewater samples from each WTP with known quantities of a *Ascaris summ* egg suspension.

Enterovirus

For enterovirus analysis, raw wastewater samples (2 L) were concentrated by adsorption on aluminum hydroxide, and samples (40 L) treated by electronegative filter adsorption–elution method (APHA 2005). The viruses were eluted with 3% beef extract in 0.05 mol/L glycine buffer (pH 9.0) and the eluate of the treated water sample was concentrated by organic flocculation. After decontamination with gentamicin sulfate and penicillin G potassium, the concentrated sample was assayed for enteroviruses by observation of cytopathic effects on rhabdomyosarcoma (RD) cells. Samples with high turbidity were cleaned up employing Vertrel (DuPont). The IPR of the method was determined by spiking known titers of poliovirus Sabin 1 suspension in four samples of purified water.

RESULTS AND DISCUSSION

During this study a total of 24 samples of raw sewage and 24 samples of treated sewage taken from four different WTP facilities were tested for pathogenic parasites and enteroviruses.

The individual results and geometrical average for *Giarda* sp. cysts, *Cryptosporidium* sp. oocysts, enteroviruses and viable *Ascaris* sp. eggs for raw and treated samples from the four WTPs are presented in Table 2.

Giardia sp. cysts were detected in all raw wastewater samples in concentrations ranging from 30 (WTP 2) to 1.9×10^4 (WTP 4), and in 79.2% of the treated samples in concentrations variable from <0.05 (WTP 4) to 109.6 cysts/L (WTP 1). It is interesting to observe that the five negative treated wastewater samples were from WTP 4, where *Giardia* sp. cysts were detected only in the sample collected in August 2009, in low densities (0.25 cysts/L). A smaller percentage of positive samples were detected for *Cryptosporidium* sp. (58.3% of the raw wastewater samples) in concentrations ranging from <2.5 (WTP 1 and 3) to 2.7×10^3 oocysts/L (WTP 4) and in 25.0% of the treated samples in concentrations ranging from <0.05 to 1.5 oocysts/L (Table 2).

Other authors have also reported *Giardia* sp. cyst occurrence more frequently in environmental samples. A study conducted by Cantusio Neto *et al.* (2006) in a WTP at Campinas City, São Paulo State, Brazil, found *Giardia* spp. cysts in 90.5% of 53 influent samples (mean densities: 1.0×10^5 cysts/L ± 8.7). *Cryptosporidium* spp. oocysts were detected in 6.4% of the raw wastewater samples (mean 6.0×10^4 oocysts/L ± 2.8). Robertson *et al.* (2006) evaluated the sewage influent from 40 Sewage Treatment Works (STW) in Norway and found that 80% of STW were *Cryptosporidium* positive and 93% of STW were *Giardia* positive,

Table 2 | Concentrations of pathogenic microorganisms in 24 raw and 24 treated samples from four WTPs

Month of collection	Type of sample	*Giardia* sp. (cysts/L)	*Cryptosporidium* sp. (oocysts/L)	Enteroviruses (PFU/L)	Viable *Ascaris* sp. eggs (eggs/L)
WTP 1					
March	Raw	1.4×10^3	80	120	0.4
	Treated	13.80	<0.05	<0.03	<0.10
April	Raw	9.3×10^3	80	22	1.2
	Treated	1.50	<0.05	0.10	<0.10
July	Raw	5.0×10^3	60	20	<0.2
	Treated	109.60	<0.20	<0.03	<0.10
August	Raw	2.4×10^3	12.50	2.1	0.8
	Treated	19.90	0.60	0.75	<0.10
October	Raw	1.3×10^3	3.30	17	1.4
	Treated	37.30	<0.14	0.18	0.20
December	Raw	2.3×10^3	65	27.6	0.4
	Treated	17.70	1.10	<0.03	<0.10
Geometrical average	Raw	2.8×10^3	32	19	0.87[a]
	Treated	18	0.81	NC	NC

(*continued*)

Table 2 | continued

Month of collection	Type of sample	*Giardia* sp. (cysts/L)	*Cryptosporidium* sp. (oocysts/L)	Enteroviruses (PFU/L)	Viable *Ascaris* sp. eggs (eggs/L)
WTP 2					
March	Raw	1.4×10^3	<10	3	0.2
	Treated	20	<0.05	0.025	<0.10
April	Raw	7.9×10^3	<4	550	0.2
	Treated	1.60	<0.05	0.77	<0.10
June	Raw	1.0×10^4	45	12	<0.2
	Treated	28.60	<0.05	<0.03	<0.10
September	Raw	30	<2.5	11	<0.2
	Treated	1.50	<0.05	<0.05	<0.10
November	Raw	410	<2.5	16	<0.2
	Treated	7	<0.05	<0.03	<0.10
December	Raw	1.1×10^3	<2.5	12	<0.2
	Treated	4	<0.05	0.08	<0.10
Geometrical average	Raw	1.1×10^3	NC	19	NC
	Treated	6	NC	NC	NC
WTP 3					
February	Raw	1.4×10^3	<10	26	3
	Treated	4	0.20	<1	<0.10
April	Raw	4.4×10^3	120	140	3.40
	Treated	2	<0.05	0.83	0.10
June	Raw	4.8×10^3	15	190	2.40
	Treated	8	<0.13	0.80	0.10
August	Raw	1.1×10^4	<2.50	15	3.20
	Treated	1	<0.05	0.03	<0.10
November	Raw	7.2×10^3	55	78	0.60
	Treated	5.80	1.50	<0.025	<0.10
December	Raw	1.5×10^3	32.5	5.45	<0.20
	Treated	3.60	1	0.03	<0.10
Geometrical average	Raw	3.9×10^3	12	41	2.1[a]
	Treated	3	0.67	NC	NC
WTP 4					
March	Raw	1.2×10^4	40	35	<0.2
	Treated	<0.05	<0.05	<0.25	<0.10
April	Raw	6.5×10^3	<20	260	<0.2
	Treated	<0.05	<0.05	<0.03	<0.10
June	Raw	1.9×10^4	25	88	<0.2
	Treated	<0.05	0.05	<0.03	<0.10
August	Raw	1.8×10^4	<2.5	0.5	<0.2
	Treated	0.25	<0.05	0.06	<0.10
October	Raw	1.7×10^3	<2.5	82	<0.2
	Treated	<0.05	<0.05	<0.03	<0.10
December	Raw	1.4×10^4	2.7×10^3	4.4	<0.2
	Treated	<0.05	0.15	<0.03	<0.10
Geometrical average	Raw	9.3×10^3	12	23	NC
	Treated	NC	0.09	NC	NC

PFU: plaque forming units.
[a]Arithmetic average.

demonstrating that giardiasis occurs more frequently than cryptosporidiosis in Norway. Fu *et al.* (2010) investigated the performance of three WTPs in Beijing, China, by determining the concentrations of fecal indicators and pathogenic protozoa. According to their study, *Cryptosporidium* and *Giardia* densities in untreated wastewater varied from 33 to 600 oocysts/L and 130 to 3,600 cysts/L, respectively. A compilation of literature data carried out by these authors report that in raw effluent samples *Giardia* and *Cryptosporidium* concentrations range from 1 to 42,000/L and 1 to 1,100/L, and in treated effluent samples from 0.01 to 1,462/L and 0 to 82/L. McCuin & Clancy (2006) analyzed *Cryptosporidium* oocysts in the wastewater from ten facilities across the USA, employing USEPA Method 1622 with some modifications. The authors emphasize the importance of the study taking into account the increased need for wastewater reuse. *Cryptosporidium* oocysts were detected in 30% of raw influent samples in concentrations ranging from <2 to 24 oocysts/L and in 19% of tertiary effluents in concentrations variable from <0.008 to 0.226 oocysts/L. During a study performed by Rose (2007), *Giardia* concentrations ranged from 10^2 to 10^6 cysts/100 L and *Cryptosporidium* densities varied from 10^1 to 10^4 oocysts/100 L in the influents of six wastewater reclamation facilities.

Regarding the quality control data, IPR assay results demonstrated a mean recovery percentage and relative standard deviation of 45.8 and 15.5%, respectively for *Giardia* sp. and corresponding values of 54.1 and 13.6% for *Cryptosporidium* sp.; values acceptable by the quality control criteria established by Method 1623 (USEPA 2005). The MS assay results met USEPA criteria for *Giardia* sp. (9–82% and 35–81% in raw and treated effluent samples) but not for *Cryptosporidium* sp. (2–45% and not detected to 22% for raw and treated effluent samples). A study performed by Ottoson *et al.* (2006) reported recovery percentages of 22 ± 1.5% and 25 ± 12% for *Giardia* sp. cysts in raw and treated wastewater ($n = 3$), respectively, and corresponding values of 15 ± 4.6 and 39 ± 13% for *Cryptosporidium* sp. oocysts. McCuin & Clancy (2006) reported *Cryptosporidium* sp. recovery percentages of 29.2 ± 12.8% (raw sewage samples) and of 53.0 ± 19.2% and 67.8 ± 4.4% for secondary and tertiary effluents respectively.

Regarding viable *Ascaris* sp. eggs (Table 2), out of 24 treated wastewater samples, only three were positive, two of them from WTP 3 (0.1 egg/L collected in April and June 2009) and one from WTP 1 (0.2 egg/L collected in October 2009). Concentrations of viable *Ascaris* sp. eggs in the positive raw influent samples from WTPs 1, 2 and 3 ranged from 0.2 to 3.4 eggs/L. The lowest density was detected in WTP 2, which is located in an upper middle class region. On the other hand, the highest density was observed in WTP 3, which receives domestic sewage from a larger area of the Metropolitan Region of São Paulo city and thus includes a population of different income levels and education. All the raw influent samples from WTP 4 were negative. Literature data for developing countries reported raw influent densities of 166–202 helminths eggs/L (Jimenez *et al.* 2007), but it should be mentioned that these figures refer to total eggs and a viability rate of 80% is assumed (Jimenez, personal communication).

In the present study, the method developed for the US Environmental Protection Agency for sewage sludge samples (USEPA 2003) was modified to be used with wastewater samples. The quality control assays demonstrated recovery percentages variable from 49 to 87% for the raw and treated wastewater samples from the four WTPs. There are no established criteria for the performance of this method, but Bowman *et al.* (2003), employing the same method for sewage sludge samples could obtain recovery percentages superior to 60%.

Enteroviruses were detected in all the raw effluent samples in densities variable from 2.1 to 550 PFU (plaque forming units)/L and in 46% (11) of the treated effluent samples in the range of <0.025–0.8 PFU/L (Table 2). Greening *et al.* (2002) also detected enteroviruses in 100% of raw effluent samples using ICC-PCR (integrated cell culture polymerase chain reaction), direct PCR and cell culture assay. Enteric viruses were found in 100% of untreated wastewater samples of six wastewater reclamation facilities in concentrations variable from 10^2 to 10^4 MPN (Most Probable Number)/100 L and in 31% of disinfected effluent in concentrations in most cases below 1 MPN/100 L (Harwood *et al.* 2005). Ottoson *et al.* (2006) analyzed enteroviruses in samples from a wastewater pilot plant and obtained positive results in 18 out of 23 samples with average concentrations of 10,000 PCR units. Petrinca *et al.* (2009) evaluated enteroviruses in the raw effluent samples from three WTPs and according to their results 78–89% of the samples were positive, in densities variable from 2.3 to 140 MPN/L. During a study conducted to evaluate indicator and pathogenic microorganisms in reclaimed water, Costán-Longares *et al.* (2008) detected enteroviruses in 24 out of 48 samples of secondary effluents (average concentrations of 1.9 log 10 units/100 L), whereas in tertiary effluents these values were 0.7 log 10 units/100 L. Although there are no established performance criteria for enterovirus enumeration in cell

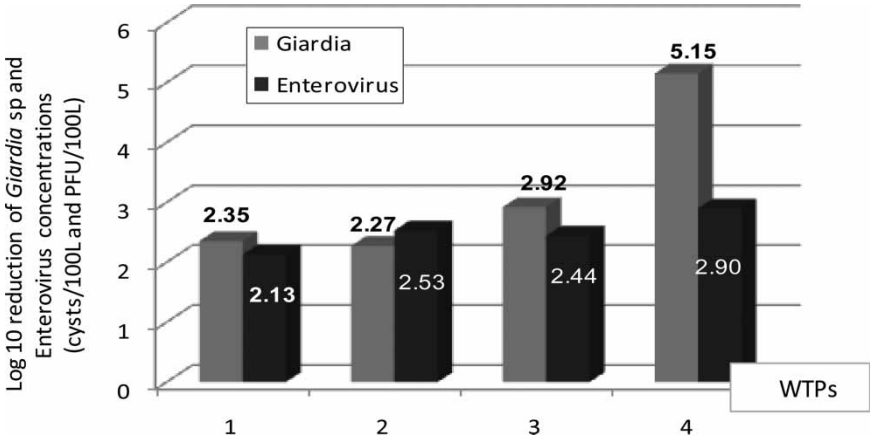

Figure 1 | Log 10 reduction of *Giardia* sp. cysts and enterovirus concentrations in the four WTPs evaluated.

culture, IPR tests for treated wastewater samples were also run: the results demonstrated an average percentage recovery of 27.7% and standard deviation of 6.2%.

The log 10 reductions of *Giardia* sp. cysts and enteroviruses from all WTPs are shown in Figure 1. The treatment was effective for about 2 to 5 log 10 reduction for *Giardia* and 2 to 3 log 10 for enterovirus, with the best efficiency for WTP 4 with stabilization ponds treatment. Rose (2007) observed a 3 log 10 reduction of pathogens (*Giardia* and enteric viruses) at final effluents (biological treatment, filtration and disinfection) from six wastewater reclamation facilities.

CONCLUSIONS

According to the results obtained in this study, WTP 4, whose treatment procedures include a series of lagoons and trickling filtration, presented the best pathogen efficiency removal for *Giardia* sp. cysts and enteroviruses, whereas the other plants were able to reduce 2.27 to 2.92 log 10 and 2.13 to 2.53 log 10, respectively of the pathogen concentrations. The densities of pathogenic protozoa *Giardia* sp. and *Cryptosporidium* sp. in raw and treated samples agree with the data reported in the literature. On the other hand, the viable *Ascaris* egg densities in raw influent samples are below the results reported for developing countries. *Giardia* sp. concentrations attained elevated densities in WTP 1 (geometrical average of 18 cysts/L and a two order magnitude maximum concentration of 109.6 cysts/L for the sample collected in June 2009) and also in WTP 3 (geometrical average of 5.8 cysts/L and maximum concentration of 8 cysts/L for the sample collected in June 2009). Taking into account that both WTPs also produce reclaimed water, a Quantitative Microbiological Risk Assessment (QMRA) should be conducted to evaluate the human health risk for the different water reuse applications. The data obtained will also be useful to compose a database about pathogen densities in raw and treated wastewater samples to be employed in QMRA studies and establish a Brazilian regulation.

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REFERENCES

American Public Health Association 2005 Section 9510. In: *Standard Methods for the Examination of Water and Wastewater*, 21st edn. APHA/AWWA/WEA, Washington, DC.

American Public Health Association 2006 Section 9020. In: *Standard Methods for the Examination of Water and Wastewater On Line*. Washington, DC. http:www.standardmethods.org (accessed 5 November 2012).

Bowman, D. D., Little, M. D. & Reimers, R. S. 2003 Precision and accuracy of an assay for detecting *Ascaris* eggs in various biosolid matrices. *Water Research* **37**, 2063–2072.

Cantusio Neto, R., Santos, J. U. & Franco, R. M. B. 2006 Evaluation of activated sludge treatment and the efficiency of the disinfection of *Giardia* species cysts and *Cryptosporidium*

oocysts by UV at a sludge treatment plant in Campinas, southeast Brazil. *Water Science and Technology* **54** (3), 89–94.

Costán-Longares, A., Montemayor, M., Payan, A., Méndez, J., Jofre, J., Mujeriego, R. & Lucena, F. 2008 Microbial indicators and pathogens: removal, relationships and predictive capabilities in water reclamation facilities. *Water Research* **42**, 4439–4448.

Fu, C. Y., Xie, X., Huang, F. F., Zhang, T., Wu, Q. I., Chen, J. N. & Hu, H. Y. 2010 Monitoring and evaluation of removal of pathogens at municipal wastewater treatment plants. *Water Science and Technology* **61**, 1589–1599.

Greening, G. E., Hewitt, J. & Lewis, G. D. 2002 Evaluation of integrated cell culture-PCR (ICC-PCR) for virological analysis of environmental samples. *Journal of Applied Microbiology* **93**, 745–750.

Harwood, V. J., Levine, A. D., Scott, T. M., Chivukula, V., Lukasik, J., Farrah, S. R. & Rose, J. B. 2005 Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. *Applied and Environmental Microbiology* **71**, 3163–3170.

Jimenez, B., Maya, C. & Galvan, M. 2007 Helminths ova control in wastewater in sludge for advanced and conventional sanitation. *Water Science and Technology* **56** (5), 43–51.

McCuin, R. M. & Clancy, J. L. 2005 Methods for the recovery, isolation and detection of *Cryptosporidium* oocysts in wastewaters. *Journal of Microbiological Methods* **63**, 73–85.

McCuin, R. M. & Clancy, J. L. 2006 Occurrence of *Cryptosporidium* oocysts in US wastewaters. *Journal of Water and Health* **4**, 437–452.

National Research Council 2012 *Water Reuse: Expanding the Nation's Water Supply through Reuse of Municipal Wastewater*. The National Academic Press, Washington, DC, 2012. http://www.nap.edu/catalog.php?record_id=13303 (accessed 9 November 2012).

Ottoson, J., Hansen, A., Bjorlenius, B., Norder, H. & Stenstrom, T. A. 2006 Removal of viruses, parasitic protozoa and microbial indicators in conventional and membrane processes in a wastewater pilot plant. *Water Research* **40**, 1449–57.

Petrinca, A. R., Donia, D., Pierangeli, A., Gabrieli, R., Degener, A. M., Bonanni, E., Diaco, L., Cecchini, G., Anastasi, P. & Divizia, M. 2009 Presence and environmental circulation of enteric viruses in three different wastewater treatment plants. *Journal of Applied Microbiology* **106**, 1608–1617.

Robertson, L. J., Hermansen, L. & Gjerde, B. K. 2006 Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in sewage in Norway. *Applied and Environmental Microbiology* **72**, 5297–5303.

Rose, J. B. 2007 Water reclamation, reuse and public health. *Water Science and Technology* **55** (1), 275–282.

USEPA (United States Environmental Protection Agency) 2003 Test method for detecting, enumerating and determining the viability of *Ascaris* ova in sludge. In: *Environmental Regulations and Technology. Control of Pathogens and Vector Attraction in Sewage Sludge*. EPA/625/R-092/013 US Agency for International Development, Washington, DC; US Environmental Protection Agency, Office of Water, Washington, DC/Office of Research and Development, Cincinnati, OH, Jul. 2003.

USEPA (United States Environmental Protection Agency) 2004 *Guidelines for Water Reuse*. EPA/625/R-04/108. US Agency for International Development, Washington, DC. http://www.epa.gov/nrmrl/smallsystems/pubs/625r04/08.pdf (accessed 9 November 2012).

USEPA (United States Environmental Protection Agency) 2005 *Office of Water*. Method 1623: Cryptosporidium and Giardia in water by filtration/IMS/FA. EPA 815-R-05–002. Dec. 2005.

Ware, M. W., Wymer, L., Lindquist, A. & Schaefer, F. W. 2003 Evaluation of an alternative IMS dissociation procedure for use with Method 1622: detection of *Cryptosporidium* in water. *Journal of Microbiological Methods* **55**, 575–83.

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