

Quantification of *Giardia* and *Cryptosporidium* in surface water: a risk assessment and molecular characterization

M. O. Bataiero, R. S. Araujo, A. C. Nardocci, M. H. Matté, M. I. Z. Sato^{IMA}, M. S. Lauretto and M. T. P. Razzolini^{IMA}

ABSTRACT

This study aimed to estimate the annual probability of *Giardia* and *Cryptosporidium* infection for a population supplied by contaminated drinking water sources. Parasites were quantified by the USEPA Method 1623.1/2012. Annual risk was estimated using the quantitative microbial risk assessment (QMRA) approach. Genotyping was performed using specific primers based on the 18S rRNA gene for *Cryptosporidium* and *gdh* gene for *Giardia*. *Giardia* was detected in 83.3% of the samples (<0.1 to 8.6 cysts/L) and *Cryptosporidium* in 37.5% (<0.1 to 2 oocysts/L). In general, annual risk values for *Giardia* were 1 log higher when compared with those obtained for *Cryptosporidium*. *Giardia intestinalis* A and B were present as well as *C. hominis* and *C. parvum*. The lack of protection measures for the water supply catchment point put the population's health at risk. The results provide data to support decision-makers to take actions to improve environmental quality resulting in a positive impact on consumers' health.

Key words | *Cryptosporidium*, *Giardia*, infection risk, QMRA, water supply

M. O. Bataiero
Sanitary Surveillance Center,
Division of Actions Regarding the Environment,
Avenida Dr Arnaldo 351 - Anexo III, Cerqueira
Cesar, São Paulo,
Brazil

A. C. Nardocci
M. H. Matté
M. T. P. Razzolini ^{IMA} (corresponding author)
School of Public Health,
University of São Paulo,
Av. Dr Arnaldo 715, 1º andar, 01246-904 São Paulo,
Brazil
E-mail: razzolini@usp.br

R. S. Araujo
M. I. Z. Sato ^{IMA}
Department of Environmental Analyses,
Environmental Company of the State of São Paulo,
Av. Prof Frederico Hermann Jr, 345, São Paulo,
Brazil

M. S. Lauretto
School of Arts, Science and Humanities,
University of São Paulo-Brazil,
R. Arlindo Bettio, 1000, São Paulo,
Brazil

A. C. Nardocci
M. I. Z. Sato
M. S. Lauretto
M. T. P. Razzolini
NARA – Center for Research in Environmental Risk
Assessment,
Av. Dr Arnaldo 715, 01246-904 São Paulo,
Brazil

INTRODUCTION

Contaminated drinking-water supplies are a public health concern worldwide, especially in areas with poor sanitation in which the population is exposed to waterborne pathogens that cause health problems (WHO 2017). According to the Brazilian Institute of Geography and Statistics, water supply services have a wider coverage than the sewage collection services; only 40% of municipalities have a sewage disposal system of which only 40.8% is treated (Ministério das Cidades/Ministry of Cities 2016).

A significant portion of Brazilian water sources utilized for public supplies are compromised by sewage discharges that carry pathogens and, therefore, pose risks to human health. Moreira & Bondelind (2017) ran an extensive literature review on global waterborne outbreaks between 2000 and 2014. The authors reported that surface raw water contamination was the probable cause of 13 outbreaks in this period, with etiological agents including *Cryptosporidium* and *Giardia*.

Among the waterborne pathogens that cause acute diarrhoeal diseases (ADD), *Giardia* and *Cryptosporidium*, have been of great concern for public health. Rosado-García et al. (2017) carried out a review referring to waterborne pathogens in Latin America and cited that Brazil leads the reports of waterborne pathogens in South America. Several studies have shown *Giardia* and *Cryptosporidium* occurrence in surface water sources used by public water systems after treatment (Sato et al. 2013; Burnet et al. 2014; Ramo et al. 2017). Franco et al. (2016) found *Cryptosporidium* in two sources of water with frequencies of 42.8% and 85.7% and *Giardia* in 100% of surface water samples analyzed.

Sato et al. (2013) surveyed *Giardia* and *Cryptosporidium* in surface water used as drinking water sources by public water systems in four densely urbanized regions of São Paulo State, and also estimated the probability of infection. *Giardia* was detected in 49.5% of the samples ($n = 206$), while *Cryptosporidium* was in 9.2%, with maximum concentrations of 97.0 cysts/L and 6.0 oocysts/L, respectively. Annual risk of *Giardia* infection, for adults and children, ranged from 0.29% to 2.47% and from 0.08% to 0.70%, respectively, while for *Cryptosporidium* it ranged from 0.15% to 0.29% for adults and from 0.04% to 0.08% for children.

The most important concern in water supplies contaminated with these parasites is the source of contamination and their resistance to the disinfection process of chlorination, usually used in DWTPs (WHO 2017).

Since not all species of *Cryptosporidium* and *Giardia* genotypes are human pathogens it is necessary to identify those that cause human disease.

A giardiasis outbreak in Bergen, Norway, between 2004 and 2005, indicated that most isolates of *Giardia intestinalis* were related to subgenotype BIII with recognized zoonotic potential. According to the authors a leakage of sewage from a residential area was the likely source of contamination of water resources (Robertson et al. 2006), while Widerström et al. (2014) reported an outbreak of *Cryptosporidium hominis* in Sweden associated with public water supply.

This study aimed to estimate the annual probability of infection for both parasites and to genotype *Giardia* and to identify species of *Cryptosporidium* in surface water used as drinking-water supply.

MATERIALS AND METHODS

Sampling and characterization of the study area

Sampling was performed in the intake point of a drinking water treatment plant (DWTP), which supplies drinking water for a population of 35,000, approximately (CETESB 2015). The treatment process applied is as follows: pre-oxidation followed by pre-chlorination, pre-alkalization, coagulation/flocculation, flotation, slow sand filtration, chlorination, fluoridation and alkalization. Samples ($n = 48$) were collected weekly from May 2013 to May 2014. Two 10-litre bottles were used, one addressed to parasite enumeration and the second one to molecular assays.

Giardia and *Cryptosporidium* quantification

Parasites were quantified according to USEPA Method 1623.1 (USEPA 2012). Briefly, water samples were concentrated through FiltaMax[®] foam filter (IDEXX[®]) and immunomagnetic separation was performed with Dynal Dynabeads[®] Crypto-Giardia Combo reagents and equipment. The dissociation of the complex beads was performed by heat dissociation. The slides were stained using fluorescein-conjugated monoclonal antibodies for both parasites (Waterborne, New Orleans, USA) and DAPI (4',6-diamidino-2-phenyl-indole, Sigma). The (oo)cysts were identified and counted by immunofluorescence reaction and confirmed by DAPI fluorescence and DIC (differential interference contrast microscopy) using an Olympus B52 epifluorescence microscope equipped with bright field, phase contrast DIC and epifluorescence optics. Negative and positive control slides were also prepared. The recovery efficiency of the method was determined by spiking 10 L purified water samples with EasySeed, according to the instructions of the manufacturer (BTFbio, Australia). The detection limit (DL) of 0.1 (oo) cyst/L was calculated considering the volume of sample concentrated (10 L) and that the entire packed pellet was assayed. The recovery rate was obtained according to USEPA 1623.1 (2012). The second sample was carried out until the step of dissociation of the complex beads and (oo)cysts and, then, for running the molecular analysis as described in the section below on *Giardia* and *Cryptosporidium* genotyping.

Risk assessment procedure

The annual risk of infection by water ingestion was estimated using the quantitative microbial risk assessment (QMRA) approach, considering the parameters as follows.

Concentration of *Giardia* and *Cryptosporidium* in raw water

Parasite concentrations (Table S1 in the Supplement, available with the online version of this paper) were modeled and the best-fitted distribution for each parasite was chosen for risk assessment. Considering samples below the DL, an adapted version of the maximum likelihood method for left-censoring was used, which has the advantage of precluding the need to assume arbitrary values for samples below the DL (Govaerts et al. 2005). Log-normal, gamma and Weibull distributions were adjusted using the R package *fitdistrplus* (Delignett-Muller & Dutang 2015), while the triangular distribution was adjusted using a routine developed by the authors. All (oo)cysts were considered viable and infectious. The genotypes of the protozoa parasites were not considered for the calculation of the risk of infection because samples were collected at the same time but analyzed separately for the quantification and for the genotyping of the parasites.

Log₁₀ removal by the treatment process

Removal efficiency of protozoans by the conventional treatment processes was modeled on the basis of a two-year classic study conducted by Nieminski & Ongerth (1995). *Giardia* and *Cryptosporidium* (oo)cyst removal by filtration at a full-scale water treatment plant and a pilot plant both operated under conventional treatment and direct filtration regimes. We considered only removal data from conventional treatment and grouped samples from both full-scale and pilot plants, as Student's *t*-test did not show significance between log₁₀ removal means ($p > 0.05$). The log₁₀ removal efficiency yielded a normal distribution with mean and standard deviation fitted from grouped raw data: (3.37, 0.62) for *Giardia* and (2.78, 0.67) for *Cryptosporidium*.

Dose–response model

The exponential dose–response model was used for both parasites, where the probability (k) was modeled as a triangular distribution with values of 0.01982 (CI 95% of 0.009798–0.03582) for *Giardia* (Rose et al. 1991) and of 0.00419 (CI 95% of 0.0022 to 0.0085) for *Cryptosporidium* (Dupont et al. 1995).

Ingestion rate

The water ingestion rates for the southeast region Brazilian population were modelled on the basis of the Kahn & Stralka (2009) study, which provided mean and 90th percentiles of total water ingestion based on body weights (mL/kg/day). These estimates were multiplied by the weighted average of body weights in São Paulo State (IBGE 2008), to provide the mean and 90th percentile estimates for daily water ingestion per person (mL/day). A log-normal distribution was fitted for each age category: children under 5 years old (meanlog = 6.36, sdlog = 0.40) and adults over 18 years old (meanlog = 7.22, sdlog = 0.36), yielding average daily ingestion of 0.62 L/d for children (90th percentile of 0.93 L/d) and 1.5 L/d for adults (90th percentile of 2.2 L/d).

Risk calculation

The daily risk of infection (P_i) was calculated using the equation:

$$P_i = 1 - \exp(-k C IR 10^{-TR}) \quad (1)$$

where:

- k = dose–response model parameter;
- C = concentration of parasites ((oo)cysts/L);
- IR = ingestion rate (L/day);
- TR = log₁₀ treatment removal.

Monte Carlo simulation was performed using the R computing environment (R Core Team 2016), where each scenario consisted in drawing 365 values for k , C , IR , TR and computing the corresponding value of $P_i^{(j)}$ according to Equation (1). Annual risk of infection (P_a) is

computed by:

$$P_a = 1 - \prod_{j=1}^{365} [1 - P_i^{(j)}]. \quad (2)$$

This procedure was repeated 50,000 times to provide the distribution of annual risk and the corresponding values of empirical mean, median and 95-percentile. The uncertainties were evaluated and characterized via sensitivity analysis, measuring the rank correlation coefficient between each parameter and the computed infection risk.

***Giardia* and *Cryptosporidium* genotyping**

Extraction of genomic DNA from *Giardia* cysts and *Cryptosporidium* oocysts was performed using the commercial QIAamp DNA Stool Mini[®] kit (Qiagen, Venlo, the Netherlands) according to the instructions provided by the manufacturer with modifications in heating time with buffer lysis, which was carried out at 95 °C for 10 minutes for all samples, and storage of purified DNA in a freezer at –20 °C until the time of use.

Molecular analysis of *Cryptosporidium*

Genotyping was performed by nPCR (nested polymerase chain reaction) and sequencing, using specific primers based on the 18S rRNA gene for *Cryptosporidium*, followed by sequence analysis of the amplified product. Briefly, the first nPCR reaction was performed using external primers: forward SCL15'-CTG.GTT.GAT.CCT.GCC.AGT.AG-3' and reverse CPB-DIAGR5'-TAA.GGT.GCT.GAA.GGA.GTA.AGG-3' (Coupe et al. 2005), amplifying a fragment of approximately 1,035 bp. The reactions were incubated in a thermocycler (Mastercycler gradient, Eppendorf[®]), as follows: initial denaturation at 94 °C for 5 minutes, followed by 40 cycles of denaturation at 94 °C for 45 seconds, annealing at 60 °C for 45 seconds, extension at 72 °C for 90 seconds, and a final extension at 72 °C for 10 minutes. For removing possible amplicon inhibitors, 1,035 bp fragments were submitted to 10-fold dilutions (1:10, 1:100, 1:1,000 and 1:10,000). The second reaction was performed using internal primers: forward 5'-GGA.AGG.GTT.GTA.TTT.ATT.AGA.TAA.AG-3' and reverse

5'-AAG.GAG.TAA.GGA.ACA.ACC.TCC.A-3' (Xiao et al. 2000), which amplified a fragment of 826 bp. PCR cycling conditions were as described above, except for a 58 °C annealing temperature. Nested PCR amplified fragments were purified using a commercial kit (Ilustra GFX[™] PCR DNA and Gel Band Purification, GE[®] Healthcare, UK) and subjected to sequencing in an external laboratory (Genomic Engenharia Molecular, São Paulo, Brazil). The 826 bp sequences were aligned (Bioedit Sequence Alignment Editor) and subjected to BLAST (Basic Local Alignment and Search Tool) analysis for comparison with available sequences of *Cryptosporidium* in the GenBank database (<http://www.ncbi-nml.nih.gov/genbank>).

Molecular analysis of *Giardia*

For confirming the presence of assemblages A and B of *Giardia intestinalis* the primers used were those described by Ulloa-Stanojlović et al. (2016). For detecting the assemblage A of *G. intestinalis* a 170 bp fragment from the *gdh* gene was amplified with the forward primer GiaAF 5'-CGG.GCT.ATG.GCG.CTG.TC-3' and the reverse primer GiaAR 5'-TTG.TCG.ACA.ATG.GTC.CCG.T-3'. For assemblage B, a 143 bp fragment was amplified with the forward primer GiaBBF 5'-GAT.ATT.GGC.GTC.GGC.GG-3' and the reverse primer GiaBBR 5'-AGC.TCC.ATA.CCC.TGT.GGC.CT-3'. Reactions were performed using SYBER[®] Green Master Mix (Applied Biosystems[®]), with 5 µL of template DNA to a final volume of 25 µL. Samples were amplified in One Step Plus – Real Time PCR System (Applied Biosystems[™]), using pre-established conditions for SYBER[®] Green Reagent, fast run (40 minutes), followed by analysis of melting curves. In order to determine the specificity of real-time PCR reactions in detecting *G. intestinalis* A and B, genomic DNA (gDNA) from other microorganisms was used as negative controls, including *Cryptosporidium parvum*, *Giardia muris*, *Escherichia coli* and *Ascaris suum*. The detection ability of primers at different DNA concentrations (sensitivity) was assessed by submitting to real-time PCR reactions serial 10-fold dilutions of each target fragment containing the sequences of specific sizes used in the assays. The whole validation procedure was performed in accordance with the Minimum Information Guide for Quantitative PCR Publication – MIQE (Bustin et al. 2009).

RESULTS

Giardia were detected in 83.3% (40/48) of the samples from the catchment point before the DWTP, with concentrations ranging from <0.1 to 8.6 cysts/L (Table S1), while for *Cryptosporidium* it was 37.5% (18/48) with concentrations from <0.1 to 2 oocysts/L (Table S2). (Tables S1 and S2 are available with the online version of this paper.)

Giardia and *Cryptosporidium* concentration data were adjusted. For both parasites the log-normal distribution yielded the best fitting, with mean and standard deviation (SD) of 1.05 and 2.2 cysts/L for *Giardia* and 0.15 and 0.33 oocyst/L for *Cryptosporidium*.

The distribution of the estimated annual risk of infection (mean, median and 95-percentile) by direct ingestion of treated water in adults (over 21 years old) and children (under 5 years old) is shown in Table 1.

Results show that annual risk values for adults were lower by around 1 log for *Cryptosporidium* when

compared with *Giardia* (Table 1). Figure 1 presents the sensitivity analysis of simulated parameters for annual risk for both parasites, in which parasite concentration and removal efficiency play the main role in risk computation.

Giardia intestinalis genotype A was detected in 33.4% (16/48) and genotype B in 16.6% (8/48) of all the samples analyzed; both were detected in the same sample on three occasions, and, when combined, were observed in almost half of the samples (21/48) (Table S1). Other genotypes besides A and B could be present but the focus of this study was on the *G. intestinalis* genotypes A and B.

Regarding *Cryptosporidium*, out of 48 water samples analyzed by nPCR, the parasite was detected in 37.5% (18/48) (Table S2), in which it can be observed that *C. hominis* was identified in four samples and *C. parvum* in five samples, but at different times. All these sequences were deposited in GenBank under Accession Numbers KX151732 and KX151740.

Table 1 | Mean, median and 95th percentile of annual risk of infection pppy (per person per year) by *Giardia* and *Cryptosporidium* in adults (over 21 years old) and children (under 5 years old) by direct ingestion of treated water, 2013–2014

Annual risk of infection (P_a)	Adults			Children		
	Mean	Median	95th percentile	Mean	Median	95th percentile
<i>Giardia</i>	1.3×10^{-2}	1.3×10^{-2}	1.9×10^{-2}	4.1×10^{-3}	3.6×10^{-3}	7.5×10^{-3}
<i>Cryptosporidium</i>	2.2×10^{-3}	2.0×10^{-3}	3.6×10^{-3}	6.3×10^{-4}	5.3×10^{-4}	1.2×10^{-3}

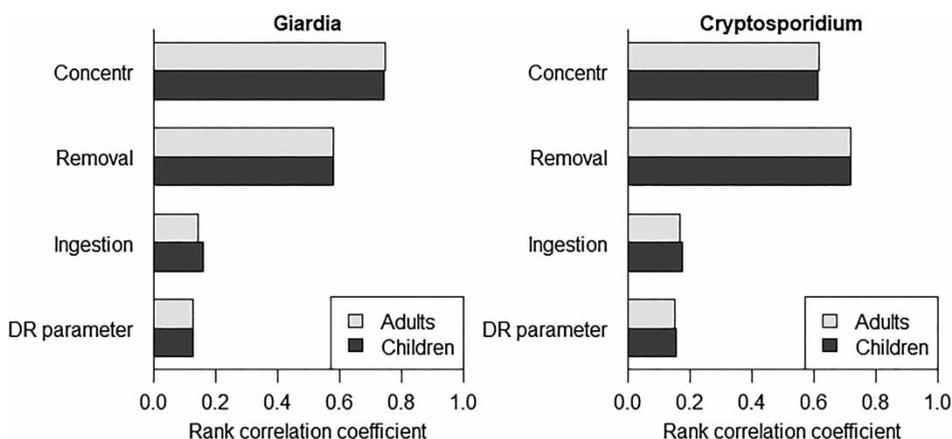


Figure 1 | Sensitivity analysis of the simulated parameters for annual risk of infection by *Giardia* and *Cryptosporidium* due to ingestion of drinking water.

DISCUSSION

The presence and concentration of (oo)cysts of *Giardia* and *Cryptosporidium* at the intake point of a DWTP reinforces the data published by other studies conducted in urban streams in Brazil (Fernandes *et al.* 2011; Sato *et al.* 2013) and sets a worrying scenario, which represents a health risk to the exposed population.

It is interesting to note that, throughout the year, parasites were found frequently. A plausible explanation is the fact that sources of contamination were identified during the collection campaigns as launching sewage from a state penitentiary and from an irregular settlement established along the mainstream of the river. The samples of water collected at this period presented high values of turbidity (varying from 6 to 505 uT) indicating high organic load, probably due to the rough treatment given to the sewage generated by the state penitentiary, which consists of grating, decantation and flotation. It is often verified that there are leakages of raw sewage towards to the river, impacting the water quality and consequently the DWTP operation, which is about one mile away. At the beginning of September 2013, there was observed to be an overflow of the sewage treatment plant (STP) of the penitentiary, leading to an increase in the use of chemical products by the DWTP, which was operated in contingency during this period, even paralyzing its operation for several hours. The vulnerability of treatment plants was pointed out by Burnet *et al.* (2014), when there are peaks of contamination in water supply.

Regarding the annual risk estimates, the values found were high (Table 1) and the sensitivity analysis (Figure 1) highlights the urgency for addressing strategies for source water protection and set targets for water treatment. The need is evident for a water safety plan (WSP) implementation with a multiple barriers approach to prevent, reduce, eliminate or minimize water contamination. Thinking from the perspective of the WSP it is necessary to improve the epidemiological surveillance of waterborne diseases in Brazil that would permit the establishment of tolerable risk values for setting up priorities to improve the quality of water catchment sources and to improve health protection.

The removal of *Giardia* and *Cryptosporidium* in conventional water treatment may be insufficient to ensure infection risk reduction by these pathogens, and the need is evident to prevent the degradation of water sources and to establish a risk management process for providing more effective sanitary barriers, from the catchment to the tap. It is crucial that there is integrated management and collaboration among the multiple agencies that have responsibility for specific aspects of the water cycle, such as the environmental agency and the surveillance institutions, advancing beyond potability and the operational characteristics of the supply system.

The lack of an established tolerable annual risk value for Brazil leads us to use the USEPA's values for comparison. Comparing with USEPA's annual tolerable risk (1×10^{-4}) the values found in this study are higher by approximately 1 log unit.

Regarding the impact on the population's health, if it is assumed that the frequency of disease followed by infection is in the range of 50% to 67% for *Giardia* and 40% to 70% for *Cryptosporidium* (Haas *et al.* 2014), we would have, respectively, about six and two cases of giardiasis and cryptosporidiosis in 1,000 adults (over 21 years) per year. In children under 5 years, we would have, approximately, one case of giardiasis and cryptosporidiosis in 1,000 children each year.

Another aspect addressed in this study was the detection of the presence of genotype and species of protozoa in source water samples capable of causing ADD: in the case of *Giardia*, almost half of the samples (21/48) were characterized as *G. intestinalis* (genotypes A and B) (Table S1) and, in the case of *Cryptosporidium*, 18.75% (9/48) were successfully sequenced and characterized as *C. parvum* and *C. hominis* (Table S2). Previous studies detected genotypes of *G. intestinalis* and species of *Cryptosporidium*, such as Araújo *et al.* (2011), whose results showed the presence of *C. hominis*, *C. meleagridis* and *C. andersoni* in water sources; and Fernandes *et al.* (2011) that reported *Giardia intestinalis*, genotypes A and B, in several sources of water.

Also, the lack of a systematic epidemiological notification of the etiological agents of the ADD is a reality in Brazil, making it difficult to establish a direct relationship

between the presence of these pathogenic protozoa and the occurrence of ADD in the studied area.

The genotyping/speciation analysis brought important information that may indicate contamination of water sources with genotypes/species affecting human health. It is noteworthy that separate samples were collected for this analysis (one for Method 1623 and one for the molecular methods) and this may limit the link between the two types of data generated from these two separated samples, given the heterogeneity in the water column.

CONCLUSIONS

This study pointed out the human health concerns of *Giardia* and *Cryptosporidium* circulating in the drinking-water catchment. Besides that, the results obtained showed elevated risk infection and illness caused by these parasites through the ingestion of (oo)cysts from drinking water, extrapolating risk levels considered not tolerable by the USEPA. This scenario is of concern for sanitary, environmental and health authorities, both from the perspective of treatment and drinking-water supply, and also the degradation and contamination of the watershed, indicating that to set a tolerable risk it would be beneficial for policy makers to define priority strategies in order to protect water sources and furthermore human health.

ACKNOWLEDGEMENTS

We would like to thank FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo for the financial support and technical trainee scholarship (2012/12750-1).

REFERENCES

- Araújo, R. S., Dropa, M., Fernandes, L. N., Carvalho, T. T., Sato, M. I. Z., Soares, R. M., Matté, G. R. & Matté, M. H. 2011 Genotypic characterization of *Cryptosporidium hominis* from water samples in São Paulo, Brazil. *The American Journal of Tropical Medicine and Hygiene* **85** (5), 834–838.
- Burnet, J.-B., Penny, C., Ogorzaly, L. & Cauchie, H.-M. 2014 Spatial and temporal distribution of *Cryptosporidium* and *Giardia* in a drinking water resource: implications for monitoring and risk assessment. *Science of the Total Environment* **472**, 1023–1035.
- Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J. & Wittwer, C. T. 2009 The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry* **55** (4), 611–622.
- Companhia Ambiental do Estado de São Paulo – CETESB (Environmental Company of the State of São Paulo – CETESB) 2015 *Qualidade das águas Superficiais do Estado de São Paulo (Surface Water Quality for State of São Paulo)*. CETESB, São Paulo, Brazil.
- Coupe, S., Sarfati, C., Hamane, S. & Derouin, F. 2005 Detection of *Cryptosporidium* and identification to the species level by nested PCR and restriction fragment length polymorphism. *Journal of Clinical Microbiology* **43** (3), 1017–1023.
- Delignette-Muller, M. L. & Dutang, C. 2015 Fitdistrplus: an R package for fitting distributions. *Journal of Statistical Software* **64** (4), 1–34.
- Dupont, H. L., Chappell, C. L., Sterling, C. R., Okhuysen, P. C., Rose, J. B. & Jakubowski, W. 1995 The infectivity of *Cryptosporidium parvum* in healthy volunteers. *The New England Journal of Medicine* **332** (13), 855–859.
- Fernandes, L. N., de Souza, P. P., de Araújo, R. S., Razzolini, M. T. P., Soares, R. M., Sato, M. I. Z., Hachich, E. M., Cutolo, S. A., Matté, G. R. & Matté, M. H. 2011 Detection of assemblages A and B of *Giardia duodenalis* in water and sewage from São Paulo state, Brazil. *Journal of Water and Health* **9** (2), 361–367.
- Franco, R. M. B., Branco, N., Amaro, B. C. T., Neto, R. C. & Fiuza, V. R. S. 2016 *Cryptosporidium* species and *Giardia* genotypes detected in surface water supply of Campinas, Southeast Brazil, by molecular methods. *Journal of Veterinary Medicine and Research* **3** (3), 1053–1059.
- Govaerts, B., Beck, B., Lecoutre, E., le Bailly, C. & Eeckaut, P. V. 2005 From monitoring data to regional distributions: a practical methodology applied to water risk assessment. *Environmetrics: The Official Journal of the International Environmetrics Society* **16** (2), 109–127.
- Haas, C. N., Rose, J. B. & Gerba, C. P. 2014 *Quantitative Microbial Risk Assessment*, 2nd edn. Wiley, Hoboken, NJ, USA.
- IBGE (Brazilian Institute of Geography and Statistics) 2008 Estimativas populacionais das medianas de altura e peso de crianças, adolescentes e adultos, por sexo, situação do domicílio e idade – Brasil e grandes regiões (Population estimates of median of height and weight of children, adolescents and adults, by sex, household condition and age – Brazil and major regions). <https://sidra.ibge.gov.br/tabela/2645>.
- Kahn, H. D. & Stralka, K. 2009 Estimated daily average per capita water ingestion by child and adult age categories based on USDA's 1994–1996 and 1998 continuing survey of food intakes by individuals. *Journal of Exposure Science and Environmental Epidemiology* **19** (4), 396–404.
- Ministério das Cidades (Ministry of Cities) 2016 *Sistema Nacional de Informações Sobre Saneamento: Diagnóstico dos Serviços de*

- água e Esgotos – 2014 (National Information System on Sanitation: Diagnosis of Water and Sewage Services – 2014). Brasília, Brazil.
- Moreira, N. A. & Bondelind, M. 2017 Safe drinking water and waterborne outbreaks. *Journal of Water and Health* **15** (1), 83–96.
- Nieminski, E. C. & Ongerth, J. E. 1995 Removing *Cryptosporidium* and *Giardia* by conventional treatment and direct filtration. *Journal – American Water Works Association* **87** (9), 96–106.
- Ramo, A., Del Cacho, E., Sánchez-Acedo, C. & Quílez, J. 2017 Occurrence of *Cryptosporidium* and *Giardia* in raw and finished drinking water in north-eastern Spain. *Science of the Total Environment* **580**, 1007–1013.
- R Core Team 2016 *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. <https://www.R-project.org> (accessed 4 March 2015).
- Robertson, L. J., Hermansen, L., Gjerde, B. K., Strand, E., Alvsvåg, J. O. & Langeland, N. 2006 Application of genotyping during an extensive outbreak of waterborne giardiasis in Bergen, Norway, during autumn and winter 2004. *Applied and Environmental Microbiology* **72** (3), 2212–2217.
- Rosado-García, F. M., Guerrero-Flórez, M., Karanis, G., Hinojosa, M. D. C. & Karanis, P. 2017 Water-borne protozoa parasites: the Latin American perspective. *International Journal of Hygiene and Environmental Health* **220** (5), 783–798.
- Rose, J. B., Haas, C. N. & Regli, S. 1991 Risk assessment and control of waterborne giardiasis. *American Journal of Public Health* **81** (6), 709–713.
- Sato, M. I. Z., Galvani, A. T., Padula, J. A., Nardocci, A. C., Lauretto, M. d. S., Razzolini, M. T. P. & Hachich, E. M. 2013 Assessing the infection risk of *Giardia* and *Cryptosporidium* in public drinking water delivered by surface water systems in Sao Paulo State, Brazil. *Science of the Total Environment* **442**, 389–396.
- Ulloa-Stanojlović, F. M., Aguiar, B., Jara, L. M., Sato, M. I. Z., Guerrero, J. A., Hachich, E., Matté, G. R., Dropa, M., Matté, M. H. & de Araújo, R. S. 2016 Occurrence of *Giardia intestinalis* and *Cryptosporidium* sp. in wastewater samples from São Paulo State, Brazil and Lima, Peru. *Environmental Science and Pollution Research* **23** (21), 22197–22205.
- USEPA 2012 *Method 1623.1: Cryptosporidium and Giardia in Water by Filtration/IMS/FA*. EPA 816-R-12, United States Environmental Protection Agency, Office of Water, Cincinnati, OH, USA. <http://www.epa.gov/safewater> (accessed 1 January 2012).
- WHO 2017 *Guidelines for Drinking-Water Quality*, 4th edn incorporating the 1st addendum. World Health Organization, Geneva, Switzerland.
- Widerström, M., Schönning, C., Lilja, M., Lebbad, M., Ljung, T., Allestam, G., Ferm, M., Björkholm, B., Hansen, A., Hiltula, J., Långmark, J., Löfdahl, M., Omberg, M., Reuterwall, C., Samuelsson, E., Widgren, K., Wallensten, A. & Lindh, J. 2014 Large outbreak of *Cryptosporidium hominis* infection transmitted through the public water supply, Sweden. *Emerging Infectious Diseases* **20** (4), 581–589.
- Xiao, L., Alderisio, K., Limor, J., Royer, M. & Lal, A. A. 2000 Identification of species and sources of *Cryptosporidium* oocysts in storm waters with a small-subunit rRNA-based diagnostic and genotyping tool. *Applied and Environmental Microbiology* **66** (12), 5492–5498.

First received 4 October 2018; accepted in revised form 19 March 2019. Available online 4 April 2019