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Cryptosporidium in small water systems in Puerto Rico: a pilot study

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ABSTRACT

A pilot study was undertaken to investigate the occurrence of *Cryptosporidium* in four very small drinking water systems supplying communities in rural Puerto Rico. Water samples (40 L) were collected and oocysts were concentrated by calcium carbonate flocculation, recovered by immunomagnetic separation and detected by immunofluorescence microscopy. *Cryptosporidium* oocysts were identified in all four systems. This is the first report of evidence of the potential public health risk from this chlorine-resistant pathogen in Puerto Rican small water systems. Further work is warranted to fully assess the health risks that *Cryptosporidium* and other protozoa pose to populations served by community-managed small drinking water systems.

Key words | *Cryptosporidium*, calcium carbonate flocculation, Puerto Rico, small water systems

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INTRODUCTION

The protozoan *Cryptosporidium* is a well-documented cause of waterborne gastroenteritis and outbreaks (Baldursson & Karanis 2011). In developed countries, risks from *Cryptosporidium* and other waterborne pathogens in large water systems managed and operated by water authorities is lower than in independent supplies, where management of water quality is poor or non-existent (Hunter *et al.* 2010; Hunter *et al.* 2011). More than 150 million people in high-income countries, mainly in rural areas and already possibly disadvantaged by geographical and economic isolation, are at increased risk of exposure to waterborne pathogens because of the lower regulatory standards applied (Bridge *et al.* 2010). Human cryptosporidiosis has been reported worldwide and is endemic in tropical countries (Fayer 2008). In Latin America and the Caribbean, the prevalence range of cryptosporidiosis has been reported as 4–60% (Lindo *et al.* 1998; Raccurt *et al.* 2008). In Puerto Rico, the prevalence of infection and incidence of disease remains unknown, despite a *Cryptosporidium hominis* outbreak in 2007 with 107 laboratory-confirmed cases linked to a recreational water park (Hlavsa *et al.* 2011). There are no data on *Cryptosporidium* occurrence in small water systems on the island.

Puerto Rico is politically and economically linked to the United States and as such the majority of water supplies are of high quality due to management by the island's water and wastewater authority (PRASA) within the United States Environmental Protection Agency water treatment rules. At least 200 of the 370 recorded small water supplies (serving <3,300 persons) are operated by local communities. Treatment, when present, is limited to (often inconsistent) chlorine disinfection (Minnigh & Ramírez Toro 2004). A positive impact of educational intervention, leading to improved water management and chlorine treatment operation, has

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been demonstrated by reduction in diarrhoeal disease (Hunter *et al.* 2010) but residual risk may remain from chlorine-resistant pathogens like *Cryptosporidium*.

There are several standard methods for the detection of *Cryptosporidium* in water samples, and selection depends on the type of water and resources available. As cartridge filters are very expensive, a cheaper alternative for a proof-of-concept study is calcium carbonate flocculation (Vesey *et al.* 1993), incorporated in standard methods ISO 15553 (Anon 2006) and 'The Microbiology of Drinking Water – Part 14′ (Anon 2010).

To investigate the occurrence of *Cryptosporidium* in small water systems in Puerto Rico, we undertook a minimally-funded proof-of-concept sample survey.

METHODS

Study sites and sampling

Four very small water systems (A–D) within the municipality of Patillas, south-east Puerto Rico, were selected (Table 1 and Figure 1).

A total of 36 raw water samples (40 L, collected in 4 × 10 L carboys) were taken at the point of intake to the water system during different weeks between September 2009 and December 2010 and transported to the laboratory in San Germán (approximately 130 km) at ambient temperature.

Recovery and detection of *Cryptosporidium*

Calcium carbonate flocculation, recovery and detection of *Cryptosporidium* were undertaken as described in Anon (2010). Flocs were allowed to settle overnight before dissolving, combining and concentrating further by centrifugation. *Cryptosporidium* oocysts were recovered by immunomagnetic separation (IMS) (Dynal, Life Technologies, Foster City, CA, USA) and 50% was deposited onto single microscopy slides with 9-mm wells (Life Technologies, Foster City, CA, USA). The remaining 50% was saved for attempted molecular characterisation. *Cryptosporidium* oocysts were stained with a fluorescein isothiocyanate (FITC)-labelled antibody (Crypto-Cel, Cellabs, Brookvale, Australia) and sporozoite nuclei were stained with 4′,6′-diamidino-2-phenylindole (DAPI) (Sigma, Ronkonkoma, NY, USA) (Anon 2010). Slides were examined using an epifluorescence microscope and oocysts showing typical, confirmatory features (size, internal contents including up to four sporozoites) were enumerated as oocysts per litre.

Recovery from matrix-spiked samples was monitored: an additional 10 L carboy of water from each of Systems A–C and one from each storage tank at System D was seeded

Table 1 | Characteristics of small water systems studied

Attribute	System A	System B	System C	System D
Households	200	175	250	75
Population	800	700	1,000	300
Landscape	Forested, mountains	Forested, mountains	Forested, mountains	Forested, mountains
Upper watershed land use	Fishing and hunting; pasture; wildlife	Some residences; wildlife	No housing; wildlife	Cattle; wildlife
Source water	Surface	Ground and surface	Surface	Surface
Storage tank(s) (litres)	113,500	378,500 and 170,500	227,000	113,500 and 113,500
Distribution piping	4′ cast and ductile iron	Mostly 4′ ductile iron; some 2–4' PVC	Mostly 2–4′ PVC	Mostly 2–4′ PVC; some 4–6′ cast and ductile iron
Treatment	Chlorine	Chlorine	Chlorine	Chlorine
Trained operators[a]	CAP (enhanced)	CAP (enhanced)	40 hours provided by local government	CAP

[a]CAP – Part of the Cooperativa de Acueductos de Patillas (Hunter *et al.* 2010) providing volunteer operator training in basic sciences, mathematics and water treatment operation. CAP (enhanced) – providing about 1,200 hours training, including disinfection and continued support.

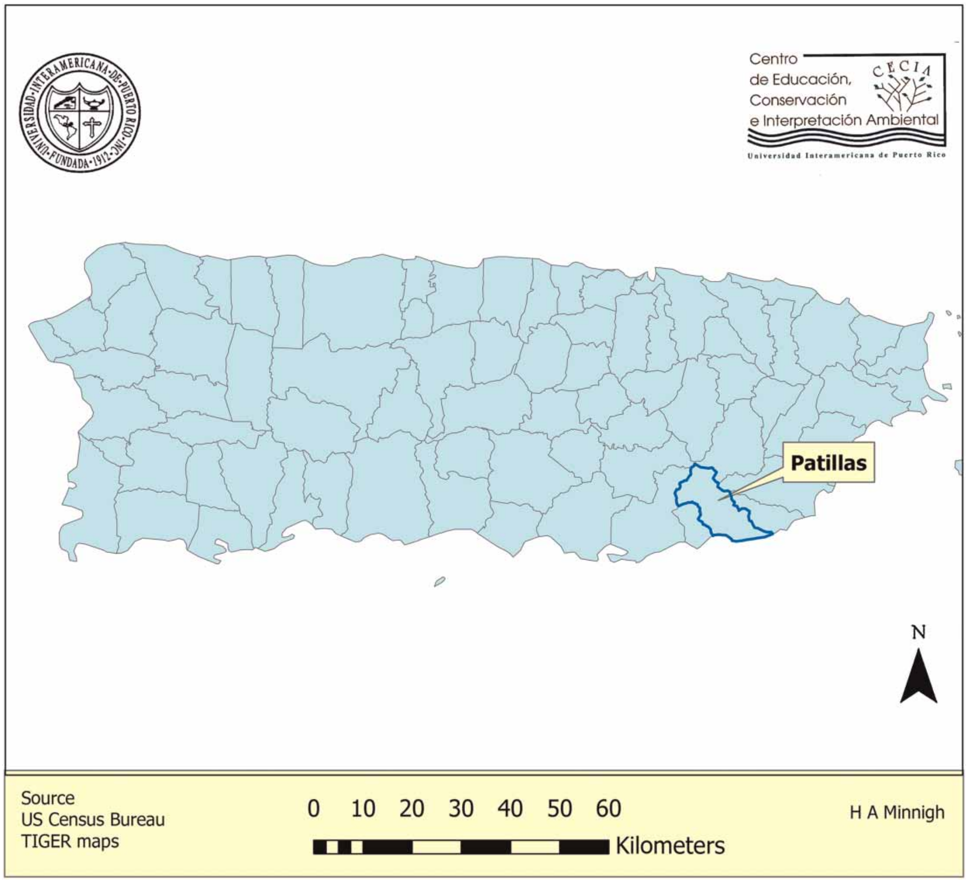

Figure 1 | Map of Puerto Rico showing the location of the municipality of Patillas (shaded area) (http://commons.wikimedia.org/wiki/File:Locator_map_Puerto_Rico_Patillas.png#).

with a mean of 286 (SD 69) oocysts (EasyPC, BTF, Sydney, Australia), accurately enumerated by 10 counts, and the recovery rate calculated following processing as described above. Although not approved for seeding water samples in regulatory laboratories, the EasyPC oocyst suspension was used due to cost restrictions in this minimally-funded pilot study. To check the concentration of the suspension remained stable, the EasyPC suspension was counted prior to each spike.

Cryptosporidium DNA was extracted and attempts made to characterise the species at the SSU rRNA gene, as described previously (Chalmers *et al.* 2010; Robinson *et al.* 2011).

Statistics

The null hypothesis that median of counts of *Cryptosporidium* did not differ between water systems was tested using the Kruskal–Wallis test for independent samples, using SPSS version 18.

RESULTS

The mean *Cryptosporidium* oocyst recovery from five matrix spikes was 168 (59%, range 118–202, SD 35). All systems

Table 2 | Frequency of detection and concentration of *Cryptosporidium* oocysts in samples from four small water systems in Puerto Rico

Number of oocysts seen in 50% of the IMS concentrate[a] (oocysts/litre)	System A ($N = 11$)	System B ($N = 10$)	System C ($N = 6$)	System D ($N = 9$)
0 (<0.05)	8	7	1	8
1 (0.05)	2	2	1	0
2 (0.10)	1	1	2	1
3 (0.15)	0	0	0	0
4 (0.20)	0	0	2	0
Total number of positive samples	3	3	5	1

[a]Equivalent to 20 L sample.

were positive for *Cryptosporidium* on at least one occasion (Table 2). Overall sample positivity was 12/36 (33%) with oocysts detected more often in System C (5/6 samples, 83%) than in System A (3/11, 27%), B (3/10, 30%) or D (1/9, 11%). The sample distribution of oocyst counts differed significantly between water systems ($p = 0.006$) with System C also yielding the highest median and range of counts. Although *Cryptosporidium* oocysts were confirmed using specific antibodies and identification of typical internal structures, characterisation of species by polymerase chain reaction (PCR) was not successful.

DISCUSSION

We undertook a pilot study to investigate whether small water sources in Puerto Rico are contaminated with *Cryptosporidium*. Acceptable recovery of *Cryptosporidium* oocysts by calcium carbonate flocculation and IMS was demonstrated from matrix spikes (mean 59%), comparable with other studies (69–79%, Vesey *et al.* (1993) and 50.0–61.8%, Stanfield *et al.* (2000)), and within the 13–111% acceptable range for regulatory water monitoring laboratories (Anon 2005). Although the duration of the study was short, trends in oocyst detection were observed; all systems were vulnerable to contamination with *Cryptosporidium*, and one system (C) significantly more so than the others. This was the first investigation of the occurrence of *Cryptosporidium* in small water systems in Puerto Rico. However, data from the USEPA's Long-term Stage 2 Enhanced Surface Water Treatment Rule (LT2), which monitors water sources used to supply populations of >10,000, show 402/1,594 (25%) of samples (8.8–11 L) taken between October 2008 and February 2012 were positive for *Cryptosporidium* (http://water.epa.gov/lawsregs/rulesregs/sdwa/lt2/upload/cryptodatareported.csv). This is higher than that reported nationally for the USA (7% positivity) (Ongerth 2013), but lower than the occurrence found in our study of rural small water systems (33%), showing that small water systems are contaminated more frequently. Of the positive LT2 samples, the vast majority contained single oocysts from approximately 10 L of water, similar to the positive detections in our study (Table 2). However, the frequency of detections in small water systems which, unlike the large systems, do not have appropriate treatment, is of concern.

Although the operation and monitoring of municipal systems by PRASA ensures that water supplied to the majority of the inhabitants is of high quality, many community-operated water systems are not well monitored, serve very poor communities and are structurally prone to environmental contamination. The potential for poor quality water puts the local communities at risk from diarrhoeal illness. Improved education on system management and operation has demonstrated a positive and sustainable health benefit in these distributions, but the risk from protozoan parasites was not previously known and may account for residual diarrhoea burden (Hunter *et al.* 2010). Detection of *Cryptosporidium* in all four systems in this study suggests a potential health risk, especially where operator training is minimal such as at System C.

Unfortunately, we were unable to identify the *Cryptosporidium* species present, which would have improved our assessment of potential public health risk. This was most likely due to the small numbers of oocysts in each subsample. Amplification of less than a third (31.7%) of samples containing one or two oocysts using the same PCR has been reported (Nichols *et al.* 2010). Nevertheless, the potential for *Cryptosporidium* to enter these water systems is apparent. Wild animals and livestock are found upstream and human infection with *Cryptosporidium* species from these hosts has been reported in other Latin American and Caribbean countries (Raccurt *et al.* 2006; Bushen *et al.* 2007; Cama *et al.* 2008). Detection of a human-specific *Bacteroides* 16S rRNA marker in System D suggests sewage contamination also occurs (Ryan 2012). Sewage from the households within each system remains on the watershed in onsite structures made of cement blocks, which are generally porous but are not septic systems.

The presence of *Cryptosporidium* in drinking water is of concern, especially the potential risk to the health of young children who are at most risk of increased fatality, severe disease and sequelae (Hunter *et al.* 2011).

CONCLUSIONS

This study confirms for the first time the presence of *Cryptosporidium* in small water systems in Puerto Rico and potential risk to public health from this chlorine-resistant pathogen. While educational intervention in communities operating and managing their own supplies is likely to have a health benefit, studies are warranted to properly assess the extent and sources of contamination, the exposure and health risks in the communities supplied and appropriate interventions.

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REFERENCES

Anon 2005 *Method 1623: Cryptosporidium and Giardia in Water by Filtration/IMS/FA*. United States Environmental Protection Agency Office of Water, USA. http://www.epa.gov/nerlcwww/1623de05.pdf.

Anon 2006 *ISO 15553: Water quality – Isolation and enumeration of Cryptosporidium oocysts and Giardia cysts from water*. International Organization for Standardization, Geneva, Switzerland.

Anon 2010 *The Microbiology of Drinking Water (2010) - Part 14 - Methods for the isolation, identification and enumeration of Cryptosporidium oocysts and Giardia cysts*. Environment Agency, Bristol. http://www.environment-agency.gov.uk/static/documents/Research/Part_14-oct20-234.pdf.

Baldersson, S. & Karanis, P. 2011 Waterborne transmission of protozoan parasites: review of worldwide outbreaks – an update 2004–2010. *Water Res.* **45**, 6603–6614.

Bridge, J. W., Oliver, D. M., Chadwick, D., Godfray, H. C., Heathwaite, A. L., Kay, D., Maheswaran, R., McGonigle, D. F., Nichols, G., Pickup, R., Porter, J., Wastling, J. & Banwart, S. A. 2010 Engaging with the water sector for public health benefits: waterborne pathogens and diseases in developed countries. *Bull. World Health Organ.* **88**, 873–875.

Bushen, O. Y., Kohli, A., Pinkerton, R. C., Dupnik, K., Newman, R. D., Sears, C. L., Fayer, R., Lima, A. A. M. & Guerrant, R. L. 2007 Heavy cryptosporidial infections in children in northeast Brazil: comparison of *Cryptosporidium hominis* and *Cryptosporidium parvum*. *Trans. R. Soc. Trop. Med. Hyg.* **101**, 378–384.

Cama, V. A., Bern, C., Roberts, J., Cabrera, L., Sterling, C. R., Ortega, Y., Gilman, R. H. & Xiao, L. 2008 *Cryptosporidium* species and subtypes and clinical manifestations in children, Peru. *Emerg. Infect. Dis.* **14**, 1567–1574.

Chalmers, R. M., Robinson, G., Elwin, K., Hadfield, S. J., Thomas, E., Watkins, J., Casemore, D. & Kay, D. 2010 Detection of *Cryptosporidium* species and sources of contamination with *Cryptosporidium hominis* during a waterborne outbreak in north west Wales. *J. Water Health* **8**, 311–325.

Fayer, R. 2008 General Biology. In: *Cryptosporidium and cryptosporidiosis*, 2nd edn (R. Fayer & L. Xiao, eds). CRC Press, Boca Raton, USA, pp. 1–42.

Hlavsa, M. C., Roberts, V. A., Anderson, A. R., Hill, V. R., Kahler, A. M., Orr, M., Garrison, L. E., Hicks, L. A., Newton, A., Hilborn, E. D., Wade, T. J., Beach, M. J. & Yoder, J. S. 2011 Surveillance for waterborne disease outbreaks and other health events associated with recreational water – United States, 2007–2008. *MMWR Surveill. Summ.* **60**, 1–32.

Hunter, P. R., Ramírez Toro, G. I. & Minnigh, H. A. 2010 Impact on diarrhoeal illness of a community educational intervention to improve drinking water quality in rural communities in Puerto Rico. *BMC Public Health* **10**, 219.

Hunter, P. R., de Sylor, M. A., Risebro, H. L., Nichols, G. L., Kay, D. & Hartemann, P. 2011 Quantitative microbial risk assessment of cryptosporidiosis and giardiasis from very small private water supplies. *Risk Anal.* **31**, 228–236.

Lindo, J. F., Levy, V. A., Baum, M. K. & Palmer, C. J. 1998 Epidemiology of giardiasis and cryptosporidiosis in Jamaica. *Am. J. Trop. Med. Hyg.* **59**, 717–721.

Minnigh, H. A & Ramírez Toro, G. I. 2004 *Regulation and Financing of Potable Water Systems in Puerto Rico, A Study in Failure in Governance*. A report of the American Water Resources Association and International Water Law Research Institute/ University of Dundee International Speciality Conference *Good Water Governance for People and Nature: What Roles for Law, Institutions, Science and Finance*, Dundee, Scotland, UK.

Nichols, R. A. B., Connelly, L., Sullivan, C. B. & Smith, H. V. 2010 Identification of *Cryptosporidium* species and genotypes in Scottish raw and drinking waters during a one-year monitoring period. *Appl. Environ. Microbiol.* **76**, 5977–5986.

Ongerth, J. E. 2013 LT2 *Cryptosporidium* data: what do they tell us about *Cryptosporidium* in surface water in the United States? *Environ. Sci. Technol.* **47**, 4029–4038.

Raccurt, C. P., Brasseur, P., Verdier, R. I., Li, X., Eyma, E., Stockman, C. P., Agnamey, P., Guyot, K., Totet, A., Liautaud, B., Nevez, G., Dei-Cas, E. & Pape, J. W. 2006 Human cryptosporidiosis and *Cryptosporidium* spp. *in Haiti*. Trop. *Med. Int. Health* **11**, 929–934.

Raccurt, C. P., Fouche, B., Agnamey, P., Menotti, J., Chouaki, T., Totet, A. & Pape, J. W. 2008 Presence of *Enterocytozoon bieneusi* associated with intestinal coccidia in patients with chronic diarrhea visiting an HIV center in Haiti. *Am. J. Trop. Med. Hyg.* **79**, 579–580.

Robinson, G., Chalmers, R. M., Stapleton, C., Palmer, S. R., Watkins, J., Francis, C. & Kay, D. 2011 A whole water catchment approach to investigating the origin and distribution of *Cryptosporidium* species. *J. Appl. Microbiol.* **111**, 717–730.

Ryan, M. O. 2012 Microbial source tracking of human and animal waste pollution of diverse watersheds and of urban drainage systems using molecular methods, PhD thesis, Drexel University, Philadelphia. https://idea.library.drexel.edu/islandora/object/idea%3A3994.

Stanfield, G., Carrington, E., Albinet, F., Compagnon, B., Dumoutier, N., Hambsch, B., Lorthioy, A., Medema, G., Pezoldt, H., de Roubin, M. R., de Lohman, A. & Whitmore, T. 2000 An optimised and standardised test to determine the presence of the protozoa *Cryptosporidium* and *Giardia* in water. *Water Sci. Technol.* **41**, 103–110.

Vesey, G., Slade, J. S., Byrne, M., Shepherd, K. & Fricker, C. R. 1993 A new method for the concentration of *Cryptosporidium* oocysts from water. *J. Appl. Bacteriol.* **75**, 82–86.

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