Giardia and Cryptosporidium in Pristine Protected Catchments in Central Eastern Australia

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Giardia and Cryptosporidium in Pristine Protected Catchments in Central Eastern Australia

We report the first confirmed records of the pathogenic protozoa Giardia lamblia and Cryptosporidium parvum from small remote streams in pristine protected areas in Australia, beyond the reach of urban or agricultural contamination.

INTRODUCTION

Giardia lamblia and Cryptosporidium parvum are unicellular protozoa which can survive for an extended period outside their animal hosts, as cysts or oocysts, respectively, in soils or water (1). They are a significant cause of severe gastrointestinal disease; are widespread in developing nations where watercourses commonly suffer human faecal contamination; and are a major cause of travellers’ diarrhoea (2). They are also widespread in the developed nations of Europe, North America and South Africa, even in small streams in protected catchments (3–10).

Their dispersal mechanism has been controversial. In North America it was ascribed initially to native mammals; later, to humans (11–13). It remains unresolved, because the organisms were already widespread in that continent long before techniques were developed to detect their (oo)cysts in waterbodies, at concentrations around 10⁻² g⁻¹, rather than in animal faeces at around 10⁸ g⁻¹ (1, 3–10).

Australia provides a unique opportunity to examine their distribution and dispersal. It is remote from other continents, and has a relatively high standard of public health and effective protection of drinking-water catchments. In Australia, Giardia and Cryptosporidium are widespread in watercourses subject to urban or agricultural contamination (14). Clinical cases of giardiasis or cryptosporidiosis have been recorded for many decades (1), but generally in individuals who have travelled overseas. There have been very few outbreaks of giardiasis or cryptosporidiosis in Australia, all associated with young children and their parents and carers, and all ascribed to direct faecal-oral or localized groundwater transmission (15–17). During 1998, the municipal water supply for Sydney, Australia’s largest city, was threatened by Cryptosporidium contamination, forcing over 3 million people, a sixth of the nation’s population, to boil drinking water for several weeks (18).

Unlike in North America and New Zealand, where remote areas have been screened for the presence of (oo)cysts (12, 19), the distribution of Giardia and Cryptosporidium in undisturbed water catchments in Australia remains unknown. Whilst human-infective Giardia cysts have been isolated from native fauna in Australia (20), there have been no rigorous analyses for presence or absence of Giardia or Cryptosporidium (oo)cysts in pristine watercourses. Relatively few Australian watercourses are completely free from human contamination (21). Here, we identified a set of rivers whose entire catchments are included in longstanding national parks upstream of all human infrastructure and with no feral stock; and sampled a series of tributary streams, successively smaller and closer to springs and sources, and with fewer opportunities for potential contamination even by occasional backcountry hikers.

MATERIALS AND METHODS

We sampled 10 rivers in 7 national parks in the Central Eastern Rainforest Reserve Australia World Heritage Area (CERRA WHA), between 28° 17’ 00” and 29° 31’ 17” S and 152° 18’ 42” and 153° 43’ 36” E. Samples were taken between January and May 1998. Stream characteristics were as follows: flow velocity 0.5–2.3 m s⁻¹, turbidity 1–2 NTU, mean temperature 18.5°C, pH 5.5–8.0, mean PO₄ 0.034 mg L⁻¹, NO₂ + NO₃ 0.12 mg L⁻¹, NH₄ 0.005 mg L⁻¹.

Each watercourse was sampled by direct micropore filtration of 100–140 L on site, using specially-designed portable battery-powered apparatus carried on foot. We used 2 different filtration techniques. In the first, we pumped 100–140 L samples, in 20-L subsamples, through separate flat circular Sartorius® nitrocellulose membrane filters of 3 µm pore size and 142 mm diameter; a total filter area of 100 x 10⁵ mm². The filters were preceded by 10 µm Gelman® prefilters. In the second, we filtered 140 L samples through Gelman® Envirocheck® folded-cartridge polyethersulfone membranes of 1 µm pore size, with an effective filtration area of 130 x 10⁵ mm². Up to 3 cartridges were needed for each sample depending on stream turbidity. All filters and prefilters were kept on ice after use, and treated within 72 hrs. Giardia cysts are oval, 8–12 µm long and 7–10 µm wide. Cryptosporidium oocysts are spherical, 4–6 µm in diameter. The 1 µm Envirocheck® cartridges trap both. The 3 µm flat filters trap Giardia cysts and also trap most Cryptosporidium oocysts. A few of the smaller Cryptosporidium oocysts may pass through the 3 µm flat filter. All sites were therefore also sampled using the Envirocheck® cartridges. Positive records of Cryptosporidium oocysts are of course equally valid for both filtration types.

Analytical protocols were based on an amalgam of methods from previous published studies, as below, and coincide closely with US EPA Standard Method 1622 (5, 22–26), which was adopted shortly after our samples were analyzed. Flat filters and prefilters were scraped and washed 3–4 times with 1% polyoxymethylene sorbitan monooleate (Sigma-Aldrich® Tween 80®). Cylindrical filters were shaken twice for 10 min in 240 ml Gelman® elution buffer (8 g NaCl, 0.2 g KCl, 0.2 g KH₂PO₄, 2.9 g hydrated Na₂HPO₄, 0.1 g sodium laurel sulfatate, 3 µl Tween 80, 150 µl Sigma® Antifoam B, 1.0 L deionized distilled water, adjusted to pH 7.4). All samples were centrifuged at 3000 x g for 10 min, resuspended and purified using a percoll-sucrose gradient. For purification, samples were centrifuged in 250 ml tubes at 3000 x g for 10 min using a refrigerated fixed-angule centrifuge; resuspended in phosphate buffered saline (PBS) at pH 7.4 with 0.1% dodecyl sulfate and 0.1% Tween 80; centrifuged down to 10 ml; resuspended in 5 ml PBS underlayered with percoll-sucrose (SG 1.5); and centrifuged at 1050 x g for 10 min in a swinging-bucket rotor. The upper aqueous layer and percoll-su-
Cryptosporidium parvum from surface waters in pristine pro-

csses. Results

40% of each 100 L sample was examined completely.

208 1.5 \mu g \text{ wet-weight from } 0.03–0.13 \text{ mg per } 100 \text{ L sample. A total of }

covery rates were 53 ± 5.5% for the flat filter technique, simi-

lar to those for previous studies; and 64 ± 4% for Gelman®

Envirocheck® cylindrical filters.

For each sample, successive 1.5 \mu g slide wells of the stained

purified pellet were examined, until either i) both \textit{Giardia} cysts

and Cryptosporidium oocysts were detected; ii) 20 wells were

counted; or iii) the entire pellet was used. Purified pellets ranged in

weight from 0.03–0.13 mg per 100 L sample. A total of

208 1.5 \mu g slide wells were counted (Table 1). On average, over

40% of each 100 L sample was examined completely.

RESULTS

\textit{Giardia} cysts and/or Cryptosporidium oocysts were found in

every stream, no matter how small, remote and pristine, and how

close to the source (Table 1). Overall mean densities were 3.4

\textit{Giardia} cysts and 1.8 Cryptosporidium oocysts per 100 L. Of

the 208 slide wells counted, 2 contained 2 \textit{Giardia} cysts, 15 con-
tained 1, and 191 contained none (Table 1). Similarly, 10 con-
tained 1 Cryptosporidium oocyst and 198 contained none. The

frequencies of cysts and oocysts are slightly higher in the na-
national parks of northernmost NSW (10/64, 4/64, respectively),

than in those of southeast Qld (6/84, 4/84) and mid-north

NSW (3/60, 1/60). The difference is significant at p < 0.05 for

\textit{Giardia} but not Cryptosporidium.

DISCUSSION

These are the first published records of \textit{Giardia lamblia} and

Cryptosporidium parvum from surface waters in pristine pro-
tected areas in Australia. Indeed, they are the first records from

such small protected watercourses worldwide. The closest

equivalent study in North America found that these protozoa

were not present in the headwaters of protected montane catch-

cments, though they were further downstream (3). Cyst concen-

trations detected in the CERRA WHA are as high as those in

protected areas in North America (3–10).

The watercourses tested here are as well or better protected

from contamination by \textit{Giardia} and Cryptosporidium as any oth-

ers worldwide, except perhaps for those in remote oceanic or

subantarctic islands. The Australian continent has historically

been free from many of the world’s otherwise widespread

waterborne pathogens (20); and within Australia, there are very

few water catchments which are equally undisturbed. Most pro-
tected areas either have roads and tracks, a history of logging,
grazing or horse pasture, or feral dogs, cats, pigs, cattle, goats,
horses, and donkeys, which routinely carry \textit{Giardia} and Crypto-
sporidium (27–31). Hence, the results reported here indicate that

\textit{Giardia} and Cryptosporidium are probably present in every suit-
able habitat worldwide.

The mechanism by which they have reached these protected

streams is likely to involve several factors. Whilst \textit{Giardia} cysts

and Cryptosporidium oocysts are robust and could potentially

be carried as high-altitude atmospheric particles, the concentra-
tions reported here are orders of magnitude too high for this to

be the only source, and indicate a contaminated faecal input di-
rectly within the catchments concerned. Humans are an unlikely

vector in this case, even though 1–3% of the human population

in this region carries \textit{Giardia} asymptotically (Wild, C. and

Vogt, S., unpubl. data), because the sampling sites were specifi-
cally selected to exclude them. The most likely mechanism is

that native marsupials or possibly feral cats, both of which can

carry \textit{Giardia} (11, 20), may have transmitted it from adjacent

catchments with domestic stock, or from areas contaminated by

humans further downstream in the same catchments. A wide va-

riety of small-to medium-sized marsupials and other mammals

are present in the study areas. Birds are another possible vector,

but avian isolates may not be cross-infective to mammals (32,

33).

An expert inquiry into the 1998 Sydney water contamination

event (18) identified several possible sources of Cryptospor-
idium, some pre- and some post-treatment. The results reported

here indicate that most or all of the catchments for municipal

water supply in Australia, and indeed worldwide, almost cer-
tainly contain \textit{Giardia} cysts and Cryptosporidium oocysts at low

concentrations. From a public health perspective the issue is now

one of risk management. Broadly, treatment systems with

\begin{table}
\centering
\begin{tabular}{|l|l|l|l|l|}
\hline
National Park & River & Number of Total & Total & Total \\
& & 1.5 \mu g wells & \textit{Giardia} cysts in & Cryptosporidium & \\
& & counted for & entire sample & oocysts in & \\
& & this sample & & entire sample & \\
\hline
Lamington & Canurgra & 8 & 1 & 2 & \\
Lamington & Canurgra & 16 & 1 & 0 & \\
Lamington & Canurgra & 16 & 1 & 0 & \\
Lamington & Canurgra & 12 & 1 & 0 & \\
Lamington & Parragulli & 16 & 1 & 1 & \\
Lamington & Nixor & 16 & 1 & 1 & \\
Border Ranges & Brindle & 8 & 4 & 1 & \\
Toonumbah & Iron Pot & 20 & 2 & 1 & \\
Mount Warning & Korumbryn & 16 & 3 & 1 & \\
Whian Whian & Minyon & 20 & 1 & 1 & \\
Gibraltar Range & Surveyor & 20 & 1 & 1 & \\
Gibraltar Range & Dandahra & 20 & 2 & 0 & \\
Washpool Coombadjha & 20 & 0 & 1 & \\
\hline
\end{tabular}
\caption{Abundance of \textit{Giardia} cysts and Cryptosporidium oocysts in water samples isolated from different watercourses at test sites.}
\end{table}
floculation or filtration steps remove protozoan cysts, whereas those with chlorination only do not (5–10).

Large holding dams with multi-day residence times may allow cysts to settle to bottom sediments; but these can be resuspended during storm floods, and there have been several major instances of cryptosporidiosis and associated gastrointestinal infection through ingesting water from lakes used for recreation (34, 35). The costs of retrofitting or replacing water treatment systems which do not currently remove protozoan cysts will actually occur in practice will depend on funding priorities by local authorities. Whether this actually occurs in practice will depend on funding priorities by public health and water-supply authorities.

Giardia and Cryptosporidium are already significant threats to human health worldwide (40), and likely to become much more so in future. Without basic information on distribution and concentration of both protozoa, public health responses are likely to be hampered and ineffective. Now that field sampling techniques for remote areas are available, it is feasible to compile for empirical data for Australian water catchments. Whether this technology is testable using the 4,6-diamino-2-phenylindole (DAPI) method, and reverse transcription-PCR respectively as applied by Johnson et al., and Mayer and Palmer, Stinear et al., and Kauffman and Stinear (36–39).

References


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