Practical Paper

The occurrence of *Cryptosporidium* and *Giardia* in the Lake Baroon catchment, Queensland, Australia

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ABSTRACT

We evaluated the occurrence of *Cryptosporidium* and *Giardia* in the Lake Baroon catchment, Queensland, Australia. A total of 132 water samples were collected from 6 sites as well as from raw influent and treated effluent from a local sewage treatment plant (STP), over a 12-month period. Three new sites close to the entrance of the lake were added following initial assessment. The prevalence of faecal indicator organisms, *E. coli* and enterococci was also examined. There was no clear relationship between the level of indicator organisms, rainfall or the detection of *Cryptosporidium* and *Giardia* for most sites. Of the five sites showing levels of contamination with *Cryptosporidium*, only three coincided with elevated rainfall. The highest level of *Cryptosporidium* oocysts detected in the raw influent from the STP, coincided with the occurrence of an outbreak of cryptosporidiosis in the local area but no *Cryptosporidium* was detected in surface water samples during that period. Our results indicate that while the human contribution of *Cryptosporidium* is minimal, the high level of faecal indicator bacteria in surface water was not always associated with the presence *Cryptosporidium* in this catchment.

Key words | catchment, *Cryptosporidium*, *Giardia*, health risk

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INTRODUCTION

Cryptosporidium and *Giardia* are single-celled parasites excreted in the faeces of infected humans and animals and cause protozoan diarrhoea. *Cryptosporidium* is potentially life threatening to immunocompromised individuals (Byleveld *et al.* 1999) and is responsible for many water-borne outbreaks in the United States (Craun *et al.* 1998), the United Kingdom (Craun *et al.* 1998) and other countries (Clancy & Hansen 1999). They cannot reproduce outside a host, but can survive in oocyst/cyst form in fresh water for up to six months (Thomas *et al.* 2000). Infection occurs as the result of consuming contaminated water, food or contact with contaminated faeces. Infected faeces can contain millions of oocysts/cysts per gram (Hayes *et al.* 1989).

Several studies have been conducted to detect these parasites in surface waters (Smith & Rose 1998). LeChevallier *et al.* (1991) showed contamination levels ranging from 0.07 to 484 oocysts l^{-1}. Water samples collected from rivers and creeks in rural Australia have shown contamination levels of 0.6 oocysts oocysts l^{-1} (Thurman *et al.* 1998). As drinking water supplies are most frequently obtained from surface and ground water, it is important to monitor any seasonal fluctuation of these parasites present in the catchment. This information assists water authorities to prepare treatment processes that are sufficient to remove or inactivate these parasites. Animal activities in a catchment should be considered as they pose a major threat to surface

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water quality (Graczyk *et al.* 2000; Bodley-Tickell *et al.* 2002).

Indicator organisms such as *E. coli* and enterococci, have been used for many years to determine water quality and may be present where there has been faecal contamination originating from warm-blooded animals (National Water Quality Management Strategy 2004). Water treatment procedures used routinely to inactivate these bacteria (e.g. chlorine) may not be sufficient to remove or inactivate *Cryptosporidium* and/or *Giardia* (Korich *et al.* 1990), which may be present in the absence of indicator organisms. Many methods to detect indicator organisms have been developed over the years since *E. coli* was first identified in 1885 (Tortorello 2003). For this survey, we used a defined substrate technology system to assess the level of indicator bacteria and to provide results within 24 h of sampling.

We evaluated the occurrence of *Cryptosporidium* and *Giardia* in surface waters of the Lake Baroon catchment and assessed the qualitative risk of contamination from a local sewage treatment plant and run-off from catchment farms.

MATERIALS AND METHODS

The catchment

Lake Baroon in the Maleny region is approximately 100 km north of Brisbane on the Sunshine Coast of Queensland, Australia. The catchment is within the Maroochy and the Caloundra Shires, with a population of approximately 6,000, with 1,500 people living within the Maleny township. There are approximately 2,385 properties within the catchment, with only 800 (34%) properties connected to the sewage system and 1,563 (66%) having home sewage treatment plants or septic tank systems, less than 1% is covered by national park and vacant blocks of land. Surface run-off and ground water are the main sources of water supply within the catchment (75 km^2). The catchment consists of three sub-catchments; Bridge Creek, Obi Obi Creek and Walkers Creek (Figure 1). Typical land usage within the catchment at the commencement of the study was 55% rural activities, 24% vegetation, 10% residential and 11% miscellaneous water storage, roads, shops and

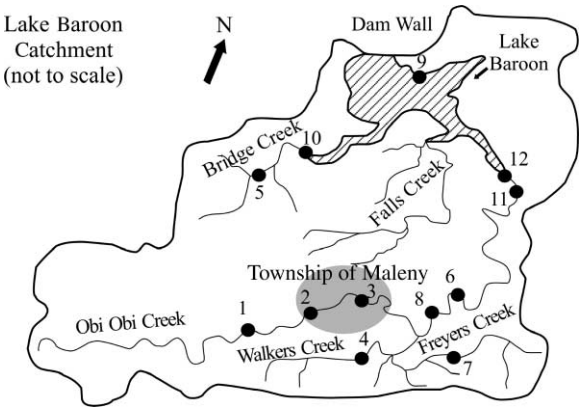

Figure 1 | Map of Lake Baroon Catchment showing sample collection sites 1–12. Sites 2 and 3 are within the Maleny Township and site 8 is the sewage treatment plant (STP).

services. The lake is frequently used for recreational activities.

Sites and sampling

Rainfall data for the catchment area was collected from the Australian Bureau of Meteorology for the 24 h prior to sampling. In some instances, no rainfall occurred within 24 h, so cumulative rainfall data for the seven days prior to sampling was also collected. Where a rainfall event was documented (i.e. >20 mm runoff) samples were collected within 24 h of this event.

Water-quality data on the level of faecal indicator bacteria was collected from local water authorities. Initially six sites, with a history of elevated levels of faecal coliforms, were selected for surveillance over a 12-month period. From February 2001 these sites included; Site 1 (six kilometres upstream from the township, dairy grazing); Site 2 (upstream from main urban area and a local water treatment plant, rural-residential and horticultural practices within township); Site 3 (urban storm-water runoff, livestock yards, within township); Site 4 (rural-residential, septic tanks and dairy farms); Site 5 (rural-residential, septic tanks, vegetation, dairy and beef cattle grazing with steep slopes); Site 6 (rural-residential, recreational area, dairy farms and downstream of sewage treatment plant), see Figure 1. Site 1 consistently demonstrated negative results and was replaced with Site 7 (septic tanks, vegetation, dairy and beef rural-residential cattle grazing) in October 2001, where there was suspected contamination from residential

septic tanks. From March 2001, samples from a local sewage treatment plant (STP) were also collected (Site 8) which included raw influent (8^r) and treated effluent (8^e). The STP is located six kilometres downstream from the township (see Figure 1). Based on preliminary data from sites 3 (19/03/01), 5 (05/03/01 and 02/04/01) and the STP (8^e, 19/03/01), we also assessed water from the lake at the intake tower of the dam (Site 9). Consequently, nine samples were collected from this site.

From the 17 April 2001, the prevalence of two faecal indicator organisms, *E.coli* and enterococci were also examined from each site (except STP).

In November 2002, two previous sites (i.e. 5 and 8) were reassessed and three new sites (10–12) downstream from previously contaminated sites were evaluated (see Figure 1).

During the rainfall season (i.e. December-May), fortnightly samples from each site (where possible) were collected ($n = 58$). During the dry season (i.e. June-November) samples were collected at monthly intervals with extra samples taken to coincide with rainfall (if any) ($n = 74$). A period of drought coincided with this survey. Water samples (10 l) were collected from each site, transported to the laboratory and analyzed within 24 h of collection. Between February 2001 and February 2002, a total of 132 water samples were analyzed from all sites. A further 24 samples were analysed between November 2002 and March 2003 totalling 156 water samples.

Water analysis

Water samples (10 l) were filtered through an Envirochek HV filter (Pall Life Sciences, New South Wales, Australia), backwashed and eluted as described previously (Wohlsen *et al.* 2004). Eluates were concentrated by immunomagnetic separation (IMS) (Dynal Biotech Pty Ltd, Victoria, Australia).

Viability procedure

The acid-dissociate fractions (0.1 N HCl) obtained from the IMS procedure were transferred into 1.5 ml microfuge-tubes containing 5 μl of NaOH. The CRY1 FISH probe (5′ CGG TTA TCC ATG TAA GTA AAG 3′), as described by Vesey *et al.* (1998), designed to target a specific sequence 18S rRNA in 128–148 bp region of *C. parvum* was used. A 100 μl mixture of 50% ethanol-50% phosphate buffered saline (PBS pH 7.4) was added and tubes incubated at 80°C for 20 min. The *Cryptosporidium* probe (CRY1 probe, GeneSet Singapore Biotech Pty. Ltd., Singapore) was suspended in hybridisation buffer (0.9 mol l^{-1} NaCl, 20 mmol l^{-1} Tris-HCL, 0.05% sodium-dodecylsulphate, pH 7.2) to a final concentration of 1 ρmol μL^{-1}. Then 10 μl of probe was added to each tube, which was then vortexed. The samples were incubated at 48°C for 2 h before being air-dried onto Spot-on-slides (Dynal Biotech Pty Ltd, Victoria, Australia), and fixed in methanol. No viability test was performed for *G. lamblia,* but cysts were detected using monoclonal antibodies as described below.

Monoclonal antibody

Slides were stained with EasyStain, containing fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies specific to *Cryptosporidium* IgG_1 and *Giardia* IgG_1 (Biotechnology Frontiers, New South Wales, Australia), and 4′, 6′-diamidino-2-phenylindole dihydrochloride hydrate (DAPI) (Sigma, New South Wales, Australia) and examined using an epifluorescent microscope. A working strength of DAPI was prepared by mixing 10 μl DAPI stock solution (2 mg ml^{-1}) with 10 mL PBS (pH 7.4) and filter-sterilized using a 0.22 μm pore size membrane filter.

Microscopic examination

A Zeiss Axioskop2 epifluorescence microscope was used to examine the stained slides under 200× magnification and confirmation was performed at 400× magnification. Microscope filter cubes appropriate for FITC (wave length 450–590 nm, FT 510, LP 515), Texas Red (wave length 530–585 nm, FT 600, LP 615) and DAPI (wave length 365 nm, FT 395, LP397) were used. Oocysts were located using the FITC filter set and identification was assisted by using the DAPI filter set and differential interference contrast (DIC) to visualise nuclei and sporozoites. Oocyst viability by FISH was determined by red fluoresce when viewed with the Texas Red filter set.

Quality control test

A quality control test was performed for each sampling round to determine percentage recovery of *C. parvum* and *G. lamblia*. A ColorSeed tube (Biotechnology Frontiers, New South Wales, Australia) containing 100 oocysts and 100 cysts, was added to one duplicate water sample collected from each site. ColorSeed oocysts/cysts are modified with a Texas Red label, producing red fluorescence when viewed by epifluorescence microscopy. This red fluorescence differentiates them from oocysts/cysts found in environmental water, which only fluoresce green (Warnecke *et al.* 2003). As FISH relies on Texas Red fluorescence, it could not be performed on duplicate samples. Quality control samples were processed in the same manner as test samples and percentage recovery of seeded oocysts/cysts was then calculated. Each sampling site was assessed at least once throughout the 12-month survey.

Faecal indicator bacteria

Commencing April 2001, additional 250 ml samples were collected from each sampling site in sterile containers and analysed for the presence of *Escherichia coli* and enterococci using the Colilert and Enterolert defined substrate technology systems in accordance with the manufacturer's instructions (IDEXX Laboratories, New South Wales, Australia). The number of bacteria was calculated using a semi-automated quantification method based on the Most Probable Number (MPN) model provided by IDEXX (Edberg *et al.* 1991).

Qualitative risk analysis

A qualitative risk analysis approach for the presence of *Cryptosporidium* oocysts was used to describe the impact of water contamination and its likelihood of happening at each site. Analysis was performed in accordance with the Environmental Risk Management – Principles and Process (Australian Standard HB 203 2000). Based on this guideline, sample sites that demonstrated no oocyst contamination during the 12-month survey were rated a likelihood of "Rare", i.e. contamination may occur only in exceptional circumstances. The likelihood of sites demonstrating contamination on only one occasion was rated as "Unlikely", i.e. contamination could occur at some time. Sites that demonstrated contamination on two to three occasions, were rated a likelihood of "Possible", i.e. contamination might occur at some time and finally sites that demonstrated contamination on four to five occasions, were rated a likelihood of "Likely", i.e. contamination will probably occur in most circumstances. If greater than five occurrences of contamination were demonstrated, then a likelihood of "Almost Certain" would be assigned, i.e. contamination is expected to occur. Qualitative assessment of risks associated with sites as used in this study can be used as a model in microbial ecology studies investigating the occurrence of *Cryptosporidium* or other pathogens in surface waters.

Illness notification

Cryptosporidium is a notifiable pathogen in Queensland. In order to investigate a possible relationship between the occurrence of *Cryptosporidium* in the catchment and the cases of illness in the community, we collected notification data from Queensland Department of Health. It was found that between 13th and 28th of August 2001, there was an outbreak of cryptosporidiosis in the Sunshine Coast region including the catchment area, involving eight children. On this occasion, the source of the illness was traced to the consumption of un-pasteurised cows' milk (Harper *et al.* 2002).

Statistical analysis

A spearman non-parametric correlation test was done to calculate correlation between abundance indicator bacteria and *Cryptosporidium* and *Giardia* in the catchment.

RESULTS

The percentage recovery of the filtration technique for detection of *C. parvum* oocysts and *G. lamblia* cysts in water samples ranged between 10% and 54% for oocysts and 13% to 42% for cysts (Table 1). All water samples

Table 1 | The percentage recovery of *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts in water samples from each site, as determined by the addition of a ColorSeed tube. Percentage recovery was not performed on samples collected from STP (8^r, 8^e)

Site	*n* *	% oocysts	% cysts
1	1	12	15
2	3	11	16
3	2	21	21
4	2	16	13
5	3	20	16
6	2	46	34
7	1	10	13
8^r and 8^e	0	NT	NT
9	1	54	42
10	1	36	32
11	1	29	27
12	1	44	36

*n = Number of samples seeded with ColorSeed tube.

collected from each site showed the presence of *E. coli* and/or enterococci with the highest numbers detected in water samples from sites 4, 5 and 7 for *E. coli* and sites 4, 5 and 6 for enterococci (Table 2). The number and date of positive samples for *Cryptosporidium*, *Giardia*, *E. coli* and enterococci collected from each site and the parameters monitored on each sampling occasion together with rainfall data are given in Table 3. Of the sites where *E. coli* and/or enterococci were detected, the risk category of only one site (site 5) was rated as "likely" for contamination from *Cryptosporidium*.

Oocysts and/or cysts were found in water samples collected from four sites on at least one occasion during the survey. The highest number of oocysts (i.e. 140 oocysts) were found in raw water from Maleny STP (site 8^r) during August 2001, and from site 3 (45 oocysts) during March 2001 (Table 3). The highest number of cysts (>5000 cysts) detected during the survey was also at site 8r during the month of August 2001. Oocysts were detected from site 5 on five occasions. All oocysts detected were viable by the FISH protocol unless otherwise stated. There was not a detectable number of *Cryptosporidium* in water samples from the other surveyed sites during the period of outbreak (August 2001), but there was an elevated level of *Cryptosporidium* and *Giardia* in the raw water samples collected from Maleny STP (site 8^r) (Table 3). The risk category of site 8^r was rated as "likely". No *Cryptosporidium* or *Giardia* were detected in water samples collected from the opening of the pipeline supplying raw water into a water treatment plant (site 9) or in any of the water samples collected from the sites re-assessed from November 2002 to March 2003 (Table 3).

Rainfall data collected throughout the survey showed that highest rainfalls were recorded in February (91 mm) and November (117 mm), 2001 (Table 3). The level of faecal indicator bacteria in sampling sites 2 to 7 did not correspond with the occurrence of rainfall in the region or to the detection level of *Cryptosporidium* and *Giardia* from these sites (Table 3).

DISCUSSION

Methods used to enumerate both *Cryptosporidium* and *Giardia* in surface and treated waters all underestimate the concentration of oocysts and cysts present (Shepherd & Wyn-Jones 1996; Hsu *et al.* 2001; Simmons *et al.* 2001; US EPA Method 1623 2001). The matrix variation and complexity of the procedures could explain these varying results (DiGiorgio *et al.* 2002; Wohlsen *et al.* 2004). The National Association of Testing Authorities, Australia, accepts a recovery rate of 10%–110% for laboratory accreditation. The quality control tests performed for each sample during this survey ranged between 10% and 54%, which were within the accreditation requirements (National Association of Testing Authorities 2002). Other ecological investigations studying the presence of *Cryptosporidium* in surface waters have not presented quality control data.

It is generally accepted that the concentrations of oocysts should increase during extreme rainfall events (Atherholt *et al.* 1998) and that rainfall is normally associated with waterborne *Cryptosporidium* outbreaks (Atherton *et al.* 1995; Curriero *et al.* 2001). We found that irrespective of rainfall, there was no constant background

Table 2 | The minimum (Min), Maximum (Max) and mean number of *Escherichia coli* and enterococci obtained from water samples at each sampling site. Indicator bacteria was not assessed from STP (8^r, 8^e), sites 10, 11, and 12

		No. of *E. coli*/100 ml			No. of enterococci/100 ml		
Sample site	***n***	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
1	4	130	280	190	9	110	49
2	12	18	460	110	1	390	59
3	12	17	1,700	340	5	820	130
4	12	38	>2,400	480	30	2,000	280
5	12	10	>2,400	530	13	>2,400	260
6	12	39	460	160	13	1300	150
7	8	9	2,400	380	1	130	42
9	9	–	–	2,400*	–	–	2,400*

*Tested on one occasion only.
n = number of samples.

level of *Cryptosporidium* at most sites. Similar results have been found by Kistemann *et al.* (2002), showing no significant correlation between oocyst concentration and rainfall or river flow at 10 sites examined in Germany. Similarly, Thurman *et al.* (1998) analysed several reservoirs and creeks within Australia for interactions between land use, rainfall and water quality parameters and found no significant correlation between these parameters and detection of parasites. Where there was a background level of oocysts and cysts, highest concentrations were detected in samples collected 48 h after the rainfall event (Thurman *et al.* 1998). Due to access restrictions to some of our sampling sites, samples were only collected 24 h after a rainfall event. Higher levels may have been detected if samples were collected after 48 h. Other data such as water depth, river flow, turbidity and pH could have helped interpret these data (Kistemann *et al.* 2002).

We found that increased levels of oocysts due to rainfall was site specific. Oocysts were detected at site 5 on three occasions between March and September 2001 with an increased level of oocysts after rainfall events. This was not the case with other sites. At site 3, there was only one occurrence of contamination when 45 oocysts were detected in a water sample collected in the absence of rainfall. Similar results were found with indicator bacteria with high levels of *E.coli* and/or Enterococci in the absence of rainfall. In our study, we did not find an increase in the number of *Cryptosporidium* or indicator bacteria in surface waters 24 h after rainfall. A small number of samples (12 per site) or limited rainfall data could partly be responsible for this observation. Assessment of the hydrologic conditions of the receiving streams could also help explain this finding.

Some researchers have demonstrated that the use of HCl (0.1 N) treated oocysts retain their viability for in vitro cell culture while others have found that a decrease around 50–70% can be observed (Di Giovanni *et al.* 1999; Rochelle *et al.* 1999).

Xiao *et al.* (2000) used an rRNA-based PCR procedure to detect different genotypes of *Cryptosporidium* and differentiated between human and animal sources of contamination in storm water. The FISH probe utilised in our study targets a sequence of the 18S rRNA gene purportedly specific for *C. parvum* (Vesey *et al.* 1998). However, alignment of this probe with 18S sequence data from *Cryptosporidium* species in the Genbank database indicated a 100% match of probe sequence to human *Cryptosporidium* (i.e. *C. hominis*; previously known as *C. parvum* genotype 1), cattle *Cryptosporidium* (i.e. *C. parvum*, previously known as *C. parvum*

Table 3 | The number of *Cryptosporidium* oocysts, *Giardia* cysts, *Escherichia coli* and enterococci found in samples collected from each site including rainfall data. All oocysts were viable unless otherwise specified. Site 1 was replaced with Site 7 in August 2001

Sample no.	Site description	Rainfall (mm)*	Date of sampling	Oocysts/10 l	Cysts/10 l[a]	*E.coli* CFU/100 ml	Enterococci CFU/100 ml	Risk category
1	Dairy grazing		Feb 01–Aug 01	0	0			Rare
2	Rural-residential, horticultural, within the township	47	07 Dec 01	9	0	460	7	Unlikely
3	Urban stormwater runoff, livestock yards, within township	47	19 Mar 01	45	0	–	–	Possible
			07 Dec 01	0	1	340	110	
4	Rural-residential, septic tanks and dairy farms		Feb 01–Feb 02	0	0			Rare
5	Rural-residential, septic tanks, vegetation, dairy and beef cattle grazing with steep slopes	69	05 Mar 01	2[b]	0	–	–	Likely
		41	02 Apr 01	5	0	–	–	
		15	25 Sep 01	3	0	17	24	
		117	12 Nov 01	10	0	2,400	2,400	
		16	19 Feb 02	7	2	10	56	
			Nov 02–Mar 03	0	0			
6	Rural-residential, recreational area, dairy farms and downstream of STP		Feb 01–Feb 02	0	0			Rare
7	Rural-residential, septic tanks, vegetation, dairy, beef cattle grazing		Aug 01–Feb 02	0	0			Rare
8[r]	Maleny sewage treatment plant – raw influent	14	17 Apr 01	0	14	–	–	Likely
		9	08 Aug 01	0	28	–	–	
		18	29 Aug 01†	140	160	–	–	
		6	11 Sep 01	0	>5,000	–	–	
		16	19 Feb 02	22	23	–	–	
			Nov 02–Mar 03	0	0	–	–	
8[e]	Maleny sewage treatment plant–effluent	47	19 Mar 01	3	0	–	–	Possible
		9	08 Aug 01	0	2	200	2,400	
			Nov 02–Mar 03	0	0	–	–	
9	Intake tower of the dam		Jul 01–Feb 02	0	0	–	–	Rare
10	Downstream site 5		Nov 02–Mar 03	0	0	–	–	NA
11	Downstream site 6 & 8		Nov 02–Mar 03	0	0	–	–	NA
12	Downstream site 6 & 8 at entrance to lake		Nov 02–Mar 03	0	0	–	–	NA

*Rainfall data 7 days prior to sample collection – Rainfall events within 24 h of sample collection: 7-Feb-02 (91 mm), 12-Nov-01 (29 mm) and 19-Feb-02 (44 mm); [a]Viability only performed on oocysts; [b]Non-viable by FISH protocol; NA = Not applicable; – = Not Tested.; † Time of the outbreak of cryptosporidiosis in the region.

genotype 2) and *C. parvum* isolated from dogs, pigs and kangaroos (Carey *et al.* 2004). Incidents of this probe reacting with *C. meleagridis* and *C. wrairi* have also been reported (Smith *et al.* 2004). Therefore, we could not differentiate between *C. hominis* (genotype 1) and *C. parvum* (genotype 2) and no conclusions can be made regarding the specific source of *Cryptosporidium* oocysts in the catchment. However, as non-human sources of *C. baileyi*, *C. canis*, *C. felis*, *C. hominis*, *C. meleagridis*, *C. muris* and *C. parvum* have been reported to also infect humans (Fayer 2004), the presence of *C. hominis or C. parvum* in surface waters has public health significance, in particular for the immunocompromised patient. Although rRNA has a short half-life, it may remain for some time after cell death. It is therefore possible that the use of FISH to detect rRNA may overestimate viability. Current research recommends the use of an RNase pre-treatment step before FISH to destroy residual rRNA to minimise this effect (Smith *et al.* 2004).

Although high levels of *Cryptosporidium* were detected in STP (8^{r}) during the outbreak, we did not find any oocysts in the treated effluent or in samples collected downstream of the STP. The effluent is treated through a process of flocculation ($Al_2SO_{4(3)}$), aeration, clarification and chlorination before being discharged to a settlement pond and was sufficient to remove oocysts during the outbreak. Based on these findings we postulate that while human defecation was the cause of the high level of oocysts at this site, *Cryptosporidium* contamination of surface water in this catchment was unlikely to be due to human contribution. From the public health point of view, it is conceivable that any failure of the STP during the course of an outbreak, would have a major impact on water quality in the catchment and therefore the performance of the STP at these times should be carefully monitored.

No *Cryptosporidium* oocysts or *Giardia* cysts were detected during the re-sampling of previously contaminated or newly added sites from November 2002 to March 2003. The maximum rainfall during this period was 39 mm and the lack of *Cryptosporidium* detected during this period further emphasises the lack of a relationship between oocyst/cyst detection and rainfall in this catchment. This also supports our conclusion that the high level of *Cryptosporidium* at STP was from the reported outbreak.

Other sites from which *Cryptosporidium* and/or *Giardia* were isolated consisted of rural-residential, horticultural (site 2), urban storm water runoff and livestock yards (site 3), rural-residential, septic tanks, vegetation, dairy and beef cattle grazing with steep slopes (site 5). These sites were rated a risk category of "unlikely", "possible" and "likely" respectively. No oocysts/cysts were detected at sites 1, 4, 6, 7 or 9, which were rated as "rare" (Australian Standard HB 203:2000, 2000). At no time during the survey were *Cryptosporidium* or *Giardia* detected in the water entering the water treatment plant (Site 9). This is probably due to the dilution of oocysts/cysts in the creeks, the natural filtration process of bedding material and an increased sedimentation rate due to the oocyst-particle association (Searcy *et al.* 2005), therefore reducing the numbers of oocysts present in surface waters.

The occurrence of *Cryptosporidium* and the level of faecal indicator bacteria in surface waters were assessed. *E. coli* are widely accepted as reliable indicator bacteria because they are not normally pathogenic, easy to detect and culture, and are found at concentrations much higher than other pathogens in surface waters (National Water Quality Management Strategy 2004). The presence of these bacteria in water indicates recent faecal contamination from humans or animals (National Water Quality Management Strategy 2004). Enterococci are also considered ideal faecal indicator bacteria because they survive in natural environments for lengthy periods (Sinton *et al.* 1993). The continuous high level of these bacteria in our samples indicates potential sources of faecal contamination within the catchment independent of *Cryptosporidium* or *Giardia* loads i.e. animals defecating in the catchment did not carry *Cryptosporidium* at the time of this study. Except on one occasion where the high levels of *E. coli* and enterococci coincided with the occurrence of *Cryptosporidium* and *Giardia*, there was no or very little correlation between the presence of these microorganisms and *Cryptosporidium* ($r = 0.4638$ for *E.coli* and $r = -0.1912$ for enterococci) or *Giardia* ($r = -0.5864$ for *E.coli* and $r = 0.3914$ for enterococci) in water samples, suggesting that indicator bacteria may not always be a suitable means for a proxy measure of *Cryptosporidium* and/or *Giardia* contamination in this catchment. This low level of correlation in our study was shown to be non-site specific as water samples from the same sites yielded contradictory results. For example,

while the presence of 10 oocysts at site 5 was accompanied with high levels of *E. coli* (>2,400 cfu/100 ml), 7 oocysts were detected at the same site with extremely low levels of these indicator bacteria (i.e. 10 cfu/100 ml).

Risk analysis studies should ideally be performed over a five-year period to better reflect variations in climate and environmental conditions and increase the sample numbers for each site assessed (O'Connor *et al.* 2005).

CONCLUSION

Despite the limited number of samples and rainfall data, our results suggest that a high level rainfall or faecal indicator bacteria do not necessarily relate to the presence of *Cryptosporidium* or *Giardia* in this catchment. Animals and farming activities appear to be the main source of contamination, although high levels of *Cryptosporidium* detected in raw influent to the STP indicates that a failure of the sewage treatment plant during the course of an outbreak could pose a major risk to the catchment. Therefore, catchment management plans should include communication with health authorities. Furthermore, applying a qualitative risk analysis approach as done in this study is a useful tool to describe the impact of water contamination and should be used in studying the occurrence of *Cryptosporidium* in surface waters. However, this should be done over an extended period of the sampling for each site.

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