Relative importance of the various environmental sources of Cryptosporidium oocysts in three watersheds
Dawn A. T. Phillip, Samuel C. Rawlins, Shrimatee Baboolal, Radha Gosein, Claudette Goddard, George Legall and Armanath Chinchamee

ABSTRACT
This study was conducted to guide the prioritisation of efforts to manage Cryptosporidium contamination of drinking water supplies in Trinidad, W.I. The main objective was to investigate the relative importance of three main types of sources of Cryptosporidium oocysts: urban, agriculture and wildlife. Weekly surface water samples were collected from 19 sites distributed among three watersheds, and examined for the presence of oocysts. A stratified random sampling design was used with each watershed representing one of the three main sources of oocysts listed above. Results showed a significant association between watershed and the occurrence of positive samples ($\chi^2 = 16.523, d.f. = 2, p = 0.000$), indicating that land use influenced the presence of oocysts. Urban and forested lands were the two most important sources of oocysts. There was no apparent association between agriculture and the presence of oocysts, and there was no significant difference between the percentage of positive samples at sites below agricultural facilities and sites not associated with agriculture within a single watershed ($\chi^2 = 2.45, d.f. = 1, p = 0.117$). We conclude that urban and wildlife are the main types of sources of Cryptosporidium contamination of surface water, whereas the contribution of agriculture is minor.

Key words | Cryptosporidium, environmental, oocysts, river, sources, watershed

INTRODUCTION
Watershed approaches have apparently concluded that agriculture is the primary environmental source of Cryptosporidium oocysts found in drinking water supplies since they tend to focus on the removal of the threat from agricultural sources such as cattle grazing on land in the catchment of a drinking water source (e.g. U.S. E.P.A. 1994); however the validity of this assumption needs to be tested.

Cryptosporidium is a coccidian pathogen that inhabits the epithelial cells lining the digestive tract and respiratory organs of humans and other vertebrates. It has been implicated as a causative agent of gastro-intestinal tract (GIT) manifestations in both humans and animals. Cryptosporidiosis, the disease caused by Cryptosporidium, is a self-limiting disease in immuno-competent persons, causing fever, abdominal cramps (MacKenzie et al. 1994), and copious, watery, often debilitating diarrhoea that lasts for 1 or 2 weeks (Baxby et al. 1985). In many of the immuno-deficient – e.g. infants, the elderly, persons undergoing chemotherapy, HIV-AIDS and organ transplant patients – remittance is achieved only with the removal of the causes of immuno-suppression (Guerrant 1997). The disease may be life-threatening for persons with HIV-AIDS. It is

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transmitted via the faecal-oral route through ingestion of contaminated food (CDC 1996, 1997; Laberge et al. 1996) or water (Bell et al. 1993; CDC 1994; MacKenzie et al. 1995; Kramer et al. 1996), or by contact with affected persons (Cordell & Addis 1994) and environmental surfaces (e.g. swimming pools (Puech et al. 2001)).

*Cryptosporidium* is endemic worldwide and can be found in surface water environments, such as rivers and lakes, and groundwater in “pristine”, agricultural and urban settings. The average percent of water samples found positive for *Cryptosporidium* in seven surveys conducted on surface waters in the United States ranged from 0.9% to 100% (Rose et al. 1997). A study of ground water showed a 12% positive rate for *Cryptosporidium* and/or *Giardia* spp. (Hancock et al. 1997).

There are many possible sources of the *Cryptosporidium* oocysts found in environmental samples since *Cryptosporidium* has numerous animal reservoirs. It has been found in over 155 mammalian species (Fayer 2004) as well as birds, reptiles and fish. *Cryptosporidium parvum* is the most widespread of the 15 species in the genus, infecting over 150 species of mammals (Fayer et al. 2000). In a number of independent studies, *Cryptosporidium* has been confirmed in a number of agricultural animals such as cattle in the US (Garber et al. 1994), Canada (Mann et al. 1986) and the UK (Reynolds et al. 1986); pigs, sheep (Xiao et al. 1993); goats (Mason et al. 1981; Johnson et al. 1999; Delafosse et al. 2003) and poultry (Current et al. 1986). Oocyst shedding from farm animals has been estimated to last from 3 to 12 days with faecal concentrations that may exceed 10¹⁰ oocysts per gram of manure (Casemore et al. 1997). Oocysts are also shed in wastes from other species of livestock, as well as poultry, companion animals and wildlife. It occurs in wildlife species (Sundberg et al. 1982; Heuschele et al. 1986; Klesius et al. 1986; Snyder 1988; Fayer et al. 1990; Ungar 1990; Atwill et al. 1997). It is estimated that mice can contribute as many as 3x10⁶ oocysts/L to surface runoff (Mager et al. 1998). There is evidence that even dead animals can continue to contribute oocysts to the environment (Duke et al. 1996).

Humans also contribute oocysts to the environment. Infected persons shed up to 10⁶ to 10⁷ oocysts/g of faeces (Goodgame et al. 1993) both during infection (Tzipori et al. 1983; Hunt et al. 1984; Hart et al. 1984) and up to 5 weeks (Baxby et al. 1985) to 2 months, in some cases (Jokipii & Jokipii 1986), after the cessation of diarrhoea. Asymptomatic shedding in apparently healthy humans has been reported by Current et al. (1983). Sewer overflows during heavy rains and flooding can also contribute high levels of *Cryptosporidium* oocysts to the environment. In the United States, sewage overflows have been cited as one of the major causes of pathogen contamination (Weis 1997). Both sewage overflows and “treated” sewage can contain high levels of *Cryptosporidium* (States et al. 1997).

There is therefore a risk that drinking water contamination may occur when oocysts from these various sources get into water systems that feed into surface and sub-surface drinking water stores. Contamination of drinking water sources is a major concern because of the potential to cause large outbreaks such as occurred in Milwaukee in 1993 in which over 400,000 persons became ill and 100 died (Kramer et al. 1996). Morgan et al. (2000), in a study of 22 *Cryptosporidium* isolates from HIV-infected patients, found 64% of the patients were infected with *C. parvum* human and cattle genotype, 27% with *C. felis* (found in cats), and 9% with *C. meleagris* (birds are the usual hosts). There has been one reported case of *C. baileyi* (usually associated with cats) in an AIDS patient, but epidemiological data on mode of transmission is weak (Jurakic 1999).

The pathogen in the environment is resistant, thus representing a health risk. It is able to survive and remain infective after over one month in manure (Jenkins et al. 1997) and river water (Robertson et al. 1992). Conventional water treatment processes are not effective in removing the risk of infection (MacKenzie et al. 1994). As few as 9 oocysts can cause infection in healthy experimental human subjects (Okhuysen et al. 1999). The infectious dose is expected to be fewer in immuno-compromised patients.

Management of this risk of infection due to the presence of *Cryptosporidium* in drinking water supplies can take place at the source of oocysts to the environment (i.e. at the watershed level), the water treatment process, or at the point of consumption. Management at the watershed level can be complex, time-consuming and expensive. Correct identification and prioritisation of management issues, such as the major sources of oocysts, are essential for the effective deployment of resources to this end. Although *Cryptosporidium* has been found in streams draining
watersheds under agricultural land use, the relative importance of agricultural, human and wildlife sources of oocysts is unclear. Madore et al. (1987) found Cryptosporidium oocysts at densities ranging from 0.005 to 18 oocysts/L in pristine watersheds, indicating that wildlife contributions to surface waters, though variable, can be substantial. They found higher densities (127 oocysts/L) in recreational river water, which may imply shedding of oocysts by humans. Variable results have been reported for surface water under the influence of cattle farming. Densities of 5,800 oocysts/L were obtained in irrigation canal water which drained through cattle pastures (Madore et al. 1987), 13.5 oocysts/L in river water below a cattle ranch (Ong et al. 1996). Another study found no difference between rivers in protected watersheds and those influenced by agriculture (LeChevallier et al. 1991). The present paper therefore attempts to establish associations between potential sources of Cryptosporidium oocysts and contamination of the surface water supplied to public water treatment facilities. This information will be important for prioritising environmental clean-up and/or control of contamination of water supplies.

METHODOLOGY

The study was carried out in the Republic of Trinidad & Tobago, which is the southernmost country in the Caribbean chain of islands (Figure 1). The study sites were located in the Northern Range Mountains of the larger island, Trinidad. The Northern Range runs along an east-west orientation across the entire northern coast of the island. It is dissected by a series of north-south oriented valleys. Whereas the northern slopes are almost uniformly rural, the southern slopes generally increase in urbanisation progressing in a westerly direction. Urban settlement is concentrated along the foothills but penetrates up the valleys, especially in the western portion of the mountain range. Valleys in the mid region of the Northern Range are also typified by agriculture, especially in their upper slopes. Eastern slopes are minimally impacted by humans and may be considered to be ‘pristine’.

Trinidad’s location between 10° and 11° N of the equator means that it is a tropical island. Meteorologically, it experiences two main seasons: wet and dry. The dry season typically runs from January to May, and the wet season from May/June to December, with a 2-week dry spell, the petite carême, in September. The distribution of rainfall also varies geographically, being highest in the northeastern part of the island. Total annual rainfall may vary from 3,048 mm in the northeast to 1,524 mm in the north- and south-west, with seasonal differences of approximately 1,000 mm in the wettest parts of the island, and 400 mm in the driest areas (Berridge 1981).

During heavy rainfall, surface run-off may cause sewers (including pit-latrines and makeshift sanitary facilities) to overflow. This overflow can reach waterways thus causing contamination of the water supply. Also impacting on some waterways are discharges from leaking and malfunctioning sewers (Rodrigues-Atwell 2000).

Research has shown that Cryptosporidium occurs in Trinidad. Oocysts were collected from pigeons (Kaminjolo et al. 1988) and livestock (Kaminjolo et al. 1993), and cases of cryptosporidiosis were found among children and HIV-infected adults (Tikasingh et al. 1986; Rawlins & Baboolal 1996). Subsequent to this, a survey conducted by Rawlins et al. (2000) found that Cryptosporidium oocysts were present in raw (source) and treated drinking water (24% and 17% of samples examined respectively) at public drinking water treatment plants (WTPs) in Trinidad and Tobago.

Site selection and description of study areas

Initially 17 sampling sites were assigned in three watersheds selected from among those that had yielded Cryptosporidium oocysts in the survey by Rawlins et al. (2000). Two of these sites were relocated during the study yielding a total of 19 sites (Figure 2a, b). It was assumed that the likelihood of contamination by oocysts from a particular source type was a function of the relative proportion of that land use type in the watershed. Thus, land use characteristics differed among the watersheds chosen allowing us to test for associations between types of reservoirs of Cryptosporidium (i.e., urban, agriculture and wildlife) and the presence of oocysts in surface waters. The watersheds, Maraval, Aripo and Hollis, are located among the southern slopes of the Northern Range (Figure 1b).
Maraval Watershed (6 sampling points)

The Maraval watershed is highly urbanised throughout almost its entire length (Figure 2c; pers. obs.). On its steep slopes, the mountains are forested and sparsely populated. Except for the extreme northern end of the valley, which is occupied by a golf course, densely populated areas are concentrated along the floor and foothills. Five sampling sites were placed along the river, from a leaky sewage treatment plant (STP) near the golf course to the intake of the Maraval Waterworks (MWW). An additional site was placed on a tributary to the main Maraval River (Figure 2a).

Aripo Watershed (6 sampling points)

Aripo watershed (Figure 2b) is the easternmost of the largest river basin draining the northwestern portion of the
island. Land use in the catchment of the Aripo Water Works (AWW) comprises secondary forest, agriculture (predominantly vegetable and tree crop farming, but with some chicken farming), and sparse rural human settlement (Figure 2d). The area below the AWW is used primarily for livestock farming. One sampling point (AR6, Figure 2d) was assigned below the effluent discharge of a pig farm on one bank and a livestock farm rearing cattle, sheep, horses and dogs on the other bank, to determine whether livestock farming in this watershed contributes Cryptosporidium oocysts to the Aripo River.

**Hollis Watershed (7 Sampling Points)**

The Hollis watershed can be considered ‘pristine’ since human activities in its catchment are severely restricted.
Except for occasional hunters who venture over from the neighbouring Aripo Valley to hunt game animals (e.g. monkeys, deer, and wild hog), the catchment remains relatively undisturbed from human influence. The main feature of this watershed is the Hollis Reservoir (Figure 2b, d), which has a maximum surface area of 66 hectares, maximum depth of 18.3 metres, and an approximate capacity of 4,700,000 m³. During the sampling period, water levels had fallen by 3 to 8 m below its maximum capacity exposing large expanses of the reservoir bed. The reservoir is fed by six main streams draining the surrounding densely forested mountains. A sampling point was established at the point of entry of each river into the reservoir and another sampling point was assigned to the draw off.

**Land use measurement**

Land use composition of each of the three selected watersheds was calculated from a GIS database held by the Department of Surveying and Land Information at The University of the West Indies in Trinidad. The primary data set used was a Landsat ETM + satellite image for Trinidad, acquired on January 29th 2001 with spatial resolution of 30 m. To provide information on areas obscured by clouds in this image, two additional Landsat ETM + images of 1999 and 2000 were also used (Al-Tahir et al. 2006). These Landsat satellite images were classified using Idrisi32, as raster base GIS software. The images were geometrically and atmospherically corrected, clouds were removed, and the issue of terrain shadow was addressed (Baban et al. 2006; Baban & Wan-Yusof 2001). Ground coordinates were extracted from a set of 1:25,000 topographic maps of Trinidad. A root mean square (RMS) error of 14 m was obtained. Following these stages, the image was classified with the aid of a false colour composite image, which assigned ETM + bands 3, 4 and 5 to the blue (B), green (G) and red (R) image band respectively. The classification scheme consisted of six forest classes: Evergreen Forest/Lastrow, Grassland/Savanna, Mangrove, Shrub-forest, Teak and Pine, in addition to Agriculture, Urban and Water. Finally, a 3x3 size mode filter was applied to remove some of the inevitable noise, smooth the classified output and reveal only the dominant classification (Lillesand & Kiefer 2000). The overall accuracy of the land use/cover was 92% after applying the 3x3 filters (Baban et al. 2006).

**Field sampling**

Water samples were collected weekly over 4½ months from March to August 2001. At each sampling point, a 10 L sample of water was collected at the water surface for determination of Cryptosporidium. Samples were chilled in a cooler and processed on the same day. Cryptosporidium was determined at the Caribbean Epidemiology Centre's (CAREC) Parasitology Laboratory.

**Laboratory analyses**

Oocysts were concentrated by flocculation according to Vesey et al. (1993). The concentrated, precipitated, fine solids were mounted as a thin smear on slides, fixed in methanol and stained using a modified Ziehl-Neilson acid fast stain. Each slide was examined for 30 minutes under oil at ×1000 magnification. Oocysts appeared as densely stained, thick-walled, red spheres ranging in size from 3 to 7 µm, which contained four sporozoites, dark granules and a central vacuole. All suspected positive identifications were read by a second reader and identifications were considered negative when there was disagreement. There was high inter-reader agreement.

**Data analyses**

Descriptive and inferential statistics, the latter mainly Chi-squared tests of association between location and categories of oocytes, were performed using SPSS (Version 13) for Windows. Minitab (Version 13) for Windows was used to perform logistic regressions.

**RESULTS**

A total of 243 raw water samples were analysed for Cryptosporidium oocysts. 15% of these produced no definite result for the presence/absence of Cryptosporidium oocysts (i.e. no data or presence uncertain) and were omitted from
further analysis. Thirty eight (18.3%) of the remaining 208 samples yielded positive results (Table 1).

Land use classification confirmed that the Maraval, Aripo and Hollis watersheds differed in the proportions of the three land use classes: urban, agricultural and forested (Figure 2c, d). The occurrence of oocysts also varied among the three watersheds. Maraval had the highest percentage of positive samples (33%), whereas Aripo had the lowest (6%). This association between watershed and the occurrence of positive samples was significant ($\chi^2 = 16.523$, d.f. = 2, $p = 0.000$) indicating that land use influenced the presence of oocysts in streams draining the watershed. Chi-squared tests showed associations between the presence of oocysts and total area (ha) devoted to agriculture ($\chi^2 = 8.6$; d.f. = 1; $p = 0.003$), size of forested area ($\chi^2 = 16.53$ d.f. = 2 $p = 0.000$) and size of urban area ($\chi^2 = 8.6$; d.f. = 1; $p = 0.003$).

Positive samples were obtained from 17 (90%) of the 19 sites used in this study. All sites sampled within the Hollis and Maraval watersheds yielded positive samples (Table 1). There was an association between the location of sites in the

<table>
<thead>
<tr>
<th>Watershed</th>
<th>Site</th>
<th>No. of samples</th>
<th>No. positive</th>
<th>% positive</th>
</tr>
</thead>
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<tr>
<td>Hollis</td>
<td>QR1</td>
<td>12</td>
<td>2</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>QR2</td>
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<td>9.1</td>
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<td>QR3</td>
<td>10</td>
<td>1</td>
<td>10.0</td>
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<td></td>
<td>QR4</td>
<td>12</td>
<td>1</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>QR5</td>
<td>12</td>
<td>2</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>QR6</td>
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<td>18.2</td>
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<td></td>
<td>QR7</td>
<td>11</td>
<td>3</td>
<td>27.3</td>
</tr>
<tr>
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<td>AR1</td>
<td>13</td>
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<td>12</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>AR4</td>
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<td>1</td>
<td>22.0</td>
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<tr>
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<td>AR5</td>
<td>11</td>
<td>0</td>
<td>0.0</td>
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<tr>
<td></td>
<td>AR6</td>
<td>12</td>
<td>1</td>
<td>8.3</td>
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<tr>
<td>Maraval</td>
<td>MR1</td>
<td>12</td>
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<tr>
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<td></td>
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<td></td>
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<td>63</td>
<td>4</td>
<td>6.3</td>
</tr>
<tr>
<td>Maraval</td>
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<td>All Watersheds</td>
<td>19</td>
<td>208</td>
<td>38</td>
<td>18.3</td>
</tr>
</tbody>
</table>

* Samples taken from a tributary of the main Maraval River
Maraval watershed and the presence of oocysts ($\chi^2 = 11.1$, d.f. = 1, $p = 0.001$). Sites in the upper watershed (MR1, MR2 and MR7) contained a higher percentage of Cryptosporidium oocysts than sites further downstream (Table 1).

The percentage of positive samples obtained from stations located at farms was compared with sites located away from agricultural establishments to test for associations between type of site (i.e. agricultural) and the presence of oocysts in surface water. This was done using the Aripo watershed since land use in the valley was primarily either agriculture or forest (Figure 2d). Positive samples were obtained from four sites on the Aripo River associated with agricultural activities: two poultry farms, one vegetable farm which used chicken manure as fertiliser, and one with livestock farms on both banks. Although the four agricultural sites were the only ones yielding positive samples (Table 1), there was no association between sites and the presence of oocysts in Aripo ($\chi^2 = 2.45$, d.f. = 1, $p = 0.117$).

### DISCUSSION

The results of the present study indicate that the two most important sources of Cryptosporidium contamination in the Northern Range, Trinidad, were urban and wildlife. There was no association between agricultural sources and the presence of oocysts; agricultural contributions of Cryptosporidium oocysts appeared to be minor. There was also no significant difference between the percentage of positive samples at sites below agricultural facilities and sites not associated with agriculture within a single watershed.

This is surprising since Kaminjolo et al. (1993) found Cryptosporidium oocysts in calves, kids, lambs and piglets in Trinidad. A possible explanation is that there are geographic differences within the island in the prevalence of the disease. Although cattle are known to be a major source of oocysts in some watersheds (States et al. 1997; Starkey et al. 2005), this may not always be the case. For instance, Ong et al. (1996) found Cryptosporidium oocysts in water under the influence of cattle farms but all cattle tested yielded negative results. In another local study, one sample each of fresh cattle and horse droppings collected at one of the livestock farms in Aripo yielded samples positive for Cryptosporidium oocysts (Chang unpubl. data). It is possible therefore that oocysts shed by livestock did not reach the river, either due to insufficient rainfall for transport or due to degradation of oocysts by soil fauna. With the exception of June, rainfall throughout the entire study period was markedly below the average recorded for this time of year (Table 2).

The high percentage of positive samples from Maraval may be due to the discharge of sewage effluent at the uppermost site. There was an association between location of sites in the Maraval watershed and the presence of Cryptosporidium oocysts; sites within 1.25 km of the STP yielded a higher percentage of positive samples than those further away. Sewage effluent is known to be an important source of oocysts. States et al. (1997) found Cryptosporidium oocysts in 33 percent of samples of treated sewage effluent. Crockett & Haas (1997) found that sewage treatment plants were a continuous source of oocysts.

Possible sources of the oocysts found in Maraval are humans and companion animals. Cryptosporidium has been shown to occur in Trinidadians (Tikasingh et al. 1986; Rawlins & Baboolal 1996), and Kaminjolo et al. (1988) reported Cryptosporidium in local pigeons collected near the study area. Although its occurrence in these animals has not been investigated locally, in other countries Cryptosporidium oocysts have been isolated from dogs (Fayer et al. 2001; Lefebvre et al. 2005) and cats (Sargent et al. 1998), the most common companion animals found in residential areas of Trinidad.

The public health risk associated with the high prevalence of Cryptosporidium oocysts from STPs in a stream feeding an urban drinking water supply can be significant.

<table>
<thead>
<tr>
<th>Month</th>
<th>Mean rainfall (1986–1998) (mm)</th>
<th>Monthly total (2001) (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>March</td>
<td>55.1</td>
<td>8.5</td>
</tr>
<tr>
<td>April</td>
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<td>138.0</td>
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<tr>
<td>June</td>
<td>264.9</td>
<td>274.2</td>
</tr>
<tr>
<td>July</td>
<td>295.0</td>
<td>190.2</td>
</tr>
</tbody>
</table>
The largest recorded outbreak of cryptosporidiosis, affecting some 403,000 persons (MacKenzie et al. 1994) and resulting in the death of 100 residents (Kramer et al. 1996), was traced by DNA analysis to Cryptosporidium parvum (Peng et al. 1997) (now recognised as a separate species C. hominis (Morgan-Ryan et al. 2002)) implying contamination of the drinking water supply by human faeces (Fayer 2004). The potential for such wide-ranging human health disasters is especially high for developing countries where centralised sanitary facilities serving densely populated areas are often poorly maintained. A survey of 181 sewage treatment plants in Trinidad, for example, found that only 50 (28%) were functioning satisfactorily and that a number were spewing improperly treated sewage onto roadways, footpaths and watercourses (Rodriguez-Atwell 2000).

The high occurrence of oocysts at the Hollis Reservoir is not unusual. Le Chevallier et al. (1997) examined water storage reservoirs and found that the occurrence and density of oocysts at several reservoirs they monitored were significantly higher in effluent samples than in influent samples, despite the fact that the water had already been treated. They cited the long retention times of reservoirs as a factor which can contribute to the higher occurrences and densities of oocysts in the effluent water. The long persistence of Cryptosporidium oocysts, up to 176 days in the environment (Rose et al. 1997) can also be a factor.

The sources of contamination were most likely wild animals in the catchment to the reservoir. Cryptosporidium oocysts have been recorded in a variety of wildlife including snakes (Brownstein et al. 1977), lizards (Koudela & Modry 1998), wild mice (Klesius et al. 1986), and birds (Ryan et al. 2003). Although there are no similar studies for wildlife in Trinidad, Tikasingh et al. (1986) and Kaminjolo et al. (1988) did find Cryptosporidium oocysts in pigeons here. The contribution of oocysts by wildlife to the environment can be substantial. Atwill et al. (2001, 2004) estimated that California ground squirrels shed an average of 53,875 and 44,482 oocysts per gram of faeces respectively. The mean environmental loading was 57,882 oocysts squirrel$^{-1}$ day$^{-1}$ (Atwill et al. 2004).

In summary, results of the present study indicate that Cryptosporidium oocysts, ubiquitous in surface waters in Trinidad, may have multiple environmental sources on the island with urban and wildlife sources being the most important. The differences between the results from the three study areas imply that watershed characteristics are important considerations in assessing the potential risk of contamination of surface water by Cryptosporidium oocysts. Other studies (e.g. Crockett & Haas 1997) have come to similar conclusions for river systems in the United States. They concluded that it is difficult to interpret the results of monitoring data for Cryptosporidium without an understanding of the watershed at a fine level of detail, as general features were inaccurate predictors of the occurrence of oocysts.

The implications for the health of the public, especially high-risk groups such as the immuno-compromised are significant. When the diversity of the socio-cultural uses of untreated surface waters (only some of which were mentioned in the foregoing) by the population is considered, what becomes obvious is that there is a multitude of routes/opportunities for infection. The main route of concern for the present study, treated drinking water, was shown to be a potential transmission route (Phillip et al. unpubl. data) requiring a change in the approach to the management of this vital resource. The solution to the management of contamination of surface waters will not be a simple one.

**CONCLUSIONS AND RECOMMENDATIONS**

1. Agriculture, especially the poultry and livestock industries, may not be an important source of Cryptosporidium oocysts in Trinidadian rivers. A closer examination of the agriculture industry is recommended.

2. A significant human health risk is posed by leakage of inadequately treated sewage effluent into surface waters which are used for contact recreation and for potable water. Thus, management of the risk of Cryptosporidium infection through contact or ingestion of affected water should focus on the functioning and operation of sewage treatment plants, particularly those located upstream of water treatment plants and recreational sites.

3. The contribution of wildlife is significant. Control of contamination of water sources by this route is an impossible task; thus, management of this risk to human health from wildlife must be undertaken at the water treatment plant and the resource/user interface.
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