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Cryptosporidium and *Giardia* as Determinants for Selection of an Appropriate Source of Drinking-water in Southern Sri Lanka

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ABSTRACT

Four different water sources (irrigation canals, small reservoirs, shallow wells, and tubewells), used for domestic purposes, in an irrigated area in southern Sri Lanka, were tested for *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts. Identification of these parasites in water sources is important as these are increasingly recognized as causative agents of waterborne diarrhoeal disease. All the four sources of water were contaminated with cysts and oocysts. The sources of surface-water contained a greater number of protozoa compared to tubewells and shallow wells ($p<0.05$). The results indicate a reduction of high parasite loads by natural filtration as the water moves from canals to shallow wells through the soil profile. This could present an opportunity to reduce the burden of diarrhoeal disease due to protozoa by selecting an appropriate source of drinking-water and identifying those water sources that require treatment solutions.

Key words: *Cryptosporidium*; *Giardia*; Parasites; Diarrhoeal diseases; Drinking-water; Water supply; Water pollution; Irrigation; Sri Lanka

INTRODUCTION

The protozoal parasites—*Giardia* spp. and *Cryptospo ridium* spp.—are increasingly recognized as important aetiological agents of waterborne diarrhoeal disease. Diarrhoeal disease is a major cause of morbidity and mortality among children in Sri Lanka and other southeast Asian countries (1,2). In industrialized nations, recent water-transmitted outbreaks of *Giardia* spp. and *Cryptosporidium* spp. have increased interest in these two intestinal parasites as they are more resistant to conventional water-treatment systems than other pathogens (3). Several studies in Sri Lanka have identified these parasites in stools of humans or animals, and results have shown prevalence between 6% and 55% (2,4-9). However, no studies have been conducted in Sri Lanka on the prevalence of *Giardia* cysts and *Cryptosporidium* oocysts in surface-water or deep wells. The role that drinking-water plays in the transmission of these two intestinal parasites is not known.

Sri Lanka is an intensively-irrigated country with an irrigated area of 570,000 ha, covering 30% of the cultivable land (10). These irrigation systems provide easy access to water across the region and are often used as a source of domestic water supply. Domestic uses of water include drinking, cooking, dish-washing, laundry, bathing, and sanitary purposes. Recent studies in the dry zone of Sri Lanka have shown that 90-100% of rural people extract water for their domestic and other needs from the irrigation system, either directly from canals and irrigation reservoirs (locally called 'tanks'), from municipal systems fed by irrigation canals, or from shallow wells (11-15). Therefore, as supply of drinking-water is closely linked to the irrigation systems, studies by the International Water Management Institute (IWMI)

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focus on how the irrigation systems can be managed for improving domestic access to water supply, quality, and subsequent health benefits.

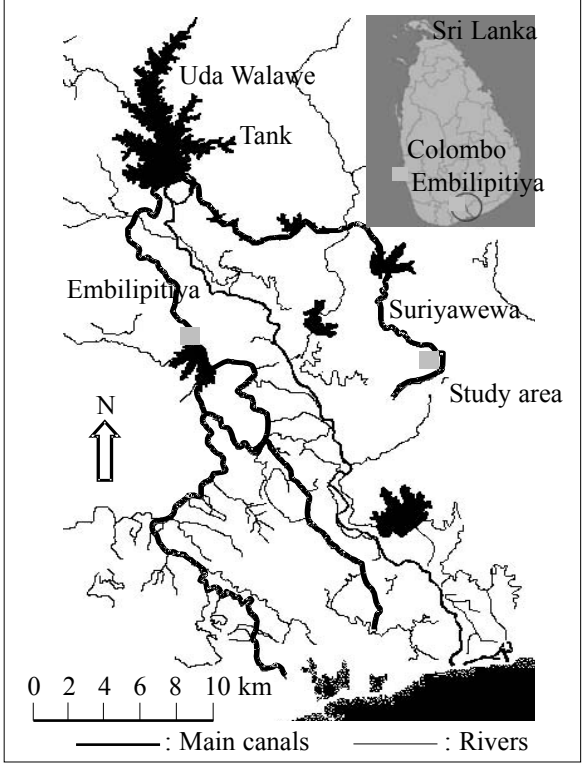
This study was designed to determine if *Giardia* cysts and *Cryptosporidium* oocysts were present in an irrigated area of southern Sri Lanka and to identify different sources of drinking-water that pose potential health risks. Second, the levels of faecal contamination of various water sources were determined by measuring the number of thermotolerant coliform units. The numbers of thermotolerant coliforms were compared with the numbers of parasites to determine if thermotolerant coliform levels can be used as an indicator of contamination by these two parasites. In combination with findings of other studies that have been done in the same area, the results were expected to enable informed decision-making about selection of different sources of drinking-water.

MATERIALS AND METHODS

Study area

The Uda Walawe irrigation scheme, fed by water from the Uda Walawe reservoir, is located in the southern region of Sri Lanka (Fig. 1). The area straddles the wet/dry zone boundary and generally receives rain during October-February. There are two main cropping seasons —May-September and November-March. In 2000, daily flow of water in canals ended on 30 September and resumed later than usual after November. The study area is situated along the left bank canal near the town of Suriyawewa. Two secondary canals were surveyed for *Giardia* cysts, *Cryptosporidium* oocysts, and thermotolerant coliforms.

Fig. 1. Location of the Uda Walawe reservoir and irrigation scheme in southern Sri Lanka. The study area near Suriyawewa is indicated by a circle



Water sources

Availability of water in the dry zone of Sri Lanka is limited due to unreliable seasonal rainfall and poor groundwater resources (16). In the study region, tubewells, which exploit water from a deep aquifer, were not heavily used due to their high concentrations of salts, iron, and fluoride (15,17). Originally, the southern region of Sri Lanka has very limited sources of groundwater. Therefore, the primary sources of domestic water are shallow wells, canals, and reservoirs, all of which derive their water from the irrigation system. Irrigation reservoirs and canals were generally used for laundry and bathing. Shallow wells, exploiting groundwater, recharged by seepage water from canals and fields (Fig. 2), were generally used for drinking, cooking, and dish-washing. Water was mostly consumed without any treatment. However, some families boiled shallow-well water prior to drinking, particularly if water was meant for the very young, old, or diseased persons.

Parasite sampling

To study the presence of parasites, water samples were collected from a shallow well, a canal, a reservoir, and a tubewell. The sampling was carried out in the first week of August 2000 and repeated in September and November of the same year. In total, 12 samples (4 sites x 3 months) were analyzed.

Fig. 2. Shallow well with protective concrete wall, located 3.7 m away from the earthen canal, Suriyawewa, Sri Lanka

The water samples were filtered using a hand-pump with a flow rate of approximately 5 litres per minute. For each sampling, 49 litres of water were pumped using only one cylindrical filter. The filtering apparatus consisted of an inlet hose, plastic filter-holder with 25-cm long yarn-wound polypropylene filter having a porosity of 1 µm, manufactured by Triosin Corporation (Mirabel, Québec). A concentrated specimen of 200 mL, recovered from the filter used for passing 49 litres of water, was kept for the study of parasites and preserved in 10% formalin. The concentrated specimen was obtained by washing and scraping the filter with distilled water in a squirt bottle and a scalpel. The filter was not disassembled. However, some filter material was scraped into the concentrated sample.

Processing of samples

The concentrated specimens were stored at room temperature (23 ^{o}C) until these were processed for microscopic analysis. The specimens were further concentrated by centrifugation to a volume of 5 mL (containing all the sediment visually detectable in the original 200 mL). Morphological characteristics, such as dimensions of the oocysts and cysts, were used for classifying the protozoa; this technique was used due to the non-availability of a microscope equipped with ultra-violet light allowing the kits for the specific detection of *Giardia* cysts and *Cryptosporidium* oocysts.

***Giardia* spp.**

Giardia cysts were identified using light microscopy without staining. A Pasteur pipette was used for dropping approximately 0.1 mL of concentrated specimen across two microscope slides (0.05 mL per slide) (18). A cover slip was placed over the specimens, and these were examined with a light microscope at 400x magnification. In total, 50 fields were examined on the two microscope slides. These fields covered the entire surface of both the slides (0.1 mL of concentrated specimen). Positive fields were registered, along with the total number of cysts counted in the 50 fields. The number of cysts was multiplied by 50 to report per 5 mL, which is the total volume of sample concentrated by centrifugation, although this actually represents 49 litres of raw water. The cysts appeared as elongated structures with visible flagella inside. They had a mean size of 12 µm.

***Cryptosporidium* spp.**

To identify *Cryptosporidium* oocysts, 5 mL of sucrose with a specific gravity of 1.103 were pipetted into a 16-mL glass centrifuge tube. Three mL of the concentrated sample were layered on top of the sucrose solution. After centrifugation at 200 g for 10 minutes, all oocysts were assumed to be in the 1-mL cloudy layer located at the interface. This cloudy layer was removed with a Pasteur pipette and washed three times in distilled water. A total volume of 0.1 mL was allowed to dry on two microscope slides at room temperature (23 ^{o}C), fixed with methanol and stained with a Ziehl-Neelsen acid-fast stain. These were examined with a microscope at 1000x magnification (oil immersion). In total, 100 fields were examined, which covered both slides and entire 0.1-mL concentrated specimen. Positive fields were registered along with the total number of oocysts counted in the 100 fields. The number of cysts was multiplied by 50/3 to report per 5 mL, which is the total volume of sample concentrated by centrifugation, although this actually represents 49 litres of raw water. The multiplication accounts for the analysis of only 3 mL of the 5 mL concentrated sample and 0.1 mL of the 1 mL cloudy layer. The oocysts appeared in red, and their dimensions had a mean size of 5.0 µm x 4.5 µm.

Thermotolerant coliform sampling and analysis

For thermotolerant coliform bacteria, sampling was done on the same sites plus 26 additional sites—12 shallow wells, 8 canal sites, 3 reservoir sites, and 3 tubewells. The thermotolerant coliform unit sampling was done in the first week of each month over five months (August-December 2000). At each sampling site, three samples were taken to give a total of 390 samples (3 samples per site x 26 sites x 5 months). Water samples for studying thermotolerant coliforms were always collected in the morning using sterile 200-mL plastic bags. Approximately, 150 mL of water were collected per sample. All samples were placed in a cool box filled with ice to keep samples at 5 ^{o}C and were analyzed within nine hours after collection. The samples were analyzed using the membrane-filter technique as outlined by the American Public Health Association (19) and Csuros and Csuros (20).

Statistical analysis

Linear regression analysis was used for determining whether type of water source, time of year, and level of thermotolerant coliform bacteria could predict numbers of parasite cysts or oocysts. Due to the limited numbers of samples, the authors recognize that the conclusions of the analysis are limited to trends only. Data were analyzed using the SAS software (SAS Institute, Inc., Cary, North Carolina). P values less than 0.05 were considered to be statistically significant.

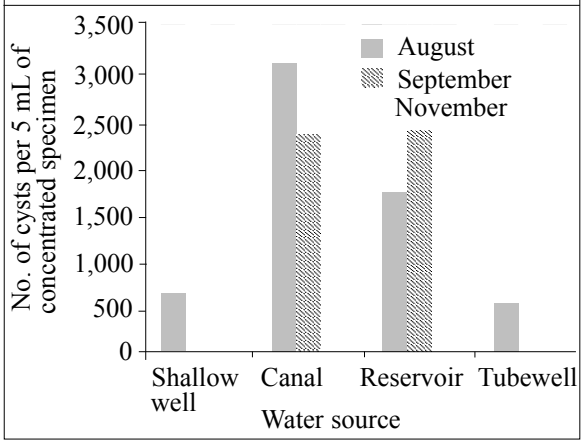
RESULTS

***Giardia* spp.**

Giardia cysts were found in all the water sources. However, fewer cysts were found in shallow wells and tube-

wells, to the extent that no cysts were found in September in either of the wells. Samples from irrigation reservoir and canal contained, on average, 2,133 *Giardia* spp. cysts per 5 mL of concentrated specimen (Fig. 3). Samples from tubewells and shallow wells contained, on average, 350 *Giardia* cysts per 5 mL of concentrated specimen (Fig. 3). The 5 mL of concentrated specimen represents 49 litres of collected raw water. The numbers of *Giardia* cysts in all the sites showed no observable pattern over the study period (August-November). Results of regression analyses showed that the type of water source was a significant factor to predict numbers of *Giardia* cysts ($p<0.05$); the coefficient of variability was 41.90%. The monthly variation factor was not significant in the prediction of numbers of *Giardia* cysts over the study period (August-November 2000). These results agree with the results of other studies reporting no definite seasonal variations in spreading of *Giardia* cysts in water (21,22).

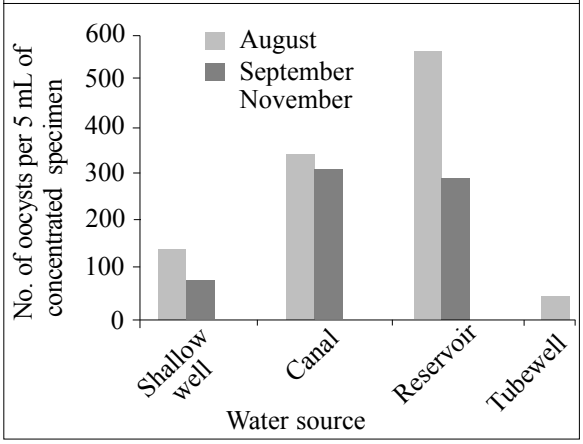
Fig. 3. Number of *Giardia* cysts per 5 mL of concentrated specimen in four different water sources used by Sri Lankans for domestic purposes. The concentrated samples were from 49 litres of raw water taken around Suriyawewa during August-November 2000



Cryptosporidium spp.

Cryptosporidium oocysts were found in all the water sources and were most numerous in surface-water. Samples from irrigation reservoir and canal showed, on average, 395 *Cryptosporidium* oocysts per 5 mL of concentrated specimen (Fig. 4). Tubewells and shallow wells showed an average of 64 *Cryptosporidium* oocysts per 5 mL of concentrated specimen (Fig. 4). The 5 mL of concentrated specimen represents 49 litres of collected raw water. Although the numbers of oocysts were moderate (<150 per 5 mL) in both the types of wells, the mere presence of one pathogenic organism would make these water sources unacceptable for drinking according to the WHO standards (23). The numbers of oocysts in all the sites ranged slightly over three months (August, September, and November) with no observable pattern over the study period. Results of regression analyses showed that the type of water source was a significant factor to predict numbers of *Cryptosporidium* oocysts ($p<0.05$); the coefficient of variability was 38.40%. Monthly variation was not a significant factor in the prediction of numbers of *Cryptosporidium* oocysts over the study period (August-November 2000).

Fig. 4. Number of *Cryptosporidium* spp. oocysts per 5 mL of concentrated specimen in four different water sources used by Sri Lankans for domestic purposes. The concentrated samples were from 49 litres of raw water taken around Su-riyawewa during August-November 2000



Thermotolerant coliforms

The numbers of thermotolerant coliforms were dependent on the type of water source with high counts in canals and reservoirs and low counts in shallow wells and tubewells (Fig. 5). The average number of thermotolerant coliform units per 100 mL found in canal was well above 1,000 thermotolerant coliform units per 100 mL which represents the Sri Lankan norm for bathing-water (dotted line, Fig. 5).

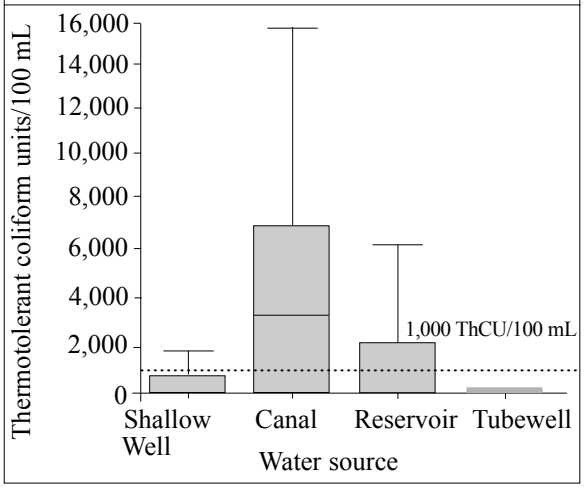
Numbers of thermotolerant coliform were independent of season over the study period (August-December). This pattern of strong dependence on water source and independence of monthly variation was displayed for both thermotolerant coliform and parasites. As reported in other studies, bacterial levels were not a significant factor for predicting numbers of parasites in water (24,25).

DISCUSSION

Giardia cysts and *Cryptosporidium* oocysts were found in irrigation water and wells in the Uda Walawe irriga-

tion system around Suriyawewa in southern Sri Lanka. Of all the samples, 92% (11/12) contained protozoa. The numbers of cysts and oocysts were dependent on the type of water source with fewer parasites in shallow wells and tubewells than in canals and irrigation reservoirs. The results of thermotolerant coliforms were in line with that of protozoa, with the majority of water samples testing positive for bacteria. More than 80% (273/338) of the samples analyzed contained thermotolerant coliform bacteria. The results of thermotolerant coliforms in the canals were significantly higher compared to any other sources and were well over the Sri Lankan norm established for bathing (1,000 thermotolerant coliform units per 100 mL). The type of water source is, as with protozoa, a determinant of high levels of thermotolerant coliforms (canal and reservoir) or low levels of thermotolerant coliforms (shallow well and tubewell). Levels of parasites were not predicted by thermotolerant coliforms, although they showed similar trends. The numbers of both *Giardia* cysts and *Cryptosporidium* oocysts were independent of the time of sampling over the study period (August-November).

Fig. 5. Concentrations of thermotolerant coliform bacteria (units per 100 mL) in four different water sources used by Sri Lankans for domestic purposes. The samples were taken from around Suriyawewa during August–December 2000. Shaded area represents 50% of data points, whiskers represent extreme values (excluding outliers), and the solid horizontal line represents the median. The horizontal dotted line at the value of 1,000 thermotolerant coliform units per100 mL indicates the Sri Lankan norm for bathing-water



This is the first study on the presence of *Giardia* cysts and *Cryptosporidium* oocysts in surface-water and groundwater in Sri Lanka. Our findings confirm the findings of clinical studies that have shown the presence of these two parasites in the population. In this study, we are reporting a high number of cysts and oocysts per 49 litres of raw water. It is likely that canal, reservoir, and shallow wells are sources of contamination for the population. Both *Giardia* spp. and *Cryptosporidium* spp. are known to cause gastroenteritis and are considered two of the leading causes of waterborne diseases in the United States as reported by the Centers for Disease Control and Prevention (26-29).

Overall, the levels and concentrations of *Giardia* spp. and *Cryptosporidium* spp. in Sri Lanka were higher than what has been reported in recent studies from other countries (23,30-35). Possible sources of faecal contamination in this study area include both human and animal (water buffalo), since animal sources are known to be important in the introduction of these protozoa to a water system (23). Water buffalos are common in the study area and are often observed to be bathing and drinking in canals. A further study to differentiate between faecal contamination by human and animal would indicate the extent to which water buffaloes or other domestic animals are contributing to the contamination. Methods to reduce buffalo faeces in water from entering the system could then be developed, such as designing special crossings and separate bathing sites for cattle. If the source of contamination is mainly human, methods to improve pit-latrines may be useful in reducing the protozoan load in the local water supplies.

We found only two published papers that examined the biological water quality in Sri Lanka (11,36). These papers reported on a study in the northwest region of the country. Thermotolerant coliform bacteria were measured in tubewells and shallow wells, but surface waters, such as irrigation canals and reservoirs, were not included. Faecal contamination, in terms of the number of samples positive for thermotolerant coliforms, was the greatest for unprotected shallow wells and lowest in tubewells. Our bacterial counts are comparable with results of studies by Mertens *et al*. (11,36). Moreover, *Cryptosporidium* oocysts showed similar trends with higher numbers of oocysts in shallow wells than in tubewells. Studies by Mertens *et al*. linked bacterial water quality in different water sources to childhood diarrhoeal morbidity, thus indicating the importance of transmission of waterborne diarrhoeal diseases in Sri Lanka.

Studies in Pakistan by the IWMI have also looked at the interactions and effects of selection of water sources in irrigated areas and levels of thermotolerant coliforms on incidence of diarrhoea (37). Similar to the present study, water sources with the lowest levels of faecal con-

tamination exploited groundwater from canal seepage (shallow wells). Higher levels of contamination were found in canals. This suggests that some level of purification is achieved by the passage of water through soil as it seeps slowly from canals into shallow wells. The results emphasize the benefit of human health gained by the availability of seepage water, where the soil has acted as a natural filter. It also emphasizes the need for studies on removal of protozoan cysts and oocysts through slow sand-filtration.

While the protozoan results followed the thermotolerant coliform results, there is certainly a need for additional indicators, especially for *Cryptosporidium*. The World Health Organization has calculated a guideline value of 1 *Cryptosporidium* spp. oocyst per 1,600 litres of drinking-water to achieve a health-outcome target of 10-6 DALYs per person per year (23). Due to the complexity of measuring the numbers of parasites, an indicator organism would facilitate the identification of water sources unsuitable for drinking.

In basins, such as in the south of Sri Lanka, contamination of supply sources of drinking-water with protozoan parasites should be a matter of concern for providers of drinking-water and policy-makers. Shallow wells, although not free of protozoa, represent a relatively better source of drinking-water compared to sources of surface-water. The preferred source of water would be shallow wells protected from surface inflow by a wall (15) used in conjunction with low-cost treatment methods. Although tubewells have low levels of protozoa, these may be chemically polluted with fluoride as a result of the local geology (17). Shallow wells are currently threatened by ongoing rehabilitation work that includes the lining of canals, leading to reduced recharge of shallow groundwater (14). An integrated approach to rural water supply is needed, considering the needs of both agricultural and domestic water, to safeguard adequate, reliable, and healthy water supply for the rural population in Uda Walawe.

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