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 Cryptosporidium 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)
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.

**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](image)
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 °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 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.

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 °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 12 µm.

RESULTS

Giardia spp.

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

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).

**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).

**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 ther-
motolerant coliform bacteria. The results of thermoto-
lerant 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 thermo-
tolerant 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 Cryptos-
poridium oocysts were independent of the time of sam-
ping over the study period (August-November).

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 find-
ings 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 popu-
lation. 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 conta-
mination in this study area include both human and ani-
mal (water buffalo), since animal sources are known to
be important in the introduction of these protozoa to a
water system (23). Water buffaloes are common in the
study area and are often observed to be bathing and drink-
ing in canals. A further study to differentiate between
faecal contamination by human and animal would indi-
cate the extent to which water buffaloes or other domes-
tic animals are contributing to the contamination. Methods
to reduce buffalo faeces in water from entering the sys-
tem 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 pro-
azoan 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 num-
ber 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 wa-
ter quality in different water sources to childhood diar-
rhoeal 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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