OCCURRENCE OF *GIARDIA* CYSTS AND *CRYPTOSPORIDIUM* OOCYSTS IN THE TEMUAN ORANG ASLI (ABORIGINE) RIVER SYSTEM

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Abstract. A survey of the river water frequently used by the Temuan Orang Asli (aborigine) indicated that 66.7% of the river water samples were *Giardia* cyst positive and 5.6% were *Cryptosporidium* oocyst positive. Although *Giardia* cysts were detected in samples from all the sites (*eg* upstream, midstream, and downstream), *Cryptosporidium* was only present in one river water sample taken from downstream from a village. The only sample of upstream water which contained *Giardia* cysts had a concentration of 0.7 cysts/l. All samples taken from midstream contained cysts with a mean concentration of 9.8±6.6 cysts/l (range = 1-20 cysts/l). Eighty-three point three percent of the samples collected from downstream contained cysts and 16.7% had oocysts. The average concentration of cysts was 12.9±16.4 cysts/l (range = 0-44 cysts/l), whereas the oocyst concentration was 0.4 oocysts/l. All river samples tested positive for the presence of *E. coli*, indicating fecal contamination. The results of this study imply that the river system is contaminated with fecal-oral transmitted parasites. The river water, used by the Orang Asli, is a probable route for *Giardia* and *Cryptosporidium* transmission in this community. Long term strategies, incorporating health education regarding personal hygiene, and provision of toilets and the importance of their proper usage, need to be embraced by this community in order to control the spread of these parasites.

INTRODUCTION

The dramatic emergence of waterborne zoonoses, such as *Giardia* and *Cryptosporidium*, has caused great public concern in the international community. Waterborne outbreaks by these two protozoa are well documented with more than 120 outbreaks infecting over 600 million people worldwide (Smith and Lloyd, 1997; Smith and Rose, 1998; Fayer *et al*, 2000). The largest *Cryptosporidium* outbreak happened in the early spring of 1993 in Milwaukee, Wisconsin, USA. An estimated 1.5 million consumers were exposed to *Cryptosporidium* contamination in the public water supply. Out of this, 403,000 became ill and 104 died (most of them were immunocompromised) (MacKenzie *et al*, 1994).

Both *Giardia* cysts and *Cryptosporidium* oocysts are found in the aquatic environment, in low numbers, depending on the contributing factors in the catchment area (Smith and Rose 1990; Anonymous 1995; Smith *et al* 1995, 1997). The size of a community, as well as the rate of infection within the population, will influence the numbers of cysts and oocysts discharged from that community. Communities with more susceptible hosts (human beings and livestock) will discharge higher densities of cysts and oocysts into the environment than those with less susceptible hosts.

Because the minimum infectious dose for *Giardia* and *Cryptosporidium* is low, and cysts and oocysts can remain viable for long periods of time at low temperatures, low contamination levels of potable water can potentially result in infection of numerous consumers. *Giardia* cysts and *Cryptosporidium* oocysts excreted by infected humans and other animals can contaminate surface waters either though feces, sewage effluent, slurry discharges, or run-off from the land.

The occurrence and concentration of these organisms in surface waters is likely to be higher in developing countries where contamination of water by human and animal waste is more likely to be prevalent. To date, there have been relatively few studies of the presence of *Giardia* cysts and *Cryptosporidium* oocysts in river water in Malaysia (Ahmad, 1995). The first report of these proto-

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zoa in Malaysian water resources was in early 1992 (Ahmad and Chan, 1994). Before this, giardiasis and cryptosporidiosis were largely unknown hazards. In a collation of various water sample studies carried out by Ahmad (1995) in Malaysia, 19.7% were positive for *Giardia* cysts and 10.5% for *Cryptosporidium* oocysts. Other studies have also documented the presence of *Giardia* cysts and *Cryptosporidium* oocysts in the Malaysian river water that supplies water for water treatment plants (Ahmad *et al*, 1997; Tan, 1998).

In Malaysia, rivers play an important role as arteries that channel water to local riverine communities, which include the Orang Asli communities. A majority of the Temuan Orang Asli are permanent settlers living near rivers. These Orang Asli communities usually concentrate themselves in the upper reaches of rivers and their tributaries. Rivers not only provide them with a livelihood and sustenance, but also with ample water for their daily activities.

Although the Temuan community living in Kampung Kuala Pangsun, Hulu Langat are provided with free basic amenities, such as electricity, pipe water (treated water) and pour flush toilets, the river still plays a vital role as a source of water for them. Various activities are carried out at the river. These range from washing clothes, bathing, playing, swimming, cooking, and drinking. Unfortunately, it is common for the river to have a dual role, both as a source of 'clean' water, and at the same time as a huge, convenient drain for a potpourri of Orang Asli wastes. This is especially true in this particular community, as some of the inhabitants have admitted to defecating along river banks or in the river; and using river water to clean themselves. It is well known that waterborne diseases are spread by drinking or washing in contaminated water. Hence, there is a need to investigate the level of contamination with *Giardia* cysts and *Cryptosporidium* oocysts in the river water used by the members of this community. The data collected from this study will enable an assessment of the role of the river as a transmission route.

MATERIALS AND METHODS

Detection of *Giardia* cysts and *Cryptosporidium* oocysts

A six-month survey was conducted on the Sungai Lopo river, which is situated just behind the Orang Asli village. Most of the houses are scattered along the bank of Sungai Lopo. Bathing, washing clothes and defecating are done directly in the river. River water, from three different sites (*eg* upstream, midstream, downstream to the community), was sampled by filtering 50 liters of water directly from each of the three locations of the river through a polypropylene fiber-wound depth cartridge filter (Ametek, Wisconsin, USA, nominal pore size = 1 μm) with a flow rate of 1.5 liters per minute (using a flow restrictor). Filters were removed from the housing, placed into plastic bags and transported to the laboratory in an ice box (4ºC to 8ºC) for processing.

River water samples were processed according to the procedures recommended by the UK Standing Committee of Analysts (SCA), Department of the Environment (Anonymous, 1990) with some minor modifications. The cartridge filter, whose matrix contained entrapped (oo)cysts was cut longitudinally and teased apart. The filter fibers were then divided into two halves. Each half was placed into double-layered plastic bags and mechanically agitated in 1 liter of 0.1% Tween 80 (Sigma Chemical, Missouri, USA) for 10 minutes using a stomacher (Seward Model 3500, Ohio, USA) to release the entrapped organisms and particulates from the filter matrix. This procedure was repeated once. The eluate was then stored at 4ºC, if it was not processed immediately.

The supernatant of the filter eluate was filtered through a 1.2 μm pore size cellulose ester (combination of nitrate and acetate) membrane filter (Catalog no. RAWP 142 SO, 142 mm diameter, Millipore, Bedford, Massachusetts) until the meniscus was 3 cm from the base of the container. The rest of the sediment was concentrated by centrifugation (Jouan B3-11, Saint-Herblain, France) at 1,500*g* for 10 minutes in 50 ml conical bottomed centrifuge tubes. Membrane filters were eluted using 0.1% Tween 80 and the wash water was concentrated by centrifugation (1,500*g* for 10 minutes). Pellets from the sedimentation and the membrane filter were pooled, centrifuged, resuspended and brought down to 20 ml. The 20 ml suspension was divided into two equal portions. Portion A was used for the detection of *Giardia* cysts and portion B for the detection of *Cryptosporidium* oocysts.

Before the clarification step, by discontinuous density gradient sucrose flotation, portion A was added to an equal volume (10 ml) of 2%

Tween 80 and portion B to 2% Tween 80 in 2% sodium dodecyl sulphate (SDS) (Sigma Chemical, Missouri, USA) to reduce aggregation. The detergents act by disrupting hydrophobic and electrostatic interactions between the (oo)cysts and the sediment. The samples were vortexed for 30 seconds and centrifuged (1,500*g* for 10 minutes). After centrifugation, the supernatants were aspirated down to 10 ml and the pellets resuspended by vortexing.

Samples were slowly underlayered with 10 ml of cold sucrose solution [a solution with a specific gravity of 1.18 (May and Baker, Dagenham, England)] and centrifuged (1,000*g*, 5 minutes). The principle of this step is that the particles which are more dense will sink to the base whereas cysts and oocysts which are less dense than the sucrose solution will float (Fricker, 1995). The entire supernatant, including the interface, was recovered without disturbing the pellet and decanted gently into clean 50 ml conical centrifuged tubes. The residual sucrose was removed by washing three times in distilled water (for portion A) or pH 7.2 phosphate buffered saline (PBS) (Oxoid, Hampshire, UK) for portion B. The final concentrated sample was reduced to a final volume of 1 to 5 ml, depending on how turbid the samples were. The equivalent volumes of sample examined for *Giardia* and *Cryptosporidium* were maintained between 2.5 and 10 liters which was 5% to 20% of the original filtered volume.

Twenty-five µl aliquots of each sample concentrate were placed in each of the four wells of a teflon®-coated microscope slide and air dried (room temperature = 24º±1ºC). Samples were methanol-fixed by dropping 50 µl of absolute methanol onto each well and leaving it to dry. An equal volume of bovine serum albumin (BSA) was spotted into each well and left at room temperature for 15 minutes. The BSA was then aspirated. Each well containing concentrates of samples A or B was overlaid with an equal volume (25 µl) of commercially available fluorescein isothiocyanate (FITC)-labelled anti-*Giardia* cysts monoclonal antibody (mAb) (Waterborne, Inc, New Orleans, Los Angelas, USA) (spotted onto concentrates of sample A), or (FITC)-labelled anti-*Cryptosporidium* oocysts mAb (spotted onto concentrates of sample B), which recognizes (oo)cyst-specific surface-exposed epitopes. These monoclonal antibodies were used because they were more sensitive than conventional stains (McLauchlin *et al* 1987; Erlandsen *et al* 1990). Slides were incubated in a humidity chamber for 30±5 minutes at 37ºC. This was to prevent the monoclonal antibodies from drying out, since dried out monoclonal antibodies form crystals.

Excess antibody was removed by rinsing the slides twice with 50 µl of PBS dropped into each well. A 2 mg/ml DAPI (Sigma Chemical, Missouri, USA) solution (prepared in absolute methanol) was diluted 1/5000 in PBS to yield a dye concentration of $4x10^{-4}$ mg/ml. The presence of sporozoite nuclei was highlighted by staining them with aliquots (25 µl) of $4x10^{-4}$ mg/ml DAPI (4′, 6-diamidino-2-phenylindole) solution for 2 minutes and later rinsed with distilled water. Twenty µl of mounting medium (PBS:glycerol, 1:1 v/v) was inserted into each well and coverslips were applied to the slides. They were then examined under a x400 blue filter epifluorescence microscope (Carl Zeiss Axioscope, Jena, West Germany, excitation BP 450-490 nm, beamsplitter FT 510 and emission LP 520 nm) to detect the FITC stain, with a UV filter (excitation G 365 nm, beamsplitter FT 395 and emission LP 420 nm) for the DAPI stain and Nomarski-DIC optics to observe the internal structures. Positive control slides containing cysts and oocysts and negative control slides were included in the analysis.

Giardia cysts are ovoid in shape, and 8 to 14 µm long and 7 to 10 µm wide. *Cryptosporidium* oocysts are spherical in shape with a diameter ranging from 4 to 6 µm (Anonymous, 1990). Enumeration of the cysts and oocysts in the water sample was based on the following formula (Anonymous, 1990):

$$\text{No. of (oo)cysts per liter} = \frac{N \times C}{A \times {}^{1}/_{2}F}$$

N = number of (oo)cysts observed on the slide
A = analysed volume (µl)
C = concentrated volume (ml)
F = filtered volume (l)

The recovery efficiency of the method when 50 liters of tap water were seeded with 10^4 *C. parvum* oocysts, ranged from 7-11% (mean = 9.6±1.6%). The minimum detection levels ranged from 1 oocyst in 350 ml to 1 in 5 liters, depending on the final volume of the concentrate.

Detection of fecal coliforms

Fecal coliforms were isolated and enumerated using the membrane filtration technique, as described by the Bacteriological Examination of

Drinking Water Supplies, 1982, Department of the Environment, Department of Health and Social Security and Public Health Laboratory Service, UK (Anonymous, 1983). The organisms produce characteristic colonies on membrane lauryl sulphate agar, both recognized by the production of yellow colonies at 44.5ºC±0.5ºC for fecal coliforms. Water samples were collected in sterile 500 ml Schott bottles, which were transported to the laboratory in a coolbox and processed immediately after sampling.

Samples were filtered through cellulose acetate membranes (Millipore, Bedford, Massachusettes, USA, 0.45 μm porosity, 47 μm diameter) held in a filter holder (Nalgene, New York, USA, 300-4050 Filter Holder, PSF) and the membranes were placed on membrane lauryl sulphate (Oxiod, Hampshire, UK) agar plates. All dilution of river water samples (whenever necessary, between 0.1 ml and 100 ml) was done with sterile Ringer's Quarter Strength solution (Oxoid, Hampshire, UK). Plates were sealed with parafilm, placed into airtight plastic bags and incubated at 30ºC for 4 hours to resuscitate injured bacteria before they were immersed in a 44.5ºC water-bath (Grant, Cambridge, England) for 14 hours.

The organisms grow selectively on membrane lauryl sulphate agar at 44.5ºC. Production of acid by lactose-fermenting colonies changed the color of the phenol red indicator in the medium from blood red to yellow. The yellow fecal coliform colonies (regardless of size) were enumerated. Results were reported as a mean of two replicates, as colony forming units per 100 ml (CFU/100 ml). Confirmation of the presence of *Escherichia coli* was done using the Colisure® assay.

Measurement of physical parameters

In this study, the physical parameters measured were pH, temperature, conductivity and turbidity. These parameters were measured *in situ* using the relevant apparatus. The pH and temperature were determined using a Water Quality Test Kit (Ciba Corning Diagnostics Limited, Essex, England). Turbidity was measured using a turbidity meter (ICM, Oregon, USA, 16800 model) and conductivity was measured using a conductivity meter (Ciba Corning Diagnostics Limited, Essex, England).

Statistical analyses

Analysis of varians were performed using statistical functions in Minitab®, Release 12.1. The same statistical software package was used to calculate the *t*-values and the levels of significance (p). Significance was defined as $p<0.05$.

RESULTS

Occurrence of *Giardia* cysts, *Cryptosporidium* oocysts and fecal coliforms in the Sungai Lopo River

Upstream. It was found that only one river water sample (16.7%) from upstream was contaminated with *Giardia* cysts (Table 1). The mean density of *Giardia* cysts per liter was 0.1±0.3 (range = 0-0.7 cysts/l). There were no *Cryptosporidium* oocysts found in any of the samples. All the water samples were contaminated with feces, as shown by the presence of fecal coliforms, with a mean of $2.5 \times 10^3 \pm 3.1 \times 10^3$ CFU/100 ml (range = 5.4×10^2-8.7×10^3 CFU/100 ml).

Midstream. Cysts were routinely recovered from the water samples taken from midstream. The mean concentration of *Giardia* cysts per liter was 9.8±6.6 (range = 1-20 cysts/l) (Table 2). No *Cryptosporidium* oocysts were found in any of the samples. The water was contaminated with fecal coliforms at a mean concentration of $4.0 \times 10^4 \pm 3.2 \times 10^4$ CFU/100 ml (range = 7.2×10^3 -8.7×10^4 CFU/100 ml). Statistical analysis showed that there was no significant correlation ($p>0.05$) between the occurrence of *Giardia* cysts and fecal coliforms.

Downstream. All samples, except one (83.3%), taken from downstream were found to contain cysts. *Giardia* cysts were not found in that one sample probably due to debris which was obscuring the cysts. On 4/8/97, only 20 liters of sample was collected due to high turbidity reading (48.32 NTU). The mean concentration of *Giardia* cysts per liter was 12.9 ± 16.4 (range = 0-44 cysts/l) (Table 3). Only one sample (16.7%) was contaminated with *Cryptosporidium* oocysts. The concentration of oocysts per liter was 0.4 oocysts/l. The water was contaminated with fecal coliforms at a mean concentration of $3.0 \times 10^4 \pm 2.5 \times 10^4$ CFU/100 ml (range = 5.6×10^3 -7.5×10^4 CFU/100 ml). There was no significant correlation ($p>0.05$) between the occurrence of *Giardia* cysts and fecal coliforms.

Table 1
Occurrence and density of cysts, oocysts, and fecal coliforms in river water samples upstream from the village.

Sampling	Date	*Giardia* (cysts/l)	*Cryptosporidium* (oocysts/l)	Fecal coliform counts (CFU/100 ml)
1	6/5/97	0	0	9.8×10^2
2	12/6/97	0	0	8.7×10^3
3	8/7/97	0	0	5.4×10^2
4	4/8/97	0	0	1.3×10^3
5	2/9/97	0	0	1.4×10^3
6	31/10/97	0.7	0	1.9×10^3
Mean ± SD		0.1 ± 0.3	0	$2.5 \times 10^3 \pm 3.1 \times 10^3$
Range		0-0.7	0	5.4×10^2 -8.7×10^3

Table 2
Occurrence and density of cysts, oocysts, and fecal coliforms in river water samples midstream from the village.

Sampling	Date	*Giardia* (cysts/l)	*Cryptosporidium* (oocysts/l)	Fecal coliform counts (CFU/100 ml)
1	6/5/97	1	0	7.2×10^3
2	12/6/97	13.2	0	8.7×10^4
3	8/7/97	20	0	6.2×10^4
4	4/8/97	6.8	0	1.2×10^4
5	2/9/97	11.6	0	5.5×10^4
6	31/10/97	6.0	0	1.8×10^4
Mean ± SD		9.8 ± 6.6	0	$4.0 \times 10^4 \pm 3.2 \times 10^4$
Range		1-20	0	7.2×10^3 -8.7×10^4

Physical parameters

Upstream. The physical parameters of the upstream water samples is shown in Table 4. The mean temperature of the river water upstream was 24.75ºC ± 0.82ºC (range = 23.5 - 26.0ºC). The pH values ranged from 5.86 to 6.67 (mean ± SD = 6.24 ± 0.34). The mean conductivity and turbidity were 28.73 mS/cm ± 3.66 mS/cm (range = 25.2-35.2 mS/cm) and 8.28 NTU ± 0.29 NTU (range = 7.96-8.74 NTU), respectively.

Midstream. The physical parameters of the river water samples midstream are shown in Table 5. The temperature of the river water midstream ranged from 24.0 to 26.0ºC (mean ± SD = 25.25ºC ± 0.76ºC). The pH values ranged from 5.96 to 8.12 (mean ± SD = 6.88 ± 0.80). The mean of conductivity was 29.95 µS/cm ± 2.30 µS/cm (range = 26.2-31.7 µS/cm). Meanwhile the mean turbidity was 8.66 NTU ± 0.75 NTU (range = 8.14-10.15 NTU).

Downstream. The physical parameters of the river water samples downstream are shown in Table 6. The mean temperature of the river water downstream was 26.5ºC ± 1.0ºC (range = 25.5-28.0ºC). The pH values ranged from 6.40 to 8.22 (mean±SD = 7.48 ± 0.67). The mean of conductivity was 27.90 µS/cm ± 2.34 µS/cm (range = 24.4-31.3 µS/cm). The mean turbidity was 15.11 NTU ± 16.29 NTU (range = 7.76-48.32 NTU).

The turbidity of the water samples from all the sites was in Class IIB (moderately polluted river) of the Interim National Water Quality Standard (INWQS) level of 50 NTU for Malaysia. This means there was no apparent turbid wastewater discharge from agricultural activities or industries. It is interesting to note that the recorded turbidity downstream showed a peak in turbidity

Table 3
Occurrence and density of cysts, oocysts, and fecal coliforms in river water samples downstream from the village.

Sampling	Date	*Giardia* (cysts/l)	*Cryptosporidium* (oocysts/l)	Fecal coliform counts (CFU/100 ml)
1	6/5/97	12.4	0	8.6×10^3
2	12/6/97	3.6	0	7.5×10^4
3	8/7/97	44	0.4	3.0×10^4
4	4/8/97	0	0	3.5×10^4
5	2/9/97	15.6	0	2.8×10^4
6	31/10/97	1.8	0	5.6×10^3
Mean ± SD		12.9 ± 16.4	0.1 ± 0.2	$3.0 \times 10^4 \pm 2.5 \times 10^4$
Range		0 - 44	0 - 0.4	5.6×10^3-7.5×10^4

Table 4
Physical parameters of river water samples upstream from the village.

Sample	Date	Temperature (ºC)	pH	Conductivity (μSiemens/cm)	Turbidity (NTU)
1	6/5/97	26.0	6.67	25.2	8.74
2	12/6/97	25.0	6.17	26.3	8.08
3	8/7/97	23.5	6.06	27.0	8.14
4	4/8/97	24.5	6.64	35.2	8.48
5	2/9/97	24.5	5.86	30.6	7.96
6	31/10/97	25.0	6.03	28.1	8.30
Mean ± SD		24.75 ± 0.82	6.24 ± 0.34	28.73 ± 3.66	8.28 ± 0.29
Range		23.5 - 26.0	5.86 - 6.67	25.2 - 35.2	7.96 - 8.74

on the 4th of August 1997. This was because it had rained the day before.

DISCUSSION

All three sampling points contained *Giardia* cysts, but only one downstream river sample contained *Cryptosporidium* oocysts. Sixty-seven percent of the river water samples were cyst positive and 6% were oocyst positive. All the samples tested positive for fecal coliforms. The presence of *Escherichia coli* was confirmed by the Colisure® assay.

As long as there is contamination with fecal coliforms, there is a risk that *Giardia* or *Cryptosporidium* may be present, because *Giardia* and *Cryptosporidium* are spread through the fecal-oral route. The fact that the samples were contaminated with *Giardia* cysts implies that some person or animal must have defecated there. Statistical analysis showed that there was no significant correlation between the occurrence of *Giardia* cysts and fecal coliforms. Fecal coliforms have been shown to have no correlation with *Giardia* or *Cryptosporidia* (Smith, 1992; Wallis, 1994; Robertson, 1995).

In a study to determine the occurrence of *Giardia* cysts and *Cryptosporidium* oocysts at the Orang Asli village, it was found that 22% of the rats caught on the compound and 35.3% of the house rats harbored *Giardia* cysts and *Cryptospridium* oocysts in their feces. With such a high occurrence rate, the possibility that these rats contaminated the river is high (Lim and Ahmad, 2001).

With the possibility of human and animal contamination, it is important to differentiate the human-pathogenic *Giardia* cysts and *Cryptosporidium* oocysts from those that do not infect humans, and to track the source of these (oo)cysts.

Table 5
Physical parameters of river water samples midstream from the village.

Sample	Date	Temperature (ºC)	pH	Conductivity (µS/cm)	Turbidity (NTU)
1	6/5/97	26.0	6.40	26.2	8.52
2	12/6/97	26.0	5.96	28.2	8.29
3	8/7/97	24.0	6.43	31.7	8.14
4	4/8/97	25.0	6.91	31.7	8.60
5	2/9/97	25.0	7.47	31.7	8.24
6	31/10/97	25.5	8.12	30.2	10.15
Mean ± SD		25.25 ± 0.76	6.88 ± 0.80	29.95 ± 2.30	8.66 ± 0.75
Range		24.0 - 26.0	5.96 - 8.12	26.2 - 31.7	8.14 - 10.15

Table 6
Physical parameters of river water samples downstream from the village.

Sample	Date	Temperature (ºC)	pH	Conductivity (µS/cm)	Turbidity (NTU)
1	6/5/97	27.5	6.40	24.4	8.24
2	12/6/97	28.0	7.27	27.8	9.79
3	8/7/97	26.0	7.97	26.5	7.99
4	4/8/97	26.0	7.14	28.3	48.32
5	2/9/97	26.0	7.87	29.1	7.76
6	31/10/97	25.5	8.22	31.3	8.56
Mean ± SD		26.5 ± 1.0	7.48 ± 0.67	27.90 ± 2.34	15.11 ± 16.29
Range		25.5 - 28.0	6.40 - 8.22	24.4 - 31.3	7.76 - 48.32

The use of the rRNA-based PCR-restriction fragment length polymorphism (RFLP) technique to detect and differentiate *Cryptosporidia* of human and animal genotypes has showed promising results, which might be employed to elucidate this dilemma (Xiao *et al*, 2001).

Survival studies have shown that *C. parvum* oocysts in the rivers of Malaysia can survive up to 3 months (Lim *et al*, 1999). With such a long survival period, *C. parvum* oocysts in the river could be a source of transmission to other village members. This is important because *Cryptosporidium* is known to have a low infectious dose.

A spiking phenomenon of *Giardia* cysts (cyst concentration higher than the overall trend) was observed on 8/7/97 in the downstream water sample. A parallel increase was also observed in the midstream sample. One possible cause for why this happened could be that there was a greater concentration of cysts per gram of feces excreted by members of the community, or more people who had gone to ease themselves that morning (a norm practice in the village) were infected with giardiasis. An analysis of peak turbidities and cyst concentrations for these two sites showed that peak turbidity values did not occur during times of peak cyst concentrations. Studies have shown that turbidity levels did not correlate with the presence of *Giardia* (Wallis, 1994).

The failure of coliform testing and turbidity measurements to predict the presence of *Giardia* cysts has been proven in other studies (Wallis, 1994; Okun, 1996; Ho and Tam, 1998). Therefore, direct detection of *Giardia* cysts and *Cryptosporidium* oocysts in the water is necessary because classical bacterial indicators (*eg E. coli*, fecal coliform) cannot be relied upon to indicate their presence (Akin and Jakubowski, 1986; Rose *et al*, 1988). This is true because bacterial indicators are more susceptible to environmental stresses (Oliveri, 1982), and chlorination (WHO, 1984; Sobsey, 1989; Smith, 1992) compared with

robust protozoan cysts and oocysts.

It was reported that cysts were not detected in downstream water on 4/8/97 when the turbidity reached its highest peak (48.32 NTU). A large amount of particulate matter was concentrated in the sample, making it difficult to detect either cysts or oocysts. The increased volume of concentrate means that a smaller equivalent volume of the original sample was examined, thereby reducing the detection rate. High turbidity values (particularly clay content), organic matter and algae concentration can affect cyst and oocyst detection rates (Rose *et al,* 1989; Thompson and Boreham, 1994; Wallis, 1994; Rodgers *et al,* 1995). Cysts and oocysts can be obscured by debris, resulting in false negatives (Bifulco and Schaefer, 1993; Smith *et al,* 1995; Feng *et al,* 2003).

Interfering autofluorescent debris, such as plant and algae particles, can complicate the procedure making identification of cysts and oocysts very difficult (Vesey *et al,* 1994; Rodgers *et al,* 1995). Such problems are becoming well recognized in the analyses for cysts and oocysts in turbid river water samples (Rose *et al,* 1989; Smith and Rose, 1990; Robertson and Smith, 1992). The data collected can underestimate the true concentrations of *Giardia* cysts and *Cryptosporidium* oocysts present. In our study, a similar problem was encountered. In the future, other methods will have to be used. Emerging technologies, such as the use of flow cytometry, immuno-magnetizable separation and dielectrophoresis for the detection of these protozoa have been described by other scientists (Smith, 1995; Watkins *et al,* 1995). Molecular techniques which are rapid and have improved specificity and sensitivity should be employed to further enhance the findings in environmental water samples, in which densities of *Giardia* and *Cryptosporidium* are very low (Jellison *et al,* 2002; Sturbaum *et al,* 2002; Guy *et al,* 2003; Nichols *et al,* 2003).

Outbreaks which occurred in Ayrshire, Swindon, and Bradford in the UK and in Milwaukee in the USA had oocysts detected in treated water at densities of less than 0.4 per liter (Smith *et al,* 1989; Richardson *et al,* 1991; MacKenzie *et al,* 1994; Atherton *et al,* 1995). If outbreaks can occur at the concentrations which were found in the downstream water (0.4 oocysts/l), then there is a risk of an outbreak if people drink the river water while bathing, playing or swimming.

This study proves that the river water is contaminated with feces which contain *Giardia* cysts and *Cryptosporidium* oocysts. It has been reported that this community is infected with *Giardia* and *Cryptosporidium* at 18.9% and 5%, respectively (Lim *et al,* 1997). From the questionnaire, it was discovered that some of the Orang Asli who harbored *Giardia* and *Cryptosporidium* (oo)cysts defecated in the river. It is common for Orang Asli to defecate in the river because they feel uncomfortable using a toilet.

The river is also used for bathing, washing, playing, swimming, drinking, and cooking. Although 95% of households are supplied with chlorinated water from the Water Supply Department (JBA), the remaining households get their water from the river (unchlorinated) which is supplied to their houses through gravity-feed pipes. On days when the water supply from the JBA is cut, the Orang Asli resort to the use of river water. The practice of defecating in the river and using the river for other purposes enables transmission of these parasites to the Orang Asli through the fecal-oral route. Recent advancements in molecular techniques to detect and genotype *Giardia* and *Cryptosporidium* may be useful in this context to elucidate the source of the contamination (Xiao *et al,* 2000; 2001; Amar *et al,* 2002; Tanriverdi *et al,* 2002).

The findings of this study reveal that other rivers in Malaysia may be contaminated with fecal-oral transmitted parasites. This is because many Orang Asli communities are at the upper reaches of rivers, and they use the river for many activities, including defecation. Some of the rivers are used as sources of drinking water for urban communities. If water treatment is inadequate, the drinking water may be contaminated with sufficient numbers of parasites to cause illness. Rivers may be one of the transmission routes for fecal-oral transmitted parasites. Long term strategies incorporating health education regarding personal hygiene, the provision of toilets and the importance of their use need to be adopted by Orang Asli villages in order to control the spread of these parasites.

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