Tropical Biomedicine 26(3): 289–302 (2009)

Occurrence of *Giardia* and *Cryptosporidium* (oo)cysts in the river water of two recreational areas in Selangor, Malaysia

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Received 19 August 2009, received in revised form 7 October, accepted 8 October 2009

Abstract. This study is the first report on the occurrence of *Giardia* and *Cryptosporidium* (oo)cysts in recreational rivers water from Malaysia. It was carried out in water samples at two rivers, 'Sungai Congkak' and 'Sungai Batu', located in Selangor State. The occurrence of both *Giardia lamblia* and *Cryptosporidium parvum* (oo)cysts was higher in Sungai Congkak (50% or 15/30 and 10% or 3/30 respectively) than Sungai Batu (16% or 5/30 and 3.3% or 1/30 respectively). The mean density of cysts/L was 0.72 in Sungai Congkak and 0.023 in Sungai Batu, and that of oocysts/L was 0.023 in Sungai Congkak and 0.0033 in Sungai Batu, showing that the occurrence of *Giardia* was higher and more frequent than *Cryptosporidium* in both rivers. Sungai Congkak also showed higher faecal coliforms count (ranging from 0.48×10^3 to 73×10^3 CFU/100 mL) than Sungai Batu (0.41×10^3 to 16×10^3 CFU/100 mL). On the other hand, the *Giardia* and *Cryptosporidium* (oo)cysts and faecal coliforms were more concentrated at the downstream station, followed by midstream and upstream stations which might be due to human factors where settlements and recreation areas were located around and between midstream and downstream stations. The (oo)cysts and faecal coliforms also increased during public holidays due to the significantly higher number of visitors (bathers) compared with the week days. All the parameters (physical, faecal coliforms and rainfall) did not show consistent significant correlation (based on r values of Pearson correlation analysis) with both protozoa, therefore these parameters are not suitable as indicator for the presence of *Giardia* and *Cryptosporidium* (oo)cysts in both rivers.

INTRODUCTION

The occurrence of the (oo)cysts of *Giardia lamblia* and *Cryptosporidium parvum* in water supplies is globally acknowledged as a public health problem, with many documented outbreaks of waterborne diseases. The (oo)cysts of these organisms can pose considerable danger to the public because they have a long survival period in water and low infectious dose. To-date, there is no safe and effective treatment for giardiasis and cryptosporidiosis, especially if infections occur in young children and immunocompromised patients. Most giardiasis and cryptosporidiosis cases or outbreaks have been reported involving drinking water, swimming pools, spas or similar recreational-water environments, but the threat posed by these organisms in rivers-based recreational water has not been studied extensively. Since the potential contributing factors (infected animals and human faeces/sewage) are present in the vicinity, the possibility of these organisms to contaminate rivers-based recreational water and causing outbreaks of waterborne illness always exist. In this study, two recreational rivers were selected to study the occurrence of the (oo)cysts and possible contributing factors and parameters of the rivers water, for a period of ten months from August 2004 to May 2005.

MATERIALS AND METHODS

Sampling site and collection of water samples

Two recreational rivers, Sungai Congkak and Sungai Batu, located in Selangor State, Malaysia, were selected. Three (3) sampling stations – upstream, midstream and downstream – of each river were selected. The distance between each sampling station was about 3000 meters. The surface water samples of about 11 litres were collected in sterile bottle at each sampling station, once a month for 10 months continuously, starting from August 2004 to May 2005. Out of these 10 visits, 5 were carried out during the week days and another 5 during weekends or public holidays.

Measurement of the water physical parameters

The physical parameters of water were measured *in situ* during every sampling activities. These parameters included turbidity (NTU) by using turbidity meter (model 2100P), temperature (°C) by laboratory thermometer, conductivity (mS/cm) by SCT meter (model YSI 30), pH by portable pH meter and dissolved oxygen (mg/L) by oxygen meter (model YSI 58). The parameters data obtained from each sampling activity were recorded and accumulated for the correlation analysis with the amount of the (oo)cysts detected.

Measurement of the rainfall data

Rainfall data from two rainfall stations were obtained from the Department of Meteorology, Petaling Jaya, Selangor, Malaysia. These two stations, the Ulu Langat Dam and Ulu Gombak, are the nearest to the study areas of Congkak and Batu rivers respectively.

Detection of *Giardia* and *Cryptosporidium* (oo)cysts

The detection of (oo)cysts involved, firstly, concentration of water sediments. A 10 litres water sample was filtered by using a filter membrane 1.2 mm pore size (mixed cellulose ester, Millipore) which was set up with holder and peristaltic pump (Millipore). The filter membrane was replaced with a new one when there was ~~a~~ blockage of the water flow. The filter membrane(s) were collected and placed in sterile petri dish(es). The sediments were scrapped using plastic scrapper (32 cm, Nunc) and eluted with 40 mL of 0.1% Tween-80 solution. The membrane was then carefully lifted up, folded, cut into smaller pieces and placed in 50-mL conical tube. It was then washed at least three times with 15 mL Tween-80 (0.1%) followed by shaking for 10 minutes for each wash. The eluate and washed solutions were collected in sterile 50-mL conical tubes and centrifuged at 4000 rpm for 15 minutes in swinging-bucket rotor (KUBOTA 2010). The supernatant was removed leaving 10 mL above the pellet. The pellet was then resuspended, pooled and concentrated several times by centrifugation until 5 mL concentrated suspension was obtained out of 10 litres original water sample. Secondly, (oo)cysts were separated from water sediments. All the above 5 mL concentrated suspension was added with a 5 mL of A-IMS buffer™ from the immunomagnetic separation (IMS) kit (Aureon Biosystem GmbH, Vienna, Austria) and the methods as described in the method 1623 of United States Experimental Protection Agency (U.S. EPA) were followed. Thirdly, (oo)cysts in IMS product were detected by using fluorescent-antibody (FA) test kit and 4',6-diamidino-2-phenylindole (DAPI) counter-staining (EasyStain™, BTF), following the manufacturer's procedure. Finally, the (oo)cysts were confirmed by observation under epifluorescence with differential interference contrast microscopy (Olympus BX51). The observation was first carried out under 200 X magnification with a blue filter block (490 nm excitation wave length and 510 nm emission wavelength), followed by 400 X with UV filter block (400 nm excitation wavelength and 420 nm emission wavelength) for visualization of 4'6-diamidino-2-phenyl indole (DAPI). The internal morphology of (oo)cysts was determined by using Normaski differential interference contrast (DIC) at magnifications of 400 X and

1000 X. The appearance and size of the (oo)cysts were then compared with the positive and negative control samples.

Detection of faecal coliforms

Water sample were collected in 500 mL sterile Schott-bottles from sampling stations. It was placed in a cool box, transported to the laboratory and processed within 8 hours of sampling. Faecal coliforms were then detected by using membrane filtration technique established by Anon (1983). Briefly, the bottle was shaken to resuspend the water sample and then sieved with sterile stainless sieve to separate from large particles. Only 100 mL of this sieved water sample was used in this experiment, while the rest was kept (as stock) in 4ºC for repeat experiment (if necessary). Each of 50 mL (out of 100 mL) was further filtered through a cellulose ester membrane (0.45 mm pore size, 47 mm diameter, Millipore), fitted on its glass filter holder (Nalgene), using a vacuum pump. Filtered membrane (with debris) was carefully lifted and placed on top of the surface of a lauryl sulphate membrane (Oxoid) which was first placed on the sterile plate. The plate was closed, sealed with parafilm and incubated at 30ºC for 4 hours to resuscitate injured bacteria before they were further incubated in a 44.5ºC incubator for 14 hours. Faecal coliforms (yellow colonies) were counted, recorded as mean (of two replicates plates) colony forming unit per 100 mL (CFU/0.1L). For those samples with heavy colonies (or uncountable), this experiment was repeated by using 100 mL of similar water sample from the stock, diluted (between 0.1 to 1.0 dilution) with sterile Ringer's solution (Oxoid) before filtration.

Statistical analysis

Linear Regression analysis was carried out by using a statistical software package (SPSS version 11.5).

RESULTS

Physical parameters data

The date of sampling and measurements of the dissolved oxygen (DO), pH, temperature, conductivity and turbidity of the water collected from the upstream, midstream and downstream stations of the studied rivers, Sungai Congkak and Sungai Batu, are respectively shown in Table 1 (A & B).

Rainfall data

Rainfall measurements at Ulu Langat Dam (nearest to Sungai Congkak) ranged from 522.7 mm^3 (highest, November 2004) to 1.6 mm^3 (lowest, January 2005) with the overall mean ± SE of 181.22 ± 86.28 mm^3. For the Ulu Gombak (nearest to Sungai Batu), the range was 521.4 mm^3 (November 2004) to 13.5 mm^3 (January 2005) with the mean ± SE of 184.06 ± 73.63 mm^3 (Table 2).

(Oo)cysts and faecal coliforms count

The highest (oo)cysts and faecal coliforms were detected in the water samples collected from the downstream, followed by midstream and upstream stations. More samples were positive for *Giardia* cysts compared to *Cryptosporidium* oocysts. Faecal coliforms were detected in all the water samples. Both (oo)cysts and faecal coliforms were higher and more frequent in Sungai Congkak than Sungai Batu (Table 3).

Occurrence of (oo)cysts and faecal coliforms during the week-day and public holiday

Observation during the week days showed lower (oo)cysts concentration than public holidays, with higher mean density per litre of *Giardia* cysts than *Cryptosporidium* oocysts in both rivers. The contamination of water by faecal coliforms during the week days ranged from 480-33000 and 410-7800 CFU/0.1L in Sungai Congkak and Sungai Batu respectively, which were lower than the public holidays with 600-73000 CFU /0.1L and 540-16000 CFU/0.1L. The occurrence of the (oo)cysts and faecal coliforms is shown in Table 3.

Correlation of the (oo)cyst with faecal coliform, physical parameters and rainfall

For Sungai Congkak, *Giardia* cysts showed significant correlations with temperature

Table 1. Physical parameters data of the water at up-, mid- and down-streams stations of: A. Sungai Congkak and B. Sungai Batu

A. SUNGAI CONGKAK					
Sampling date	DO (mg/L)	pH	Tem (ºC)	Con (µS/cm)	Tur (NTU)
UPSTREAM STATION					
04-08-04	9.30	7.10	23.60	28.10	2.07
05-09-04	8.40	6.50	23.70	48.50	4.30
07-10-04	8.30	6.70	22.30	29.50	4.03
07-11-04	8.45	6.70	22.60	30.40	3.10
16-12-04	8.55	6.70	22.10	29.30	2.90
09-01-05	8.35	6.50	23.00	30.00	1.90
03-02-05	8.36	6.30	23.00	38.90	3.30
06-03-05	8.37	6.60	24.00	41.80	3.40
06-04-05	8.50	6.90	24.00	52.20	4.30
08-05-05	8.80	7.10	23.00	28.60	1.90
Mean±SE	**8.53±0.15**	**6.68±0.13**	**23.05±0.29**	**35.73±4.50**	**3.12±0.46**
MIDSTREAM STATION					
04-08-04	8.70	7.02	23.00	34.50	2.90
05-09-04	8.50	6.38	23.40	42.90	5.40
07-10-04	8.48	6.56	22.70	27.40	3.24
07-11-04	8.60	6.67	23.20	27.90	3.24
16-12-04	8.60	6.54	22.80	26.60	3.36
09-01-05	8.50	6.50	24.00	29.00	10.70
03-02-05	8.80	6.20	23.00	36.00	2.80
06-03-05	8.70	6.40	24.00	41.00	14.00
06-04-05	8.90	6.90	24.00	49.00	2.90
08-05-05	8.80	7.00	24.00	27.00	12.40
Mean±SE	**8.65±0.07**	**6.60±0.14**	**23.43±0.27**	**34.17±3.92**	**6.09±2.22**
DOWNSTREAM STATION					
04-08-04	8.50	6.97	25.50	44.00	2.83
05-09-04	8.30	6.41	25.50	44.90	4.00
07-10-04	7.92	6.59	24.20	29.90	6.00
07-11-04	8.39	6.65	24.60	28.70	3.00
16-12-04	8.28	6.61	24.40	27.90	4.90
09-01-05	8.13	6.40	27.40	31.00	4.50
03-02-05	8.71	6.21	24.80	37.40	4.00
06-03-05	8.60	6.41	26.80	41.70	7.90
06-04-05	8.60	6.87	25.80	56.40	4.24
08-05-05	8.53	7	25.20	29.10	8.20
Mean±SE	**8.39±0.12**	**6.61±0.13**	**25.42±0.51**	**37.10±4.74**	**4.96±0.93**

B. SUNGAI BATU					
Sampling date	DO (mg/L)	pH	Tem (ºC)	Con (μS/cm)	Tur (NTU)
UPSTREAM STATION					
16-08-04	8.70	6.99	22.90	24.80	4.80
19-09-04	8.10	6.29	22.80	29.30	8.80
22-10-04	9.11	6.56	22.50	26.20	4.10
28-11-04	9.27	6.54	22.80	25.10	3.30
22-12-04	8.50	6.51	22.80	29.90	1.60
23-01-05	8.89	6.70	24.00	27.00	2.50
23-02-05	8.50	6.50	24.00	35.20	3.10
20-03-05	9.00	6.60	24.00	40.20	6.63
20-04-05	8.85	6.80	24.00	25.00	7.30
22-05-05	9.30	6.50	22.00	23.20	183.00
Mean±SE	**8.82±0.18**	**6.60±0.10**	**23.09±0.27**	**28.60±2.67**	**22.51±8.21**
MIDSTREAM STATION					
16-08-04	8.71	6.90	23.60	24.10	2.50
19-09-04	7.80	6.29	23.10	28.60	16.80
22-10-04	8.90	6.57	23.10	25.90	11.30
28-11-04	8.36	6.54	23.10	25.00	6.40
22-12-04	8.50	6.46	23.40	32.80	2.50
23-01-05	8.60	6.40	24.00	28.00	14.40
23-02-05	8.60	6.50	24.00	35.00	3.40
20-03-05	9.10	6.50	24.00	41.00	15.10
20-04-05	9.00	6.80	24.00	26.00	9.60
22-05-05	9.30	6.40	23.00	23.00	111.00
Mean±SE	**8.68±0.21**	**6.54±0.08**	**23.52±0.27**	**28.97±2.89**	**19.25±6.25**
DOWNSTREAM STATION					
16-08-04	8.34	6.60	24.00	25.10	4.00
19-09-04	8.50	6.37	23.40	28.90	15.10
22-10-04	8.88	6.55	23.50	27.50	10.70
28-11-04	8.54	6.50	23.40	25.70	7.60
22-12-04	8.40	6.57	24.10	37.90	2.10
23-01-05	8.79	6.51	24.60	29.10	7.70
23-02-05	8.50	6.56	24.60	37.10	4.20
20-03-05	8.80	6.63	24.70	42.00	17.70
20-04-05	8.80	6.79	24.40	26.30	10.80
22-05-05	9.34	6.45	22.90	23.60	96.70
Mean±SE	**8.72±0.14**	**6.54±0.05**	**23.95±0.33**	**30.9±3.21**	**17.66±4.10**

DO = Dissolved oxygen, Tem = Temperature, Con = Conductivity,
Tur = Turbidity, NTU = Nephelometric turbidity unit, SE = Standard error

Table 2. Rainfall data at Ulu Langat Dam (near to Sungai Congkak) and Ulu Gombak (near to Sungai Batu) rainfall stations

Measurement Date (month, year)	Rainfall volume (mm^3) at Ulu Langat Dam	Rainfall volume (mm^3) at Ulu Gombak
August, 2004	69.10	92.70
September, 2004	212.00	253.60
October, 2004	308.20	245.60
November, 2004	522.70	521.40
December, 2004	42.80	36.00
January, 2005	1.60	13.50
February, 2005	34.30	125.00
March, 2005	82.80	163.80
April, 2005	160.80	126.40
May, 2005	377.90	262.60
Mean ± SE	181.22 ± 86.28	184.06 ± 73.63

SE = Standard error

($r = 0.561$, $p < 0.01$) and faecal coliforms count ($r = 0.641$, $p < 0.01$), whereas *Cryptosporidium* oocysts showed no significant correlations with faecal coliforms, physical parameters and rainfall. For Sungai Batu, the occurrence of *Cryptosporidium* oocysts showed significant correlation with conductivity ($r = 0.422$, $p < 0.05$) and *Giardia* cysts did not show any significant correlations with faecal coliforms, physical parameters and rainfall (Table 4).

DISCUSSION

The detection and presence of *Giardia* cysts and *Cryptosporidium* oocysts in recreational waters and their outbreaks have been previously reported in the United States (Craun *et al.*, 2005). In Malaysia, the recreational water research is still lacking and this study is the first information on the occurrence of these two protozoa in recreational water. Both of these protozoa were more frequently detected in the water samples collected from Sungai Congkak (50% or 15/30 cysts and 10% or 3/30 oocysts) than Sungai Batu (16.6% or 5/30 cysts and 3.3% or 1/30 oocysts). Additionally, more protozoan (oo)cysts were detected at the downstream followed by midstream and the least was at the upstream of these two studied rivers.

The upstream stations of both rivers were near to the natural source of water, originated from the hill (water catchment area) and located far away from human contamination. It is least exposed to any forms of human contaminant but there were stray pigs, oxen, tapir and porcupine living in the jungle surrounding this area of the Sungai Congkak. Thus, water samples from the upstream of Sungai Congkak showed no *Cryptosporidium* oocysts (0.0 ± 0.0 per litre) and very low contamination by *Giardia* cysts (0.04 ± 0.04 per litre). At Sungai Batu, oocyst was also not detected and 0.01 ± 0.01 cyst/litre of *Giardia*. In pristine environment, wildlife can be a significant source of *Giardia* and *Cryptosporidium* (oo)cysts since these protozoa were noted to be common in most animals, although the zoonotic potential of non-human-adapted *Giardia* is poorly understood (Olson & Guselle, 2000; Savioli *et al.*, 2006).

The presence of *Giardia* cysts and *Cryptosporidium* oocysts at the midstream and downstream stations of Sungai Congkak and Sungai Batu were associated with the size of human settlements, recreational and

Table 3. Number of (oo)cysts and faecal coliforms at the up-, mid- and down-stream stations of the study rivers

Sungai Congkak				Sungai Batu			
Sampling Date	No. of cysts/L	No. of oocyst/L	CFU/ 0.1L x 10^3	Sampling date	No. of cysts/L	No. of oocyst/L	CFU/ 0.1L x 10^3
UPSTREAM STATIONS							
04-08-04	n	n	0.68	16-08-04	n	n	1.0
05-09-04	n	n	2.6 0	19-09-04	n	n	0.65
07-10-04	n	n	0.51	22-10-04	n	n	0.41
07-11-04	n	n	0.60	28-11-04	n	n	0.54
16-12-04	n	n	0.48	22-12-04	n	n	0.60
09-01-05	0.10	n	1.60	23-01-05	0.10	n	1.40
03-02-05	n	n	1.40	23-02-05	n	n	3.40
06-03-05	n	n	1 0.0	20-03-05	n	n	0.91
06-04-05	n	n	2.60	20-04-05	n	n	1.30
08-05-05	0.30	n	1.30	22-05-05	n	n	4.00
Mean±SE	**0.04±0.04**	**n**	**2.1±1.4**		**0.01±0.01**	**n**	**1.4±0.62**
MIDSTREAM STATIONS							
04-08-04	1.30	0.20	1.40	16-08-04	n	n	4.90
05-09-04	n	n	3.90	19-09-04	0.10	n	5.10
07-10-04	n	n	1.40	22-10-04	n	n	0.71
07-11-04	n	n	3.10	28-11-04	n	n	3.40
16-12-04	n	n	1.20	22-12-04	n	n	1.80
09-01-05	n	n	11.00	23-01-05	n	n	6.40
03-02-05	1.40	n	8.80	23-02-05	n	n	4.90
06-03-05	0.10	n	12.00	20-03-05	n	n	5.20
06-04-05	0.10	n	3.30	20-04-05	n	n	1.40
08-05-05	n	n	13.00	22-05-05	n	n	6.80
Mean±SE	**0.29±0.28**	**0.02±0.03**	**5.9±2.3**		**0.01±0.01**	**n**	**4.0±1.0**
DOWNSTREAM STATIONS							
04-08-04	0.20	n	8.10	16-08-04	n	n	4.50
05-09-04	0.70	0.40	24.00	19-09-04	0.10	n	4.70
07-10-04	n	n	12.00	22-10-04	n	n	0.95
07-11-04	1.60	n	20.00	28-11-04	n	n	7.80
16-12-04	1.20	n	17.00	22-12-04	0.10	n	3.10
09-01-05	12.0	0.10	73.00	23-01-05	0.30	n	11.00
03-02-05	0.80	n	33.00	23-02-05	n	n	7.80
06-03-05	0.60	n	63.00	20-03-05	n	0.10	8.70
06-04-05	0.60	n	31.00	20-04-05	n	n	1.80
08-05-05	0.50	n	53.00	22-05-05	n	n	16.00
Mean±SE	**1.82±1.80**	**0.05±0.06**	**33.0±11.0**		**0.05±0.04**	**0.01±0.01**	**6.60±2.20**
Σmean± ΣSE	**0.72±0.71**	**0.023±0.03**	**13.0±4.90**		**0.023± 0.02**	**0.0033± 0.003**	**4.0±1.30**
Mean±SE of (oo)cysts and faecal coliforms during week day							
Mean±SE	**0.37±0.26**	**0.01±0.02**	**8.20±5.40**		**0.01±0.01**	**n**	**2.50±1.00**
Mean±SE of (oo)cysts and faecal coliforms during public holiday							
Mean±SE	**1.06±1.53**	**0.03±0.05**	**19.0±11.0**		**0.04±0.04**	**0.01±0.01**	**5.50±2.10**

[shaded box] = Data during holiday, L = Litre, Faecal coliform, measuring in CFU /0.1Lx10^3, CFU = Coliform forming unit, n = Nil, S = Total, SE = Standard error.

Table 4. Correlations between *Giardia* or *Cryptosporidium* and physical parameters, faecal coliforms and rainfall in study rivers

Parameter	Sungai Congkak		Sungai Batu	
	Giardia	Cryptosporidium	Giardia	Cryptosporidium
Dissolved oxygen	r = - 0.33 p> 0.05, NS	r = - 0.22 p> 0.05, NS	r = - 0.18 p > 0.05, NS	r = 0.04 p > 0.05, NS
pH	r = - 0.17 p> 0.05, NS	r = - 0.03 p> 0.05, NS	r = - 0.17 p > 0.05, NS	r = 0.08 p > 0.05, NS
Temperature	r = 0.561** p< 0.05, S	r = 0.25 p> 0.05, NS	r = 0.28 p > 0.05, NS	r = 0.33 p > 0.05, NS
Conductivity	r = - 0.01 p> 0.05, NS	r = 0.24 p> 0.05, NS	r = 0.05 p > 0.05, NS	r = 0.422* p < 0.05, S
Turbidity	r = - 0.07 p> 0.05, NS	r = - 0.07 p> 0.05, NS	r = - 0.11 p> 0.05, NS	r = - 0.01 p > 0.05, NS
Faecal coliforms	r = 0.641** p< 0.05, S	r = 0.17 p > 0.05, NS	r = 0.29 p >0.05, NS	r = 0.24 p > 0.05, NS
Rainfall	r = - 0.23 p> 0.05, NS	r = - 0.08 p > 0.05, NS	r = - 0.26 p >0.05, NS	r = - 0.02 p > 0.05, NS

r = Correlation coefficients, p = Probability level, S = Significant, NS = Not significant
** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

agriculture areas, which were all located between midstream and downstream sampling stations of the rivers. At the Sungai Congkak, there were higher number of local people, living and carrying out their daily activity together with higher number of visitors coming for recreational activity, making this area more crowded compared to Sungai Batu.

From our observation and related authoritative information, there were about 2000 and 1000 visitors coming for recreational activity during every holiday (weekend and public holiday) at Sungai Congkak and Sungai Batu respectively. Our results also showed that the occurrences of these two protozoa are increased during holidays than weekdays at Sungai Congkak and Sungai Batu respectively. Nevertheless, the concentrations of (oo)cysts per litre water are unlikely to be different between holidays and weekdays, except during a holiday on 9 of January 2005 (Table 3). This may be due to the sedimented (oo)cysts resulting from contamination by local communities, that floated at water surface and moved further downstream, due to the agitation of rivers bed by many bathers and swimmers during holiday. Also 9 of January 2005 fell in the driest month of the year, when the rainfall volume was as low as 1.60 mm^3 (Table 2), that caused less volume and slow flow of the river water, which may have contributed to accumulation of more (oo)cysts on the rivers bed. Visitors (especially infected people) through their water-based activities were also believed to be important sources of contamination for both treated and untreated recreational waters (Craun *et al.*, 2005). When the infected people continue to swim, this increases the likelihood of contamination

(CDC, 2001). Ongerth *et al.* (1995) showed that there was statistically significant relationship between the increased human use of recreational water and the prevalence of *Giardia* in surface water.

There were no "animals breeding activities' at or around the sudy sites or nearby areas, but there were agriculture activities and disposal of wastewater and sewage (main source of (oo)cysts) by the local people into the rivers through pipes and drainage. Meinhardt *et al.* (1996) have reported that *Cryptosporidium* oocysts maybe present in wastewater in varying amounts depending on the size of the community, and the prevalence of cryptosporidiosis within that community. High number of toddlers and children from the local community and visitors through their bathing and other water-based activities, were likely to contribute to the contamination of river water by protozoa (oo)cysts because of their lower awareness of personal hygiene. At the same time, they were at greater risk to exposure to these protozoa infections because their body immunity system was weaker compared to the adults. Giardiasis rates are highest among children from 0-5 years of age, due to frequent use of recreational water (Furness *et al.*, 2000). Through the water flowing from up- to downstream, the accumulation of these protozoa (oo)cysts became higher in downstream area. This result is in accordance with Ono *et al.* (2001) who found *C. parvum* oocysts did not decrease in number by settling out while moving downstream, instead the intensity of contamination increased downstream, because of the confluence of contaminated tributary streams.

In the present study, *Giardia* cysts occurred more frequently (20/60 or 33.3%) and at higher density than *Cryptosporidium* oocysts (4/60 or 6.7%) in water samples from both the rivers. This result concurs with previous reports (Kfir *et al.*, 1995b; Ahmad *et al.*, 1997) that found cysts were more frequently found in aquatic environment and at higher density compared to oocysts. The more frequent finding of *Giardia* cysts in raw water could suggest that giardiasis is more widespread and occurred with greater intensity than cryptosporidiosis. This is supported by several studies in Malaysia that indicated giardiasis was more common among communities and had higher prevalence rates (Che Ghani *et al.*, 1987, 1993; Lai, 1992; Penggabean, 1998) compared to cryptosporidiosis (Che Ghani *et al.*, 1984; Lai, 1992; Menon *et al.*, 2001). *Giardia* cysts have thicker walls which contribute to higher resistance than *Cryptosporidium* oocysts. Furthermore, several characteristics of the oocysts could also cause their detection to be less than usual. Oocysts are smaller in size (approximately 4-6 µm) and able to change its shape which could squeeze through the pores of membrane filter during vacuum pump (Mayer & Palmer, 1996), therefore filtration technique was claimed to be more efficient in detecting *Giardia* cysts (approximately 8-12 µm) than *Cryptosporidium* oocysts (Falk *et al.*, 1998), although this is the only technique available to separate the (oo)cysts from the water sample. Filtrations also could form the compaction of particles on, around, to attach and aggregate to the oocysts, and this makes it difficult to detect the oocysts (Farias *et al.*, 2002). It was reported to be less effective to detect (oo)cysts in river water compared to distilled water (Tan, 1998). The steps involved during water samples processing (e.g. filtration, elution or scrapping the membrane surface, centrifugation etc) might also cause oocysts loss at the final recovery (Vesey & Slade, 1990; Rochelle *et al.*, 1999). Oocysts could be destroyed and epitopes lost during centrifugation or pipetting (Versey & Slade, 1990) resulting in insufficient material for detection using monoclonal antibody (Vesey *et al.*, 1993). In addition, it was also reported that there was low recovery of (oo)cysts using DNA-amplified in PCR-based detection after the IMS separation (Johnson *et al.*, 1995; Deng *et al.*, 1997).

In general, the density of *Giardia* and *Cryptosporidium* (oo)cysts detected in both rivers was low over the period of the study. The concentrations of these protozoa were either truly low or possibly due to the small volume of water samples (10 litres) compared with other studies which permitted the water sample filtration until 100 to 1000 litres. A study conducted by Kfir *et al*. (1995a) indicated that the use of cartridge filters for the processing of large volume of water samples (100 litres) showed a slightly better recovery efficacy than the (flat-bed) membrane filtration technique which limits sample volume to about 10 litres. Apart from weaknesses in IMS technique, the commercially available IgM-type monoclonal antibody (MAbs) against both protozoa had a low affinity since it had not undergone affinity maturation or isotype switching (WHO, 2002). Therefore, during immunomagnetic separation, not all (oo)cysts are attached to the beads (IgM MAb) up to the final step of experimental procedure. Repeated rinsing and aspiration during slide preparation can also lead to loss of the (oo)cysts together with supernatant (Bukhari *et al*., 1998).

Although, the density of the (oo)cysts was low the dose-response to cause infection was also relatively low, possibly as low as 1 (oo)cyst (DuPont *et al*., 1995). The outbreak which occurred in Ayrshire, Swindon, Bradford (UK) and Milwaukee (USA) had *Cryptosporidium parvum* oocysts detected in treated water at densities of less than 0.4 per litre (Richardson *et al*., 1991; MacKenzie *et al*., 1994; Atherton *et al*., 1995). Therefore, with the concentration found in the midstream and downstream (0.1-0.4 oocyst/ litre) of these rivers, the unintentional ingestion of a single mouthful of contaminated water while swimming and bathing could pose a possible outbreak, even in non-outbreak setting. Low level contamination of surface waters with *Cryptosporidium parvum* oocysts is normal in environmental water samples and has been reported in many countries (Ongerth & Stibbs, 1987; LeChevallier *et al*., 1991) and is a potential risk to public health (Haas & Rose, 1995).

Pearson correlation analysis revealed that only three significant relationships occurred which were, between *Giardia* cysts and temperature ($r = 0.561$, $p < 0.01$) and *Giardia* cysts and faecal coliforms ($r = 0.641$, $p < 0.01$) in Sungai Congkak, and between *Cryptosporidium* oocysts and conductivity in Sungai Batu ($r = 0.422$, $p < 0.05$). These significant correlations do not express the true description of the association between these protozoa and physical/microbiological parameters of river water because it was not consistent in both study rivers. Thus, although the relationship between *Giardia* cysts and faecal coliforms was significant in Sungai Congkak the relationship was not significant in Sungai Batu. The same scenario also happened to the relationship between *Giardia* cysts and temperature (significant in Sungai Congkak but not in Sungai Batu) and between *Cryptosporidium* oocysts and conductivity (significant in Sungai Batu but not in Sungai Congkak). Furthermore, Pearson correlation analysis showed significant correlation between *Cryptosporidium* oocysts and conductivity in Sungai Batu even though data obtained showed that there was only one water sample positive for *Cryptosporidium* oocysts. Based on r (correlation coefficients) values, all the above mentioned significant correlations showed weak associations, which might be not meaningful because there are less positive water samples with protozoa (oo)cysts. Therefore, all the physical and microbiological parameters could not be used as indicator for the occurrence of these protozoan (oo)cysts in water from both rivers. Similar result was also reported by many other studies of river water (Rose *et al*., 1988; LeChevallier *et al*., 1991; Ashbolt & Veal, 1994; Ahmad *et al*., 1997) and water treatment plant (Tan, 1998).

In the assessment of water quality, the screening for pathogenic parasites is an important adjunct because the lack of faecal coliforms does not necessarily imply the absence of protozoa (Rose *et al*., 1986). The lack of correlation with the occurrences of faecal coliform contamination may be due to the differences in resistance to environmental stresses and the mean survival time of this microbe in water (Oliveri, 1982; Basualdo *et al*., 2000). Therefore, microbiological parameter (faecal coliforms) could not be used to show the occurrence of these protozoa (oo)cysts in water. Anyway, as long as faecal contamination is detected (shown by the availability of faecal coliforms), the (oo)cysts might also be present in these rivers water because these protozoa are intestinal parasites which are transmitted through faecal oral route.

Additionally, all residents living around Sungai Batu and most of those around Sungai Congkak were not provided with water supply facilities. Those residents who were provided with water supply complained of getting rusty water, thus leading these local people to use the river water for their daily activities such as swimming, bathing, washing, drinking and cooking. Therefore, there was a high possibility of these local people to be infected with *Giardia* and *Cryptosporidium* (oo)cysts through consumption of this contaminated water. Furthermore, we had seen several times local people defaecating and cleaning their child's bottom (after defaecating), and children disposable diapers with faeces floating in these rivers.

Giardia and *Cryptosporidium* (oo)cysts were also not significantly correlated with rainfall data ($p > 0.05$) for both rivers. Therefore, the source of contamination with these protozoa was not from the non point source like soil surface run-off. The findings, in part, agreed with Svoboda *et al.* (1999), that no significant association was found between rainfall and *Cryptosporidium* oocysts in the Lutzel river, Switzerland. Another study conducted by Jarmey-Swan *et al.* (2001) in KwaZulu-Natal populations also showed that the incidence of *Cryptosporidium* and *Giardia* did not correlate with rainfall.

Since Sungai Congkak and Sungai Batu ended in Sungai Langat and Batu Dam respectively, the presence of *Giardia* and *Cryptosporidium* (oo)cysts in these two rivers might render a risk to the public, because both Sungai Langat and Batu Dam are used as sources of water supply for several nearby districts. Despite water supply source was treated before been distributed to the public risk still exist, as according to Parker (1993), *Giardia* and *Cryptosporidium* (oo)cysts maybe present in drinking water after conventional treatment. *Cryptosporidium* oocysts are often present in raw and treated drinking water, even when the drinking water meets United States Environmental Protection Agency (EPA) treatment standards (Rose *et al.*, 1986; Ongerth & Stibbs, 1987; LeChevallier *et al.*, 1991). Furthermore, there are several reports in the literature of giardiasis and cryptosporidiosis waterborne outbreaks involving treated drinking water (Smith *et al.*, 1995).

Acknowledgement. The authors of this paper would like to thank Academician Yong Hoi Sen for his comments, the University Malaya for the financial support, Metereological Department of Petaling Jaya and Forestry Department of Selangor for technical supports.

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