Tropical Biomedicine 22(2): 89–98 (2005)

Contribution of cattle farms towards river contamination with *Giardia* cysts and *Cryptosporidium* oocysts in Sungai Langat Basin

Farizawati, S.[1], Lim, Y.A.L[2], Ahmad, R.A.[1], Fatimah, C.T.N.I[3] and Siti-Nor, Y.[3]
[1] School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia.
[2] Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[3] University Veterinary Hospital, Universiti Putra Malaysia, 43400 UPM Serdang

Abstract. A study to determine the contribution of *Giardia* cysts and *Cryptosporidium* oocysts from cattle farms was carried out at the Langat Basin. This study investigated the contribution of cattle farms, located near Sungai Langat and Sungai Semenyih, towards river contamination with these cysts and oocysts. The findings showed that out of 24 samples of water taken from Sungai Semenyih, 4.2% was positive for *Giardia* cysts with a concentration of 1.3 cysts/L and 20.8% were positive with *Cryptosporidium* oocysts with a range of 0.7 – 2.7 oocysts/L. At Sungai Langat, from the 43 samples taken, 23.3% were positive for *Giardia* cysts with a range of 1.5 – 9 cysts/L whereas 11.6% were positive with *Cryptosporidium* oocysts with a range of 2.5 – 240 oocysts/L. Isolation of cysts and oocysts in bovine faecal materials revealed that 14.6% of faecal samples were positive for *Giardia* cysts which had a range of 75 – 1.3×10^4 cysts/g and 25% were positive for *Cryptosporidium* oocysts with a range of 50 - 3.9×10^5 oocysts/g. From the cattle wastewater, 98% were positive with oocysts and 6.7% with cysts. The concentrations were between 20 – 3.1×10^3 oocysts/mL for *Cryptosporidium* and 4 – 75 cysts/mL for *Giardia*. Given that the prevalence of *Cryptosporidium* and *Giardia* are high amongst the cattle and the positive findings of the (oo)cysts in the river samples, it could be deduced that there is a very high possibility of the cattle farms contaminating the river with *Giardia* cysts and *Cryptosporidium* oocysts. Viability study of *Cryptosporidium* oocysts in the surrounding soil and pond within the cattle farm showed that the viability of *Cryptosporidium* oocysts decreased with time. It was estimated that it will take 52 days for all the oocysts from both environment to be non-viable. With a viability rate of approximately 2 months in a cattle farm setup, river water contaminated with *Cryptosporidium* oocysts has a high chance of acting as an agent of transmission. As cattle farms are also inhabited by the owners and their families, this problem may pose a threat to humans (e.g. children) especially if they are dependent on the river water as their source of water for their daily activities.

INTRODUCTION

Cryptosporidium and *Giardia* have emerged to be pathogens of medical and veterinary importance. Although *Cryptosporidium* and *Giardia* infections have been reported for 79 species of mammals, the most frequently reported and most widely recognized host species other than humans are the ruminants (O'Donoghue, 1995). In cattle, these parasites can cause diarrhea and poor performance, predominantly in younger animals.

Cryptosporidium is most commonly found in young calves a few weeks of age. This parasite can cause diarrhea in calves between 1 and 3 weeks of age. Clinically normal calves can also shed *Cryptosporidium* oocysts in their faeces and serve as a source of infection for other calves and the environment (Becher *et al.*, 2004). However, *Giardia* is found in cattle of all ages. It is rarely diagnosed as a cause

of clinical diarrhea in calves. A few studies suggest that infection may be associated with reduced performance (Olson *et al.*, 2004).

Economic loss in the cattle farming industry because of cryptosporidiosis has been linked to neonatal diarrhea which causes dehydration, inhibits normal development and even death (de Graaf *et al.*, 1999). In Belgium, mortality caused by neonatal diarrhea is between 5 to 10% and *Cryptosporidium parvum* is the enteropathogen commonly found in the calves' first few weeks of live (de la Fuente *et al.*, 1998).

Control of cryptosporidiosis and giardiasis in cattle has become important, not only to reduce the risk of disease in cattle, but also to reduce the risk of infection in humans. If the manure from cattle contaminates water supplies and the water is not properly cleaned or if fresh manure is used on produce, humans may become infected and develop diarrhea. Calves that are infected have been reported to be able to shed 10^6-10^7 oocysts per gram of faeces (Garber *et al.*, 1994; McCluskey *et al.*, 1995). High rate of oocysts and cysts excretion has great potential in contaminating the environment either directly or through its role as manure in the agricultural sector.

The aim of this study was to investigate the contribution of *Giardia* cysts and *Cryptosporidium* oocysts from cattle farms, located near Sungai Langat and Sungai Semenyih towards river contamination. In addition, viability study of *Cryptosporidium* oocysts was also carried out in the surrounding soil and pond within a cattle farm to ascertain how long *Cryptosporidium* oocysts can survive in the farm environment.

MATERIALS AND METHODS

Isolation of *Cryptosporidium* and *Giardia* (oo)cysts from river water

River water from Sungai Semenyih and Sungai Langat which were adjacent to cattle farms were sampled by filtering 10 - 25 L of water through a polypropylene fiber-wound depth cartridge filter (Ametek, Wisconsin, USA, nominal pore size = 1 µm) with a flow rate of 1.5 litres per minute (using a flow restrictor).

River water samples were processed according to the procedures recommended by the U.K. Standing Committee of Analysts (SCA), Department of the Environment (Anonymous, 1990) with some minor modifications. The cartridge filter whose matrix might contained entrapped (oo)cysts was cut longitudinally and teased apart. The filter fibres were eluated with 0.1% Tween 80 (Sigma Chemical, Missouri, USA) for 10 min using a stomacher (Seward Model 3500, Ohio, USA).

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, Massachusettes). Membrane filters were eluted using 0.1% Tween 80 and the wash water was concentrated by centrifugation (1500 x g for 10 min) before being 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 discontinous density gradient sucrose flotation, portion A was added with an equal volume (10 mL) of 2% Tween 80 and portion B with 2% Tween 80 in 2% sodium dodecyl sulphate (SDS) (Sigma Chemical, Missouri, USA). The samples were centrifuged (1500 x g for 10 min) and the supernatants were aspirated down to 10 mL.

Samples were slowly underlayered with 10 mL of cold sucrose solution and centrifuged (1000 x g, 5 min). The entire supernatant including the interface was recovered without disturbing the pellet and decanted gently into a clean 50 mL conical centrifuged tube. 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.

Isolation of *Cryptosporidium* and *Giardia* (oo)cysts from faecal samples

Seven cattle farms within the Langat Basin were identified for cattle faecal and wastewater samples. Water-ether technique was employed for the concentration and purification of (oo)cysts from the faecal samples. Each faecal suspension containing 1 gram of faeces was made up to 50 mL with distilled water and vortexed for 20 sec. The resulting suspension was filtered through a sieve into a 250 ml conical flask. The filtrate was concentrated by centrifugation at 1050 x g for 5 min and the supernatant was aspirated down to 10 mL.

Two mL of diethyl-ether was added to the washed faecal suspension and the sample was vortexed for 20 sec and then centrifuged (1050 x g, 5 min). Following the centrifugation of each faecal sample, the fat layer was discarded, pellet was resuspended and sufficient distilled water was added to bring the volume up to 50 mL. Sample was vortexed for 20 sec and centrifuged (1050 x g, 5 min). The faecal washing procedure (as described earlier) was repeated two times and then the pellet was resuspended in distilled water to a final concentrated volume (between 3 to 5 mL depending on the amount of particulate matter).

Isolation of *Cryptosporidium* and *Giardia* (oo)cysts from wastewater samples

Two hundred ml of wastewater was transferred to a clean 500 mL Schott bottle. Then 2 mL of Tween 80 (10%) was added to the sample. The bottle was closed very tightly and shaken for a few minutes. Twenty ml of sample was then transferred to a centrifuge tube and topped up to 50 mL with distilled water and filtered through a muslin cloth and the filtrate was collected in a conical flask. Distilled water was used to rewash the big particles and filtered through till the volume reaches 500 mL. The filtrate was then transferred to 50 mL centrifuge tubes and concentrated at 1050 g for 15 minutes. The concentration step was repeated till the whole sample was contained in the final 10 mL.

Sample was slowly underlayered with 10 mL of cold sucrose solution and centrifuged (1050 x g, 15 min). The entire supernatant including the interface was recovered without disturbing the pellet and decanted gently into a clean 50 mL conical centrifuge tube. The residual sucrose was removed by washing three times in distilled water. The final concentrated sample was reduced to a final volume of 1 to 5 ml, depending on how turbid the sample was. The sample was further concentrated using the Immuno-magnetisable Separation (IMS) technique. The IMS technique employed followed the protocol suggested by the Dynal manual which used Dynabeads® anti-*Cryptosporidium* (Dynal® A.S., prod. No. 730.01, Oslo,Norway).

Detection and enumeration of *Cryptosporidium* and *Giardia* (oo)cysts from river water, faecal and wastewater samples

Twenty five µL aliquots of each sample concentrate was placed onto each of the four wells of a teflon®-coated microscope slide and air dried (room temperature = 24 ± 1ºC). Each well containing concentrates of sample was overlaid with 25 µL of commercially available fluorescein isothiocyanate (FITC)-labelled anti-*Cryptosporidium* oocysts mAb or 25 µL of commercially available fluorescein isothiocyanate (FITC)-labelled anti-*Giardia* cysts mAb (Waterborne™ Inc, USA). Slides were incubated in a humidity chamber for 30 ± 5 min at 37ºC.

Excess antibody was removed by rinsing the slides twice with 50 µL of PBS dropped onto each well. DAPI (4’, 6-diamidino-2-phenylindole) stain (Sigma Chemical, Missouri, USA) was then applied to stain the four nucleus sky-blue. Twenty µL of mounting medium (PBS:glycerol, 1:1

v/v) was mounted onto each well and coverslips were applied to the slides which 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 FITC stain and Nomarski-DIC optics to observe the internal structures.

(Oo)cysts which were stained with FITC-mAb exhibited bright apple green fluorescence typically concentrated on the periphery of the oocysts when observed under the epifluorescent microscope. Putative *Giardia* cysts are ovoid in shape, and are 8 to 14 µm long and 7 to 10 µm wide, with 2-4 DAPI stained sky-blue nuclei contained within the labelled cyst under the UV filter block. Whilst *Cryptosporidium* oocysts are spherical in shape with a diameter ranging from 4 to 6 µm with 4 sky-blue nuclei contained within the labelled oocyst.

Assessment of oocysts viability using vital dye assay in soil and pond environments

The design of the semipermeable container which contained the parasites was developed to allow oocysts to be in contact with defined environments and to allow regular sampling with minimal risk of bacterial contamination (Robertson *et al.*, 1993).

Prior to injecting oocysts into these containers, the containers were thoroughly sterilized in a 1% hypochlorite solution which was then rinsed by repeated immersion of the containers into deionised water. Oocyst suspensions were treated overnight with an antibiotic solution (4 µL of gentamicin (40µg/µL) and 50 µL of penicillin G (1µg/µL) per mL of oocyst suspension) before being injected into the containers.

Approximately 3 x 10^5 oocysts were spiked into each container and the containers were then placed in defined environments (eg. soil and pond in one of the cattle farms). For the control, oocysts were injected into the container which stored 4 litres of deionised water at 4°C in the dark.

Oocyst viability was based primarily on the viability assay described by Campbell *et al.* (1992), which is dependent on the morphology and the inclusion or exclusion of two fluorogenic vital dyes, 4' 6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI), by the oocysts.

RESULTS

Detection of *Cryptosporidium* and *Giardia* (oo)cysts from river water

The results of this study showed that out of 24 samples of water taken from Sungai Semenyih, 1 sample (4.2%) was positive for *Giardia* cysts with a concentration of 1.3 cysts/L and 5 samples (20.8%) were positive with *Cryptosporidium* oocysts with a range of 0.7 – 2.7 oocysts/L (Table 1 and 2). At Sungai Langat, 10 samples (23.3%) from 43 samples taken were positive for *Giardia* cysts (range = 1.5 – 9.0 cysts/L) whereas 5 samples (11.6%) were positive with *Cryptosporidium* oocysts (range = 2.5 – 240 oocysts/L) (Table 1 and 2).

Table 1. Occurrence of *Giardia* cysts in river water samples at Langat Basin

Location	No. of samples	No. of positive sample(s)	Percentage of positive sample(s)	Range of concentration of cysts/L
Sungai Semenyih	24	1	4.2	1.3
Sungai Langat	43	10	23.3	1.5 – 9

Table 2. Occurrence of *Cryptosporidium* oocysts in river water samples at Langat Basin

Location	No. of samples	No. of positive sample(s)	Percentage of positive sample(s)	Range of concentration of oocysts/L
Sungai Semenyih	24	5	20.8	0.7 – 2.7
Sungai Langat	43	5	11.6	2.5 – 240

Table 3. Occurrence of *Giardia* cysts and *Cryptosporidium* oocysts in cattle faecal samples

Parasite	No. of samples	No. of positive sample(s)	Percentage of positive sample(s)	Range of concentration of (oo)cysts/L
Cryptosporidium	96	24	25	50 – 3.9×10^5
Giardia	96	14	14.6	75 – 1.3×10^4

Isolation of *Cryptosporidium* and *Giardia* (oo)cysts from cattle faeces
Isolation of cysts and oocysts in faecal materials revealed that 14.6% of faecal samples were positive for *Giardia* cysts (range = 75 – 1.3×10^4 cysts/g) and 25% were positive for *Cryptosporidium* oocysts (range = 50 - 3.9×10^5 oocysts/g) (Table 3). There were 5 samples which had double infections of cryptosporidiosis and giardiasis (Table 4). Occurrence of double infections has also been reported in Sweden among the cattle (Bjorkman *et al.*, 2003).

Table 4. Occurrence of double infections of cryptosporidiosis and giardiasis in cattle faeces

Sample	Concentration of *Giardia* cysts/L	Concentration of *Cryptosporidium* oocysts/L
1	1.5×10^2	1.5×10^2
2	1.0×10^2	1.0×10^2
3	1.2×10^2	2.8×10^2
4	2.5×10^3	8.0×10^2
5	1.5×10^2	75

Detection of *Cryptosporidium* and *Giardia* (oo)cysts from cattle wastewater
From the cattle wastewater, 98% were positive with oocysts and 6.7% with cysts. The concentrations were between 20 - 3.1×10^3 oocysts/mL for *Cryptosporidium* and 4 - 75 cysts/mL for *Giardia* (Table 5). Given that the prevalence of *Cryptosporidium* and *Giardia* are high amongst the cattle and the positive findings of the (oo)cysts in the river water and wastewater samples, it could be deduced that there is a very high possibility of the cattle farms contaminating the river with *Giardia* cysts and *Cryptosporidium* oocysts.

Assessment of oocysts viability using vital dye assay in soil and pond environments
Viability study of *Cryptosporidium* oocysts in the surrounding soil and pond

Table 5. Occurrence of *Giardia* cysts and *Cryptosporidium* oocysts in cattle wastewater

Parasite	No. of samples	No. of positive sample(s)	Percentage of positive sample(s)	Range of concentration of cysts/L
Giardia	45	3	6.7	4 – 75
Cryptosporidium	45	44	98	20 – 3.1 x 10^3

within the cattle farm showed that the viability of *Cryptosporidium* oocysts decreased with time. It was estimated that it will take 52 days for all the oocysts from both environment to be non-viable (Figure 1, 2 & 3).

DISCUSSION

The results from this study indicated that the rivers were highly contaminated with *Cryptosporidium* oocysts and *Giardia* cysts (range = 0.7 – 240 (oo)cysts/L). Outbreaks which occurred in Ayrshire, Swindon, Bradford in UK and Milwaukee in USA had oocysts detected in treated water at densities from less than 0.4 per litre (Smith *et al.*, 1989; Richardson *et al.*, 1991; MacKenzie *et al.*, 1994; Atherton *et al.*, 1995). A concentration of 240 oocysts/L at Sungai Langat is 600 times higher than the concentration of 0.4 oocysts/L which was known to have caused outbreaks.

These data revealed that there is a risk of a possible outbreak if people were to accidentally drink the river water while bathing, playing or swimming. Moreover, the low infectious doses of both cryptosporidiosis and giardiasis would definitely facilitate the transmission of these parasites. Faecal coliform test carried out on these river water samples showed that there was contamination of

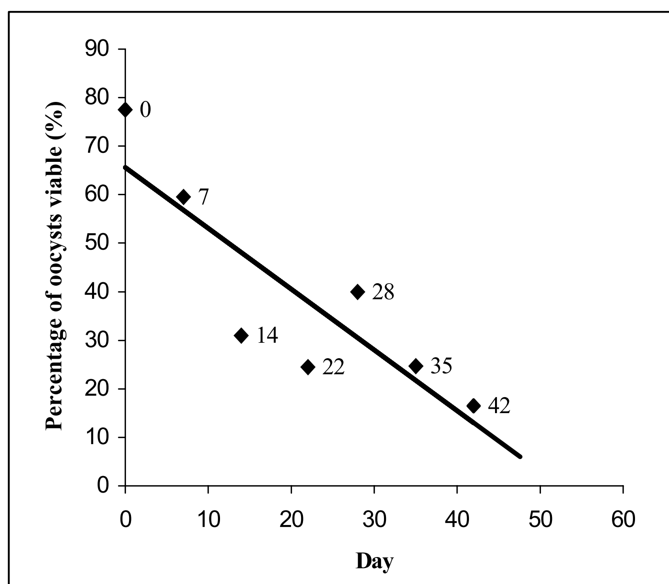

Figure 1. Linear regression graph showing correlation (r=0.86) between percentage of viable oocysts and duration of days oocysts exposed to soil.

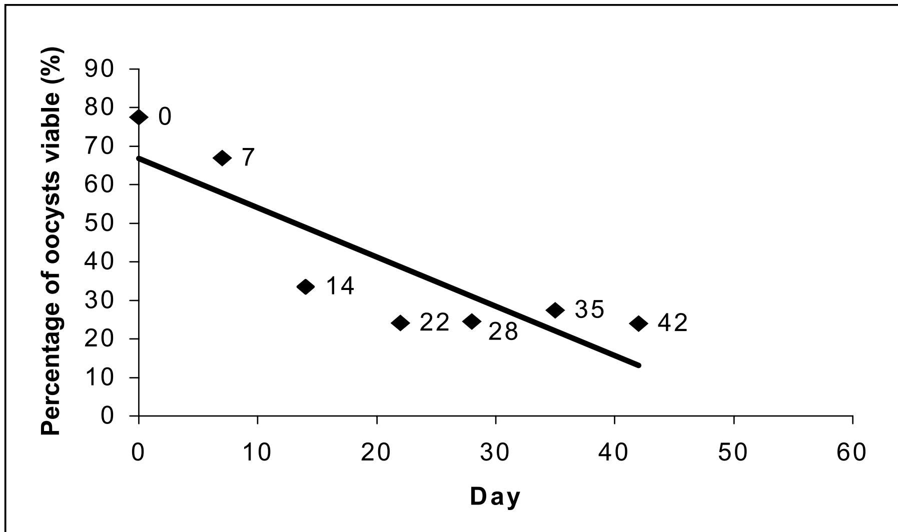

Figure 2. Linear regression graph showing correlation (r=0.85) between percentage of viable oocysts and duration of days oocysts exposed to pond water.

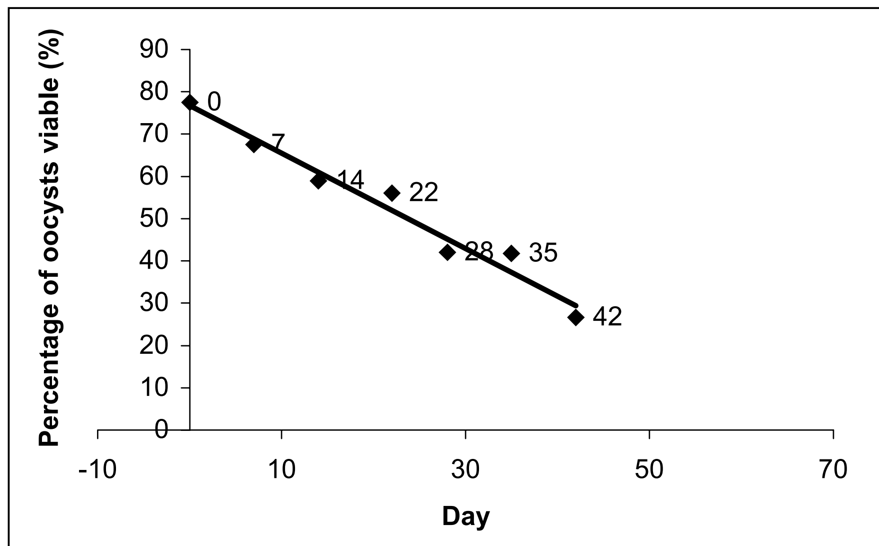

Figure 3. Linear regression graph showing correlation (r=0.98) between percentage of viable oocysts and duration of days oocysts exposed to the controlled environment (deionised water at 4°C)

faeces into the river water. However, the origin of the faeces whether they were human or animal could not be determined.

The excretion of *Cryptosporidium* and *Giardia* (oo)cysts in the cattle faeces was discovered to be between 50 to 3.9 x 10^5 (oo)cysts/g. A calf is said to be able to excrete 40 kg of faeces in a day. Therefore if the concentrations of (oo)cysts detected were to be converted to per 40 kg, it would mean that there would be 2 million to 16 billion of (oo)cysts excreted in a day by a cattle. This finding re-emphasis the high degree of contamination that can occur if

these (oo)cysts contaminates the environment. It becomes hazardous to human health when contamination happens in rivers that are used for abstraction for drinking water supplies.

There is a high probability of this event happening in this study sites because results from the wastewater of the cattle farms suggested that the wastewater do contain (oo)cysts (range of concentration = 4–3110 (oo)cysts/L). Wastewater was seen being discharged into the river signifying contamination of (oo)cysts from the cattle farms into the river system. The viability assay indicated that the oocysts can survive up to 52 days in soil and pond environment. With a long survival period, the presence of *C. parvum* oocysts in the river water could be a probable source of transmission to humans. This is especially important because *Cryptosporidium* is known to have a low infectious dose. In another survivality study conducted in Malaysia, it was shown that seeded *C. parvum* oocysts in the river environment in Malaysia could survive up to 3 months (Lim *et al*., 1999). Therefore this further confirms that oocysts can survive for about 2 to 3 months in Malaysian climate.

It is interesting to note that besides cattle in the cattle farm other animals can also be sources of *Cryptosporidium* and *Giardia*. Szostakowska *et al*. (2004) reported that filth flies associated with a cattle barn and a municipal landfill were tested positive by combined immunofluorescent antibody and fluorescent *in situ* hybridization(FISH) for *C. parvum* and *Giardia lamblia* on their exoskeletons and in their guts. It was discovered that more pathogens were carried by flies from the cattle barn than from the landfill. Besides that it was also found that 81% of *C. parvum* and 84% of *G. lamblia* pathogens were presumptively viable (Szostakowska *et al*. 2004).

Although this present study did not identify the species of the parasites, it is known that cattle are frequently parasitized with *Giardia duodenalis* (=*lamblia*), *C. parvum* and *Cryptosporidium andersoni*. In a study by Merle *et al*.(2004), they discovered that most *G. duodenalis* from cattle (Assemblage E) are different from those in humans (Assemblages A and B), and *C. andersoni* does not infect humans. However, molecular tools have shown that humans can be infected with zoonotic *C. parvum*, as well as anthroponotic *Cryptosporidium hominis* (Merle *et al*. 2004).

As cattle farms are also inhabited by the owners and their families, contamination of river water with *Cryptosporidium* oocysts and *Giardia* cysts by cattle farms may pose a threat to humans (e.g. children) especially if they are dependent on the river water as their source of water for their daily activities. Therefore, in order to reduce the risk of human infection from cattle faeces, it is recommended that owners of cattle farms practice good manure management practices. Practices should prevent leaching, erosion, and run-off from manure treated fields, pens, and catch basins into lakes, rivers, streams, and ditches. Direct access of cattle to watercourses should be controlled (e.g. rotational grazing, off-site watering, access ramps, fencing). Workers should practice good personnel hygiene. Drinking water for humans should be filtered and chlorinated. Only composted manure should be used as fertilizer for fresh vegetable and fruit crops. These steps should be taken seriously so as to curb contamination of parasites from cattle farms into the river.

Acknowledgements. This study was supported by the IRPA 08-02-02-0035 grant, from the Ministry of Science, Technology and Environment. The authors would also like to thank Mr. Mansor Osman and staff of Pejabat Haiwan Daerah Hulu Langat, Kajang for their kind assistance.

REFERENCES

Anonymous (1990). Isolation and identification of *Giardia* cysts, *Cryptosporidium* oocysts and free living pathogenic amoebae in water,

etc. 1989. In *Methods for the examination of waters and associated materials*. Department of the Environment, Standing Committee of Analysts. 1990; 6-14. London: HMSO.

Atherton, F., Newman, C.P.S. & Casemore, D.P. (1995). An outbreak of waterborne cryptosporidiosis associated with a public water supply in the UK. *Epidemiology and Infection* **115:** 123-131.

Becher, K.A., Robertson, I.D., Fraser, D.M., Palmer, D.G. & Thompson, R.C. (2004). Molecular epidemiology of *Giardia* and *Cryptosporidium* infections in dairy calves originating from three sources in Western Australia. *Veterinary Parasitology* **123**(1-2):1-9.

Bjorkman, C., Svensson, C., Christensson, B. & de Verdier, K. (2003). *Cryptosporidium parvum* and *Giardia intestinalis* in calf diarrhoea in Sweden. *Acta Veterinaria Scandinavia* **44**(3-4):145-152.

Campbell, A.T., Robertson, L.J. & Smith, H.V. (1992). Viability of *Cryptosporidium parvum* oocysts: correlation of *in vitro* excystation with inclusion or exclusion of fluorogenic vital dyes. *Journal of Applied and* Environmental *Microbiology* **58**(11): 3488-3493.

de-Graaf, D.C., Vanopdenbosch, E., Ortega, M.L.M., Abbassi, H. & Peeters, J.E. (1999). A review of the importance of cryptosporidiosis in farm animals. *International Journal of Parasitology* **29:** 1269-1287.

de la Fuente, R., Garcia, A. & Ruiz Santa Quiteria, J.A. (1998). Proportional morbidity rates of enteropathogens among diarrheic dairy calves in central Spain. *Preventive Veterinary Medicine* **36:** 145-152.

Garber, L.P., Salman, M.D., Hurd, H.S. & Keefe, T. (1994). Potential risk factors for *Cryptosporidium* infection in dairy calves. *Journal of American Veterinary Medicine Association* **205:** 86-89.

Lim, Y.A.L., Ahmad, R.A., Osman Ali & Zulkeflie, Z. (1999). Survival of *Cryptosporidium parvum* oocysts in river and soil environments. *Tropical Biomedicine* **16:** 7-15.

MacKenzie, W.R., Hoxie, N.J., Proctor, M.E., Gradus, M.S., Blair, K.A., Peterson, D.E., Kazmierczak, J.J., Addiss, D.G., Fox, K.R., Rose, J.B. & Davis, J.P. (1994). A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *New England Journal of Medicine* **331:** 161-167.

McCluskey, B.J., Greiner, E.C. & Donovan, G.A. (1995). Patterns of *Cryptosporidium* oocysts shedding in calves and a comparison of two diagnostic methods. *Veterinary Parasitology* **60:** 185-190.

Merle, E., Olson Ryan, M., O'Handley, Brenda, J., Ralston, Timothy A., McAllister & R.C. Andrew Thompson. (2004). Update on *Cryptosporidium* and *Giardia* infections in cattle. *Trends in Parasitology* **20:** 185-191.

Olson, M.E., O'Handley, R.M., Ralston, B.J., McAllister, T.A. & Thompson, R.C. (2004). Update on *Cryptosporidium* and *Giardia* infections in cattle. *Trends in Parasitology* **20**(4):185-191.

O'Donoghue, P.J. (1995). Cryptosporidium and cryptosporidiosis in man and animals. *International Journal of Parasitology* **25:** 139-195.

Richardson, A.J., Frankenberg, R.A., Buck, A.C., Selkon, J.B., Colbourne, J.S., Parsons, J.W. & Mayon-White, R.T. (1991). An outbreak of waterborne cryptosporidiosis in Swindon and Oxfordshire. *Epidemiology and Infection* **107:** 485-496.

Robertson, L.J., Campbell, A.T. & Smith, H.V. (1993). A low cost, low technology container for studying the survival of transmission stages of parasites and other pathogens in water-related environments. *Water Research* **27**(4): 723-725.

Smith, H.V., Patterson, W.J., Hardie, R., Greene, L.A., Benton, C., Tulloch, W.J., Gilmour, R.A., Girdwood, R.W.A., Sharp, J.C.M. & Forbes, G.I. (1989). An outbreak of waterborne cryptosporidiosis caused by post-treatment contamination. *Epidemiology and Infection* **103:** 703-715.

Szostakowska, B., Kruminis-Lozowska, W., Racewicz, M., Knight, R., Tamang, L., Myjak, P. & Graczyk, T.K. (2004). *Cryptosporidium parvum* and *Giardia lamblia* recovered from flies on a cattle farm and in a landfill. *Applied Environmental Microbiology* **70**(6): 3742-3744.