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RESEARCH ARTICLE

Aquatic biomonitoring of *Giardia* cysts and *Cryptosporidium* oocysts in peninsular Malaysia

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Abstract An aquatic biomonitoring of *Giardia* cysts and *Cryptosporidium* oocysts in river water corresponding to five villages situated in three states in peninsular Malaysia was determined. There were 51.3 % (20/39) and 23.1 % (9/39) samples positive for *Giardia* and *Cryptosporidium* (oo)cysts, respectively. Overall mean concentration between villages for *Giardia* cysts ranged from 0.10 to 25.80 cysts/l whilst *Cryptosporidium* oocysts ranged from 0.10 to 0.90 oocysts/l. Detailed results of the river samples from five villages indicated that Kuala Pangsun 100 % (9/9), Kemensah 77.8 % (7/9), Pos Piah 33.3 % (3/9) and Paya Lebar 33.3 % (1/3) were contaminated with *Giardia* cysts whilst *Cryptosporidium* (oo)cysts were only detected in Kemensah (100 %; 9/9) and Kuala Pangsun (66.6 %; 6/9). However, the water samples from Bentong were all negative for these waterborne parasites. Samples were collected from lower point, midpoint and upper point. Midpoint refers to the section of the river where the studied communities are highly populated. Meanwhile, the position of the lower point is at least 2 km southward of the midpoint and upper point is at least 2 km northward of the midpoint. The highest mean concentration for (oo)cysts was found at the lower points [3.15±6.09 (oo)cysts/l], followed by midpoints [0.66±1.10 (oo)cysts/l] and upper points [0.66±0.92 (oo)cysts/l]. The mean concentration of *Giardia* cysts was highest at Kuala Pangsun (i.e. 5.97±7.0 cysts/l), followed by Kemensah (0.83±0.81 cysts/l), Pos Piah (0.20±0.35 cysts/l) and Paya Lebar (0.10±0.19 cysts/l). On the other hand, the mean concentration of *Cryptosporidium* oocysts was higher at Kemensah (0.31±0.19 cysts/l) compared to Kuala Pangsun (0.03±0.03cysts/l). All the physical and chemical parameters did not show significant correlation with both protozoa. In future, viability status and molecular characterisation of *Giardia* and *Cryptosporidium* should be applied to identify species and genotypes/subgenotypes for better understanding of the epidemiology of these waterborne parasites.

Keywords Biomonitoring · *Giardia* · *Cryptosporidium* · River water · Riverine indigenous communities

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Introduction

The paucity of safe water and proper sanitation can cause severe impact on the well-being and wholesome development of human health. This is evident as approximately 1.8 million child deaths occur each year due to waterborne and water-related diseases, which translates to 4,900 deaths each day due to diarrhoea (Human Development Reports 2006). Recently, the World Health Organization postulated that improvement of water, sanitation and hygiene are able to prevent at least 9.1 % of the global disease burden (in disability-adjusted life years), or 6.3 % of deaths (Prüss-Üstün et al. 2008).

The repercussion of waterborne transmission can be massive and costly as illustrated in the largest waterborne *Cryptosporidium* outbreak, which occurred in Milwaukee, USA, in the early spring of 1993. With an estimated 1.5 million consumers exposed to *Cryptosporidium* contamination of the public water supply, 403,000 became ill and 104 died (Mac Kenzie et al. 1994). This translated to an economic cost of outbreak-associated illness to approximately $96.2 million (Corso et al. 2003). In areas where intestinal parasitic infections are highly endemic such as Malaysia (Norhayati et al. 2003), prevailing conditions of scarcity of water supply and improper sanitation provide conditions which are conducive for the transmission of diseases especially via contaminated water.

Previously, there have been several studies on the contamination of these waterborne pathogens in the Malaysian water systems (e.g. rivers, well, raw and treated water from drinking water treatment plants, household storage containers and influent and effluent of sewage treatment plants) (Ahmad and Chan 1994; Lim and Ahmad 2004a; Lim et al. 2007; Lim et al. 2008; Lim et al. 2009a, Lim et al. 2009b). It was not surprising that the highest occurrence of *Giardia* and *Cryptosporidium* (oo)cysts were detected in the sewage influent (100.0 % for *Giardia* cysts; 40–50 % for *Cryptosporidium* oocysts) and effluent (83.3–100.0 % and 20.0–25.0 % respectively) (Lim et al. 2007, 2008), followed by contamination of these protozoa in the water bodies in a local zoo [94.4 % (17/18) for *Giardia* cysts] (Lim et al. 2009a) and recreational lake water [77.8 % (7/9) for *Giardia*; no *Cryptosporidium* detected] (Lim et al. 2009b). More importantly, findings with greater impact on public health are the occurrence of these parasites in raw water [46.0 % (40/87) cysts; 6.9 % (6/87) oocysts] (Ahmad et al. 1997; Tan 1998; Lim and Ahmad 2004b) abstracted for drinking water processing, community well water [17.9 % (5/28) cysts; 7.1 % (2/28) oocysts] (Ahmad and Chan 1994) and household storage water [10.0 % (2/20) cysts; 0.0 % (0/20) oocysts] (Lim and Ahmad 2004a).

In addition to these water types, a few studies have also analysed contamination in river water samples in Malaysia. A study conducted in a Temuan indigenous community demonstrated that 66.7 % (12/18) river water samples were contaminated with *Giardia* cysts whilst 5.6 % (1/18) with *Cryptosporidium* oocysts (Lim and Ahmad 2004b). In addition, there was also evidence of *Giardia* cysts and *Cryptosporidium* oocysts contamination into river water from seven cattle farms within the Langat Basin as it was demonstrated that 16.4 % (11/67) and 14.9 % (10/67) of the river water samples taken were contaminated with *Giardia* cysts and *Cryptosporidium* (oo)cysts, respectively (Farizawati et al. 2005). In 2008, Lim et al. (2008) collated all published and unpublished Malaysian studies and reviewed the current knowledge on the presence of *Giardia* and *Cryptosporidium* (oo)cysts in humans, animals, water and soil in Malaysia. The article highlighted that 39 % (67/174) river water samples in Malaysia were contaminated with *Giardia* cysts whilst 11.5 % (20/174) for *Cryptosporidium* oocysts (Lim et al. 2008). In Malaysia, river water is not only used for daily activities such as washing, cooking, bathing and drinking but also as a defecation site for humans and animals. Some of these rivers are also use for recreational activities and as shown in a latest study conducted in recreational river water (i.e. Congkak and Batu rivers) in Selangor, whereby the presence of *Giardia* cysts and *Cryptosporidium* oocysts were 66.7 % (20/30) and 13.3 % (4/30), respectively (Azman et al. 2009).

Giardiasis and cryptosporidiosis can be acquired through anthroponotic and zoonotic transmission routes (Learmonth et al. 2004; Feltus et al. 2006; Khan et al. 2011; Lebbad et al. 2011). There have been numerous studies on *Giardia* and *Cryptosporidium* human infections in Malaysia; however, studies on their contamination in river water found in the vicinity of indigenous communities are still limited. Generally, indigenous people lack adequate supplies of safe water and safe methods of disposing their wastes. Most river water contamination problems are due to their daily activities such as domestic waste disposal (household water), solid waste (garbage) dumps and agricultural activities. The lack of safe water and sanitation create ideal conditions for giardiasis and cryptosporidiosis transmission. According to Castro-Hermida et al. (2009), cysts of *Giardia duodenalis* are able to survive in water at 8 °C for 2 months and Luján et al. (1998) also stated that the *Giardia* cyst can maintain their viability for several months after released outside the host. On the other hand, the *Cryptosporidium* oocysts has been experimentally proven to survive in a wide range of temperature, for example, oocysts held at 25 to 30 °C remain viable for 3 months, survived at 4 °C up to 1 year in artificial seawater and some oocysts maintained in −5 °C can remain viable for 2 months (Fayer et al. 2000; Monis & Thompson, 2003; Carey et al. 2004; Castro-Hermida et al. 2009). Over in Malaysia, viability assay showed that *Cryptosporidium* oocysts can survive in river environment around 2 to 3 months (Lim et al. 1999; Farizawati et al. 2005). Granted that *Giardia* and *Cryptosporidium* (oo)cysts are highly resistant, riverine indigenous communities are exposed to the risk of these waterborne diseases due to environmental contamination. To date, there is limited published information on the occurrence of these waterborne parasites in the river water in indigenous communities although this has crucial implications on their health. Besides, majority of the water quality research and studies were done on physical and chemical parameters without taking into consideration the biological parameters (Arsad et al. 2012) such as protozoa and helminths. Hence, this present study was conducted to determine the occurrence of *Giardia* cysts and *Cryptosporidium* oocysts in the rivers, which are widely used for daily activities by the adjacent indigenous communities.

Materials and methods

Ethical consideration

The present study has been given the ethical considerations (i.e. MEC ref. no. 824.11) by the Ethics Committee of the University Malaya Medical Centre (UMMC) Malaysia prior to the commencement of the study.

Sampling sites and collection of water samples

A total of 10 l of grab water samples were collected from rivers adjacent to the five villages [i.e. Kuala Pangsun (101.88° E