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Research Note

Titiwangsa Lake a source of urban parasitic contamination

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Abstract. Urban recreational lake acts as a source of waterborne parasites contamination, as reported in 2013 in Petaling Jaya, Selangor, Malaysia. This scenario will lead to the transmission of waterborne diseases due to exposure of water-related activities by humans. This study was conducted to reveal the occurrence of common waterborne parasites in a urban lake (i.e. Titiwangsa Lake). The lake is situated in the city of Kuala Lumpur and is known to be overcrowded with vast activities participated by both local and tourist. Results of study showed the presence of *Cryptosporidium*, *Giardia*, free-living amoeba, and helminth-like ova from the lake.

Waterborne diseases are the leading cause of severe illnesses and fatality in humans. Cryptosporidiosis, giardiasis, amoebiasis and schistosomiasis are the most common water-related parasitic diseases that were isolated from the surface water. In humans, a total of 199 outbreaks were reported due to waterborne transmission of protozoan parasites from 2004 until 2010 (Baldursson & Karanis, 2011). Of this, *Cryptosporidium* and *Giardia* are the most common causative agents of waterborne outbreaks resulting in severe gastroenteritis (Mayer & Palmer, 1996). *Acanthamoeba* can be found from both natural and man-made aquatic environments (e.g., soil, water, etc.) and is a cause of nasopharyngeal and cutaneous infections, corneal keratitis and fatal *Acanthamoeba* granulomatous encephalitis (GAE) (Khan, 2003; Marciano-Cabral & Cabreal, 2003; Schuster & Visvesvara, 2004). The (oo)cyst of *Cryptosporidium* and *Giardia* as well as the cyst of *Acanthamoeba* can persist for a long period of time and able to survive in harsh conditions (Rezaeian *et al.*, 2008; Onichandran *et al.*, 2013). Waterborne parasites can be transmitted to the host via inhalation or accidental ingestion through nasal cavity and broken skin.

Lakes can be categorized as surface water with a weak water flow (0.001–0.01m/s) and they mostly drain into rivers or filled by the rainfalls. Titiwangsa Lake (TL) (3°10'44" North, 101°42'27" East) covers an area of 46 hectares of land in Kuala Lumpur, has attracts large numbers of visitors for recreational and water sports activities. Titiwangsa Lake also functions to accommodate runoff from two polluted rivers (Bunus River to Gombak River) when heavy rain occurs (Umar, 2012).

Pollution of surface water such as lakes may originate from effluent discharges, runoff due to rainfall/snowfall and accidental defaecation by animals from the surroundings (Fayer *et al.*, 2000; Fong *et al.*, 2007) and able to contribute to the emergence of waterborne parasites. Water-related activities have long been one of the major factors that contribute to the exposure of waterborne parasites (Kramer *et al.*, 1998; Azman *et al.*, 2009). Meanwhile, two urban lakes, located within the vicinity of Petaling Jaya, Selangor were reported to be contaminated with waterborne parasites (Onichandran *et al.*, 2013).

A total of 12 litres of water samples were collected in sterile polyethylene containers from Titiwangsa Lake at 7 different stations, namely; TL1 (near a jetty), TL2 (near a rest hut), TL3 (near water polo), TL4 (near playground), TL5 (near food stall), TL6 (in front of a picnic site), and TL7 (in front of an open place), as shown in Figure 1. Water was taken from two different points at each station; surface point (at least 5 cm beneath the surface) and deep point (bottom of the lake). Ten and two litres of water samples were filtered for the detection of protozoan (oo)cysts and *Acanthamoeba*, respectively, using a nitrocellulose membrane (0.45 µm pore size, 142 mm diameter, Millipore).

The (oo)cysts were purified via immuno-magnetic separation (IMS) technique (Dynabeads GC-Combo, Invitrogen, USA), based on the established method 1623 (USEPA 2005). The (oo)cysts were stained with commercial fluorescence iso-thiocyanate (FITC)-labelled (Crypto/Giardia Cel IF Kit, Cellabs Pty Ltd., cat. No. KR2134A1, Brookvale, Australia) and 4'6-diamidino-2-phenyl indole (DAPI) (Sigma Chemical Co., cat. No. D-9542 Louis, Missouri, USA) prior to observation at 400x magnification via epifluorescence microscope (Olympus BX51, Tokyo, Japan). The supernatant from the IMS was decanted and stained with one drop of Lugol's iodine solution for the detection of helminth ova.

According to microscopic examination, *Cryptosporidium* oocysts and *Giardia* cysts were described as round to ovoid and ovoid to spherical, respectively, as *Giardia* cyst is relatively bigger than *Cryptosporidium* oocyst (Thompson, 2004; Sunnotel *et al.*, 2006; Chalmers *et al.*, 2010; Fayer, 2010). In average, the number of (oo)cysts detected were at low concentration (0.4-6.4 (oo)cysts/L). *Cryptosporidium* oocysts were not frequently found at each station and the number of oocysts detected were lower (average of 0.8 oocyst/L) compared to *Giardia* cysts (average of 0.94 cysts/). *Giardia* cysts were recovered from all sampling stations (except TL2), showing

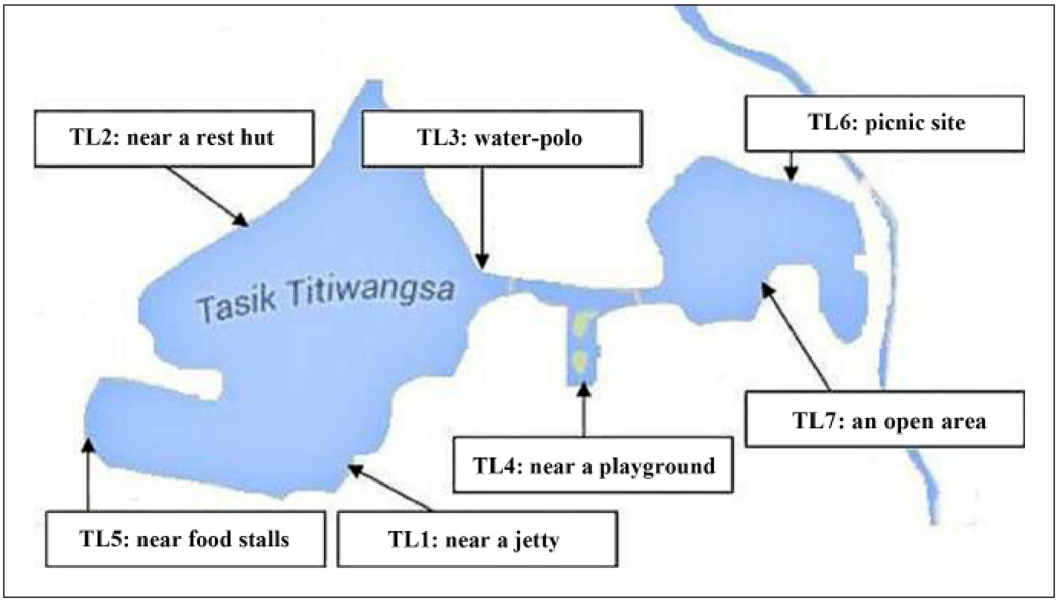

Figure 1. Location of sampling stations at Titiwangsa Lake (TL).

that high frequency of *Giardia* can be found from raw water samples (Robertson *et al.*, 2001). The number of *Giardia* cysts recovered from surface point was 3.7 times higher than the cysts obtained from deep point. This could be due to the agitation during water collection. Among the helminths, the eggs of hookworm-like and *Trichuris*-like were found in all stations, followed by *Ascaris*-like and *Schistosoma*-like eggs, as summarized in Table 1. The presence of these parasites can be associated with faecal contamination from human and animals into the water bodies (Hunter *et al.*, 2005; Xiao *et al.*, 2008). The faecal contamination in Titiwangsa Lake may have originated from Bunus River runoff or excreted by animals that were roaming around the area (presence of birds, cats, dogs, or horses was observed).

The presence of free-living amoeba in Titiwangsa Lake water was determined by plating assays on NNA medium (Figure 2). All samples were found positive for *Acanthamoeba*-like structure after a series of sub-culturing. This may be due to the presence of algae in the lake that act as food source to the amoeba, besides bacteria. The recognition of the amoeba was confirmed via two distinct life stages of protruding trophozoites (i.e. acanthopodia) and double-layered wall cysts (Khan & Tareen, 2003). Culturing on agar medium mimicked the condition of water samples collected at the lake bank, where the adhesion and movement of the amoeba can be seen during morphological determination.

The occurrence of waterborne parasites is associated by abiotic factors (i.e. physico-chemical) (Patz *et al.*, 2000; Onichandran *et al.*, 2013). The physical data can be used as preliminary results to determine the water quality and plays a vital role in reflecting the nutrient availability. The nutrients in the water may be not used directly by the parasites, but is consumed by other microbes that forms the source of food to the waterborne parasites that had been identified. Therefore, physical parameters such as turbidity, temperature, dissolved oxygen (DO), salinity (Sal), conductivity, total dissolved solids (TDS), and pH were measured *in situ* using multi-probe parameter (YSI 556 Multiprobe System, Colorado, USA). Correlation between physical parameters and the presence of (oo)cysts in the lake were determined. Values of $p< 0.05$ and $p< 0.01$ were considered as satatistically significant. *Giardia* cyst was found to be correlated with turbidity (R^2=0.936) and temperature (R^2=0.773), as shown in Table 2.

Table 1. Occurrence of waterborne parasites in Titiwangsa Lake (TL)

Station	Point	*Cryptosporidium* (oocyst/L)	*Giardia* (cyst/L)	*Acanthamoeba*-like structure	Helminth-like ova			
					Ascaris	Hookworm	*Trichuris*	*Schistosoma*
TL1	Surface	1.2	0.8	+	ND	+	+	+
	Deep	0	0					
TL2	Surface	0	0	+	ND	+	+	ND
	Deep	0	0					
TL3	Surface	0	0.4	+	ND	+	+	+
	Deep	2.0	1.2					
TL4	Surface	0	1.6	+	+	+	+	+
	Deep	0.8	1.2					
TL5	Surface	0	6.4	+	+	+	+	ND
	Deep	0	0.4					
TL6	Surface	0	0.4	+	ND	+	+	+
	Deep	0	0					
TL7	Surface	0.8	0.8	+	ND	+	+	+
	Deep	0	0					

ND = not detected
'+' = positive growth/detected

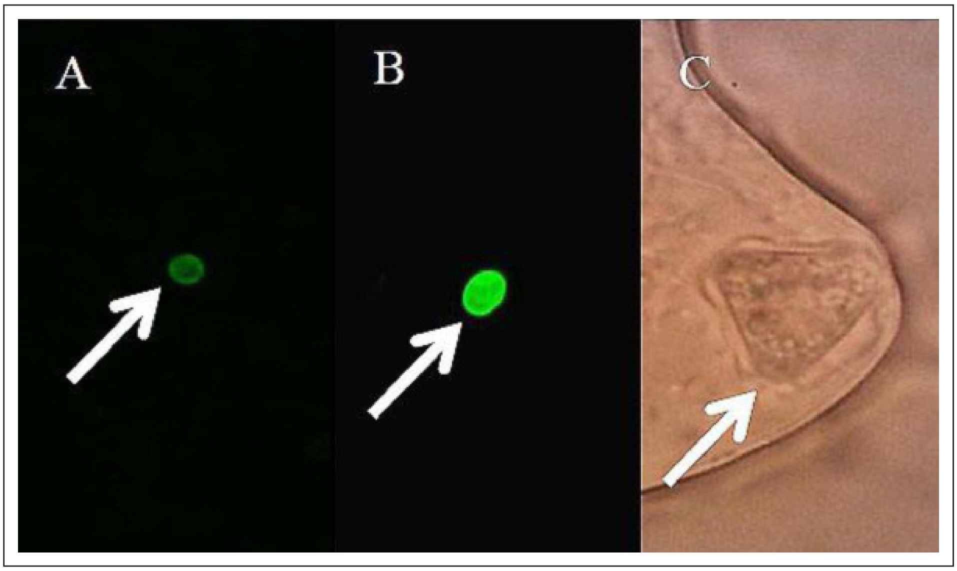

Figure 2. The isolated waterborne parasites from Titiwangsa Lake: (A) The oocyst of *Cryptosporidium* spp. (x400), (B) The cyst of *Giardia* spp. (x400), (C) *Acanthamoeba* spp. in triangular shape (x400).

Table 2. Overall mean of water physical parameters of Titiwangsa Lake (TL)

Physical parameter	Point	Mean±SD
Turbidity (NTU)	Surface	4.11±3.57**
	Deep	111.75±109.22
Temperature (°C)	Surface	30.41±0.68
	Deep	29.74±0.62*
Dissolved oxygen (mg/L)	Surface	2.10±0.41
	Deep	1.99±0.29
Conductivity (mS/cm)	Surface	0.15±0.01
	Deep	0.15±0.01
Salinity (ppt)	Surface	0.06±0.01
	Deep	0.06±0.01
Total dissolved solids (mg/L)	Surface	87.25±6.47
	Deep	88.50±8.35
pH	Surface	8.94±0.27
	Deep	8.64±0.21

SD=standard deviation
*$p<0.05$; **$p<0.01$ (correlation is significant at these levels for the presence of *Giardia* cyst)

Titiwangsa Lake, which is stagnant by nature, revealed the existence of waterborne protozoan (oo)cysts, free-living amoeba and helminth-like ova. This finding can serve as a baseline to determine the presence of common waterborne parasites in an urban water basin. The emergence of the aforementioned parasites posed a great health threat, given the frequency of visitors coming to Titiwangsa Lake is increasing. Therefore, precautions should be taken by the authorities to ensure the lake water is safe to be used.

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