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The Occurrence of *Cryptosporidium* oocysts and Physical Assessment of Selected Lakes in Kuantan, Pahang

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ABSTRACT

Parasitic infection is one of major public health problems especially in developing countries. *Cryptosporidium* is one of the common human parasites that cause waterborne disease outbreak in industrialized countries. In this present study, we aimed to determine the occurrence of *Cryptosporidium* and to determine whether a statistical association existed between the occurrences and physical parameters in water samples from selected recreational lakes in Kuantan, Pahang, Malaysia. Samples were collected from three stations of Lake TG and Lake TB for three consecutive months, from November 2014 to January 2015. The samples were processed to detect the presence of the waterborne protozoa using flat-bed membrane filtration technique. Between the two lakes, the occurrence of *Cryptosporidium* was higher in Lake TB (0.66 oocysts/L) as compared to Lake TG (0.53 oocysts/L). For monthly occurrence trend for both lakes, the number of *Cryptosporidium* oocysts present was highest in November, followed by January, and lastly December. In Lake TG, the occurrence of these protozoa was 0.27 oocysts/L during November, 0.06 oocysts/L during December, and 0.20 oocysts/L during January while in lake TB, the occurrence of *Cryptosporidium* was 0.37 oocysts/L during November, 0.06 oocysts/L during December, and 0.23 oocysts/L during January. All the physical parameters did not show any significant correlation with the protozoa based on Pearson correlation analysis where p (probability level) > 0.05. Therefore, these physical parameters are not suitable for the indicator of the presence of these parasite oocysts in both lakes. Based on the results, it is clearly shown that there is a need to implement the detection of waterborne protozoa and physico-chemical analysis in Malaysia. In, addition, the analysis of heavy metals (copper, mercury, chromium, and zinc) is also recommended for this type of study. Hence, molecular characterization and viability status of *Cryptosporidium* should be applied to identify species and genotypes for better understanding of the epidemiology of this waterborne parasite.

Keywords: Cryptosporidium, lake, physical parameters, Kuantan, Malaysia

INTRODUCTION

Waterborne parasites are ubiquitous protozoan pathogens that affect human, domestic animals and wildlife [8]. In most cases, waterborne parasites occur in countries with low socio-economic communities. The outbreaks are related to contamination of drinking water, river, lakes, and other recreational water environment.

Lake is one of the sources of freshwater, which serves as an important component to our environment. In particular, lakes function as catchment basins for almost 40% of landscape and thus serve in recreational, sporting and fishing activities [2]. From these findings, there are high possibilities for lakes in recreational areas to be contaminated with microorganisms since these areas always attract crowds of people and animals [3].

There are many types of waterborne protozoan parasites but *Giardia* and *Cryptosporidium* species

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are the most common types that have always been highlighted as significant waterborne parasites and enteric protozoa [8,13]. *Cryptosporidium* can cause human gastrointestinal illness worldwide [9]. The transmission of *Cryptosporidium* by water occurs from the interaction between human host and animal host [5,14]. In Malaysia, so far, only one study had been done on the assessment of waterborne parasites in recreational lakes, showing that there was occurrence of *Cryptosporidium* from two recreational anthropogenic lakes located in Selangor, Malaysia [12]. In other studies on rivers, they showed that river water contains high occurrence of this protozoa [4,10]. From those studies, we can assume that lake water will have higher occurrence of *Cryptosporidium* since the rate of water flow of lake is lower than river. Thus lead to higher accumulation of the protozoa. Besides, human contacts with recreational lakes for many purposes are very frequent.

Hence, this study was conducted to identify the *Cryptosporidium* in two selected lakes in Kuantan, Pahang, Malaysia since there is no study done yet in this area. Considering the seasonal factors, Pahang is located in the east-coast region of Malaysia and Kuantan is the main capital of Pahang which receive maximum rainfall during annual monsoon season. Rainy season may increase waterborne parasites trending. These conditions may be due to contamination of drinking water and flooding of rivers and lakes that could be originated from sewage or fecal contamination. Therefore, the establishment of intestinal waterborne parasite trending during monsoon season is best done during maximum rainfall, from November to January (Malaysian Meteorological Department, 2013).

In this study, samples were taken from Lake TG and Lake TB in Kuantan, Pahang, Malaysia during three consecutive months of monsoon season (November-January). The samples were then processed to detect *Cryptosporidium*. Therefore, the presence of *Cryptosporidum* in water samples obtained from recreational lakes was determined by microscopy observation of the oocysts. Physical parameters such as dissolved oxygen, pH, salinity, temperature and total dissolved solid were measured to find the significant association with the occurrence of the oocysts.

2. Objectives:

The aim of this study was to identify the occurrence of *Cryptosporidium* and to determine its association with physical parameters in water samples from two selected lakes in Kuantan, Pahang, Malaysia.

Materials and Methods

3.1. Study Area:

The present study was carried out in Kuantan, Pahang (3° 49'00''N 103°20'00''E). This district is located in east coast region of Peninsular Malaysia where it is one of the fast growing cities. Kuantan is the 9th biggest city in Malaysia with a population approximately 608,000. Fast growth in industrialization in 10 years has assisted in tremendous increase in economic growth and thus resulted in increase of its population. Kuantan receives rainfall throughout the year. The average humidity level for Kuantan is hot-humid.

3.2. Sample Collection:

Two recreational anthropogenic lakes located in Kuantan, Malaysia selected for water collection. The first recreational Lake TG (L-TG) is located at 3°48'26.356"N and 103°20'50.819''E which is within a housing and restaurant area which serve public, while second recreational Lake TB (L-TB) is located at 3°50'10.196"N and 103°17'49.731''E that is surrounded by offices, school, hotel, and shops, also serving public. Sampling stations in both lakes (Figure 1) were selected according to several criteria, where there is involvement of human recreational activities such as fishing and picnics near the inlet, middle, and outlet of each lake. Three water samples from each lake were collected from November 2014 until January 2015.

Fig. 1: Three sampling stations near the inlet (a), middle (b), and outlet (c) of both recreational Lake TG and Lake TB.

3.3 Measurement of Physical Parameters:

The physical parameters of water were recorded in situ during sampling activities. The parameters measured were dissolved oxygen (mg/L), pH, temperature (°C), and turbidity (NTU) using multi probe equipment (Hydro-Lab Quanta). All parameters data obtained from each sampling activity were recorded and accumulated for correlation analysis with the amount of cyst/oocyst of protozoa detected.

3.4. Filtration of Water Samples:

A total of 10 L water sample was collected in sterile carboyl containers at each sampling spot and stored at 4ºC prior processing. Ten litres of water samples were filtered using flat-bed membrane filtration technique. The Whatman® Sterile nitrocellulose membrane with 1.2 µm pore size were used to filter the water samples. Sediment trapped on the membrane is then filtered.

3.5. Concentration and purification of Cryptosporidium:

A commercial kit (Dynabeads GC-Combo, Invitrogen, USA) was used to separate oocysts with debris by using immunomagnetic separation (IMS) technique. The purification of oocysts technique was done by following the manufacturer's procedures. This technique was applied according to standard protocol the US EPA Method 1623. Basically, the oocysts are bound to the beads labeled with Cryptosporidium antibody which separates oocysts from the debris. At the end, purified oocysts were collected for oocysts examination.

3.6. Detection of Cryptosporidium Oocysts:

A total volume of 50 µl purified samples were stained with anti-*Cryptosporidium* monoclonal antibodies conjugated to fluorescein isothiocyanate (FITC) kit (Cellabs, Australia). Stained sample was observed under epifluorescence microscope (magnification x400). The presence of *Cryptosporidium* was calculated as; number of (oo)cysts per liter = number of oocysts on slide (contained by 50 µL)/10 L.

3.7. Statistical Analysis:

Data obtained were analyzed using version 17.0 of Statistical Package for Social Sciences software for Windows (SPSS Inc., Chicago, IL, USA). Linear regression analysis was used to evaluate the association between the number of oocyst of waterborne parasites and physical parameters. The values of $p<0.01$ and $p<0.05$ were considered as statistically significant.

Results and Discussion

4.1 Occurrence of cryptosporidium in water samples:

Both Lake TG and Lake TB were contaminated with *Cryptosporidium* in most stations (0.1–0.3 oocyst/L). Overall, the occurrence of *Cryptosporidium* oocysts was the highest during November in both lakes, followed by January and lastly, December.

Among these three months of monsoon season, the occurrence of *Cryptosporidium* oocysts has increased in months with lower rainfall volume (November and January). The high occurrence of oocysts during these months may be due to less volume and slow flow of the lake water. These conditions have contributed to accumulation of many oocysts on the lake bed [4].

According to Malaysian Meteorological Department (2014), the rainfall distribution in Kuantan during December 2014 was the highest among other monthly distribution with readings of 1806.0 mm^3.

In December 2014, high rainfall volume has caused flooding in area of Lake TB and has caused a massive increase in water volume in Lake TG. The number of protozoa per liter could have decreased in more diluted water. Furthermore, high water current produced during the flood may wash away the protozoa. High rainfall in that month has also reduced the number of visitors to the lakes and therefore less contamination from people.

Based on Figure 2, from November 2014 to January 2015, *Cryptosporidium* oocysts were detected more frequently in water samples from Lake TB compared to Lake TG. The location of Lake TB is nearer to Kuantan city centre than Lake TG. Besides, the population's size surrounding Lake TB is bigger with higher number of local people carrying out their daily activities and higher number of visitors coming for recreational activities, causing this area to be more crowded compared to Lake TG. These conditions make Lake TB highly exposed to various forms of human contamination. To support this finding, many stray dogs were observed to live in the surrounding area of Lake TB. It is believed that food wastes and garbage were the attraction factor for the animals. It could be a significant source of *Cryptosporidium* oocysts since they were known to be common in many animals [4].

4.2 Physical parameters:

The physical parameters measured are summarized in Table 1. Both lakes have a slightly acidic condition (pH 6.0) at 25.4°C in December. However, the pH was neutral (pH 7.0) at 28.9-29.1°C in other months of study for both lakes.

The DO for Lake TG was similar (4.0-4.3 mg/L) in November and December except for January (2.8 mg/L). However, the DO for Lake TB was measured at close range at each station for all three months (4.75–5.98 mg/L).

Overall, the average turbidity of Lake TB (141.9 nephelometric turbidity units (NTU) appeared to be higher than Lake TG (9.2 NTU). The turbidity of Lake TB was 120.5 in November, the highest in December with NTU of 256.8 and 48.3 in January. The high level of turbidity in Lake TB clearly exceeded the Interim National Water Quality Standard (INWQS) of Malaysia limits for Class II B waters (50 NTU). However, the highest turbidity in Lake TG was measured at station 1 (10.1 NTU) during January 2015 and the lowest at station 3 (0.1 NTU) during December 2014.

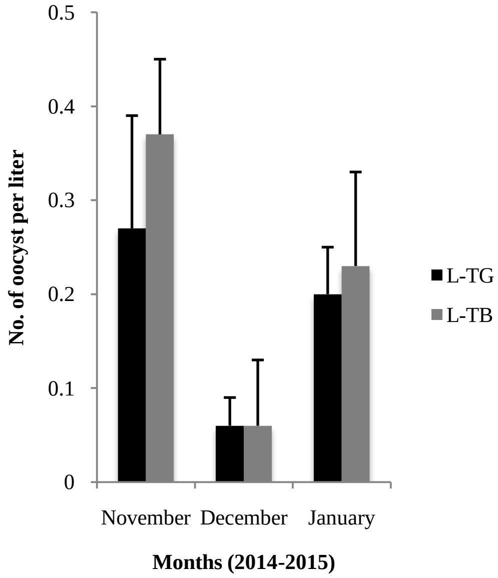

Fig. 2: Comparison of number of oocyst (per litre) found in all station from Lake TG (L-TG) and Lake TB (L-TB) within the three consecutive months in year 2014 to 2015.

4.3 Correlation between physical parameters and detected cryptosporidium oocyst:

Pearson correlation analysis revealed that there was no significant relationship between *Cryptosporidium* oocysts and DO, pH, temperature and turbidity respectively. Detailed correlation analysis between *Cryptosporidium* oocysts detected and physical parameters is presented in Table 2.

Table 1: Physical parameters from different stations of Recreational Lake TG and Lake TB.

Samples	Dissolve Oxygen (mg/L)	pH	Temperature (ºC)	Turbidity (NTU)
November 2014				
Lake TG Station 1	4.46	7.27	28.90	1.65
Station 2	4.04	6.90	29.52	3.65
Station 3	3.56	7.20	29.00	1.45
Mean	4.02	7.12	29.14	2.27
Lake TB Station 1	5.68	7.19	29.67	134.40
Station 2	6.32	7.94	29.47	114.55
Station 3	5.93	7.39	29.62	112.70
Mean	5.98	7.51	29.59	120.55
December 2014				
Lake TG Station 1	4.04	6.44	26.38	0.35
Station 2	3.25	5.89	26.01	2.85
Station 3	5.71	6.99	25.50	0.05
Mean	4.33	6.44	25.96	1.08
Lake TB Station 1	5.53	6.93	25.24	329.50
Station 2	3.52	7.02	25.57	208.50
Station 3	5.21	7.10	25.25	232.50
Mean	4.75	7.02	25.35	256.83
January 2015				
Lake TG Station 1	2.59	7.38	28.81	10.09
Station 2	2.43	7.21	28.03	4.85
Station 3	3.34	6.85	29.62	2.48
Mean	2.79	7.15	28.82	5.81
Lake TB Station 1	4.98	6.39	29.41	59.45
Station 2	4.81	6.88	28.88	40.30
Station 3	4.99	6.95	29.07	45.15
Mean	4.93	6.74	29.12	48.30

Table 2: Correlation between physical parameters and *Cryptosporidium* (r values at 95 % CI) in Lake TG and Lake TB.

Parameters	Lake TG	Lake TB
Dissolved oxygen (mg/L)	-0.37	0.90
pH	-0.93	0.58
Temperature (oC)	0.97	0.93
Turbidity (NTU)	0.42	-0.69
*p <0.05 (correlation is significant at these levels)		

These non-significant correlations express the true description of the association between *Cryptosporidium* and physical parameters of lake water since the results were consistent in both lakes. Thus, all the physical parameters could not be used as markers for the occurrence of this protozoon in water from both lakes. Same findings were also obtained in many other studies of water [1,7,11].

Although there was no significant correlation between the occurrence of the parasite with the parameters in Lake TG and Lake TB, the results could not be generalized. This is because every lake in the world has its own condition in terms of the level of pollution and also the organism content. They were major contributing factors that influence the level of physical parameters in lake water. Perhaps with more sampling stations the results could be more remarkable.

Conclusion:

The findings of this study imply that recreational lakes in Kuantan represent a risk for contamination of protozoa infection because of the *Cryptosporidium* occurrence in the water samples. Although no significant relationship was observed between the presence of the oocsyts and physical parameters being studied, this three-month survey emphasizes that there is still lack of conventional indicators for *Cryptosporidium* contamination of water. The occurrence of *Cryptosporidium* in the water samples from two lakes was possibly caused by the contamination of daily recreational activities. Due to the possible health risk, detailed inspection should be done on recreational lakes, as well as other sources of recreational water. Therefore, further action should be taken to reduce the infection of waterborne protozoa in those lakes. Malaysian citizens and relevant authorities need to improve the water safety level by maintaining the quality of recreational lakes such as appropriate biological reviving methods, routine cleaning up, and, most importantly, public education via awareness campaign programs.

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