Asian Pacific Journal of Tropical Disease

journal homepage: http://www.apjtcm.com

Original article https://doi.org/10.12980/apjtd.7.2017D7-103

The association of *Cryptosporidium* from three different points of Balok River and Kuantan River by using physico-chemical and heavy metal assessments

Fatin Khairunnisa Zainutdin[1], Mohd Aiman Barudin[1], Mohammed Abdullah Jainul[1], Muhammad Lokman Md Isa[2,3], Afzan Mat Yusof[2,3*]

[1]*Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia*

[2]*Department of Basic Medical Sciences, Kulliyyah of Nursing, International Islamic University Malaysia, Jalan Hospital Campus, 25100 Kuantan, Pahang, Malaysia*

[3]*Integrated Cellular and Molecular Biology Cluster (iMolec), Integrated Centre for Animal Care and Use, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia*

ARTICLE INFO

Article history:
Received 12 May 2017
Received in revised form 1 Jun 2017
Accepted 30 Jun 2017
Available online 31 Jul 2017

Keywords:
Water quality
River
Cryptosporidium
Physico-chemical
Metal elements

ABSTRACT

Objective: To detect the occurrence of *Cryptosporidium* oocysts and to assess the physico-chemical and heavy metal parameters in two main rivers in Kuantan.

Methods: Water samples were collected at three sampling points per river (upstream, midstream, downstream) from Kuantan River and Balok River. Samples were filtered using the continuous flow centrifugation machine followed by immunomagnetic separation technique to isolate *Cryptosporidium* oocysts and stained using fluorescein isothiocyanate staining. *Cryptosporidium* oocysts were examined using fluorescence microscope. Physical parameters were assessed *in-situ* using Cyber Scan PCD 650 multi-parameter instrument. Both chemical and heavy metal assessments were done in the laboratory following the American Public Health Association Standard Methods with slight modification. The parameters attained were compared with the Interim National Water Quality Standards (INWQS) which is the standard vital parameters used to evaluate the safety level of surface water in Malaysia.

Results: All samples were positive with *Cryptosporidium* oocysts. Results for physical parameters were within the range of INWQS in Malaysia. For chemical assessment, results for chemical oxygen demand and biological oxygen demand exceeded the INWQS range in most of the sampling points. Of 23 metal elements assessed, only 9 elements were found. Both Kuantan River and Balok River can be classified under Class III river following INWQS which requires extensive treatment.

Conclusions: This study hoped to provide new and updated information on the occurrence of *Cryptosporidium* and its physico-chemical assessment in two main rivers in Kuantan. Future study on molecular identification of *Cryptosporidium* in rivers needs to be done in order to identify the source of transmission of this waterborne parasite.

1. Introduction

Water is a vital and valuable source for all living things. Our earth is 71% covered by water where it comes from many sources such as rivers, seas, underground and ice caps[1]. However vital point is that only 2.5% consists of fresh water resources and again only 0.4% is available for use[2]. In Malaysia, Department of Environment is responsible for monitoring and controlling the water quality by enforcing the Environmental Quality Act where the quality of water is compared with the National Water Quality Standards for Malaysia[3]. In order to maintain the health of a human population, obtaining safe water supply is very important. Contaminated public water supply may possess serious human health risk and might even lead to death. People have been using water for various purposes such as for drinking, irrigation, industrial, recreational, and fisheries[4].

Human activities such as deforestation, agriculture, logging, and industrialization would really affect the water quality. Uncontrolled and illegal waste disposal into the river body can also affect the water quality, hence making it unsafe for daily usage[5]. It has led to the greatest environmental threat which needs to be controlled

*Corresponding author: Afzan Mat Yusof, Assistant Professor, Ph.D, Department of Basic Medical Sciences, Kulliyyah of Nursing, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia.
Tel: +60129656253
E-mail: afzan@iium.edu.my

Foundation Project: Supported by Ministry of Higher Education Malaysia under the grant of Fundamental Research Grant Scheme (RAGS) No. 14-1480389 and International Islamic University Malaysia Research Initiative Grant Scheme (RIGS) No. 16-301-0465.
The journal implements double-blind peer review practiced by specially invited international editorial board members.

and resolved as it may affect the quality of water resources[6]. Proper management and treatment of water supply are essential to ensure the water is safe and free from any pollutants for public consumption. Due to that, regular monitoring of water quality is important as it helps to protect the natural resources from contamination[7].

Water contamination can be defined as the alternation of physical, chemical and biological properties of water which may lead to harmful effects on living organisms and other biological aspects either directly or indirectly, or immediately or after sometimes[8]. Physical and chemical assessments of water can be done to determine the quality of water. Vital parameters such as pH, temperature, dissolved oxygen, salinity, biological oxygen demand (BOD), chemical oxygen demand (COD), heavy metals and many others are commonly used to evaluate the quality of water.

Despite the remarkable advancement in water treatment technology, the presence of waterborne parasites especially *Cryptosporidium* in water sources is still a growing problem worldwide. The occurrence of *Cryptosporidium* in water has also been reported in Malaysia[1,9-11]. *Cryptosporidium* is a waterborne parasite that commonly caused gastrointestinal diseases. This parasite can be transmitted to both human and animals where the infectious sporulated oocyst is ingested by a host causing cryptosporidiosis[12]. In immunocompetent human, infection by *Cryptosporidium* can result in acute but self-limiting diarrhea while in immunocompromised people, it can be a life-threatening disease[13].

Cryptosporidium oocysts are ubiquitous in water sources and have the ability to survive for months in the extreme environment[1,11]. Due to its thick cell wall, the oocyst is highly resistant to chlorination and ozonation[1,14,15]. Other than that, it can pass through common water filter due to its micro size (4–6 μm)[1]. Low infectious dose of *Cryptosporidium* oocysts are able to affect humans and animals[11]. Approximately 10–83 oocysts of *Cryptosporidium hominis* can cause infection to human while 132 oocysts of *Cryptosporidium parvum* are needed to be ingested for the infection to occur[16]. However, in reality, ingestion of 1 oocyst would be sufficient enough to cause cryptosporidiosis[17].

In Malaysia, studies on the occurrence of *Cryptosporidium* and physico-chemical assessment of river water have not been clearly established especially in the east coast region. Most of the studies were done in Selangor[1,9,10] and until now only one study was done in Kuantan[11]. However, their research only focused on the occurrence of *Cryptosporidium* and physical assessment in selected rivers in Kuantan. Information regarding the river quality of Pahang is not sufficient enough. Therefore, two main rivers in Kuantan were selected and screened for the occurrence of *Cryptosporidium* and tested for selected physical and chemical parameters. The concentration of heavy metal in the rivers was also assessed. This study would like to provide new and updated information on the occurrence of *Cryptosporidium*, its physico-chemical and heavy metal assessments in two main rivers in Kuantan.

2. Materials and methods

2.1. Study area

The present study was done in Kuantan, Pahang (3°49'00'' N 103°20'00'' E). Kuantan is the state capital of Pahang and is one of the main city in the east coast of Peninsular Malaysia. According to the Kuantan Local Plan, the population in Kuantan was 607 778 in 2012 and it is expected to be 642 555 in 2015. Due to the rapid development in Kuantan for the past years, there is a great increase in its population now[18]. Throughout the year, the weather in Kuantan is typically hot and humid and also received rainfall.

Two main rivers in Kuantan, *viz.* Kuantan River and Balok River were selected as the sampling sites. Kuantan River flows from Sungai Lembing through Kuantan city whilst Balok River located near the Gebeng industrial area and flows through Balok town. Both rivers flow towards the South China Sea.

2.2. Collection of water samples

Water samples were collected from two main rivers in Kuantan namely Kuantan River and Balok River. Water samples were collected at three sampling points (upstream, midstream and downstream) per river. Figure 1 shows the location of the sampling points from both Kuantan River and Balok River. The sampling points were given by the Department of Irrigation and Drainage Pahang. Downstream areas have the highest population density followed by midstream and upstream areas of rivers. Water samples were collected from both rivers from May 2016 to August 2016.

Water samples of 50 L were collected per sampling point from approximately 20 cm below the water surface of river. Collection of water samples were done using Van Dorn water sampler and transferred into sterile carboy containers. Water samples were stored in ice boxes to be preserved before transported to the laboratory for analysis and processed immediately upon arrival.

2.3. Detection of Cryptosporidium oocysts

The detection of *Cryptosporidium* oocysts was done following the established United States Environmental Protection Agency Method 1623. Water samples of 50 L per sampling point were filtered using continuous flow centrifugation machine (Scientific Methods Inc, USA). *Cryptosporidium* oocysts were isolated from the eluate by using the immunomagnetic separation technique (Dynabeads GC-Combo, Life Technologies AS, Norway) following the manufacturer's instructions. Evaluation of *Cryptosporidium* oocysts was done by immunofluorescence assay where purified oocysts were stained using a fluorescein isothiocyanate-labelled monoclonal antibody kit specific to *Cryptosporidium* oocyst (Cellabs, Pty Ltd., Australia). The oocysts were examined using Nikon Eclipse Ti Fluorescence microscope (Nikon Instruments Inc., Japan) where *Cryptosporidium* oocyst should stained in fluorescence green and appeared as ellipse, oval or spherical shapes (4–6 μm) under 400× magnification[1]. Crypto-positive control slide (Cellabs Pty Ltd., Australia) was used as a positive control while distilled water was used as a negative control.

2.4. Assessment of physical parameters

Physical parameters were measured *in-situ* by using the Cyber Scan PCD650 handheld multi-parameter instrument (Thermo Scientific, USA). The multi-probe electrode was submerged in the river and the results obtained were recorded. There were six

Figure 1. Maps showing the sampling points from both Kuantan River and Balok River.

parameters assessed which were pH, temperature, dissolved oxygen (DO), conductivity, salinity and total dissolved solids (TDS). Parameters attained were compared with the Interim National Water Quality Standards (INWQS) which is the standard vital parameters used to evaluate the safety level of surface water in Malaysia.

2.5. Assessment of chemical and heavy metal parameters

Chemical and heavy metal parameters were measured in this study. Chemical parameters assessed were COD, BOD, ammoniacal nitrogen (NH_3-N), fluoride (F), nitrate (NO_3), nitrite (NO_2) and

chloride (Cl). For heavy metal parameters, the assessment was done by using the inductively coupled plasma mass spectrometry (ICP-MS). Table 1 shows all metal elements that had been screened using ICP-MS. All parameters tested were measured following the American Public Health Association Standard Methods with slight modification. The parameters attained were then compared with the INWQS which is the standard vital parameters used to evaluate the safety level of surface water in Malaysia.

Table 1
List of metal elements screened using ICP-MS.

No.	Element
1	Silver (Ag)
2	Aluminium (Al)
3	Boron (B)
4	Barium (Ba)
5	Bismuth (Bi)
6	Calcium (Ca)
7	Cadmium (Cd)
8	Cobalt (Co)
9	Chromium (Cr)
10	Copper (Cu)
11	Iron (Fe)
12	Gallium (Ga)
13	Indium (In)
14	Potassium (K)
15	Lithium (Li)
16	Magnesium (Mg)
17	Manganese (Mn)
18	Sodium (Na)
19	Nickel (Ni)
20	Lead (Pb)
21	Strontium (Sr)
22	Thallium (Tl)
23	Zinc (Zn)

3. Results

3.1. Detection of Cryptosporidium oocysts

A total of six water samples were collected from Kuantan River and Balok River. Following the immunofluorescence assay, all samples were positive with *Cryptosporidium* oocysts. When observed under a fluorescence microscope, *Cryptosporidium* oocysts were detected as it appeared in an oval shape with approximately 5 μm in size. The cell wall of *Cryptosporidium* oocyst stained in bright green fluorescence with a dark green background. These criteria were found in all six sample slides hence confirming the occurrence of *Cryptosporidium* in both Kuantan River and Balok River.

3.2. Assessment of physical parameters

Table 2 presented the average values for physical parameters of all water samples collected from the rivers. Kuantan River showed slightly acidic condition (pH 6.12–6.50) at 26.8–30.1 ˚C. However, Balok River was a slightly alkaline condition (pH 7.20–7.79) at 28.5–30.4 ˚C except at the upstream (pH 6.54) which was slightly acidic.

DO reveals the amount of dissolved oxygen in the water. The amount of DO was ranging from 3.70–7.72 mg/L in both Kuantan River and Balok River. The upstream of Kuantan River and Balok River showed the highest DO content which was 7.72 mg/L and 7.02 mg/L respectively. The lowest DO content was at the midstream of Balok River (3.70 mg/L).

The conductivity of downstream Balok River (49.190 mS) was the highest followed by downstream Kuantan River (21.713 mS) and midstream Kuantan River (4.186 mS). Conductivities at midstream Balok River and both upstream Kuantan River and Balok River were low. For salinity, downstream Balok River (67.30 ppt) was the highest followed by downstream Kuantan River (26.40 ppt) and midstream Kuantan River (4.50 ppt).

The concentration of TDS generally described the inorganic salts and a small amount of organic matter present in the water. Results of TDS at the downstream of Balok River was the highest followed by downstream Kuantan River and midstream Kuantan River with 46.27 ppt, 20.46 ppt and 3.45 ppt, respectively.

3.3. Assessment of chemical and heavy metal parameters

The chemical parameters measured are summarized in Table 3. Each sampling site has a different amount of COD. Both downstream Kuantan River and Balok River recorded the highest COD level which was 390 mg/L and 490 mg/L, respectively. BOD level at all sampling points (8.2–51.0 mg/L) exceeded the INWQS Class III river (6 mg/L) except at the downstream of Balok River (5.1 mg/L). However, the level of ammoniacal nitrogen, nitrate and nitrite had not exceeded the safety level of Class III river. Interestingly, the level of chloride in the downstream Kuantan River and Balok River were higher (1 100 mg/L and 13 600 mg/L) compared to the upstream and midstream rivers. Chloride is commonly found in water as it leached from various rocks into the soil and water by the weathering process. The Cl^- ions is highly mobile, hence it can be found at a higher concentration in the downstream area (1 100–13 600 mg/L)

Table 2
Physical parameters of river water from Kuantan River and Balok River.

Samples		pH	Temperature (˚C)	DO (mg/L)	Conductivity (mS)	Salinity (ppt)	TDS (ppt)
Kuantan River	Upstream	6.50 ± 0.10	26.80 ± 0.06	7.72 ± 0.63	0.037 ± 0.010	0.06 ± 0.03	0.05 ± 0.03
	Midstream	6.34 ± 0.08	29.00 ± 0.45	4.12 ± 1.75	4.186 ± 1.510	4.50 ± 1.72	3.45 ± 1.62
	Downstream	6.12 ± 0.02	30.10 ± 0.06	4.49 ± 0.04	21.713 ± 0.010	26.40 ± 0.01	20.46 ± 0.04
Balok River	Upstream	6.54 ± 0.20	28.50 ± 0.12	7.02 ± 0.62	0.350 ± 0.450	0.08 ± 0.00	0.08 ± 0.00
	Midstream	7.20 ± 0.29	28.90 ± 0.23	3.70 ± 0.02	0.800 ± 0.170	0.08 ± 0.19	0.70 ± 0.17
	Downstream	7.79 ± 0.25	30.40 ± 0.83	6.74 ± 0.06	49.190 ± 0.090	67.30 ± 0.51	46.27 ± 0.80

Data were expressed as mean ± SD.

Table 3
Chemical parameters of river water from Kuantan and Balok River (mg/L).

Samples		COD	BOD	Ammonium nitrate	Fluoride	Nitrate	Nitrite	Chloride
Kuantan River	Upstream	54	24.0	ND	ND	ND	0.13	6
	Midstream	24	51.0	0.06	0.29	6.7	0.13	270
	Downstream	390	8.2	0.06	ND	ND	ND	1 100
Balok River	Upstream	56	41.0	ND	ND	5.7	ND	11
	Midstream	88	37.0	ND	ND	ND	2.33	92
	Downstream	490	5.1	ND	0.45	ND	0.24	13 600

ND: Not detected.

Table 4
Metal elements found in river water from Kuantan and Balok River

Sample		Al (ppm)	B (ppm)	Ca (ppm)	Co (ppm)	Fe (ppm)	K (ppm)	Mg (ppm)	Na (ppm)	Sr (mg/L)
Kuantan River	Upstream	0.67	0.49	2.76	ND	0.80	1.47	1.29	5.11	ND
	Midstream	1.02	0.25	4.89	ND	ND	7.48	13.83	120.90	ND
	Downstream	81.00	1.60	165.80	22.43	ND	231.10	489.10	4 181.00	1.462
Balok River	Upstream	0.15	0.14	4.56	ND	ND	2.98	1.34	4.60	ND
	Midstream	1.87	0.45	21.14	ND	2.38	7.78	3.95	120.90	ND
	Downstream	0.13	1.54	166.50	ND	ND	189.90	272.20	120.90	2.170

ND: Not detected.

compared to the midstream (92–270 mg/L) and upstream (6–11 mg/L) area as the river water flow from the upstream to the downstream area.

A total of 23 elements were screened for the occurrence of metal elements especially heavy metal using the ICP-MS. However, only nine elements were found (aluminium, boron, calcium, cobalt, iron, potassium, magnesium, sodium and strontium). Table 4 shows the elements found in Kuantan River and Balok River. It was found that majority of the elements were higher at the downstream points in both Kuantan River and Balok River followed by the midstream points and upstream points in both Kuantan River and Balok River.

4. Discussion

Despite the enforcement of Environmental Quality Act in 1974, water quality of rivers in Malaysia is still deteriorating. Many studies have been reported on the occurrence of *Cryptosporidium* oocysts in Malaysia[1-3,9-11]. Water sources can be contaminated by the oocysts from the shedding of infected human or animal faeces into the water[9]. The occurrence of *Cryptosporidium* oocysts in water can be related to agriculture, industrial and also human activities[11]. It is noted that there are several animal farms such as goat farms and cattle farms nearby the upstream of Kuantan River. The shedding from infected livestock faeces can contribute to the presence of *Cryptosporidium* in water. The midstream and downstream of both rivers are located near a residential area. Several fishing villages are also spotted at the river bank in the downstream area. Improper waste disposal was found along the downstream area. The fishing villages do not have a proper sewage system either. Effluents from houses are directly discharged into the river. Based on our observations, the communities are at high risk of getting infected by this waterborne parasite.

According to Afzan *et al*., Kuantan River and Balok River were classified under Class III river where it has economic values and also consists of tolerant species[11]. Both Kuantan River and Balok River required extensive water treatment. Based on the INWQS, DO in both midstream Kuantan River and Balok River and downstream Kuantan Rivers were within the range of INWQS which was 3–5 mg/L. However, DO in both upstream Kuantan River and Balok River were higher than 7 mg/L which was within the range for Class I. DO level is closely related to aquatic ecology. Low amount of DO value can be due to lack of aerobic organism and the aquatic plant in the water. These can be an indicator to detect water contamination[19].

Kuantan River showed a slightly acidic property while Balok River was slightly alkaline except for the upstream area. However, standard pH value in the current river flow is between 6 and 9. Hence, pH values obtained in both Kuantan River and Balok River were still safe for aquatic organisms. Temperature plays a role in the physiological process of organisms. Lower temperature can decrease the growth rate while higher temperature helps to promote growth rate of organisms. Naturally, a water temperature of the flowing river is between 0 and 30 ˚C where the temperature will gradually increase from the upstream area to the downstream area[20].

The river water flow from the upstream to downstream has caused the metal elements would more likely to accumulate in the downstream area. Heavy metals consist of essential and non-essential elements that have a significance in eco-toxicology since they are potential to be toxic to the organism[21,22]. These elements can occur in surface water through a natural process such as weathering process, rock erosion and sedimentation or by anthropogenic activities such as agricultural activities, industrial process and domestic waste[23].

Balok River is located nearby Gebeng Industrial area where many factories that process metals, plastics and petroleum are located there. Therefore, there is a higher possibility that Balok River will be contaminated with heavy metals compared to Kuantan River. Many studies have been conducted on metal pollution in Balok River and toxic heavy metals such as cadmium (Cd), chromium (Cr), lead (Pb), zinc (Zn) and nickel (Ni) were found[23-25]. The occurrence of these metal elements was due to improper wastewater discharged by the factories into the river body without any prior treatment.

The findings of this study suggest that Kuantan River and Balok River represent as a major risk of waterborne parasites and chemical contamination. Exposure to these contaminants can cause adverse health effects to human and affect the aquatic ecosystem as well. Improper waste management and poor hygiene practices can contribute to the occurrence of *Cryptosporidium* in water. Effluents from residential areas and factories could be the reasons why both Kuantan River and Balok River are polluted.

Regular monitoring of both Kuantan River and Balok River, as well as other rivers in Pahang should be done by related authorities. Other than that, raising awareness among the local communities is one of the ways to improve river water quality. Each one of us should be responsible for improving and maintaining the quality of the rivers. For future study, molecular characterization should be carried out in order to identify the specific species of *Cryptosporidium* that contaminate river water. Besides, more parameters should be added to confirm the water quality of the rivers.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This research was funded by FRGS Grant No. 14-148-0389 from the Ministry of Higher Education (MOHE) and RIGS Grant No. 16-301-0465 from International Islamic University Malaysia (IIUM).

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