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Contamination of waterborne parasites at water treatment plants and a gravity-feed system: a highlight on water safety for urban and rural communities in Kuching, Sarawak

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Abstract—Waterborne parasites, particularly *Cryptosporidium* and *Giardia*, are emerging pathogens implicating the safety level of drinking water globally. The aim of this study was to determine the distribution pattern of waterborne parasites in raw and treated water at urban and rural water treatment plants and untreated water from gravity-feed system in Kuching, Sarawak. This study focused on water treatment plants (four urban and two rural) and Bong rural community that utilise gravity-feed system in Kuching, Sarawak. A total of 69 raw and treated water samples were collected and processed before being used in detection of *Cryptosporidium* and *Giardia* using Aqua-Glo™ G/C Direct and 4′,6-diamidino-2-phenylindole stains, as well as other parasites that were detected using Lugol's iodine staining. Parameters which were temperature, pH, turbidity, dissolved oxygen, total dissolved solids, conductivity, faecal coliform of the water as well as rainfall intensity were determined. Correlation of the parameters with distribution of the waterborne parasites was analysed. Out of 69 water samples collected across all localities, 25 samples were contaminated with waterborne parasites with varying waterborne parasite concentration in the water samples. The presence of waterborne parasites in the raw and treated water of water treatment plants in this study signifies public health threats do exist despite being conventionally treated. This study also highlights that the gravity-feed system which is commonly depended by rural communities in Malaysia may facilitate waterborne parasitic infections

Keywords: *Cryptosporidium*, *Giardia*, gravity-feed system, water supply

I. Introduction

Waterborne parasites have impacted two million mortalities annually particularly among below five-year-old children [1]. Based on previous cases, *Cryptosporidium* and *Giardia* are the most common diarrhoea-causing infections and associated to numerous global waterborne outbreaks [2]. However, in majority of countries with scarce reports of waterborne parasitic infections, real number of infections are underestimated by lack of documentation systems and lower sensitivity of detection methods [3].

Sarawak is the largest state in Malaysia and located in northwest Borneo Island. In 2016, the state has 93 water treatment plants that produced 1,328 million litres per day of water production (MLD) and 474 MLD of domestic consumption [4; 5]. In the state, public water supplies are regulated by Kuching Water Board, Sibu Water Board and Sarawak Rural Water Supply Department (JBALBS) that are possessed by the Sarawak government [6]. Whereby, the government is responsible for development, operation and maintenance of water supplies [7].

Based on the recent statistics, 99% of urban and 88% of rural population in Malaysia received basic water supplies from improved sources in 2015 [8]. Within that number, there are many rural communities do not receive piped portable water since being remote from the nearest water treatment facilities. Rural communities are underprivileged from social and economic standards such as basic water supplies, thus being

exposed to various infections sourced from the untreated water. This has been highlighted in many studies that lack of safe water supply represents one of the significant risk factors of intestinal parasitism particularly among rural communities [9 – 17]. In reference to the scenario in rural Sarawak, a proportion of water is relied on untreated water from gravity-feed systems which represent the only water source used for drinking by marginalised communities. Whereas, drinking water used in urban and semi-urban areas in Sarawak is produced by urban and rural water treatment plants whose water quality being periodically maintained.

Safe water supply is a fundamental and global issue considering that water contamination constitutes high potential to massive outbreaks that can impact economic and social development [18]. There is a little focus has been put on the safety status of portable water depended by the urban and rural communities in Malaysia. Up to now, there are five local studies have tested different types of portable water involving water treatment plants, mineral, tap water and drinking water from rural community [19-23]. The findings from these studies urged for a similar investigation concerning the transmission risk of waterborne parasites in different water sources used in Sarawak. The aim of this study was to determine the distribution pattern of waterborne parasites in raw and treated water at urban and rural water treatment plants and untreated water from gravity-feed system in Kuching, Sarawak.

II. MATERIALS AND METHODS

A. Background of the studied areas

This study focused on six water treatment plants (WTPs) and a rural settlement in Kuching division, Sarawak. As shown in Figure 1 and Table 1, six WTPs in this study comprised of four urban water treatment plants (i.e. WTPs A, B, C and D) and two rural water treatment plants (i.e. WTPs E and F). WTPs A, B, C and D are in Batu Kitang. The plants treat raw water abstracted from Sungai Sarawak Kiri and supply to the urban areas in Kuching and Kota Samarahan divisions. The plants also conditionally supply water to Bau and Lundu districts in shortage of water supply. WTPs E and F treat raw water abstracted from Sungai Sarawak Kanan and supply to the rural areas in Bau (e.g. villages of Siburuh, Suba Bau and Pengkalan Bau) and Siniawan (e.g. villages of Melayu Siniawan and Kandis) districts, respectively. Other than that, a village of Bong rural settlement in Tringgus, Bau was as well focused in this study. The village is inhabited by Bidayuh community that is not supplied with piped portable water. Thus, gravity-feed system remains as the only water source that utilises the basis of gravity flow at higher elevation to channel spring water from an impoundment [24]. The impoundment is located at 150-m sea level of a hill that channels water to houses and a school without any basic water treatments. The catchment area where the system is located, has been enacted as a free-farming and cultivating zone by the Ministry of Health to preserve the water quality for safe human consumption. Pedi river, a stream from the downflow of the gravity-feed system, was included as a study point as it had close association with the daily activities of the community.

B. Water sample collection

Sample collection was conducted within October 2017 – March 2018 and carried out three times at each location. A volume of 20 L of water samples (i.e. 10 L for *Cryptosporidium* and *Giardia*, and 10 L for other parasite detection) were collected by using a pail and kept in multiple 5-L polyethylene carboys. Another 100 ml of water samples were collected at each of the sampling station for faecal coliform analysis. Water samples for faecal coliform were kept in ice box except the 20-L water samples due to space limitation. All samples were immediately transported to the Molecular Microbiology Laboratory at Universiti Malaysia Sarawak for further analysis.

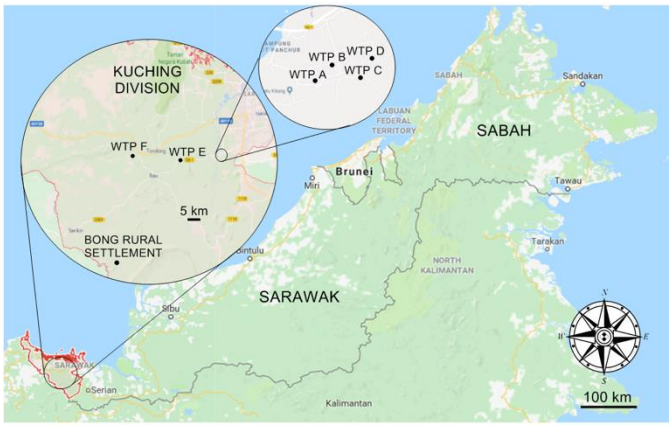

Figure 1. Sampling localities in Kuching Division, Sarawak. 'WTP' denotes water treatment plant

C. Measurement of water physicochemical parameters

Five selected physicochemical parameters of the samples (i.e. pH, temperature, turbidity, dissolved oxygen, total dissolved solids and conductivity) were measured *in-situ*. pH and temperature were measured using pH meter (WalkLAB, TI 9000), turbidity was measured using turbidity meter (Martini, Mi 415), dissolved oxygen was measured using dissolved oxygen meter (YSI, Pro 20), and total dissolved solids and conductivity were measured using total dissolved solids meter (Eutech Instrument, Cyberscan Con 11). Rainfall intensity was collected in the 24 hours period before the sampling as in Razzolini *et al.* [25]. The rainfall data was retrieved from the website of Department of Irrigation and Drainage Sarawak [26].

D. Aluminium sulphate flocculation method

This method was in accordance with the protocol in Karanis and Kimura [27]. Each 10 L water sample was processed separately (i.e. 10 L for *Cryptosporidium* and *Giardia*, and another 10 L for other parasite detection). A volume of 20 ml of $Al_2(SO_4)_3$ solution was added into each 10 litres of the sample. pH of the samples was adjusted from pH 5.4 – 5.8 by adding a few drops of NaOH solution with constant stirring. The formed flocs were left for sedimentation at room temperature for 24 h. Next, the clear solution was discarded gently until leaving approximately 300 ml of it with sediment. The mixture was centrifuged at 2,100 × g for 10 min. Then,

supernatant was discarded until leaving approximately 15 ml of it with pellet. Distilled water was added until 50 ml and proceed with vortexing before centrifugation at 2,100 × g for 10 min. Supernatant was discarded and the pellet was vortexed. A volume of 20 ml of lysis buffer (0.399 M citric acid monohydrate, 0.599 M trisodium citrate dihydrate, pH 4.7) was added to each pellet of the 20-L sample and vortexed every 15 min at room temperature for 1 h. Afterwards, distilled water was added until 50 ml and vortexed. The mixture was centrifuged at 2,100 × g for 10 min. Supernatant was discarded and the pellet was used in the sucrose floatation method.

E. Sucrose floatation technique

Sucrose floatation method was performed in accordance with the method by Ma and Soave with minor modification in increment of the specific gravity [28]. The tube was centrifuged at 400 × g for 5 min and added with extra sucrose solution (2.60 specific gravity) until a convex meniscus was formed. A glass coverslip was placed on top of the meniscus and left for 15 min and lift off. Then, the solution captured from the coverslip was washed with distilled water into a 50-ml tube. This step was performed twice.

F. Faecal coliform analysis

Faecal coliform was enumerated using membrane filtration method as described in American Public Health Association and USEPA [29 ; 30]. A volume of 100 ml water sample was filtered through 47 mm, 0.45 µm nitrocellulose membrane filter (Sartorius, Germany) with the aid of vacuum pump. Afterwards, the membrane was transferred onto a membrane faecal coliform (m-FC agar) (Merck, Germany) supplemented with 1% Rosolic acid (Merck, Germany). The agar was incubated at 37 °C for 2 h and transferred to 44 °C for 24 h incubation. Blue colonies of faecal coliforms were counted and recorded. *Escherichia coli* was included as positive control, while *Salmonella* Typhimurium was included as negative control.

G. Cryptosporidium and Giardia detection by fluorescence microscopy

This method was in accordance with the Method 1623 by USEPA and the protocol provided by Waterborne, Inc. (USA) [31]. Briefly, sample was air dried on 4 × 9 well microscope slide and fixed with a drop of methanol. Then, the slide was stained with 50 µl 4′,6-diamidino-2-phenylindole (DAPI) (Waterborne, Inc., USA) and incubated for 4 min. DAPI stain was washed with phosphate buffered saline (PBS) and incubated for 1 min. A volume of 45 µl of Aqua-Glo™ G/C Direct, FL (Waterborne, Inc., USA) was added on the sample and incubated for 20 min at 27 °C before washing with PBS for 1 min. The sample was air dried and mounted with a drop of No-Fade™ Mounting Medium (Waterborne, Inc., USA). A cover glass was sealed on the microscope slide using nail polish. Afterwards, it was viewed at 400× and 600× magnifications of IX51 fluorescence microscope (Olympus, Japan). *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts (Waterborne, Inc., USA) was used as the positive control, and distilled water was used as negative control.

H. Detection of other parasites

A drop of Lugol's iodine was added on the sample. It was observed under a BX51 compound microscope (Olympus, Japan) with 400× and 600× magnifications. Any suspects of parasite (oo)cysts, ova or larvae were confirmed with parasites image library by WHO and CDC [32 ; 33]. *Giardia lamblia* cyst was used as a positive control, while distilled water was used as a negative control.

I. Statistical analysis

The statistical analysis in this study was performed by using Social Science Package Software version 24. Concentration of (oo)cysts/ova/larvae detected were expressed per L of water sample. Correlation of the detected parasites with physico-chemical parameters, faecal coliform and rainfall data was determined by using bivariate correlation analysis with Kendall's Tau-b correlation coefficient. The correlation was considered statistically significant when P-value < 0.01 and < 0.05.

J. Spiking control

Recovery efficiency of the methods used in this study was performed utilising AccuSpike™-IR (Waterborne, Inc., USA) in accordance to the manufacturer's instructions. Briefly, the AccuSpike™-IR was uncapped and added with 0.25 ml EluMax™. The vial was recapped and vortexed for 15 sec. Then, the liquid in the vial was aspirated out and added into a 10 L distilled water. A volume of 0.75 ml deionised water was added into the vial and vortexed for 15 sec. The liquid was added into the 10 L deionised water again. This was performed twice into the 10 L water. The spiked water was processed with aluminium sulphate flocculation and sucrose floatation method before being stained with DAPI and FITC-conjugated anti *Cryptosporidium* and *Giardia* (Waterborne, Inc., USA) to be viewed under IX51 fluorescence microscope (Olympus, Japan). This process was performed three times to get the average recovery efficiency. Percentage recovery efficiency of *Cryptosporidium* was 37%, while *Giardia* was 53%, which were within the range of acceptance criteria (i.e. 11 – 100% for *Cryptosporidium* and 14 – 100% for *Giardia*) as outlined in the Method 1623.[31]

III. RESULTS

A. Overall occurrence of waterborne parasites in water samples collected from six water treatment plants and a gravity-feed system in Kuching division

Examination of *Cryptosporidium* and *Giardia* in this study using immunofluorescence assay, as in accordance with the EPA Method 1623, enabled genus-specific detection of both parasites in water samples with reduced misidentification [31]. Whereas, utilisation of the non-specific Lugol's iodine dye, that is routinely used in clinical parasitological screening, enabled broad detection of other parasites in the studied samples. In this study, out of 69 water samples collected from all localities (i.e. water treatment plants and Bong rural settlement), 25 samples were contaminated with waterborne parasites. Parasite with the highest number of occurrence was *Cryptosporidium* (20.29%; 14/69), followed by nematode

larvae (7.25%; 5/69), *Giardia* (4/69), *Enterobius vermicularis* (1/69), *Ascaris lumbricoides* (2.90%; 2/69), hookworm (2.90%; 2/69), *Clonorchis* (2.90%; 2/69) and *Schistosoma haematobium* (1.45%; 1/69).

B. Distribution of waterborne parasites in raw and treated water from urban and rural water treatment plants

A total of 36 water samples (i.e. raw and treated) were collected from six water treatment plants (i.e. WTPs A - F) in Kuching division. Ten out of 18 raw water samples (55.56%) were positive with waterborne parasites. The highest occurrence (i.e. based on the numbers of positive samples) was *Cryptosporidium* (16.67%; 3/18) and nematode larvae (16.67%; 3/18), followed by *Giardia* (11.11%; 2/18), *Ascaris lumbricoides* (11.11%; 2/18), hookworm (11.11%; 2/18) and *Schistosoma haematobium* (5.55%; 1/18). Of 18 treated water samples, five samples were positive (27.78%) with waterborne parasites. The highest occurrence in treated water samples was *Cryptosporidium* (16.67%; 3/18), followed by nematode larvae (11.11%; 2/18) and *Enterobius vermicularis* (5.55%; 1/18) as shown in Table 2.

At four urban WTPs (i.e. WTPs A, B, C and D), seven out of 12 (58.33%) raw water samples were contaminated with waterborne parasites. While, five out of 12 (0.45%) treated water samples were positive. At two rural WTPs (i.e. WTPs E and F), three out of nine (33.33%) raw water samples were positive with waterborne parasites, whereas none of the nine (0.00%) treated water samples were positive.

At WTP A, the raw water was contaminated with *Giardia* (0.1 cysts/L), nematode larvae (0.3 larvae/L), hookworm (0.1 ova/L) and *Schistosoma haematobium* (0.1 ova/L), whilst the treated water was contaminated with *Cryptosporidium* (0.2 oocysts/L) and nematode larvae (0.1 larvae/L). At WTP B, the raw water was contaminated with *Cryptosporidium* (0.1 oocysts/L) and hookworm (0.1 ova/L), whereas the treated water was contaminated with nematode larvae (0.4 larvae/L). At WTP C, the raw water was contaminated with *Giardia* (0.4 cysts/L) and nematode larvae (0.1 larvae/L), whilst the treated water was contaminated with *Cryptosporidium* (0.1 oocysts/L) and *Enterobius vermicularis* (0.1 ova/L). At WTP D, the raw water was contaminated with *Cryptosporidium* (0.1 oocysts/L), while the treated water was contaminated with *Cryptosporidium* (0.3 oocysts/L). At WTP E, the raw water was contaminated with *Ascaris lumbricoides* (0.1 and 0.2 ova/L), whereas the treated water was not contaminated with any waterborne parasites. At WTP F, the raw water was contaminated with nematode larvae (0.1 larvae/L), however the treated water was not contaminated with any waterborne parasites.

C. Distribution of waterborne parasites in water from Bong rural settlement.

A total of 33 water samples were collected from the gravity-feed system at Bong rural settlement in Bau district. Overall, ten water samples (30.3%) were positive with waterborne parasites as shown in Table 3. The highest occurrence (i.e. based on the numbers of positive samples) was *Cryptosporidium* (24.24%; 8/33), followed by *Giardia* (6.06%; 2/33) and *Clonorchis* (6.06%; 2/33).

Water samples collected from the impoundment were detected with *Cryptosporidium* (0.1 and 0.8 oocysts/L). At upstream of the Pedi river, the samples were positive with *Cryptosporidium* (0.1 oocysts/L) and *Giardia* (0.1 cysts/L). Whilst, at midstream of the river, the water was detected positive with *Cryptosporidium* (0.1 oocysts/L), whereas downstream was contaminated with *Cryptosporidium* (0.1 and 0.2 oocysts/L). At Bong rural settlement receiving untreated water from the gravity-feed system, three out of seven localities were contaminated with waterborne parasites. The contaminated locations were house 1, house 2 and house 3. No waterborne parasites were detected from school, community water filtration tank (CWFT) 1, CWFT 2 and CWFT 3. At house 1, the water was contaminated with *Clonorchis* (0.1 ova/L). At house 2, the water was detected positive with *Cryptosporidium* (0.1 oocysts/L) and *Giardia* (0.2 cysts/L). Whereas, at house 3, the water was contaminated with *Cryptosporidium* (0.1 oocysts/L).

D. Determination physicochemical parameters, faecal coliform of the water and rainfall intensity at each locality with correlation with the distribution of the detected waterborne parasites.

All 63 water samples were tested for selected physicochemical parameters (i.e. temperature, pH, dissolved oxygen, turbidity, conductivity and total dissolved solids). As shown in Table 4, among the WTPs, temperature of raw water was in the range: 26.43 – 27.08 °C, whereas treated water was 26.38 – 27.42 °C. pH of raw water was ranged 7.07 – 7.32, whilst treated water was 6.63 – 7.88. DO in raw water was 6.54 – 8.19 mg/L, while treated was 7.83 – 8.23 mg/L. Turbidity ranged 17.68 – 117.20 NTU in raw water and 1.15 – 10.81 NTU in treated water. Conductivity was measured 50.67 – 199.25 µS in raw water and 65.54 – 210.13 µS in treated water. TDS ranged 25.31 – 99.90 ppm in raw water, whilst 32.77 – 104.97 ppm in treated water. Faecal coliform was detected with 163 – 991 CFU/100 mL in raw water, while 0 CFU/100 mL in treated water. Rainfall intensity was recorded 0 – 15.17 mm.

As shown in Table 5, Bong rural settlement was recorded with temperature ranged 24.69 – 27.13 °C, pH was 6.98 – 7.87, DO 6.99 – 8.63 mg/L. Whereas, turbidity was recorded 0.05 – 2.87 NTU. Conductivity was 23.99 – 36.72 µS, TDS was 11.70 – 18.47 ppm, faecal coliform was counted 0 – 20 CFU/100 mL. Rainfall intensity was recorded 3.83 mm.

Based on the correlation analysis between the detected parasites with the selected physicochemical parameters (i.e. temperature, pH, DO, turbidity, conductivity and TDS) as well as with faecal coliform and rainfall intensity showed that *Cryptosporidium* was negatively correlated with temperature of the water (r = - 0.255), while nematode larvae were positively correlated with pH of the water (r = 0.308). *Giardia* was not correlated with any of the selected physicochemical parameters, rainfall and faecal coliform as shown in Table 15.

Correlation analysis was not able to be conducted on other waterborne parasites due to the scarce numbers of the positive samples which might produce inaccurate results.

IV. DISCUSSION

A. Survival of waterborne parasites at urban and rural water treatment plants

In raw water samples from the studied water treatment plants, a higher number of positive samples (7/12; 58.33%) with waterborne parasites was observed from urban WTPs (i.e. WTPs A, B, C and D). It was notable that the aforementioned plants abstracted raw water from Sungai Sarawak Kiri which was near to human settlement at Bunga Rampai and Lidah Tanah villages that could possibly be the source of faecal contamination. In comparison, lower number of positive samples (3/9; 33.33%) were detected from rural WTPs (i.e. WTPs E and F) that abstracted water from Sungai Sarawak Kanan. Contamination of raw water source can be attributed by several factors such as settlement and agricultural runoff, industrial pollutions, human and animal excreta, rainfall events, and leakage of septic tanks [34]. In treated water samples, higher number of positive samples (5/12; 0.45%) were from urban WTPs (i.e. WTPs A, B, C and D) compared with rural WTPs (i.e. WTPs E and F) that none of the collected treated water samples were positive. There are several factors to the presence of waterborne parasites in the treated water. High prevalence of waterborne disease outbreaks is attributed by adulteration of water sources by faecal matters, suboptimal disinfections and wastewater inflows into distribution networks [35]. Low raw water quality can cause diminution of water treatment efficacy. This was proven from the Milwaukee's cryptosporidiosis outbreak in 1993 where the treated water was tested containing high turbidity [36].

Based on the finding of this study, waterborne parasites survive in the raw and treated water of the urban WTPs as detected in the collected samples. However, different prevalence was observed between urban versus rural WTPs that might be influenced by different water sources, treatments utilised and plant designs of those plants. The rural WTPs are small scale plants that produce lesser water capacity compared to urban WTPs and both kinds are administered by different waterworks. For that reason, the treatment procedures are slightly different. For instance, treatment processes (e.g. aluminium sulphate flocculation) are carried out manually at one of the rural WTPs (i.e. WTP E) compared to urban WTPs which is machinery-operated. At urban WTPs, the water was abstracted from Sungai Sarawak Kiri that has a greater number of populations residing along the river compared to Sungai Sarawak Kanan as a raw water source for rural WTPs.

Cryptosporidium is one of the most significant waterborne parasites in public water systems due to the robust and recalcitrant nature which can survive in treated water of conventional treatment facilities [37]. *Cryptosporidium* survived in the treated water of three urban WTPs which were WTP A (0.2 oocysts/L), WTP C (0.1 oocysts/L) and WTP D (0.3 oocysts/L). Moreover, these three WTPs supply water to wide receiving areas such as Kuching, Samarahan, Bau and Lundu. Of these concentrations, public health impact does exist pertaining potential of initiating outbreaks to a massive number of populations. This is a concern as there was a cryptosporidiosis outbreak occurred in Bradford, UK in 1992 which was eventually investigated to be associated with consumption of contaminated tap water. Following examination of the public water supplies revealed the concentration of *Cryptosporidium* in the treated water was as low as 0.01-0.18 oocysts/L [38].

Successful elimination of *Cryptosporidium* remains as a primary goal and benchmark for protozoa removal in water treatment plants [37]. The parasite is robust despite being in a treatment of high chlorine concentration for 18 hours and utilising chloramines [39]. The small size of the parasite, measuring 4 to 6 μm, enables it to compromise the filtration treatment which is last barrier for impeding pathogen survival into treated water supplies. Based on an evaluation of WTPs performance target by Lo *et al* [40]. that assessed water treatment efficacy, water treatment processes at eight WTPS in Sarawak and one WTP in Peninsular Malaysia received below the required minimum of 4 log credit of performance target when compared with the Drinking-water Standards for New Zealand [41]. Two WTPs achieved 3 log credit, in contrast, seven WTPs achieved 0 log credit. The low log credits obtained by those plants were due to the real-time turbidity assessment was not administered and utilising chlorine as the only disinfectant, reflecting the risk of suboptimal treatment against microbial contamination.

This study also highlights the presence of other parasites in the treated water, namely nematode larvae at two WTPs (WTP A: 0.1 larvae/L and WTP B: 0.4 larvae/L) and *Enterobius vermicularis* ova at one WTP (WTP C: 0.1 ova/L). Although the infection potential by both parasites through water is not as high as *Cryptosporidium*, their detection in the treated water might indicate breakthrough of contaminants into the treated water. For instance, nematode larvae and *Enterobius vermicularis* survived into the treated water that might indicate suboptimal coagulation, flocculation and filtration to remove large microbial contaminants.

B. Occurrence of waterborne parasites at gravity-feed system at Bong rural settlement.

All houses including a school at Bong rural settlement in Bau receive water directly from water impoundment at 150 metres uphill of the Tringgus hill. The water is used for drinking and other domestic activities and even without being pretreated such as at Points-of-Use and Points-of-Entry. Of 11 studied points at Bong rural settlement, a water sample collected at water impoundment was detected positive with the highest concentration of *Cryptosporidium* (0.8 oocyst/L) and this holds high infection risks as the water is used for drinking and domestic purposes. The high *Cryptosporidium* concentration could be caused by the water held by the barrier that made the parasite to accumulate in the impoundment. Despite being

free-farming zone (i.e. free-contamination from faecal of domestic animals), the water at the impoundment was still contaminated with *Cryptosporidium*. As the area is on the hill and surrounded with the protected natural forest, this occurrence might be associated with the existing wild animals [42 ; 43].

Seven areas receiving water from the impoundment were also included in this study. They were three houses, a school and three community water filtration tanks as shown in Table 3. *Cryptosporidium* was detected from house 2 and house 3, *Giardia* was detected from house 2, while *Clonorchis* was detected from house 1. The presence of *Cryptosporidium* and *Giardia* from the in-house water pipes demonstrates the existing direct exposures of parasitic infections. The findings in this study are supported by a study by Nisha *et al.* that found untreated water from gravity-feed system was associated with parasitic infections among indigenous community in Selangor [44]. Extrapolating the current finding, the water can be deemed as safe for consumption if being rolling boiled, except for other purposes such as washing vegetables and hands before eating despite these may still be representing a lower risk.

This study also revealed contamination of *Clonorchis* ova which is a liver fluke trematode in two samples from House 1 (0.1 ova/L in each sample). However, the parasite is only infectious if humans ingest the metacercariae from consumption of undercooked freshwater fish [45]. Even though prevalence of such infection in Malaysia has been documented previously [46 – 51], the fluke infection among this Bidayuh population is highly unlikely as fishing is unlawful via the enacted Tagang system by the Department of Agriculture Sarawak in regard to achieving sustainable management of fishery resources [52]. This indicates low potential incidence of clonorchiasis among the population.

There were three community water filtration tanks available at Bong rural settlement which none of them was contaminated with waterborne parasites (0.00%; 0/9) in this study. Water stored in the tanks is sourced from the same gravity-feed system as mentioned before. Whenever the water is channelled into the tank, it will undergo filtration process that capable to filter out contaminants measuring as low as 15 nanometres only if the tanks being periodically maintained. Even so, the whole Bidayuh community do not rely on the water for daily use due to the non-strategic location and inconvenience of having to alternately carry personal bottles from their house to the tanks.

Of nine total samples collected from upstream, midstream and downstream of the catchment area, four samples were positive with *Cryptosporidium* (range: 0.1 – 0.2 oocyst/L) and *Giardia* (0.1 cyst/L). The water flows from the hill (including from the water impoundment) and was not abstracted for drinking and other domestic purposes, however, included as the studied points because of routine swimming especially children was evident. Swimming in contaminated water represents a major source of infection where many of such outbreaks were associated with person-to-person transmission [53 ; 54]. Swimming-associated cryptosporidiosis and giardiasis can occur if the swimmers swallow a mouthful of contaminated water. Infected swimmers should not be allowed to enter or be near to the water due to the reason that potential of contamination from stool does exist. A study found that a stool of infected individuals may contain up to $6.7 \times 10^6 – 4.1 \times 10^8$ oocysts [55]. Infected individuals may still shed oocyst of *Cryptosporidium* even though have recovered from the symptoms for weeks [56].

C. Water physicochemical parameters, faecal coliform and rainfall intensity and correlation with the waterborne parasites.

The Malaysia Drinking Water Quality standard formulated by the Ministry of Health has outlined 90 physicochemical parameters of raw and treated drinking water for ensuring safety of water supply [57]. In raw water, the acceptable value for pH is 5.5 – 9.0, turbidity is 1000 NTU and TDS is 1500 ppm. Whereas, there is no acceptable value outlined for temperature, DO, and conductivity in raw water. In treated water, the maximum acceptable value for pH is 6.5 – 9.0, turbidity is 5 NTU and TDS is 1000 ppm. Whilst, there is no maximum acceptable value outlined for temperature, dissolved oxygen, and conductivity in treated water. In this recent study, the selected physicochemical parameters (i.e. temperature, pH, turbidity, TDS, DO, and conductivity) of raw and treated water were in compliance with the Malaysia Drinking Water Quality Standard except for turbidity of treated water from two urban WTPs, namely WTP A (mean: 5.89 ± 8.72 NTU) and WTP B (mean: 10.81 ± 5.92 NTU). These trends were also uncovered by a study by Richard *et al.* where turbidity of WTP A was 18.19 ± 21.55 NTU, while WTP B was 16.57 ± 12.39 NTU [22]. Based on the finding of this study, concern should be put on the turbidity levels of both plants where they were significantly higher than the water turbidity of Howard Avenue Water Treatment Plant in Milwaukee (i.e. up to 1.5 NTU) that spiked during the massive cryptosporidiosis outbreak [36]. Following investigations found out that the Streaming Current Detector (SCD) for adjusting the dose of coagulant were incorrectly installed and the malfunctioning turbidity meters of filtered effluent [36 ; 58]. Even prior to the outbreak, high turbidity of filtered water from the water treatment plant was also correlated with high number of gastroenteritis outpatients in hospital [59]. In this recent study, high turbidity of treated water at the two WTPs should be inspected because such elevation can be an indicator of contaminant breakthroughs into treatment systems.

Analysing the correlations between physicochemical parameters, faecal coliform and rainfall intensity with parasite loads can reflect the source of contamination into water systems. As shown in Table 6, no correlation was found between the selected physicochemical parameters (i.e. temperature, pH, turbidity, TDS, DO and conductivity), faecal coliform and rainfall intensity with the presence of waterborne parasites with exemption for *Cryptosporidium* that was found positively correlated with dissolved oxygen of the water (T_b = 0.206; P<0.05) and negatively correlated with rainfall intensity (T_b = - 0.185; P<0.01), *Giardia* was found positively correlated with pH (T_b = -0.166; P<0.01), while nematode larvae was found positively correlated with turbidity (T_b =

0.185; $P<0.01$) and faecal coliform concentration of the water ($T_b = 0.181$; $P<0.01$). Other waterborne parasites detected in this study were not included in the correlation analysis due to scarce number of parasites detected positively.

With the focus on faecal coliform count in samples from urban WTPs, the highest number was recorded from raw water samples from WTP A (mean: 991 ± 152.91 CFU/100 ml). Meanwhile, from rural WTPs, the high faecal coliform count was from WTP E (mean: 671 ± 211.77 CFU/100 ml). WTPs A, B, C and D were at Sungai Sarawak Kiri. Whilst, WTPs E and F were situated near to Sungai Sarawak Kanan. Both rivers were surrounded with settlements and animal farms along them. Among them, WTPs A and E were located at the most downstream at their respective river, where high load of faecal contamination might be carried from the upper streams. At Bong rural settlement, no faecal coliform bacteria were recorded in the water utilised for drinking (i.e. water impoundment, houses, school and community water filtration tanks). Faecal coliform was observed from the samples from upstream, midstream and downstream of Pedi river where the mean counts were 7 ± 10.79 CFU/100 ml, 20 ± 26.27 CFU/100 ml and 16 ± 14.50 CFU/100 ml, respectively. None of the detected parasites were correlated with faecal coliform. However, as highlighted by Boyer and Kuczynska [60], this condition (i.e. no correlation with faecal coliform) might have been caused by the dissimilarity in sources, dissimilar die-off rates, and hydrological conditions. For instance, faecal bacteria are shed in the faeces of all warm-blooded animals, but *C. parvum* is shed only by the infected animals. Therefore, this circumstance does not deny that the detected parasites such as *Cryptosporidium* might still be from faecal sources that could have contaminated the water.

In general, temperature affects the chemical, physical and biological properties of water. High in water temperature heightens the growth of microorganisms and decreases gases solubility (e.g. oxygen and carbon dioxide) [61]. High TDS and DO indicate high precipitation and water usage, creating an unfavourable condition for *Cryptosporidium* and *Giardia*. High rainfall can indicate high non-point source after the downpour [20]. Faecal coliform indicates faecal contamination into the water source [60]. In general, high rainfall intensity could facilitate parasite contaminations in water systems through non-point source overflow and river resuspension. However, this current study found no correlation between both variables [62]. It is suggested for similar future studies to understand more about rainfall association by designing a long longitudinal study, particularly in equatorial climate countries that have high temperature and humidity all year round.

V. CONCLUSION

The presence of waterborne parasites in the raw and treated water of water treatment plants in this study signifies public health threats do exist despite after treatment processes. This study also highlights that the gravity-feed system which is commonly depended by rural communities as a sole water source in Malaysia may facilitate parasitic infection transmissions if not properly treated at least by boiling before drinking. As a future mitigation, this study suggests carrying out water catchment management controls and Quantitative microbial risk assessment (QMRA) towards waterborne parasites in the country. If there is necessity, evaluation on monitoring raw and treated water quality coupled with the inclusion *Cryptosporidium* and *Giardia* as two of the regulating parameters of water quality in Malaysia should be conducted.

ABBREVIATIONS

µS: Microsiemens; CFU: Colony-forming unit; CWFT: Community water filtration tank; DAPI: 4′,6-diamidino-2-phenylindole; DO: dissolved oxygen; JBALB: *Jabatan Bekalan Air Luar Bandar Sarawak* (Sarawak Rural Water Supply Department); LAKU: Northern Region Water Board; m-FC: Membrane faecal coliform; MLD: Million liters per day; NTU: Nephelometric Turbidity Unit; ppm: Parts per million; PBS: Phosphate-buffered saline; QMRA: Quantitative microbial risk assessment; TDS: total dissolved solids; WTP: Water treatment plant

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

Ahmad Syatir Tahar, Lesley Maurice Bilung, Kasing Apun, Lau Seng, Elexson Nillian and Hashimatul Fatma Hashim were involved in the study design; Ahmad Syatir Tahar was involved in sample collection and conducting the experiment; Ahmad Syatir Tahar, Yvonne Ai-Lian Lim and Reena Leeba Richard were involved in the analysis and interpretation of the data; all authors read and contributed to the manuscript drafting.

Table 1. Details of the sampling locations and type of samples collected

Location	Notation	Type of sample	
Batu Kitang	WTP A (Urban water treatment plant)	Raw water (*n*=3) Treated water (*n*=3)	
	WTP B (Urban water treatment plant)	Raw water (*n*=3) Treated water (*n*=3)	
	WTP C (Urban water treatment plant)	Raw water (*n*=3) Treated water (*n*=3)	
	WTP D (Urban water treatment plant)	Raw water (*n*=3) Treated water (*n*=3)	
Siniawan	WTP E (Rural water treatment plant)	Raw water (*n*=3) Treated water (*n*=3)	
Bau	WTP F (Rural water treatment plant)	Raw water (*n*=3) Treated water (*n*=3)	
Tringgus, Bau	Bong rural settlement	[a] Water impoundment (*n*=3) Pedi river upstream (*n*=3) Pedi river midstream (*n*=3) Pedi river downstream (*n*=3) House 1 (*n*=3) House 2 (*n*=3)	House 3 (*n*=3) School (*n*=3) Community water filtration tank 1 (*n*=3) Community water filtration tank 2 (*n*=3) Community water filtration tank 3 (*n*=3)

Note: "[a]" indicates "the water impoundment is located on a hill at 150 m elevation", "*n*" indicates "number of sample".

Table 2. Distribution of waterborne parasites at urban and rural water treatment plants

Water treatment plant	Waterborne parasite concentration (per L)	
	Raw water	**Treated water**
Urban WTP A	-	*Cryptosporidium* = 0.2 oocysts/L Nematode = 0.1 larvae/L
	Giardia = 0.1 cysts/L	-
	Nematode = 0.3 larvae/L *Schistosoma haematobium* Hookworm = 0.1 ova/L = 0.1 ova/L	-
Urban WTP B	*Cryptosporidium* = 0.1 oocysts/L	-
	Cryptosporidium = 0.1 oocysts/L Hookworm = 0.1 ova/L	-
	-	Nematode = 0.4 larvae/L
Urban WTP C	*Giardia* = 0.4 cysts/L	*Cryptosporidium* = 0.1 oocysts/L
	-	-
	Nematode = 0.1 larvae/L	*Enterobius vermicularis* = 0.1 ova/L
Urban WTP D	*Cryptosporidium* = 0.1 oocysts/L	-
	-	-
	-	*Cryptosporidium* = 0.3 oocysts/L
Rural WTP E	-	-
	-	-
	Nematode = 0.1 larvae/L	-
Rural WTP F	*Ascaris lumbricoides* = 0.2 ova/L	-
	-	-
	Ascaris lumbricoides = 0.1 ova/L	-

Note: "-" indicates the sample was found negative with any waterborne parasites.

Table 3. Distribution of waterborne parasites at Bong rural settlement.

Sampling location	Parasite concentration (per litre of water sample)	Sampling location	Parasite concentration (per litre of water sample)
Water impoundment	-	House 3	*Cryptosporidium* = 0.1 oocyst/L
	Cryptosporidium = 0.1 oocyst/L		-
	Cryptosporidium = 0.8 oocyst/L		-
Upstream (Pedi river)	-	School	-
	Cryptosporidium = 0.1 oocyst/L *Giardia* = 0.1 cyst/L		-
	-		-
Midstream (Pedi river)	-	Community water filtration tank	-
	Cryptosporidium = 0.1 oocyst/L		-

	-	(CWFT) 1	-
Downstream (Pedi river)	*Cryptosporidium* = 0.1 oocyst/L	Community water filtration tank (CWFT) 2	-
	-		-
	Cryptosporidium = 0.2 oocyst/L		-
House 1	*Clonorchis* = 0.1 ova/L	Community water filtration tank (CWFT) 3	-
	-		-
	Clonorchis = 0.1 ova/L		-
House 2	-		
	Cryptosporidium = 0.1 oocyst/L *Giardia* = 0.2 cyst/L		
	-		

Note: "-" indicates the sample was found negative with any waterborne parasites.

Table 4. Physicochemical parameters of raw and treated water from all studied water treatment plants (WTP A – F) in this study.

Sampling location	Physicochemical parameters	[a]Mean ± SD		Sampling location	Physicochemical parameters	[a]Mean ± SD	
		Raw water	Treated water			Raw water	Treated water
WTP A (Urban)	Temperature	27.04 ± 0.94	27.42 ± 0.50	WTP D (Urban)	Temperature	26.43 ± 0.29	26.42 ± 0.29
	pH	7.32 ± 0.31	7.76 ± 0.40		pH	7.25 ± 0.25	6.71 ± 0.04
	Dissolved oxygen	8.19 ± 0.09	8.11 ± 0.21		Dissolved oxygen	7.80 ± 0.10	7.94 ± 0.11
	Turbidity	117.20 ± 140.14	5.89 ± 8.72		Turbidity	23.35 ± 1.48	2.74 ± 0.75
	Conductivity	51.21 ± 8.86	110.24 ± 2.19		Conductivity	52.47 ± 5.69	65.54 ± 6.21
	Total dissolved solids	25.71 ± 4.59	54.77 ± 0.61		Total dissolved solids	26.19 ± 2.85	32.77 ± 3.09
	Faecal coliform	991 ± 152.91	0 ± 0		Faecal coliform	163 ± 42.52	0 ± 0
	Rainfall intensity	1.83 ± 1.61			Rainfall intensity	0 ± 0	
WTP B (Urban)	Temperature	27.08 ± 0.84	27.15 ± 0.76	WTP E (Rural)	Temperature	26.6 ± 0.20	26.95 ± 0.50
	pH	7.12 ± 0.13	7.88 ± 0.89		pH	7.07 ± 0.13	7.17 ± 0.26
	Dissolved oxygen	7.66 ± 0.76	8.05 ± 0.30		Dissolved oxygen	6.54 ± 0.91	7.83 ± 0.37
	Turbidity	29.80 ± 10.89	10.81 ± 5.92		Turbidity	17.68 ± 9.48	1.21 ± 0.85
	Conductivity	58.34 ± 3.01	98.94 ± 9.24		Conductivity	199.25 ± 45.56	210.13 ± 60.29
	Total dissolved solids	29.28 ± 1.69	49.22 ± 4.39		Total dissolved solids	99.90 ± 22.58	104.97 ± 29.82
	Faecal coliform	556 ± 163.69	0 ± 0		Faecal coliform	671 ± 211.77	0 ± 0
	Rainfall intensity	1.83 ± 161			Rainfall intensity	15.17 ± 24.14	
WTP C (Urban)	Temperature	26.44 ± 0.19	26.38 ± 0.28	WTP F (Rural)	Temperature	26.6 ± 0.06	26.51 ± 0.03
	pH	7.28 ± 0.24	6.63 ± 0.09		pH	6.91 ± 0.01	6.87 ± 0.00
	Dissolved oxygen	7.87 ± 0.06	8.20 ± 0.15		Dissolved oxygen	7.25 ± 1.47	8.23 ± 0.67
	Turbidity	22.68 ±2.93	1.15 ± 0.22		Turbidity	37.55 ± 36.71	2.22 ± 0.71
	Conductivity	50.67 ± 4.77	72.80 ± 2.78		Conductivity	109.94 ± 77.83	130.09 ± 55.92
	Total dissolved solids	25.31 ± 2.39	36.40 ± 1.33		Total dissolved solids	55.30 ± 39.55	65.15 ± 27.98
	Faecal coliform	479 ± 344.72	0 ± 0		Faecal coliform	279 ± 168.40	0 ± 0
	Rainfall intensity	0 ± 0			Rainfall intensity	2.5 ± 2.60	

Note: "SD" indicates "standard deviation", "a" indicates the mean was calculated based on three sampling visits. Data in the table above are displayed based on the units as follows: Temperature is in °C, dissolved oxygen is in mg/L, turbidity is in NTU, conductivity is in µS, total dissolved solids is in ppm, faecal coliform is in CFU/100 ml, rainfall intensity is in mm.

Table 5. Physicochemical parameters of water samples from Bong rural settlement

Sampling location	Physico-chemical parameters	[a]Mean ± SD	Sampling location	Physico-chemical parameters	[a]Mean ± SD	Sampling location	Physico-chemical parameters	[a]Mean ± SD
Water Impoundment	Temperature	24.69 ± 0.94	House 1	Temperature	27.03 ± 2.31	Community water filtration tank 1	Temperature	26.07 ± 0.44
	pH	7.34 ± 0.28		pH	7.13 ± 0.33		pH	6.98 ± 0.255
	Dissolved oxygen	8.51 ± 0.20		Dissolved oxygen	8.36 ± 0.08		Dissolved oxygen	6.99 ± 0.55
	Turbidity	1.50 ± 0.52		Turbidity	1.06 ± 0.31		Turbidity	0.05 ± 0.07
	Conductivity	27.91 ± 3.10		Conductivity	27.87 ± 3.23		Conductivity	23.99 ± 3.63
	Total dissolved	13.93 ± 1.65		Total dissolved solids	13.91 ± 1.59		Total dissolved	11.96 ± 1.82

	solids						solids	
	Faecal coliform	0 ± 0		Faecal coliform	0 ± 0		Faecal coliform	0 ± 0
	Rainfall intensity	3.83 ± 6.21		Rainfall intensity	3.83 ± 6.21		Rainfall intensity	3.83 ± 6.21
Upstream (Pedi river)	Temperature	25.13 ± 0.81	House 2	Temperature	26.83 ± 0.19	Community water filtration tank 2	Temperature	27.13 ± 1.33
	pH	7.87 ± 0.07		pH	7.18 ± 0.43		pH	7.30 ± 0.42
	Dissolved oxygen	8.63 ± 0.04		Dissolved oxygen	8.27 ± 0.18		Dissolved oxygen	7.78 ± 0.16
	Turbidity	2.60 ± 0.49		Turbidity	0.81 ± 0.50		Turbidity	0.23 ± 0.19
	Conductivity	35.68 ± 4.20		Conductivity	28.55 ± 3.48		Conductivity	24.19 ± 2.77
	Total dissolved solids	17.90 ± 2.03		Total dissolved solids	14.3 ± 1.64		Total dissolved solids	11.7 ± 1.54
	Faecal coliform	7 ± 10.79		Faecal coliform	0 ± 0		Faecal coliform	0 ± 0
	Rainfall intensity	3.83 ± 6.21		Rainfall intensity	3.83 ± 6.21		Rainfall intensity	3.83 ± 6.21
Midstream (Pedi river)	Temperature	25.69 ± 0.19	House 3	Temperature	27.00 ± 0.63	Community water filtration tank 3	Temperature	26.77 ± 2.11
	pH	7.03 ± 0.24		pH	7.02 ± 0.27		pH	7.24 ± 0.17
	Dissolved oxygen	8.37 ± 0.25		Dissolved oxygen	8.32 ± 0.15		Dissolved oxygen	7.76 ± 0.21
	Turbidity	2.87 ± 0.90		Turbidity	1.39 ± 0.40		Turbidity	0.42 ± 0.09
	Conductivity	36.72 ± 5.80		Conductivity	27.75 ± 4.21		Conductivity	36.87 ± 22.01
	Total dissolved solids	18.37 ± 2.87		Total dissolved solids	13.84 ± 2.08		Total dissolved solids	18.47 ± 11.02
	Faecal coliform	20 ± 26.27		Faecal coliform	0 ± 0		Faecal coliform	0 ± 0
	Rainfall intensity	3.83 ± 6.21		Rainfall intensity	3.83 ± 6.21		Rainfall intensity	3.83 ± 6.21
Downstream (Pedi river)	Temperature	26.45 ± 0.37	School	Temperature	26.98 ± 1.55			
	pH	7.33 ± 0.12		pH	6.99 ± 0.34			
	Dissolved oxygen	8.36 ± 0.04		Dissolved oxygen	8.12 ± 0.25			
	Turbidity	2.83 ± 0.87		Turbidity	1.68 ± 1.09			
	Conductivity	36.19 ± 4.44		Conductivity	28.32 ± 3.31			
	Total dissolved solids	18.03 ± 2.37		Total dissolved solids	14.12 ± 1.65			
	Faecal coliform	16 ± 14.50		Faecal coliform	0 ± 0			
	Rainfall intensity	3.83 ± 6.21		Rainfall intensity	3.83 ± 6.21			

Note: "SD" indicates "standard deviation", "a" indicates the mean was calculated based on three sampling visits. Data in the table above are displayed based on the units as follows: Temperature is in °C, dissolved oxygen is in mg/L, turbidity is in NTU, conductivity is in µS, total dissolved solids is in ppm, faecal coliform is in CFU/100 ml, rainfall intensity is in mm.