Detection and Identification of Waterborne Parasites in Taguibo Watershed Forest Reserve, Butuan City

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Abstract – *There exists a water crisis around the world; hence, water quality should always be a priority to maintain its safety. Due to the scarcity of reported data on waterborne parasites, the consumption of unsafe water persists. Thus, the microbial aspect of water quality, specifically waterborne parasites present in water was studied. The main objective of the study is to detect and identify the waterborne parasites of medical importance up to the genus level, in the main water source of Butuan City, the Taguibo Watershed Forest Reserve. A total of three samples, from the upstream, midstream, and downstream sites, each consisting of four liters, were collected and processed to obtain sediments. Another batch of samples were also collected to test for physical and chemical parameters. Results of temperature (22.8°C on both upstream and midstream, and 22.1°C on downstream), total dissolved solids (144 mg/L on upstream, 246 mg/L on both midstream and downstream), salinity (0 ppt on all sampling sites) and nitrate (6.50 mg/L on upstream, 5.30 mg/L on midstream and 4.80 mg/L on downstream) were all within range according to their respective classification. However, pH of the downstream area (pH 8.85) was out of range since the standard value is 6.5-8.5. This reveals that the water at Taguibo Watershed Forest Reserve passed the standards of water quality. The sediments obtained were microscopically examined using Lugol's iodine and modified Ziehl-Neelsen stain and all samples showed negative results for Giardia spp. cysts, Entamoeba spp. cysts, and Cryptosporidium oocysts. However, two out of three water samples, the midstream and downstream, were positive for free-living organisms. Thus, Taguibo Watershed Forest Reserve is negative for medically important cysts, oocysts and ova of parasites that can cause waterborne diseases.*

Keywords – *waterborne parasites, Taguibo watershed Forest Reserve*

INTRODUCTION

Water sustains human life and is vital for human health. Because of its importance, the United Nations General Assembly has explicitly recognized water as a basic human right and acknowledged that clean drinking water and sanitation are essential to the realization of all human rights. The right of water is defined as the right of everyone to sufficient, safe, acceptable and physically accessible and affordable water for personal and domestic use. States and international organization are given the responsibility to provide financial resources, promote capacity-building and technology transfer to help countries, especially developing countries, to provide safe, clean, accessible and affordable drinking water and sanitation for all [1]. However, the global population is now facing water crisis. Water scarcity is considered one of the main problems that the world is facing in the 21st century. Around 1.2 billion people, or almost one-fifth of the world's population, live in areas of physical scarcity, and 500 million people are approaching this situation. Another 1.6 billion people, or almost one quarter of the world's population, face economic water shortage [3]. Declining water quality has also become a global issue of concern. Almost 2.1 billion people globally lack safe water at home. Also, 884 millions of those do not have basic drinking water services and 159 million people drink water directly from surface sources such as streams or lakes. With the growing number of people who lack basic drinking water services, poor sanitation and direct consumption from surface water, more people are also in hazard of consuming dangerously polluted and chemically contaminated water that can cause waterborne diseases and many more [4]. Philippines, along with other countries in the world, is facing both water and sanitation crisis. According to Water.org [5], out of 101 million Filipinos, nine million rely on unimproved, unsafe, and unsuitable water resources.

Water quality is commonly defined as the chemical, physical, and biological condition of the water with respect to its suitability for a particular purpose [6]. The water quality parameters are determined by its intended use such as human consumption, industrial and agricultural use and more. It is important to take note that the nature and form of drinking water standards may vary among different countries but together aimed to provide quality drinking water that is safe for all. There are a number of threats to safe drinking water. These can be categorized into two groups--anthropological activities and naturally occurring substances that contaminate drinking water. This poses a health risk among humans. In 2004, the Environment Protection Agency (EPA) has set safe drinking water standards to ensure that each individual has access to safe drinking water. It has provided a list of standards of water quality categorized into: Microorganisms, Disinfectants, Disinfection Byproducts, Inorganic Chemicals, Organic Chemicals and Radionuclides. The World Health Organization (WHO) [4] also has similar safe drinking water standards. The World Health Organization recognizes five aspects in safe drinking water standards including microbial aspects, disinfection, chemical aspects, radiological aspects and acceptability aspects. In the Philippines, the Department of Health also includes microbiological, physical, chemical, and radiological compositions as parameters and standard requirement in determining the quality of water [7]. This study focused on the microbial aspect as a standard of water quality, specifically waterborne parasites present in water.

Globally, water that is contaminated can cause variety of illnesses causing severe pain, disability and even death. People can be infected as they consume contaminated water or have contact with contaminated surface water [9].

In a case study on South Africa's drinking water by Mackintosh, et. al [10], monitoring, management and treatment of parasites in drinking water should be taken as an important aspect in quality of drinking water. The need to test for parasites in water was based on two important considerations. It is important to understand parasites' lifespan, characteristics and its significance to human health. According to the study, parasites are more infectious at lower doses (1-10 cysts/oocysts) than infectious bacterial levels required. Parasites have the ability to withstand extreme environmental conditions and can still live up to six months outside a host. Parasites are microscopic pathogens that are resistant to chlorine disinfection. This poses a very serious threat among consumers of drinking water in a particular area because they are not affected by conventional treatment methods. The most common parasitic infections are Giardiasis and Cryptosporidiosis. Until now, there is no effective treatment for Cryptosporidiosis and when not given proper medical care, it can develop into a prolonged cholera-like illness and worse, death.

Most studies on the cases of cryptosporidiosis, giardiasis and amoebiasis in humans have been carried out in developed countries but less in other areas. Bakir et al. [11] investigated the various water samples in Ankara, Turkey for the presence of waterborne parasites. The results showed that three out of six Ankara River samples contained pathogenic parasites particularly Entamoeba histolytica cysts, Strongyloides stercoralis eggs, Trichuris trichiura eggs, Cryptosporidium parvum oocysts and Giardia lamblia cysts. The waterborne parasites were detected and identified by conventional microscopy, immunologically, and by polymerase chain reaction (PCR). With the utilization of underground water sources in the near future, it was suggested to include these sources in the future surveys and investigations of waterborne parasites that is relevant to public health.

The majority of the world's population has still no access to healthy water and that contaminated drinking water is a major threat to people in the developing world. Protozoan parasites such as Cryptosporidium sp., Giardia duodenalis and Entamoeba histolytica cause periodic outbreaks of diarrheal diseases even in developed world. Thus, various techniques and methods were reviewed in this study for the progress in technology for detection and surveillance of waterborne protozoan parasites. Cyst and oocyst detection in environmental water samples were done primarily by filtration, centrifugal concentration, flow cytometry and classical microscopy and staining. However, immunological and molecular techniques have largely replaced staining for diagnosis. New technology for waterborne pathogen detection and removal includes biosensors, DNA microarrays, polymerase chain reaction, mass spectroscopy and materials engineering. Surveillance of drinking water through these methods is of great importance to minimize contaminations and ensure continuous supply of healthy water around the world [12].

FeizHadad, et. al [13] studied the recent status of occurrence, source and human intestinal parasites in sources and tap water in Delhoran, South West, Iran. The results of the study revealed the presence of variety of protozoan parasites in the water sample sources and were mostly pathogenic. The study also revealed the significant relationships between distance of water sources from the place of contamination and type of water sample and chlorination status. For example, the contamination of groundwater can also affect the quality of surface water and vice versa. The study also showed the direct association between safe water and human health. It was highly recommended to encourage practicing test for parasites in drinking water sources.

According to Lim and Nissapatorn [14], waterborne parasitic protozoa outbreaks were mostly reported in the regions of Australia, Europe, New Zealand, and North America. The reason for this is that these regions have established surveillance and reporting systems for waterborne parasitic protozoa outbreaks. Only an estimated 1% of these outbreaks have occurred in Asia because of lack of organized mechanisms of documentation of parasitic infections. The study was conducted to provide an overview of the available data and studies on waterborne parasitic occurrences among Association of South East Asian Nations (ASEAN). The need was very immediate and timely considering the lack of access to safe drinking water to provide to billions of individuals in South East Asian countries. With the growing population, the prevalence of waterborne diseases caused by waterborne parasites posed major health threats among people in countries.

There have been minimal studies related to waterborne parasites published despite its prevalence and significance to human health. Even with growing population, increase in pollution and water scarcity, there has been no reports on waterborne outbreaks in the Philippines [15]. The study was conducted to monitor the current status of the Philippines on waterborne parasites in urban, suburban and rural areas. The water samples were taken from Manila for the urban area, Cavite for the suburban, and Batangas and Pampanga for the rural areas. The results showed that the water samples tested were positive for Giardia spp. and Cryptosporidium. The presence of the protozoan parasites could be due to the contamination of fecal matter from both animals and humans, flow of residues from nearby slaughter houses and direct discharge of effluents from residences to the nearby water sources. Based on the results of the study, the quality of water in the Philippines seemed to be contaminated and needs more improvement. The study recommended to provide more attention to waterborne parasites to provide safe drinking water to Filipino citizens.

With this problem, it is relevant and timely to provide information about the current status of the quality of water in the Philippines and to lessen the population at risk of acquiring waterborne diseases caused by protozoan parasites. To date, the reports on the prevalence of waterborne parasitic diseases outbreaks are very scarce. There have been studies on the occurrence of waterborne parasites in sources of drinking water showing its current status; however, it was only noted in the islands of Luzon. Very few studies have been conducted in the islands of Visayas and Mindanao and some were not even documented and reported. In Butuan City, waterborne diarrhea and other diseases have emerged recently due to the consumption of water in their respective households [16]. There are also no studies that had been carried out as an update to the Taguibo Watershed and Forest Reserve, particularly in detecting and identification of waterborne parasites of medical importance. Thus, this study was designed and performed to evaluate the waterborne parasite infestations, if there are any, and to fill in the gap of knowledge on the Taguibo Watershed Forest Reserve in Brgy. Anticala, Butuan City.

Objective of the Study

The main objective of the study is to detect the presence of and identify the waterborne parasites of medical importance such as Giardia, Cryptosporidium and Entamoeba up to the genus level, in the main water source of Butuan City, the Taguibo River Watershed Forest Reserve.

The study can help raise awareness on the current status of water quality to the people and also to both public and private organizations. It can help in providing safe drinking water to the consumers. More so, it can help national and local water districts to improve conventional strategies or methods in treating water so that waterborne parasites could also be given important consideration.

MATERIALS AND METHODS

Sampling Area

The study was conducted at the Taguibo River Watershed Forest Reserve (Figure 1), Brgy. Anticala, Butuan City. It is located at 125°31’ to 125°43’16”.

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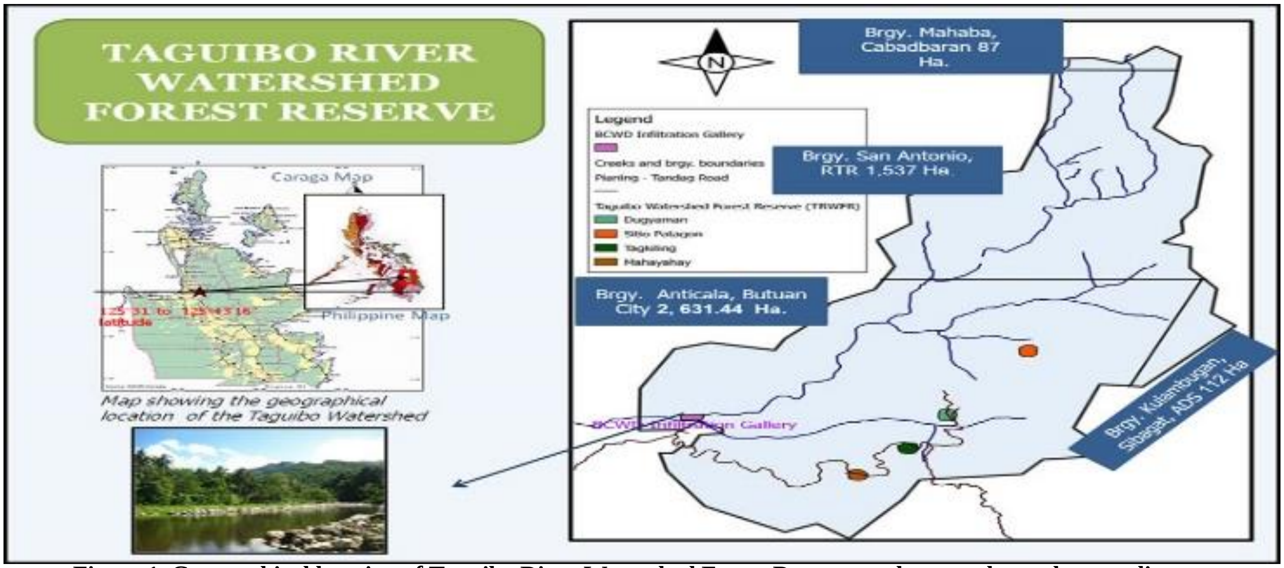

Figure 1. Geographical location of Taguibo River Watershed Forest Reserve; red arrow shows the sampling area

It is the biggest tributary of the Taguibo River with 2,634.44 hectares in size along with those in Brgy. Mahaba, Cabadbaran City, Brgy. San Antonio, RTR, and Brgy. Kalambungan, Sibagat, Agusan del Sur (Presidential Proclamation No. 1076).

It is considered as the major source of potable water in Butuan City. The drinking water is supplied by Butuan City Water District (BCWD) to over 40,000 concessionaires or more than 200,000 individuals in the city of Butuan and other nearby towns. The Watershed also supplies water to National Irrigation Administration (NIA) irrigation dams servicing vast hectares of rice lands, and a source of livelihood for occupants living near the watershed [17]. The river water is also being used for agricultural crops and aquacultures in the city of Butuan and towns of Magallanes and Remedios T. Romualdez [18].

Water Sample Collection

The sampling sites were selected based on the points set by the Butuan City Water District (BCWD) and Taguibo Aquatech Solutions Corporation (TASC) that included the following: Point 1 (Upstream), Point 2 (Midstream) and Point 3 (Downstream). The sampling was done during the months of February and April 2018. All of the three water samples were obtained during the dry season. Temperature, being an important physical parameter of water was measured in-situ using a portable water thermometer. The water samples were collected using nine one-liter, six 500-mL, and three four-liter clean screw cap polyethylene bottles and were labelled with the date, time, and site of collection. They were then placed in a cooler containing ice gel packs to prevent parasite degradation and were immediately transported and processed within 48 hours [15]. The cooler was equipped with a temperature logger to ensure the maintenance of the required temperature, which was 4° Celsius. The water samples were sent to separate institutions for testing: six one-liter samples and six 500-mL water samples from upstream, midstream and downstream areas were sent to the Department of Science and Technology – Regional Standards and Testing Laboratory for physical parameters testing; three one-liter samples were sent to the Butuan City Water District for the testing of nitrate content; and the three four-liter of water samples were sent to the clinical laboratory for the screening of waterborne parasites.

Measurement of Physico-chemical Characteristics of Water Samples from the Sampling Sites

The water samples collected were subjected to water quality analysis in terms of physical parameters such as pH, temperature, total dissolved solids (TDS) and salinity. Chemical parameters such as dissolved oxygen (DO) and nitrate were also analyzed. The physical and chemical testing were done at the Department of Science and Technology – Regional Standards and Testing Laboratory following their protocol. The pH was conducted using a pH meter; the total dissolved solids (TDS) was conducted using gravimetric method dried at 105°C; and the salinity was measured using a salt refractometer using the two one-liter water samples from each site. The dissolved oxygen was conducted using a Dissolved Oxygen meter using the two 500-mL water samples from each site. The test for water nitrate was done at the Butuan City Water

District using Cadmium Reduction method with the remaining one-liter water samples from each site.

Sample Processing

Water samples were filtered through a 47 mm diameter, 1 µm pore size membrane filter. The water samples contained in the three four-liter screw-cap polyethylene bottles were allowed to settle down for one hour before the filtration process. The supernatant was discarded, allowing the sediments to be filtered by inverting the water bottle against the 1 µm pore size membrane water filter. The process took an hour to finish due to the very small pores of the water filter [11].

Screening for Cysts or Ova of Parasites

Sediments retained by filtration were scraped using smooth-edged plastic loop (Franco, Rocha-Eberhardt, & Cantusio, 2001) and were examined microscopically in 0.9% normal saline solution (NSS) smear for parasite cysts, trophozoites and helminth eggs detection. A portion of each sample was stained with Lugol's iodine on a separate slide. Fresh preparations were examined visually at 10x and 40x objective lenses of a compound light microscope (Nikon, E100) over approximately 100 fields. Ziehl-Neelsen technique of acid fast staining was also applied for the identification of *Cryptosporidium* species, *Entamoeba* species and *Giardia* species [13].

Microscopic identification was performed using histochemical stains such as Lugol's iodine and Ziehl-Neelsen acid fast stain; and direct microscopy with the use of 0.9% normal saline solution [13].

Identification of parasites screened through light microscopy and histochemical staining were validated by two medical parasitologists.

RESULTS AND DISCUSSION

Physico-chemical Analysis

Data obtained through physical and chemical parameter analysis were compared to the regulations set by the Department of Environment and Natural Resources' (DENR) Water Quality Criteria for Water Usage and Classifications. Table 1 shows the summary table of physico-chemical characteristics of water samples.

As to temperature, water from upstream and midstream was found to have a temperature of 22.8°C, while that in the downstream was 22.1°C. This reveals that the water temperature in all sampling sites were normal as they fall within the standard temperature, which is 26-30°C. The river water temperature can affect the dissolved oxygen concentration. When river water is warmer than the normal temperature range, it has reduced ability of the river water to retain oxygen thus, affecting the aquatic organisms.

The pH of the sampling points were all within range except for the downstream which was slightly above the standard range, with a value of 8.85. The higher pH of the downstream point is due to its location, in which it is affected by anthropogenic discharge. The dumping of chemicals into the water by individuals living nearby such as detergents from clothes washing and shampoo rinse waters can cause an increase in the pH [19].

Table 1. Physico-chemical Characteristics of Water Samples from the Sampling Sites

Sampling Site	Coordinate (Latitude, longitude)	Temperature (°C)	pH	Dissolved Oxygen (mg/L	Total Dissolved Solids (mg/L)	Salinity (ppt)	Nitrate (mg/L)
Upstream	9°00' 53.0" N 125°40' 10.5 E	22.8	8.51	2.70	144	0	6.50
Midstream	9°00' 53.0" N 125°39' 43.7" E	22.8	8.53	2.77	246	0	5.30
Downstream	9°00' 53.0" N 125°39' 28.2" E	22.1	8.85	2.67	246	0	4.80

The level of dissolved oxygen in all sampling points, which are the upstream (2.70 mg/L), midstream (2.77 mg/L), and downstream M(2.67 mg/L) did not meet the minimum requirement which is 5 mg/L. This is because ground waters, a primary source of river flow during dry weather and base flow conditions, is naturally low in dissolved oxygen. During dry season, as this study was conducted in the months of February and April, the cooler ground water inflow may at first lower the dissolved oxygen concentration, but it also tends to reduce the river water temperature which improves the ability of the river to hold oxygen [20]. As to the total dissolved solids (TDS), water from the upstream was found to have 144mg/L TDS, while both midstream and downstream have 246 mg/L TDS. This indicates that the water from all sampling sites is suitable as a source of drinking water since a TDS of below 1000 mg/L is acceptable to consumers [7].

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The salinity in water samples in all sampling sites were all 0 ppt. Salinity affects and is necessary for the survival of aquatic organisms. It must also be within the normal range to be acceptable for human drinking water consumption. The normal range for the salinity of freshwaters, such as rivers, is 0-0.5 ppt. This shows that water in all sampling areas are of good quality since the data obtained were all within range.

Water from the upstream have a nitrate content of 6.50 mg/L, the midstream have 5.30 mg/L and 4.80 mg/L in the downstream.

The normal nitrate content of river waters is less than 10 mg/L thus showing that water from all sampling sites is within range, and is safe for consumption.

Microscopic and Morphologic Examination of Recovered Organisms

From the three (3) sampling points, two (2) were positive for free-living organisms: the midstream and downstream points. Presence of these organisms was observed in the midstream and downstream areas, wherein the highest presence of these organisms was detected in the downstream area.

Table 2. Microscopic Examination Findings for *Giardia* spp., *Entamoeba* spp., and *Cryptosporidium* spp.

	Direct smear			Lugol's Iodine			Modified Ziehl-Neelsen Stain		
Organism/ Sampling Site	**Up-stream**	**Mid-stream**	**Down-stream**	**Up-stream**	**Mid-stream**	**Down-stream**	**Up-stream**	**Mid-stream**	**Down-stream**
Giardia spp.	-	-	-	-	-	-	-	-	-
Entamoeba spp	-	-	-	-	-	-	-	-	-
Cryptosporidium spp.	-	-	-	-	-	-	-	-	-

Legend: (+) Present, (-) Absent

Table 2 shows the summary table of microscope examination findings for *Giardia* spp., *Entamoeba* spp., and *Cryptosporidium* spp. The findings revealed that all water samples were negative for *Giardia* spp., *Entamoeba* spp., and *Cryptosporidium* spp. Sample 1 and 2 which were the water samples from the upstream and midstream, respectively, are from lotic environments and are classified as Class A according to the Water Usage and Classification of the Department of Environment and Natural Resources; whereas Sample 3 was classified as Class B, also in a lotic environment [21]. Although pathogenic parasites are prominent in dry and summer season (Kao, et al., 2013), protozoans such as *Giardia, Cryptosporidium* and *Entamoeba* were not recovered in the sampling area due to the lotic environment wherein fast flowing waters can affect the *Cryptosporidium* oocysts viability due to the seclusion of the area that agricultural and residential activities, one of the main cause of Giardia cyst infestation, cannot take place [22] and the absence of source of contamination for *Entamoeba* cysts to persist [23].

Figure 2. Free-living organisms found in midstream

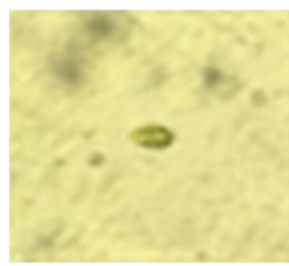
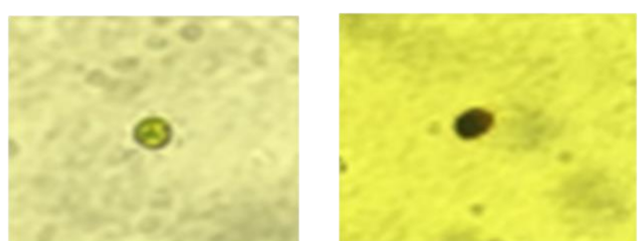


Figure 3. Free-living organisms found in downstream



Microscopic Examination Findings for Other Free-living Organisms

Table 3

Sampling Site	Direct smear	Lugol's Iodine	Modified Ziehl-Neelsen Stain
Upstream	-	-	-
Midstream	+	+	+
Downstream			

Legend: (+) Present, (-) Absent

Table 3 shows the microscopic examination findings for other free-living organisms. Sample 1, the water from the upstream area declared no free-living organisms in all detection methods. The upstream area is a secluded area and people come in this area for water sampling purposes only. Sample 2 (Figure 2), the midstream area, showed the presence of free-living organisms in all detection methods. On the upper part of the riverside in this area, there are residents living thus, proper toilets were being provided by the Taguibo Aquatech Solutions Corporation (TASC) to prevent possible fecal contamination of the river. These free-living organisms, as seen in Figure 2, were largely found at the downstream area, wherein a hanging bridge linking the main highway and the residences and households is found just above the sampling site. People usually pass through this bridge by means of walking and motorcycles. Residents nearby also wash their clothes in the river and children bathe in this portion of the river. Human activities are not controlled within this area because it is located outside the protected watershed. However, this is included in the monthly water quality monitoring.

It is noted that only free-living organisms were recovered from the water samples in the basis of their morphology and motility using the direct smear using 0.9% normal saline solution and Lugol's iodine. This means that the river water in the Taguibo watershed is unlikely to be polluted. Thus, there is little possibility of pathogenic parasitic infestation in the sampling area. However, the free-living organisms recovered were not identified by genus and species and were not classified as to being pathogenic or not. Thus, waterborne parasite surveillance is needed to prevent and mitigate the pathogenic effects of these free-living organisms.

CONCLUSION AND RECOMMENDATION

The study shows that free-living organisms are found in Taguibo Watershed Forest Reserve, the major source of drinking water in Butuan City and other nearby towns but are negative for waterborne parasites of medical importance. Whether these free-living organisms are pathogenic or not, direct consumption from this surface water is still not safe and may pose health threats to the public. As consumption of safe water is the ultimate need of the public, it should be an initiative and of great priority to maintain regular monitoring of water quality that includes parasite detection, identification, and quantification.

Despite the quality exhibited by the major source of drinking water in Butuan City and other nearby towns, waterborne diarrhea and other gastrointestinal illnesses are still prevalent. It is recommended to study the major pipelines and pump stations that lead to household faucets, and other sources of drinking water in the city such as deep wells that may become a reservoir for waterborne parasites that may cause gastrointestinal illnesses. It is also recommended to encourage the water districts and the health agencies to consider and test drinking water sources routinely for parasites in the workup. Moreover, future studies can be done such as detecting and identifying the parasites through concentration and molecular methods; and obtaining a larger sample size by collecting samples in other major tributaries of the river as well as other water sources in the city in order to provide information for risk assessment with the occurrence of parasite contamination in the water environment. This could further help create a greater awareness among public, and agencies governing water safety, as it is a key health issue in the city.

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