Identification of Pathogen Protozoans *(Cryptosporidium spp and Giardia lamblia)* from Canteen's Water-sources at Jatinangor, West Java, Indonesia

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Abstract

Background: The prevalence of intestinal protozoan infection caused by *Cryptosporidium spp* and *Giardia lamblia* has been rapidly increasing in developing countries. Contamination of water-sources by intestinal protozoans can spread infection to humans when it is consumed without any specific treatment. Jatinangor is an educational area where many universities are located and many students live in this area. This study aimed to identify the presence of intestinal protozoa (*Cryptosporidium spp and Giardia lamblia*) in water-sources used by canteens at Jatinangor area to detect the magnitude of protozoans intestinal infections.

Methods: A descriptive cross sectional study was carried out from August to September 2014. Raw water samples from reservoir tanks, water containers and tapwater were taken from all canteens located along the main road of Jatinangor. Samples were centrifuged and divided into two portions; two drops from the first test tube was stained with Lugol solution and observed by 2 observers under a light microscope for *Giardia lamblia*, and sediments from the second test tube was stained using acid fast staining then observed for Cryptosporidium spp under the light microscope.

Results: Out of 50 samples, 26 were positive for parasites, of which 22 (44%) were contaminated by *Cryptosporidium spp* and 4 (8%) were contaminated by *Giardia lamblia*.

Conclusions: Half of the raw water-sources in Jatinangor are contaminated by *Cryptosporidium spp* and *Giardia lamblia*. Proper water treatment should be implemented by the owners of the canteens before it is consumed as drinking water.

Keywords: *Cryptosporidium spp*, *Giardia lamblia*, water-source

Introduction

Parasites are known as living organisms which depends on other organism to continue living. It builds a parasitic relationship with the depending organism by living on or in the particular organism.[1] Humans are well-known hosts where these parasites choose to live in. Parasites which live in the digestive tract of humans are called intestinal parasites. Intestinal parasites cause a wide range of parasitic infections in humans.[1,2] According to the World Health Organization (WHO), intestinal parasite infections have been spreading almost throughout the world with increasing number of occurrence.[3]

Parasites are commonly divided into two major groups: Parasitic Protozoa and Parasitic Helminthes. Protozoans which are commonly found are *Cryptosporidium Spp* and *Giardia lamblia*.[2,4] Protozoan parasites can be detected through the characteristic of their cyst or oocyst.[5] Protozoan usually multiply rapidly in host and causes an acute onset of symptoms. The intestinal protozoans are one of the common human gastrointestinal infections.[3]

Intestinal parasitic infection commonly occurs through soil transmitted or water-borne parasites. The transmission mostly goes through fecal to oral route.[1] Poor sanitation and water supplies often lead to water contamination.[6,7] Water transmitted intestinal parasites often cause various water-borne diseases involving gastrointestinal tract such

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as diarrheal diseases.[8] Indonesia is a high populated country with poor water quality, sanitation and hygiene problems. Previous studies have claimed that gastrointestinal disorders particularly diarrhea has been the main problem faced by the citizens.[9]

Jatinangor is a subdistrict in Sumedang, West Java province which is known as an educational area since many universities are located in this area and many students live in this area. The populationof students has been increasing each year. Most of the students have their lunch and dinner in the canteens which are easily found along the main road of Jatinangor. These canteens can be a source of infection where intestinal protozoan can be transmitted through contaminated water and food.[10] Canteens in the student area are often cheap and easily available, however the cleanliness and hygiene has been an issue which often arises due to the location and the water supply to the canteens.

The water supply in Jatinangor area comes from various water-sources such as from the municipal tap water, river, ground water from dug-wells or bore-holes, spring water and rain water depending on the location. The type of water-source, treatment and storage management towards the water plays a role on the level of contamination of the water by intestinal parasites.[11] Therefore, the aim of this study was to identify the presence of Intestinal Parasites (*Cryptosporidium spp and Giardia lamblia*) in water-sources used in canteens in Jatinangor in order to detect the magnitude of intestinal protozoan infection.

Methods

This descriptive study used the cross sectional method and was carried out from August to September 2014. Water-sources which were used by canteens for cooking, washing and also as source of drinking water were collected, while identification of the protozoans was conducted in the Parasitology Laboratory, Faculty of Medicine, Universitas Padjadjaran.

The study took place at canteens located along the main road (Jalan Raya Jatinangor) between the the Institute of Local Government (IPDN) and Robita Dharma Corporation which lies at the eastern part of Universitas Padjadjaran was labelled as the mark point, and samples were collected approximately 500 meters to the left and right direction from the campus.

All canteens located along the main road or Jalan Raya Jatinangor were selected using the total sampling method to collect raw water samples. The canteen owners were given an informed consent letter and a questionnaire to be filled in. Data from the questionnaires described the characteristics of the canteen such as the type of water-source used, the way of storing water and the kind of water treatment method used.

The raw water samples were obtained in a 1.5 liter water container. Before collecting the water sample, the water container was rinsed three times using the water sample directly from the water-source thoroughly. This method was used for water sample collection since the identification was intended to find the cyst of the protozoans. The samples were directly brought to the Parasitology Laboratory of Faculty of Medicine of Universitas Padjadjaran and examined by 2 observers.

Next, the sample was filtered and about 10cc of sample was placed in a centrifugation tube and was centrifuged for 5 minutes at the speed of 2000 rpm. The centrifuged water was then separated into two test tubes (Test tube A & B). Furthermore, water in test tube A was stained with Lugol solution and was observed under the microscope with 40X magnificence for *Giardia lamblia*. Then, water in test tube B was stained using Modified Acid fast staining and *Cryptosporidium spp* was observed via a light microscope with the magnificence of 100X. After the results were obtained, data was presented in a table in the form of frequency and percentage. This study was conducted with the approval of the Health Research Ethics Committee, Faculty of Medicine, Universitas Padjadjaran and permission from the local authority was given by the Hegarmanah Subdistrict.

Results

About 80 canteens were identified along the main road in Jatinangor, however only fifty canteens participated in the water sample collection as described in Figure 1. The number of *Cryptosporidium spp* in raw water-source was higher than of *Giardia lamblia*. However, the number of raw water-source without any presence of *Cryptosporidium spp* and *Giardia lamblia* was slightly higher (Table 1).

Out of 50 samples of raw water collected from the canteens in Jatinangor area, 22 (44%) water samples showed positive for *Cryptosporidium spp* and 4 (8%) water samples showed positive for *Giardia lamblia*. It also showed that out of 50 canteens, 24 (48%) water samples were free from these parasites

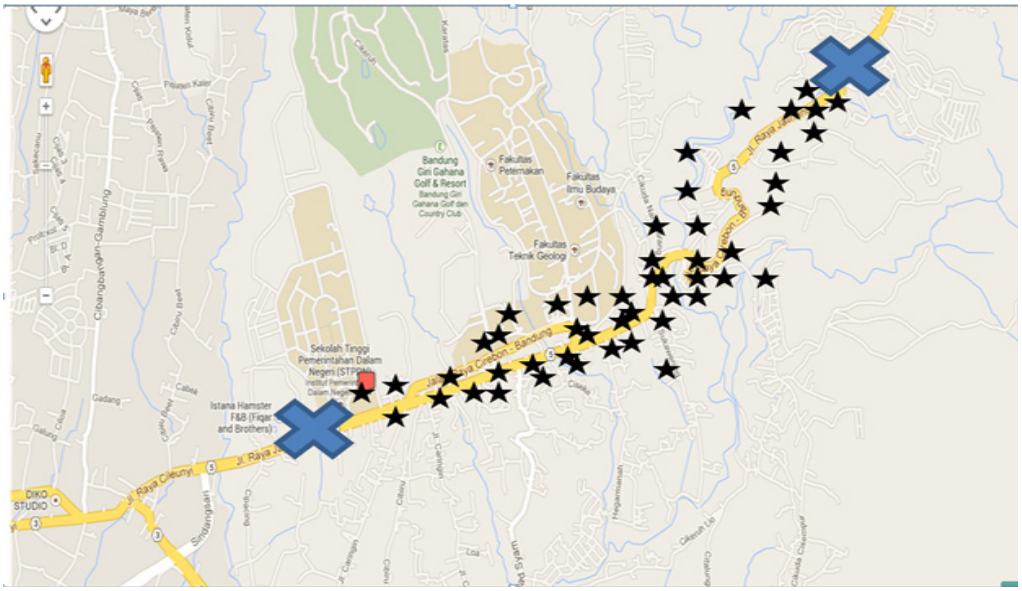

Figure 1 The Location of Study Site

(Table 1).

A high number of canteens got their water-source from deep wells either dug-well or bore-hole, while spring water was usually distributed through pipes from the area of Manglayang hill, while municipal tap water was only available in the area of the Institute of Local Government.

Moreover, 47 (94%) of the canteens got their water-source from ground water. Out of the 47 samples 20 were positive for *Cryptosporidium spp* and 4 were positive for *Giardia lamblia*. Spring water used by 2 (4%) of the canteens contained *Cryptosporidium spp*. Municipal tap water was only used by 1 (2%) canteen and it was negative for both the protozoans (Table 2).

From the type of water-source used, the water tank reservoir was mostly used to store raw water for daily usage; this type of reservoir was usually located outside the house. Followed by the water container which was placed inside the house, and also the direct tap water from the Municipal water company (Table 3).

Furthermore, 92% of the canteens stored their water in a water-tank reservoir, almost half of the water was contaminated and mostly with *Cryptosporidium spp*. All of the water in the water-container was contaminated with *Cryptosporidium spp*. However, municipal tap-water showed no contamination (Table 3).

The water-sources were used for cooking, washing and as a source of drinking water. Only 29 out of 50 canteens clean the water-container minimal once in a year. Besides, the practice of storage cleaning and water contamination showed only 34.4% of the water was contaminated if the water storage

Table 1 Frequency of Intestinal Parasites (*Cryptosporidium spp and Giardia lamblia*) Found in Water-sources used by Canteens in Jatinangor Area

Intestinal Parasites	n =50	%
Cryptosporidium spp	22	44.0
Giradia lamblia	4	8.0
Others		
Entamoeba coli	2	4.0
Naegleria Fowleri	4	8.0
Negative	18	36.0

Table 2 Type of Water-Source and Intestinal Protozoan Contamination

Type of water source (n=50)	*Cryptospordium spp* (n,%)	*Giardia lamblia* (n,%)	Negative Results (n,%)	Total (n,%)
Deep well water (n=47)	20 (40)	4 (8)	23 (46)	47 (94)
Municipal Tap water (n=1)	0 (0)	0 (0)	1 (2)	1 (2)
Spring water (n=2)	2 (4)	0 (0)	0(0)	2 (4)

Table 3 Type of Water Storage and Intestinal Protozoan Contamination

Type of Water storage (n=50)	*Cryptosporidium spp* (n,%)	*Giardia lamblia* (n,%)	Negative Results (n,%)	Total (n,%)
Water tank reservoir (n=46)	20 (40)	4 (8)	22 (44)	46 (92)
Water container (n=3)	2 (4)	0	1 (2)	3 (6)
Municipal Tap water (n=1)	0 (0)	0 (0)	1 (2)	1 (2)

was cleaned minimal once in a year, compared to 76.2% of the water contamination if the storage was never cleaned (Table 4).

Discussion

Based on the study performed, the results showed that 22 (44%) of the samples were positive for *Cryptosporidium spp.* This protozoa is not easily eliminate by chlorination, the cysts are able to withstand in chlorine water for about 3.5 to 10.6 days.[12,13] However, boiling the water is still effective as water treatment method for most protozoan cysts.

The outcome for *Giardia lamblia* was much lower compared to *Cryptosporidium spp*, which was 4 (8%) positive, since *Cryptosporidium spp* survives longer compared to *Giardia lamblia* in water.[13] *Giardia lamblia* cyst can be in any environmentally exposed water-sources but to increase the identification, a high sensitivity method and big sample size should be used.[14] Apart from that, the contamination by *Giardia lamblia* is closely related to the weather condition.[14] Throughout the period of the study, Jatinangor was experiencing a dry season which could be the reason for the altered outcome. Besides, it was noticed that out of 50 water samples 24 (48%) was negative for *Cryptosporidium spp* and *Giardia lamblia.*

The study showed that most of the canteens in Jatinangor area used deep well-water as their raw water-source. The deep well-water has higher contamination rate for *Cryptosporidium spp* and *Giardia lamblia*[15], particularly unprotected deep well can be easily contaminated by animal and human feces.[6,16] Municipal tap-water had negative for both the parasites, this might be due to the proper treatment before the water was distributed to the consumers through the pipe distribution.

Apart from that, the most common water storage used by the canteens in Jatinangor area was the water tank reservoir, which could accumulate about 1000 liter of water and was usually placed at the attics or the rooftop of the building. It was usually made out of polypropylene plastic or stain-less steel. From the data collected, it showed that the water tank reservoir had the highest level of contamination, followed by the water container inside the house which came in smaller size than the tank, and was usually easily moved and stored. Lastly, the municipal tap-water is a water system from the community water system supplied through a pipeline directly to the households which again showed zero contamination.

Additionally, water storage cleaning is essential to reduce the risk of contamination; most of the water storage which have never been cleaned regularly are known to be

Table 4 Water Storage Cleaning and Intestinal Protozoan Contamination

Water storage Cleaning	*Cryptosporidium spp*	*Giardia lamblia*	%
Minimal once in a year (n=29)	9	1	34.4
Never (n=21)	13	3	76.2

contaminated by the intestinal protozoans. *Cryptosporidium spp* and *Giardia lamblia* are resistant towards chlorination therefore, they could survive,[17] however cleaning the water storage regularly might reduce the risk of contamination. Further proper water treatment is still needed before consuming the water as drinking water.

The study was conducted with a minimal number of samples due to the time limitation, and many of the canteens refused to participate in the study. Therefore, the sample should be increased to obtain a more significant outcome. On top of that, most of the water-sources obtained were from deep wells, therefore this would have affected the outcome. In a further study, samples should be taken from more various sources. Due to the time limitation, water-sources can be only obtained from canteens located along Jalan Raya Jatinangor and not further than that. Moreover, a further study should be conducted during the whole year to identify the protozoans in the dry and wet seasons. During this study the climate in Jatinangor was not favorable, as the dry seasons reduce the risk of water contamination.[18,19]

Lastly, a further study is recommended since this study was only carried out specifically on water-sources which was used for washing, rinsing and cooking, thus identification of Intestinal Parasites in drinking water should be conducted in the future to figure out the effectiveness of water treatment. *Giardia lamblia* has been detected positive in such low percentage due to its method used; a higher sensitivity method such as Polymerase Chain Reaction (PCR) should be used to identify the intestinal parasites.

It can be concluded, that *Cryptosporidium spp* is the most protozoan that contaminates the water, and deep well-water has a higher range of contamination compared to other water-sources. Therefore, further treatment should be performed such as boiling the water until it is at rolling boil temperature, which has higher chances of eliminating the parasites.[20] Owners of a canteen should be also well guided towards maintaining better water treatment and hygiene. Besides, water-sources and cleanliness of canteens should be regularly monitored by the higher authorities. Lastly, the community should be counseled about the importance of water, hygiene and sanitation.

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