

## **EFFECT OF CHLORINE ON GIARDIA CYST ISOLATION FROM DIFFERENT WATER SOURCES**

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### **ABSTRACT**

*G. lamblia* was a binucleate flagellated protozoan parasite that infected the upper intestinal tract of human and many animal species. Giardiasis was the most frequently diagnosed water borne disease and the major public health concern of water utilities in the developed and developing nations, water is an important vehicle for the transmission of *Giardia* to human and mammals. For identified the effect of chlorine on *Giardia* cyst. To detect viability of cyst in different chlorine concentration. To determine the threshold level of chlorine concentration that caused cyst destruction. Measure the pH, chlorine concentration, filtration processes and examined by zinc sulfate centrifugal flotation technique using 50% of samples contain *Giardia* cysts which are untreated water sources. Cyst viability differ in different chlorine concentration in different period of time extend from few hours to more than twenty days. The extreme chlorine concentration which caused cyst destruction in hours is 1.6 mg/L.

**KEY WORDS:** *Giardia*, Waterborne, chlorination, filtration, chloro-meter, Reverse Osmosis.

### **INTRODUCTION**

The genus *Giardia* falls within the phylum Sarcomastigophora, class Zoomastigophora, order Diplomonadida and family Hexamitidae. It includes six valid species that inhabit the intestinal tracts of virtually all classes of vertebrates, with *G. duodenalis* being the only species found in humans (1, 2). *Giardia* has been known since the seventeenth century work by Antony van Leeuwenhoek, although for most of this time this protozoa has been considered a harmless intestinal commensal, primarily because the parasite was identified in asymptomatic individuals. It was not until 1987 that the pathogenic nature of *Giardia* was definitively confirmed in a study of infection in human volunteers (3). *Giardia lamblia* (syn. *G. duodenalis*, *G. intestinalis*) is an intestinal flagellate that infects a wide range of vertebrate hosts, such as domestic and wild mammals, including humans (4). These flagellates have a simple two stages life cycle consisting of the reproductive trophozoite stage and the environmentally robust cyst stage (4). Cysts are shed by infected hosts into the environment and these cysts can survive in the environment for months without losing their

infectivity (4). Transmission of *Giardia* is common in certain high-risk groups. Children, workers in day-care centers and /or sewage / irrigation processes, those who are exposed to contaminated water and /or certain animals, travelers and the immuno-compromised, are known to be more susceptible to infection (5). Water is responsible for a quarter of endemic *Giardia* infections in the USA and the role of contaminated municipal water supplies in epidemic outbreaks of giardiasis is undeniable (6,7). This protozoan parasite has been recognized as a frequent cause of waterborne disease (8, 9) because of their strong resistance against chlorine disinfection (10) as well as the low minimum infectious dose (11). Many of waterborne outbreaks in 1980s have been associated with the ingestion of untreated surface water by hikers and campers in the USA, Canada, England, Sweden, but giardiasis outbreaks have not been limited only to these people. Some waterborne outbreaks were reported in some municipal drinking water systems, and then the United States Environmental Protection Agency (USEPA) have regulated treatment techniques for a sufficient removal of *Giardia* cysts at water treatment plants as one of the drinking water rules (10). Microbial contamination of groundwater is a serious concern worldwide (9, 12). For many countries, groundwater provides approximately 40% of the potable water used for human consumption (9, 12). Cyst-forming protozoans such as *Giardia intestinalis* and *Cryptosporidium parvum*, viruses such as Hepatitis A, and even pathogenic bacteria such as certain *E. coli* strains can survive for extended periods of time in ground water systems with temperatures of less than 10°C, migrate significant distances, and are relatively resistant to standard municipal water system chlorination practices (12). Though dormant outside the host, as few as ten cysts can result in a human infection (12). *Giardia* is more frequently found in surface waters than *Cryptosporidium*, and it has been associated with at least 132 waterborne outbreaks worldwide (13). However, giardiasis outbreaks have not been limited only to people who ingest untreated surface water, since in many municipalities using only chlorine for disinfection, outbreaks of giardiasis have been reported after the consumption of the treated water (14). The environmental study showed that water, whether drinking water, washing and sewage come in the first place and basic of disease transmission between human and animals and vice versa compared to other modes of transmission (15). Though dormant outside the host, as few as ten cysts can result in animal or human infection, called giardiasis (12, 16, 17). *G. intestinalis* is the most frequently identified protozoan agent causing waterborne outbreaks of parasitic infections in humans in the world (18, 19).

## **METHODEOLOGY**

In this work 10 liters were collected in a sterile glass bottles tightly closed from each source of different sources of water (river water, water marshes, water pools, taped water, water purification stations and RO water) in Basrah. Conducted a measurement process for the pH proportion and chlorine concentration for each samples of water by using two devices the pH meter (Hanna/ Romania) and chloro-meter (Accue/ China).

By using a Domestic Reverse Osmosis Unit (General Master/ Korea) for the purpose of filtering each samples of water on separately ways, the steps are:

- 1- First stage: removing the particles, dust and mud.
- 2- Second stage: removing organics, odor and turbidity.
- 3- Third stage: removing organics, odor and turbidity.
- 4- Fourth stage: removing bacteria, protozoa cyst, salt, heavy metal, mineral substance and other dissolved matter.



**Figure:** Reverse Osmosis Unit

Then changing the filtering tubes for each filtration processes separately and use anew filtering tubes for each next filtering steps to get sediment in each samples and examined by zinc sulfate centrifugal flotation technique by use sediment samples collecting by filtering tubes instead off stool samples according to (20) and then investigate cyst of Giardia by direct smear with normal saline according to (21, 22).The positive samples were taken and which contained Giardia cyst.

Each positive sediment give symbols for differentiation from other as follow:

river water	water marshes	water pools	taped water	water purification stations	RO water
S 1	S 2	S 3	S 4	S 5	S 6

2 g of positive samples used to put them in different concentrations of chlorine to see the effect of sterilized rule on the cysts and to determine the concentration for Giardia cyst destruction.

## RESULTS

Each 10 liters of different water source have different chlorine concentration and pH level in table (1) below.

Table (1): Water sources and its chlorine concentration and pH.

Water source	Cl <sub>2</sub> concentration mg/L	pH
S 1	0	10.1
S 2	0	12.5
S 3	0	12.7
S 4	0.1	8.9
S 5	0.2	8.1
S 6	0.6	7.2

The microscope examination identified 50% of samples contain Giardia cysts which are S1, S2 and S3, table (2).

Table (2): Sediment samples and Giardiacysts present

Sediment samples	cyst
S 1	+
S 2	+
S 3	+
S 4	
S 5	
S 6	

Different chlorine concentration and cyst (viability and activity) among several day work show different results as in table (3).

Table (3): Chlorine concentration and Giardia cyst viability

Cl <sub>2</sub> concentration mg/L	Cyst viability	Duration of activity
≤ 0.2	+	≥ 20 days
0.3 - 0.4	+	10 – 15 days
0.5 – 0.8	+	7 – 9 days
1.0 – 1.2	+	3 – 6 days
1.4 – 1.6	+	1 – 2 days
>1.6	–	Few hours

## DISCUSSION

The results of work shown present of Giardia parasite in different raw water sources as rivers, marshes and pools, so water play important role in transmission of parasite to different hosts and this agree with (4, 18, 23, 24, 25). This is due to all these water sources are untreated. The 50% of sediment samples contain Giardia and this similar to (25, 26). There are several factors contributing to the occurrence of waterborne outbreaks. Cyst concentration in drinking water and parasite host specificity, infectivity, and virulence as well as population susceptibility are complex, interacting variables (25). Acquired immunity was noted in one study comparing attack rates of residents with those of visitors during a waterborne outbreak of giardiasis (27). Another recent community study showed that residents with laboratory-confirmed giardiasis from one waterborne outbreak were much less likely to be infected in the second outbreak (28). The viability of Giardia cyst is different in different chlorine concentration, cyst remain viable at 0.2, 0.3, 0.4, 0.5, 0.8, 1.0, 1.2 and 1.4 mg/L for a different period of time, but cyst destruction occurs when concentration reaches more than 1.6 mg/L. The prevention of *G. duodenalis* infections is a difficult task since the cysts are ubiquitous, resist environmental decomposition and remain viable for a considerable period of time (29). The disinfection and cleaning of surfaces should occur utilizing products such as organic iodine, tincture of iodine, chlorine or bleach (hypochlorite) to reduce contact with viable cysts by potential hosts (30). Systems providing drinking water should use coagulo-sedimentation and filtration as methods of purification in order to prevent waterborne giardiasis outbreak (31). Chlorination only is effective and kills most entero-pathogenic organisms, but Giardia cysts require higher concentrations and more prolonged exposure to chlorine to be killed, especially in cold water (17).

## CONCLUSION

Water is present as important vector for transmission Giardia. Disinfection processes of water not enough method for treated drinking water. Combination of chlorination, filtration and disinfection of water is more effective in treated water and in killing all parasites including Giardia.

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