A Preliminary Assessment of the Occurrence of *Cryptosporidium* and *Giardia* in the School Swimming Pool Water in Dubai, United Arab Emirates

Alia Ali Ahmad Aljanahi and Munawwar Ali Khan

***Abstract*—The aim of this study was to assess the presence of *Cryptosporidium* and *Giardia* in the water of school swimming pools located in Dubai, UAE. The swimming pool water samples from five schools were tested for *Cryptosporidium* oocysts and *Giardia* cysts using direct immunofluorescence assay. Samples were also examined for the total coliform, fecal coliform and heterotrophic bacteria using the membrane filtration technique. The majority of the tested swimming pool water samples were found contaminated with either *Cryptosporidium* oocysts or *Giardia* cysts or both. *Cryptosporidium* oocysts were found to be in the higher range (1-15 oocysts per liter) than that of *Giardia* cysts (1-4 cysts per liter). All of the swimming pool water samples were found negative for the presence of traditional bacterial indicators. However, direct DAPI staining detected several types of viable but nonculturable (VBNC) bacteria on the membrane filter. In conclusion, based on the preliminary data obtained, *Cryptosporidium* and *Giardia* were found to be present in the tested schools' swimming pool water. Future work on a long term monitoring of both parasites is highly recommended to enhance the swimming pool water quality and ensure the public health safety which will help in maintaining a hygienic and healthy recreational water environment.**

***Index Terms*—Cryptosporidium, giardia, swimming pool.**

I. Introduction

The protozoan parasites Cryptosporidium and Giardia are recognized as important waterborne disease pathogens and are associated with severe gastrointestinal illness of humans worldwide [1], [2]. These protozoan parasites are quite prevalent in swimming pools and other recreational water bodies [3], [4]. Despite frequent outbreaks, little is known about these parasites occurrence in swimming pools in the absence of outbreaks [4]. Furthermore, there is a lack of documentation of waterborne parasitic protozoan outbreaks and surveillance in developing countries [2]. Cryptosporidium and Giardia species infect humans as well as a wide range of animals causing gastroenteritis with symptoms such as nausea, weight loss, watery diarrhea and abdominal cramps [5]. In a number of countries the contamination of water by microorganisms caused remarkable outbreaks. For instance, in the United States the exposure to contaminated recreational water venues including swimming pools resulted in more than 18,500 illnesses and about 24 deaths from 1995-2004 [6]. In the past, bacteria were considered the major source of waterborne infections, but according to several studies, parasites have replaced bacterial pathogens and became the primary cause of waterborne diseases [7]. In particular, in the last decade several studies specifically in the United States, Canada, Australia and the United Kingdom stated that parasites such as Cryptosporidium and Giardia became a major source of waterborne illnesses including gastroenteritis [8].

Cryptosporidium and Giardia are found in the fecal matter of an individual or an animal that is infected by these parasites. It is transmitted to other individuals by drinking water or eating food which is contaminated by these parasites [8]. Nevertheless, it has been shown in several studies that water is considered the primary vehicle for the transmission of cryptosporidiosis which is the infection caused by Cryptosporidium as well as giardiasis which is the infection caused by Giardia [5]. In the infected host, Cryptosporidium produces oocytes which are round shaped with a diameter of 3-5 μm [5]. Oocysts are the transitive stage of the parasite that causes cryptosporidiosis. The oocyst has a critical role because it gives the Cryptosporidium parasite a double outer wall which helps the parasite to survive in the environment for extended duration of time up to several months [9]. On the other hand cysts are oval shaped with a diameter of 8-13 μm [5]. Cysts are the transitive stage of Giardia which has a similar role of the oocysts. It has an outer wall that also protects and helps Giardia to survive long durations of time in the environment as well [6]. A number of studies stated that several risk factors can increase the frequency of cryptosporidiosis and giardiasis associated with the swimming pools. For instance, risk factors including age, frequency of swimming and duration spent in a swimming pool as well as the type of swimming pool were found to have a significant role in increasing or reducing the incidences of both infections [3], [4], [7].

In several countries swimming pools are not routinely tested for the presence of Cryptosporidium oocysts or Giardia cysts and most of the incidences of cryptosporidiosis and giardiasis are undiagnosed because the diagnosis occurs mostly in the scientific institutions', therefore, the condition of the presence or absence of both parasites is unknown [10]. A similar practice is prevalent in the UAE. According to the Dubai Central Laboratory (DCL) environment section head,

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environmental safety inspectors in Dubai municipality collect swimming pool water samples from a variety of locations including water parks, schools and hotels for the monitoring of bacterial indicators [11]. However, the swimming pools are not tested for the contamination of Cryptosporidium oocysts and Giardia cysts. Therefore, the prevalence of these parasites in the swimming pool water in Dubai is unknown.

In general, there are several types of swimming pools in Dubai such as in parks, hotels, residential areas and schools which are growing in number along with the population size. Additionally, due to the hot weather in the U.A.E in general, individuals from different age groups tend to use the swimming pools more frequently; therefore, the risk of swimming pool contamination by Cryptosporidium and Giardia can increase in spite of using the most popular water purification methods including filtration and chlorination. The nature of the parasites, such as their small size, low infectious dose and chlorine resistance [9], make it important to investigate the prevalence of Cryptosporidium and Giardia in the swimming pools of Dubai.

Children and young adults are mostly affected by the presence of both parasites in the swimming pools, consequently, they are more prone to the severe consequences of cryptosporidiosis and giardiasis [4], therefore, investigating the prevalence of Cryptosporidium and Giardia in Dubai is important to know whether the water purification methods utilized by swimming pool operators are adequate to reduce the occurrences of both parasites. This study was undertaken to collect preliminary data on the occurrence of Cryptosporidium and Giardia in the selected primary schools' swimming pool water in Dubai. The school swimming pools were chosen because the swimming pools are used by children and young adults who are likely to be more prone to the infections caused by the presence of both parasites.

II. Material and Methods

A. Sample Collection

In total, 22 schools in Dubai were chosen randomly and the school administrators were contacted via emails, phone calls and fax to get their approvals for collecting swimming pool water samples. Out of 22 schools, 5 schools gave their approval and therefore, only 5 schools participated in this study. These schools are herein designated as A, B, C, D and E. The first phase of sampling was conducted in the last two weeks of November, 2012 whereas the second sampling phase was in the second week of December, 2012. During each visit the schools' representatives' were interviewed to collect information regarding the type of the swimming pool, method of water purification, age of the swimmers and frequency of swimming. A total of 10 samples was collected i.e. 2 samples from each school within a gap of 2 weeks between the two sampling phase. In order to collect the samples, the Dubai Municipality guideline that illustrates the steps of collecting swimming pool water samples was implemented [11]. During each visit 3L of swimming pool water samples were collected, each sample was taken directly from the swimming pool in a 1000 ml sterile wide- mouthed plastic bottle. While collecting the swimming pool water samples physical parameters such as the temperature and pH were recorded.

B. Detection of Cryptosporidium and Giardia

In order to detect Cryptosporidium oocysts and Giardia cysts, A100 FLK Aqua-Glo G/C Direct Comprehensive Kit was used (Waterborne, Inc., New Orleans, LA). This kit is designed to specifically to detect the presence or absence of a genus of Cryptosporidium and Giardia. Purified *G. lamblia* and *C. parvum* (oo) cyst suspensions for microscopy positive control were supplied with this kit. The kit manufacturer's protocol was adopted for the analysis of swimming pool water samples for the presence of both parasites.

Before using the kit, swimming pool water samples were filtered on cellulose-acetate filters of 0.45µm pore size and 142 mm diameter (Sartorius, Germany). The filters were scraped and washed using 25 µl of the PBS (phosphate buffer saline) to concentrate the (oo) cysts. The positive control of 5 µl in a mixed aldehyde buffer which contained a mixture of Cryptosporidium oocysts and Giardia cysts was prepared. In order to identify the Cryptosporidium oocysts and Giardia cysts appropriate filters of an epifluorescence microscope were used. Both parasites were identified as both appeared bright apple green when viewed under the fluorescein filter and bright blue when viewed using UV filter. When viewing the slide the swimming pool water sample was compared with the positive control to confirm the results. Quantification of the parasites was carried out by screening of the slides with an epifluorescence microscope (Olympus BX-51, Japan) equipped with UV and blue light and fluorescein isothiocyanate (FITC) excitation blocks.

C. Indicator Bacteria Analysis

Swimming pool water samples were analyzed for the presence of fecal coliform, total coliform bacteria and heterotrophic bacterial count. The membrane filtration procedure [12] was used to enumerate the bacterial indicators. Appropriate volumes of water samples were filtered through 0.45 mm filters (Sartorius, Germany). The quantitative analyses by membrane filtration technique were carried out in duplicate. Culture media and incubation conditions used were: 1) VRB-agar (HiMedia, India) for total Coliforms (36 ℃, 24-48 hour); 2) m-FC agar (HiMedia, India) for fecal Coliforms (at 44.5 ℃, 24-48 hour); and 3) HPC (HiMedia, India) for heterotrophic bacteria (36 ℃, 24-48 hour).

Direct DAPI (4, 6-diamidino-2-phenylindole) staining was also applied on swimming pool water samples. DAPI tends to bind with the microorganisms DNA. Implementing direct DAPI staining was an additional step in order to detect the presence of bacteria or parasites in a swimming pool water sample, therefore, 250ml of a swimming pool water sample was filtered. After that, the filter membrane was placed on sterile petri plate where 5ml of the diluted DAPI was added and left for 15 minutes. After that, the membrane filter was washed with PBS buffer and transferred into a sterile slide using a sterile forceps and viewed directly under fluorescence microscope using a UV filter block.

III. Results and Discussion

The swimming pool water of public and private schools in Dubai are not routinely tested for the contamination of Cryptosporidium oocysts and Giardia cysts which makes this study the first of its kind in the UAE to investigate the occurrence of Cryptosporidium and Giardia spp. in the swimming pools of schools in Dubai. Table I provides the general description of the school swimming pools along with the physical parameters measured during each sampling visit to the school.

TABLE I: Description of School Swimming Pools

School /pool type*		Age of swimmers (Grades)	Swimming frequency	Sampling phase	Physical parameters	
					pH	Temperature (℃)
A	I	3-5	2 classes/week	First	7.5	32.5
				Second	7	34
B	O	1-5	1 class/week	First	7.5	35
				Second	7	29
C	I	1-6	2-3 classes/week	First	7.5	34
				Second	6	35
D	I	4-11	2 classes/week + swimming competitions	First	7.5	34
				Second	7	34
E	O	7-12	1-2 classes/week	First	7	36
				Second	6.5	30

*I=Indoor; O=Outdoor

Table II summarizes the laboratory analysis results of the detection of Cryptosporidium oocysts and Giardia cysts using direct immunofluorescence technique. It also summarizes the results of bacteriological analysis of the swimming pool water samples. In general, this investigation indicated that the majority of the tested swimming pools (9/10) were contaminated with Cryptosporidium oocysts, while a number of them were found positive for Giardia cysts (4/10). Cryptosporidium oocysts in the tested swimming pool water samples were found more frequently than that of Giardia and that could be due to the actual low occurrence of Giardia cysts in the swimming pools. On the other hand, all of the swimming pool water samples were found negative for the presence of all traditional bacterial indicators except of one sample which tested positive for the heterotrophic bacterial count (Table II).

Furthermore, the results of the laboratory analysis indicated that the majority of the swimming pools were found to have less contamination with Cryptosporidium & Giardia during the first phase of sampling, whereas, the level of Cryptosporidium & Giardia contamination were found higher in the second phase of sampling with an exception of school A where the results of both sampling phases were quite similar. Cryptosporidium oocysts and Giardia cysts were observed in both samples (first and the second phase samples) and their numbers were quite similar which could be due to the smaller amount of water sample collected (Fig. 1).

TABLE II: Microbial Analysis of the Swimming Pool Water

School/pool type		Sampling phase	Cryptosp-oridium Oocysts *	Giardia cysts**	Bacteriological count***		
					FC	TC	HC
A	Indoor	First	+(2)	-(0)	-	-	-
		Second	+(4)	+(2)	-	-	-
B	Outdoor	First	-(0)	-(0)	-	-	+
		Second	+(2)	-(0)	-	-	-
C	Indoor	First	+(1)	+(1)	-	-	-
		Second	+(15)	-(0)	-	-	-
D	Indoor	First	+(3)	-(0)	-	-	-
		Second	+(7)	-(0)	-	-	-
E	Outdoor	First	+(7)	+(4)	-	-	-
		Second	+(9)	+(2)	-	-	-

*( ) Number of Cryptosporidium (oo) cysts/ 3 liter; ** () Number of Giardia cysts/3 liter, *** FC= Fecal coliform; TC= Total coliform; HC= Heterotrophic count; + = Present; - = Absent

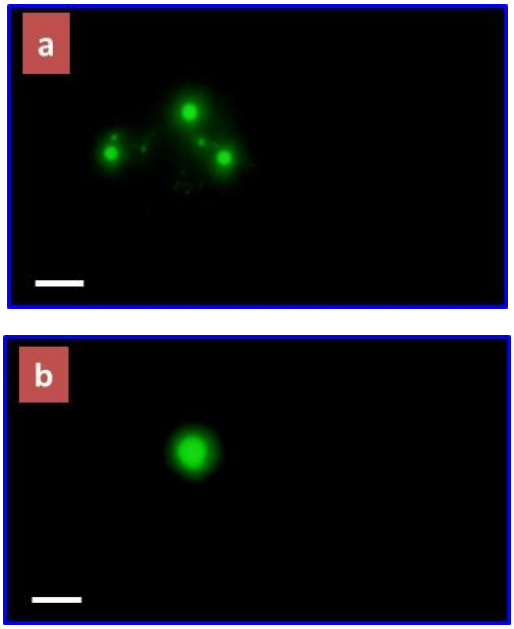

Fig. 1. Direct immunofluorescence technique positive result of (a) Cryptosporidium oocyst. (b) Giardia cyst. The bright apple green color was observed with the fluorescein isothiocyanate (FITC) filter. Bar = 10µm and applies to both photomicrographs.

The variation of the results between both sampling phases in majority of the swimming pools could be due to the fact that most of the schools were maintaining their swimming pools properly during the first sampling phase because students were having swimming classes, therefore, a good hygienic water quality was implemented whereas, at the time of the second sampling phase majority of the swimming pools were probably not maintained properly and water purification methods were not applied which contributed to the survival of the parasites specifically Cryptosporidium. For instance the results of the laboratory analysis of the

swimming pool in school B did not reveal the presence of Cryptosporidium oocysts and Giardia cysts in the first phase of sampling, while in the second sampling phase Cryptosporidium oocysts were found to be present which indicated that poor hygienic water quality was correlated with the parasite survival. This result was similar to the results of a study in Poland [10]. This study demonstrated that the prevalence of both parasites depends on the level of swimming pool water treatment. It was found that when standard water treatment protocols were implemented, the contamination by Cryptosporidium oocysts and Giardia cysts decreased significantly by > 25% compared to the swimming pools that did not implement the standard water purification methods [10].

One of the significant observations which could be related to the variability of the results between the different swimming pools is due to the type of the swimming pools. A study in Italy indicated that outdoor swimming pools tend to be more contaminated with Cryptosporidium oocysts and Giardia cysts [7]. In addition to the contamination of the swimming pool caused by humans, this study demonstrated that outdoor swimming pools were more contaminated with Cryptosporidium and Giardia compared to indoor swimming pools. In that case, birds, wide range of animals in addition to human hosts was stated as the primary sources of the swimming pool contamination by the parasites [7].

In this study, two of the tested swimming pools were outdoor swimming pools (School B & E). The major difference between the swimming pools was that school B had shaded swimming pool whereas, the swimming pool of school E was completely exposed to the outdoor environment. The laboratory analysis of school B indicated a negative result of Cryptosporidium oocysts and Giardia cysts in the first phase of sampling, while the samples of the second sampling phase indicated a positive result for Cryptosporidium oocysts which could be due to improper maintenance of the swimming pool, whereas absence of Giardia cysts in the water sample could be due to their low numbers in the swimming pool. In contrast, the swimming pool of school E was completely exposed to the open environment which probably made it prone to outdoor contaminants. The results of the laboratory analysis indicated that the number of Cryptosporidium oocysts increased in the second sampling phase whereas the number of Giardia cysts decreased slightly in the second phase sample. But unlike school B, both samples from school E were found positive for the presence of Cryptosporidium oocysts and Giardia cysts .In this case, the source of contamination is more likely to be birds because birds' fecal remaining was observed on the ground that surrounded the swimming pool. This result correlates with the results of another study [7]. This result indicates that shading the swimming pools or having indoor swimming pools might decrease the risk of outdoor contamination with Cryptosporidium oocysts and Giardia cysts.

In addition, the age of swimmers as well as the frequency of swimming was major factors that contributed to the variation of the results between the schools' swimming pools. For instance, the swimming pool in school C was used more often by younger students compared to the other swimming pools and the number of swimmers in one class was also higher than the other schools (Table I). During the first phase of sampling the students were using the swimming pool for the first time during the day; therefore, the number of Cryptosporidium oocysts was less than the number of oocysts in the second phase of sampling which could be related to the fact that the swimming pool was maintained properly during the first phase of sampling. In addition, the sampling was performed earlier during the day which indicated that not many swimmers used the pool, therefore, the level of contamination with Cryptosporidium oocysts in the first phase sample was less than the second phase sample, whereas Giardia cysts were not prevalent as much as Cryptosporidium oocysts which could be associated with their low occurrence in the water. At the second phase of sampling the water was super chlorinated by the trained school's instructors, while the pH of the water was slightly acidic (pH6). That pH value corresponds to approximately 97% of HOCl which is a key agent that kills most of the microorganisms [13]. However, the laboratory analysis results indicated an increase in the number of Cryptosporidium oocysts in the second phase samples. In fact it was the highest among all of the swimming pools. In general, an increase in the number of Cryptosporidium oocysts is not expected with increasing level of chlorine, but this could be due to the sampling time. For instance, the period between super chlorination and sample collection was very short. According to the report [14], super chlorination takes approximately 8 hours to be completely effective. At the end of that period swimming pool operator must confirm that water condition is safe and permissible for swimmers to use the swimming pool. The second phase sample was collected during the super chlorination process; therefore, it might not be completely effective at the time of sampling which might have led to the increase in the number of Cryptosporidium oocysts. In addition, it was found that swimming pools had dead spots where circulation of swimming pool water through the filters is not guaranteed [14]. Consequently, that could contribute to the increase of water contaminated with Cryptosporidium oocysts.

Correspondingly, the laboratory analysis results of the swimming pool in school D are another example of how the frequency of swimming and the number of swimmers affected the result between the first and the second phase samples (Table I). The number of Cryptosporidium oocysts was higher in the second phase sample compared to the first phase sample which could be due to the fact that swimming competitions were held in the swimming pool. In addition, at the time of second sampling phase, the swimming pool was neither used nor maintained which probably contributed to the survival of Cryptosporidium oocysts. On the other hand, Giardia cysts were not detected in the swimming pools of school D which could be due to their low occurrence in the water. Despite not finding a high level of contamination by Giardia cysts in the present study on selected schools' swimming pools in Dubai, incidences of giardiasis in Dubai schools are prevalent. According to one of the participants in the study, a nursery principal in Dubai, nearly 10 incidences of giardiasis were indicated in the nursery in June, 2009. The children in the nursery were between 14 months-4 years old.

Samples of water were collected from different locations in the nursery and tested for the presence of total Coliforms, *Pseudomonas aeruginosa*, *E. coli* and Giardia. At the same time all children and staff of the nursery were also tested. Stool samples were collected and tested for giardiasis infection. The detailed microbiological analysis reports revealed the presence of Giardia cysts specifically *G. lamblia* in the stool samples in 5 children. This result is consistent with the study that stated that reported incidences of giardiasis were correlated with the prevalence of *G. lamblia* [5]. After analyzing the provided laboratory analysis reports, it was noticeable that the nursery's staff was not affected by giardiasis. The majority of the infected individuals were children. This result supported the studies that children are at higher risk of being infected by both parasites [15]. But in the case of the nursery's incidences, children were more exposed to the contaminated water and more prone to swallowing water than adults which contributed to the increase in number of giardiasis infections among children.

As stated previously, the laboratory analysis results of the swimming pool water samples showed that Cryptosporidium oocysts and Giardia cysts were present in most of the water samples but the occurrence of Cryptosporidium oocysts was always more than that of Giardia cysts. In contrast, the prevalence of the oocysts and cysts did not indicate increasing incidences of cryptosporidiosis and giardiasis which could be due to the unreported cases. Another reason could be linked to the type of Cryptosporidium and Giardia species. This investigation targeted only the parasite genus but not the species type. Several studies have indicated nearly 14 species of Cryptosporidium but the reported incidences of cryptosporidiosis were linked mostly with the presence of only two Cryptosporidium species including *C. parvum* and *C. hominis*, whereas Giardia genus comprises 6 species [5].In most of the reported giardiasis incidences *G. duodenalis* and *G. lamblia* has been reported as the major source of giardiasis infections [5]. Therefore, the presence of both parasites in the tested swimming pools might not correlate with the incidences of cryptosporidiosis and giardiasis. Further research is required to confirm the species of the parasites.

In addition, it was stated that larger volumes of swimming pool water (> 10L) are associated with more accurate and precise results regarding the presence of oocysts and cysts [14]. Large volumes of water give more accurate results, whereas, smaller samples do not represent the entire swimming pool [7]. In this investigation only 3 L of water sample was tested for the prevalence of Cryptosporidium and Giardia. Therefore, high levels of contamination by the oocysts and cysts was not observed and this could be due to the fact that the collected samples under represented all of the pool's water, but just provided the presence or absence test for Cryptosporidium and Giardia. Other studies have also indicated that it is difficult to determine the dose of the parasites which is needed to cause harm [14], therefore, finding positive results in this investigation is probably not linked to increased risks of cryptosporidiosis and giardiasis infections. But in spite of that, several studies indicated that one of the characteristics' of both parasites is their low infectious dose; which means that infections of both parasites can occur even a small amount of the (oo) cysts is ingested [9], therefore, it is crucial that swimming pools must be tested for the presence of both parasites.

Despite the presence of Cryptosporidium oocysts and Giardia cysts in most of the swimming pool water samples which are generally caused by fecal contamination, the bacteriological analysis results were found negative. All of the laboratory analysis results have shown the absence of all conventional bacterial indicators including fecal coliform, total coliform as well as the heterotrophic bacterial count. The conventional bacterial indicators were never detected and were always negative except for the swimming pool of school B which tested positive only once for the heterotrophic bacteria in the first sampling phase. The heterotrophic bacteria positive sample indicated the presence of a wide range of bacteria that were culturable microorganisms which required organic nutrients for their growth. Examples of these bacteria are fecal Coliforms which are prevalent in the intestinal tract of humans and animals as well [16].

Nevertheless, the majority of the samples were found negative for all of the conventional bacterial indicators. That could be due to using unfavorable nutrients for these types of bacteria which prevented them from growing. Another reason could be due to the nature of the bacteria. The bacteria could be in the state of being viable but nonculturable (VBNC). Bacteria that are in this state do not grow when using conventional media. They do not form colonies or change the broth appearance but their presence could be detected by using certain methods [17]. Additionally, a wide range of bacteria including fecal Coliforms are susceptible to be in VBNC state. Studies stated that several factors can induce this state such as oxygen level, elevated nutrient, incubation as well as chlorination. Several methods including using DAPI are recommended to indicate the bacterial viability [17]. Therefore in this study, when DAPI was used several types of bacteria such as rod shaped as well as clusters of cocci were observed on 0.45 μm sized membrane filter (Fig. 2.) But their identity was not investigated; therefore their nature or pathogenicity was unknown.

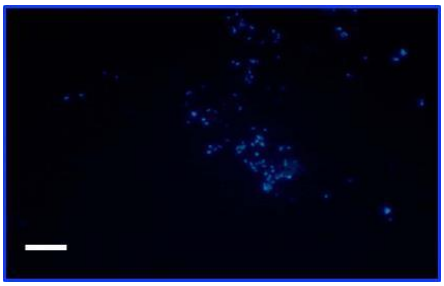

Fig. 2. Direct DAPI staining of bacterial cells on the 0.45 μm sized membrane filter. Bar = 10μm.

The pH of a majority of the samples was within the range of 7.0-7.5 which is nearly in the recommended range to ensure optimal swimmer safety, disinfection, and HOCl production which is essential in killing microorganisms in the swimming pool [13]. But in spite of that, the presence of Cryptosporidium and Giardia in the tested swimming pools yielded similar to the results of a number of studies which stated that both parasites can survive routine levels of chlorine [13]. At the time of second sampling phase in the

school C, super chlorination was implemented and water pH was around 6 which indicate a high percentage of HOCl (about 97%). But testing the sample revealed the presence of Cryptosporidium oocysts which might or might not be active. A study conducted in Atlanta, Georgia stated that the oocysts detected after super chlorination are usually inactive, therefore, non-infectious [4]. Therefore, a study should be conducted in the future to investigate the oocysts viability.

IV. Conclusion

In conclusion, the majority of the tested swimming pools were found contaminated with Cryptosporidium oocysts, whereas a number of them were found positive for Giardia cysts. It is evident that frequency of swimming classes, age of swimmers, water purification methods and types of swimming pools could have a significant impact on the variability of the results. Therefore, monitoring the swimming pools, informing the students about the risks of transmission of infectious diseases through swimming, preventing students who suffer from gastroenteritis diseases from swimming, implementing good hygiene practices in and around the swimming pool, reporting fecal incidences in the swimming pool, having a good hygienic water quality are critical to decrease the risk of contamination with Cryptosporidium oocysts and Giardia cysts. Furthermore, early detection and control by concerned authorities will help to reduce significant rates of exposure and infections.

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