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Full Length Research Paper

Detection of *Cryptosporidium parvum* oocysts contaminating hospitals drinking water supply using different techniques during winter/summer season

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Water is the principal transmission route for *Cryptosporidium spp*; most of the outbreaks are mainly the result of infected water consumption. The study was designed to identify the prevalence and viability of *Cryptosporidium spp* in the drinking water supply of Assiut university hospitals. Also, analyze places, and origin of contamination, using flow cytometry and conventional staining techniques during winter/summer seasons. Water samples covering university hospitals drinking water supply inlet, tanks, and outlets were collected throughout winter and summer. Samples were subjected to filtration, elution, and concentration. The diagnosis was completed using modified acid-faststaining, direct fluorescent antibody (DFA) techniques and flow cytometry. Nested PCR was employed for *Cryptosporidium spp* characterization. About 79% of the analyzed specimens were infected with *Cryptosporidium parvum* oocysts. Significant seasonal variation was identified as oocysts were more dominant in winter (87.5%) than in summer (70.8%), but the intensity of water contamination was greater in summer. Hospitals water inlets supplied from the local water plant were oocysts polluted, but with a lesser intensity than hospitals water tanks and outlets. It is confirmed that hospitals receive contaminated drinking water from the water plant; however, the tanks and pipes become extra polluted with additional domestic sources. The seasonal prevalence in drinking water system is the mirror of the ecological factors that should be audited to obtain an efficient control policy. Consequently, tracking the origins of infection using the flow cytometry technique will provide experts with further information to combat the *Cryptosporidium* water pollution.

Keywords: *Cryptosporidium parvum*, season, drinking water, flow cytometry, Egypt.

INTRODUCTION

Cryptosporidium is an apicomplexanan parasite which so far has more than 26 known species, as recognized by host specificity, morphology, and molecular biology studies (Bouzid et al. 2013). *C. parvum* and *C. hominis* are the predominantly infecting species of humans (Stark et al. 2011). Variability in the *Cryptosporidium* prevalence among

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different hosts and even within the same host species hinge on a number of elements comprising: age, immune status, living conditions, diagnostic methodology, region studied. In addition to factors that could influence the prevalence as season, feeding habits and urban/rural living conditions (Hamnes et al. 2007). Despite the higher prevalence of cryptosporidiosis in developing and tropical countries causing approximately 10-15% of acute diarrheal illness (Lima et al. 2011), few studies have been conducted to identify risk factors. Although information on cryptosporidiosis is deficient in Arab countries, (Ghenghesh et al. 2012), showed that prevalence rates were ranging between 1 to 43% among diarrheic immune competent pediatrics. This wide variety might be due to differences in the number of patients examined, methods used, populations studied (e.g. urban or rural), location and year of study. Baldursson and Karanis (2011) reviewed, between 2004 and 2010, about 199 reports of waterborne protozoan parasitic outbreaks published worldwide; *Cryptosporidium* was responsible for 60.3% of them. Cryptosporidiosis is contracted via consumption of *Cryptosporidium* oocysts polluting private and public water supplies and from exposure to recreational water in swimming pools or water parks. It is difficult to determine the source of *Cryptosporidium* oocysts in water; the epidemiology of the infection involves both direct transmission from animals to humans and indirect transmission through ingestion of water and food contaminated with infectious oocysts (Cacciò et al. 2005; Smith et al. 2006). The high prevalence of oocysts in surface water and even wastewater indicates that ruminants are probably a major source of *Cryptosporidium parvum* contamination (Xiao et al. 2004). Most of the animal-associated species and genotypes do not appear to be pathogenic to man except *C. parvum* that infects immune-competent humans in all age groups (Leoni et al. 2006). According to Medema et al. (2009) many factors contribute to the increased risk of *Cryptosporidium* waterborne outbreaks: small oocysts size, wide range of host specificity, monoxenous development, close associations between human and animal hosts allowing zoonotic transmission, large number of oocysts excreted /host, low infective dose, high infectivity of the parasite and robust oocysts (resistance to chlorine). The seasonal increase in the incidence of cryptosporidiosis was found to be related to increases in temperature and precipitation, besides the meteorological factors affect timing and intensity of seasonal outbreaks (Jagai et al. 2009).

Cryptosporidium waterborne outbreaks in the Arab world are scarcely reported (Areeshi et al. 2007); only sporadic studies in a few Egyptian governorates have been done on different water sources and there are no actual studies of the prevalence of *C. parvum* in drinking water in Egypt (el-Shazly et al. 2002; Shoukry et al. 2009; Khalifa et al. 2011,2014). Hence, it is very important to detect and trail the source of *Cryptosporidium parvum* oocysts contaminating the local water supply especially when cryptosporidiosis is classified in Egypt to be mainly a waterborne pathogen (Putignani and Menichella 2010). The seasonal variation also denotes ecological factors that should be considered in control measures to be applied in high-risk months to reduce the jeopardy of infection. Moreover, the studied water is delivered to hospitals and consumed by patients with uncertain immune status. We intended to get an idea about the prevalence of *Cryptosporidium spp* contaminating the University Hospitals facilities to help in the infection control planning. The present study was based according to US Environmental Protection Agency (EPA) instructions(EPA 2011); the use of newly designed apparatus beside these criteria make this study one of the first leading projects in this regard in Upper Egypt to the best of our knowledge.

MATERIALS AND METHODS

Sample collection: Forty-eight water samples were collected from Assiut University hospitals drinking water supply including the three main distributing inlets at hospital gates coming from the supplying local water treatment plant in addition to inlets, tanks, and outlets of all hospitals and faculty of medicine buildings. According to the Standard Operating Protocols (SOPs) for samples collection, developed by the US EPA, grab samples of 20 L volume were collected during two seasons (winter and summer), 24 samples in each season (Smith and Grimason 2003; EPA 2011). Samples were designed as H1-H24. Each sample was collected in clean plastic containers with 25 Lcapacity; the sample was observed for turbidity and stored at room temperature for a maximum of 3 days until the time of processing as following:

Filtration, concentration, and elution of the samples: Each 20 L water sample was passed completely through the filter of the newly designed apparatus by Gaber, 2015 at Assiut University. The apparatus is briefly composed of Vacuum Air Pump, 25 l capacity stainless steel tank, 2 stages filter cartridge and powerful housing, closely fitted for membrane filter with an equivalent pore size of 1µm supplied by IDEXX Laboratories (New South Wales, Australia). After each filtration process, the membrane filter was processed according to the manufacturer's instructions with modification. According to Pezzana et al. (2000) trials, the Laureth 12 elution buffer was replaced with a phosphate-buffered saline (PBS)-Tween-Antifoam buffer composed of 2 L of PBS buffer (PH 7.4), 300 ml of Tween 80 and 300 ml of antifoam.

Detection of the parasite in water sample

Modified Acid faststain: Modified carbolfuchsin (Kinyoun's) with methylene blue (KMb) was used to stain

each concentrated sample according to Cole (1997)instructions.

b) Direct Fluorescent Antibody Staining (DFA): It comprises the use of fluorescence microscopy and using a fluorescein isothiocyanate-conjugated anti-*Cryptosporidium parvum* monoclonal antibody (FITC-C-mAb), which recognizes surface epitopes on oocysts (Smith and Grimason 2003; Jex et al. 2008). Samples were processed according to the manufacturer's instructions.

c) Flow cytometry: The Aqua-Glo™ kit (A100FLK, Waterborne, USA) was used to detect the oocysts of *Cryptosporidium parvum* from water samples by recognizing surface epitopes on oocysts, together with other fluorochrome stains as DAPI for determining parasite viability (EPA 2005). Positive and negative control samples were used before the introduction of tested samples to calibrate the device. Positive control samples were *C. parvum* oocysts "Iowa" live isolate preparation provided from Waterborne (P102M, Waterborne, USA); and stored at 4º C until used. According to Khalifa et al. (2011, 2014), negative control samples were taken from boiled distilled water. All samples were introduced into the flow cytometer (FACS Calibur™ Becton, Dickinson), each water sample was performed twice; one as a non-stained autofluorescence sample; which was served as self-control to detect nonspecific fluorescence and the other one as a test sample. The samples were processed according to the manufacturer's instructions.

Nested-PCR Technique (nPCR)

The stored oocysts were processed for Genomic DNA extraction using Favor Prep stool DNA isolation Mini Kit (Favorgen Biotech corporation ping-Tung 908, Taiwan) according to manufacturer's guidelines.After thermal shock of the samples (5 cycles of deep freezing and boiling in water bath each for 5 min.), with continuation of incubation for one hourat 95^{o}C after 56^{o}C at 10 minutes. The extracted DNA was amplified by nPCR targeting COWP gene, using two sets of primers (Table 1).According to Spano et al. (1997), the reaction mixture and condition were completedin a total volume of 25µl. The amplified products were visualized using 1.5% agarose gel electrophoresis after being stained with ethidium bromide. PCR products were digested by *Rsa*I (Fermentas UAB, V. Graiciuno 8, LT-02241Vilnius, Lithuania). Digestion of N-COWP fragments was determined by electrophoresis in 3.2% typing-grade agarose gels containing ethidium bromide, visualization of fragments were seen by UV light to determine *Cryptosporidium* genotype.

Statistical analysis

A cross-sectional study was conducted; data were collected, tabulated statistically and analyzed using SPSS program version 11. One way ANOVA followed by Post Hoc Test. Pearson correlation and independent test were used in comparison between quantitative variables. Data were expressed as mean ± Standard deviation (SD); *P* value ≤0.05 was considered significant and *P* value ≤0.001 was considered highly significant.

RESULTS

The isolated *Cryptosporidium* oocysts have been proved to be *Cryptosporidium parvum* using n-PCR (Figure 1). *Cryptosporidium parvum oocysts* were detected in 23 out of 48 samples (47.9%) using Kinyoun's Methylene blue (KMb**)**. DFA technique raised the detection of the parasites to be found in 35 out of 48 samples (72.9%) (Table 2). Flow cytometry results for each tested water sample were analyzed as histogram and dot plots expressing each oocyst surface antigen detected as events at the gate region 1 (R1) (Figure.2 c –d) .Flow cytometry displayed that 79% tested water samples were infected with *Cryptosporidium parvum* oocysts (Table 2).Highly significant seasonal variation was found as oocysts were more prevalent in winter (87.5%) than in summer (70.8%) when recovered by flowcytometry (Table 2). On another hand, the intensity of water contamination was higher in summer with a mean of 4744 (oocysts/L) than in winter with mean of 3698 (oocysts/L) (Figure 3). The intensity of water contamination was estimated by the quantity of FITC staining ability of the parasite and expressed as total events taken by the flow cytometry device in all samples (Table 3 & Figure 2).

Moreover, hospitals' distributing water inlets supplied from the local water plant were contaminated but with a lesser oocyst's intensity than hospital water tanks, and outlets (Table 4). The highest contamination was found at the Main Hospital water supply in winter. While in summer the highest contamination was detected in the distributing inlets supplying all hospitals received from water plants. The least contamination was detected at Al-Rajhi Liver Hospital in both seasons (Figure 3). Comparing contamination of hospital inlets, tanks, and outlets during both seasons revealed that hospital outlets had the greatest oocyst's contamination (Table 4). No significant difference in oocysts viability was detected between winter and summer seasons. Similarly no statistical correlations were found between the percentage of oocysts viability and atmospheric temperature at the time of sample collection (Table 3; Figure 4).

Assessing the different techniques used, Flow cytometry proved to have highly significant ability in the detection of *Cryptosporidium* oocysts, reaching 100% and 52% sensitivity and specificity respectively in comparison to KMb as a standard stain (Table5; Figure 5).

Table 1: Primers sets used for nPCR targeting COWP gene.

Primers	Sequences	Expected product size (bp)	Annealing temp.	References
1st PCR (E-COWP = Extended COWP)				
BCOWPF	**5'-ACCGCTTCTCAACAACCATCTTGTCCTC-3'**	769	65	(Pedraza-Díaz et al. 2001)
BCOWPR	**5'-CGCACCTGTTCCCACTCAATGTAAACCC-3'**			
2nd PCR (N-COWP = Nested COWP)				
cry-15	**5'-GTA GAT AAT GGA AGA GAT TGT G-3'**	553	54	(Spano et al. 1997)
cry-9	**5'-GGA CTG AAA TAC AGG CAT TAT CTT G-3'**			

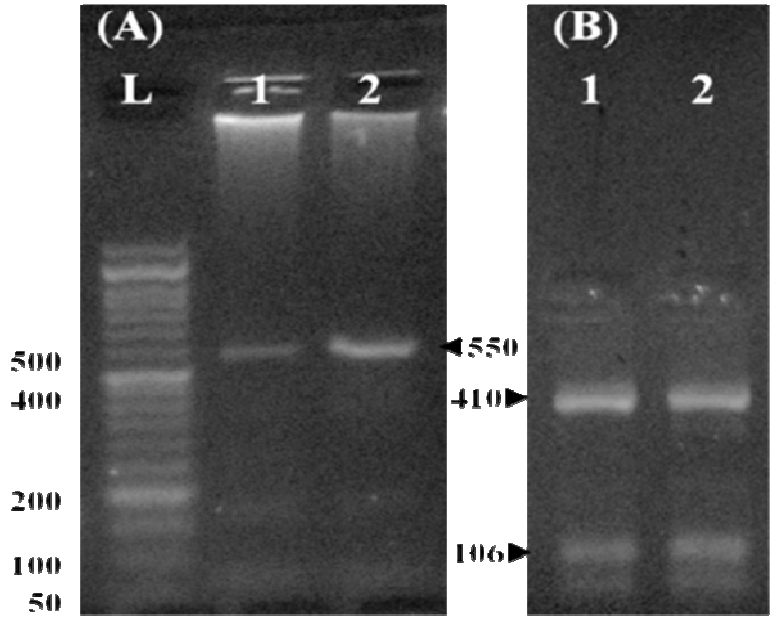

Figure 1: Agarose gel electrophoresis showing (A) the products of the nPCR targeting COWP gene of *Cryptosporidium* at 553bp. (B) RFLP products after digestion with RsaI endonuclease. A: Lane L: 50 bp DNA molecular weight marker. Lanes 1&2: Positive samples for *Cryptosporidium.* B: Lanes 1-2: *C. parvum* genotype 2 digestion products at 34, 106 and 410 bp (faint band at 34bp).

Table2: Comparison between different techniques used in the detection of Cryptosporidium *spp* in filtrated concentrated water samples.

Time of collection	KMb		DFA		Flow cytometry		P-value[a]	P-value[b]
	No.	%	No.	%	No.	%		
Winter:								
Positive	14	58.3	20	83.3	21	87.5**	0.057	0.023*
Negative	10	41.7	4	16.7	3	12.5		
Summer:								
Positive	9	37.5	15	62.5	17	70.8**	0.083	0.020*
Negative	15	62.5	9	37.5	7	29.2		
Total:								
Positive	23	47.9	35	72.9	38	79.2	0.012*	0.001##
Negative	25	52.1	13	27.1	10	20.8		

KMb: Modified Kinyoun's with methylene blue**DFA**: Direct Fluorescent Antibody Staining
[a] Comparison between **KMb** with **DFA**
[b]Comparison between **KMb** with **Flow cytometry**
* P ≤ 0.05
** P ≤ 0.001highly significant difference in prevalence of *Cryptosporidium* oocysts between seasons**.**
##P ≤ 0.001 highly significant difference**KMb**and **Flow cytometry.**

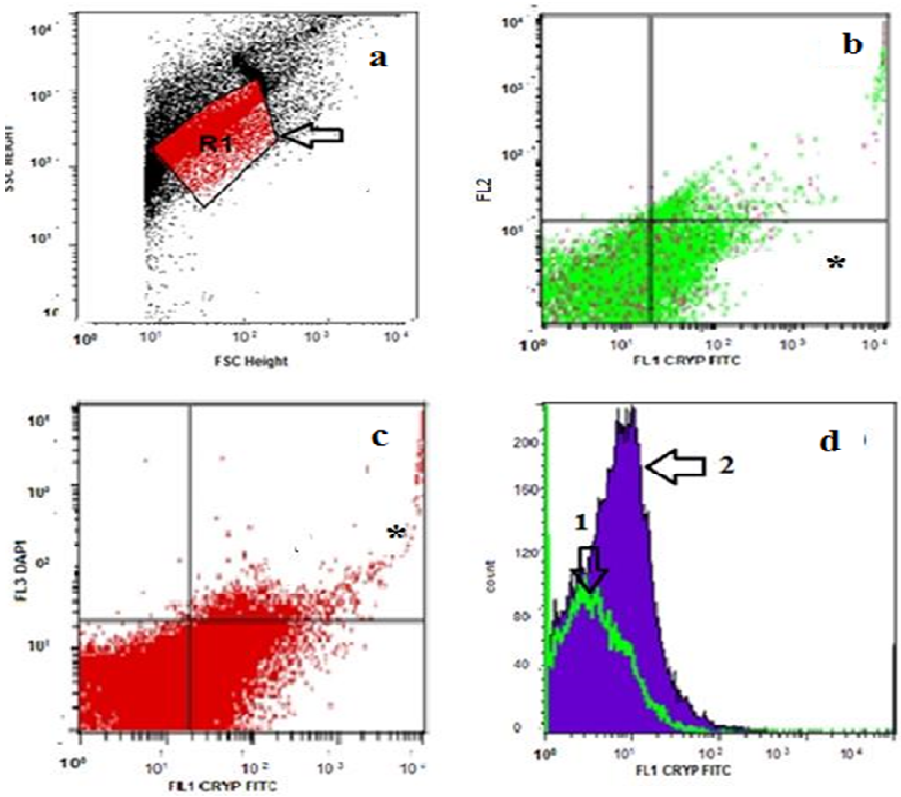

Figure 2: Flow cytometry histogram and dot plots of H6 sample at summer time showed positive result for *Cryptosporidium parvum***a:***Cryptosporidium parvum* gated region R1 (arrow) showed side-scater (SSC) against forward side-scater (FSC), **b:** Events representing *Cryptosporidium parvum* in the right lower quadrant (*) **c:** Viable *Cryptosporidium parvum* oocysts with DAPI stain were detected in the upper right quadrant FL3 (*) **d:** Histogram representing *Cryptosporidium parvum* counted by FITC (FL1) arrow2 in comparison with negative control (auto) arrow1

Table3: Seasonal variability of *Cryptosporidium spp* in term of prevalence, intensity (n° of oocysts /L) and viability detected by flow cytometry at each collection site.

Hospital Buildings	Sample Place	Winter			Summer		
		Temp C^0	Oocysts/ L	Viability %	Temp C^0	Oocysts /L	Viability %
Main inlets from Water Plant	Main Inlet	22	705.0	1.42	37	1080.0	0.34
	Entry pipe	22	8630.0	1.65	37	12990.0	1.67
	outlet	22	4702.5	0.03	38	30597.5	0.11
Outpatient Clinic	inlet	21	4475.0	0.43	39	23457.5	1.79
	tank	21	0.0	0	39	3060.0	0.98
	outlet	18	12647.5	0.26	39	8300.0	0.36
Main Hospital	inlet	17	4895.0	3.15	40	8727.5	0.30
	tank	17	8407.5	0.3	40	10202.5	7.64
	outlet	17	5937.5	0.94	40	3052.5	0.46
Children Hospital	inlet	19	6612.5	0.2	39	0.0	0
	tank	19	1995.0	1.28	39	757.5	0.4
	outlet	19	3010.0	0.08	39	0.0	0
Women Health Hospital	inlet	20	4792.5	0.86	40	3407.5	0.31
	tank	20	1637.5	0.86	40	0.0	0
	outlet	20	6532.5	0.37	40	875.0	0.78
Al-Rajhi Hospital	inlet	20	0.0	0	38	0.0	0
	tank	21	370.0	1.5	38	0.0	0
	outlet	21	0.0	0	38	0.0	0
Urology/Neurology Hospital	inlet	22	2715.0	0.38	38	1645.0	1.75
	tank	22	6870.0	0.39	39	3282.5	3.46
	outlet	22	2112.5	2.03	39	0.0	0
Faculty Of Medicine	inlet	23	605.0	0.22	39	862.5	0.3
	tank	23	835.0	0.37	40	1050.0	0.4
	outlet	23	280.0	2.18	40	525.0	0.65

Table 4: Comparison between inlets, tanks and outlets of every studied site during both seasons

Hospitals Buildings	Inlets Oocysts / L	Tanks Oocysts / L	Outlets Oocysts / L
Outpatient clinics	**27935**	**3060**	**30995**
Main H.	**13622**	**18609**	**32231**
Children H.	**6612**	**9570**	**3010**
Woman H.	**8199**	**1637**	**9836**
Al-Rajhi H.	**0**	**370**	**0**
Urology H.	**4360**	**10152**	**2112**
Faculty of Medicine	**1467**	**1885**	**3352**
Total	**62195**	**45283**	**81536**

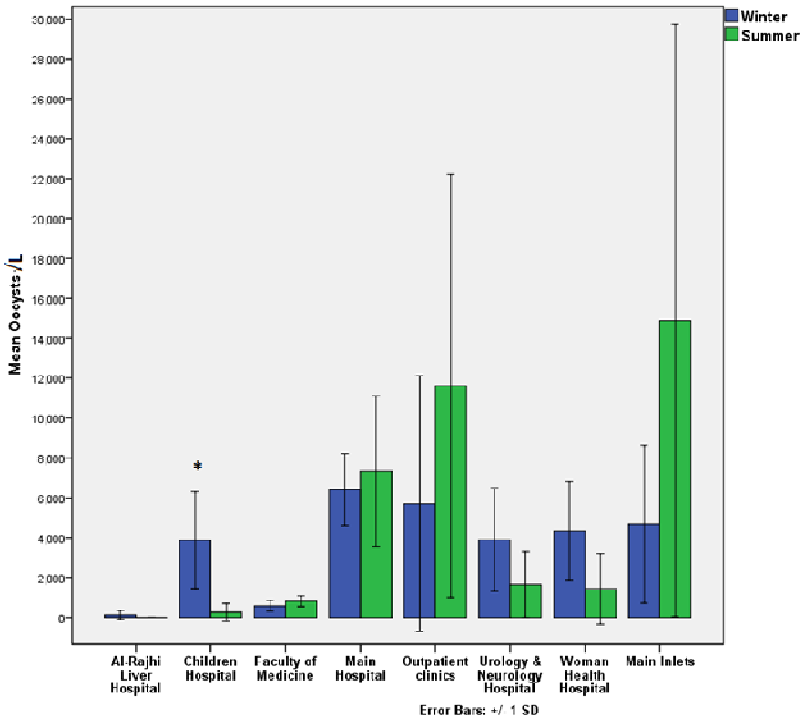

Figure 3: Mean Oocysts/L detected by Flow cytometry at different hospitals sites during winter and summer. Error bars represent SD ± 1.* Indicates a significant seasonal variation with a *p*-value of < 0.05.

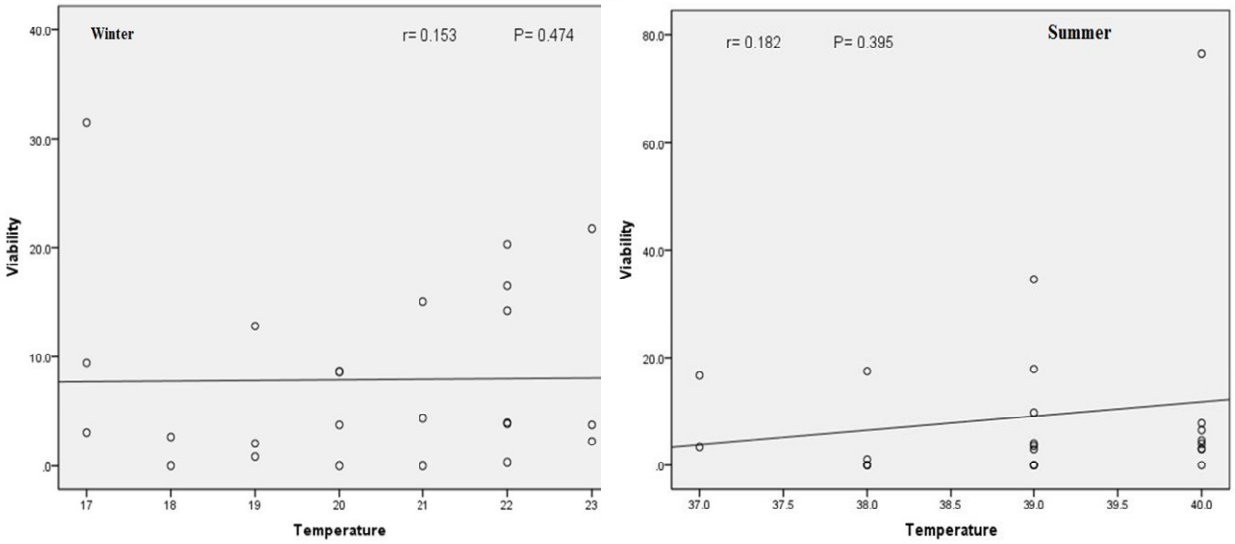

Figure 4: Correlation between *Cryptosporidium spp* viability and atmospheric temperature at time of samples collection in winter and summer using Flow cytometry

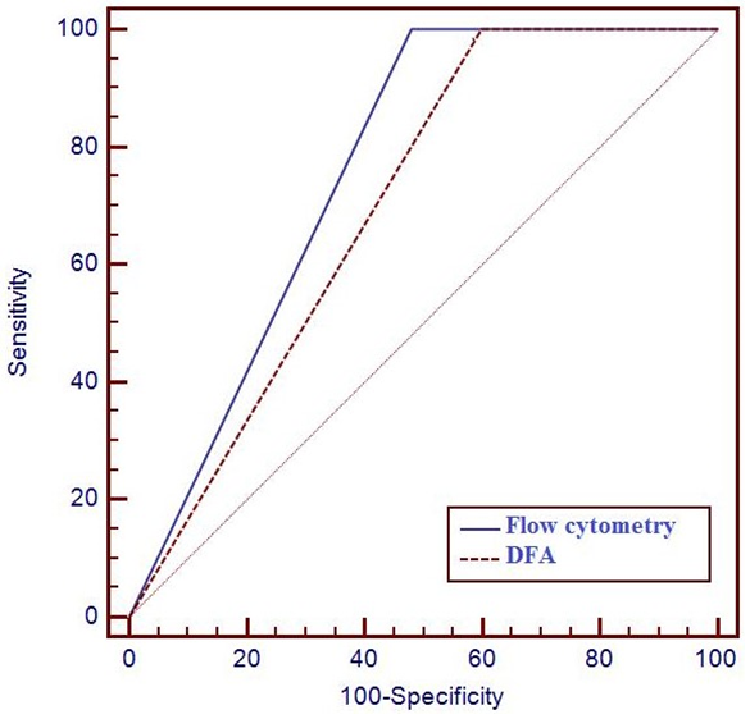

Figure 5: representing ROC curve of Flow cytometry and DFA

Table 5

Test	Sensitivity	Specificity	+PV	-PV	Accuracy	AUC	CI	P-value
Flow Cytometry	100.00	52.00	65.7	100.0	75.0	0.760	0.615-0.871	0.172
DFA	100.00	40.00	60.5	100.0	68.8	0.700	0.551-0.824	

DISCUSSION

Waterborne cryptosporidiosis is of special interest for drinking water safety due to the resistance of *Cryptosporidium* oocysts to standard methods of water disinfection. Infectivity remains active in water environments for over a year (Betancourt and Rose 2004). In addition, their low infective doses (less than 10 oocysts) represents a major challenge in the monitoring and delivery of safe drinking water (Chen et al. 2007; King and Monis 2007). The collected water samples covered all Assiut University Hospital buildings following the USEPA instructions (EPA 2011). In this way of water collection and detection of *Cryptosporidium* being used for the first time made a more precise idea about the extent of water pollution. Thus, empowering the study with advantageous points compared to other studies done in Egypt that was not following such strict terms (el-Shazly et al. 2002; Shoukry et al. 2009; Khalifa et al. 2011,2014).

The collected water was not less than 20 Lto overcome the problem of uneven distribution of oocysts as well as the low *C. parvum* oocysts infected dose. Filtration was through a newly designed water filter apparatus acted like the recommended FILTA-max apparatus. Plus the use of 100 and 25 µm pre-filters was adopted to remove any debris or algae that might interfere with parasite detection by plugging the membrane filter or cross-reacting with parasite oocysts.

About 79% of the tested water samples were proved to be infected with *C. parvum* oocysts as recognized by molecular characterization. *Cryptosporidium* oocysts were better diagnosed by flow cytometry which proved to be a

more sensitive and specific technique than DFA technique. The high sensitivity of flow cytometry could be attributed to the little minimum detection limit, as low as 112 oocysts/100 µm of water. Similarly, to Valdez et al. (1997) study , the minimum detection limit in stool samples was 100 oocysts/100 µm. Moreover, in the study carried out by Delaunay et al. (2000)flow cytometry was reported to have a tenfold higher sensitivity than immunofluorescence staining. *Cryptosporidium* oocysts detection is more difficult in stool samples than in water samples. Flow cytometry device needs more fluid media for organism detection, a character which is available more in water than stools since the latter needs more processing and washing steps which might lead to the hazard of oocysts loss, giving false-negative results (Barbosa et al. 2008). Above and beyond being a more reliable technique, flow cytometry has a great advantage in detection and quantification of *C. parvum* oocysts in water samples even at very low concentrations. Besides, it is founded on instantaneous quantitation of an internal standard of fluorescent calibrated beads for maximal precision, as well as detecting viability versus other techniques used (Barbosa et al. 2008). Many studies have already addressed the flow cytometry as a specific and time-saving alternative method for detection of *Cryptosporidium* in feces and water (Cole et al. 1999; Montemayor et al. 2005; Hsu et al. 2005). The 92% sensitivity of DFA technique and ability to detect oocysts as low as 1790 oocysts / 100 µm is much better than the results (5800 oocysts /100 µm) reported by Valdez. This great disparity could be attributed to our use of genus-specific kits which was not the case in the work of Valdez et al. (1997).

Regarding the great number of *Cryptosporidium* parvum oocysts loading the water supply in the present work, it agrees with the report of Almeida et al. (2010) that water is the major environmental transmission route for *Cryptosporidium spp.* Although information on *Cryptosporidium spp* in drinking water is deficient in Egypt, available data showed variable prevalence rates**.** Khalifa et al. (2014) had only collected 10ml of water from different water sources (canals, tanks, and tap water) from the northern part of El-Minia Governorate. They filtered it through cellulose acetate filters, and showed a53% *Cryptosporidium spp* prevalence after acid-fast staining. Khalifa et al. (2011) had proven by flow cytometry that 100% of 30 samples of tank water from different districts of Alexandria city were *C. parvum* contaminated. On the other hand, much lower *C. parvum* prevalence was identified, only 9% of 75 water tanks samples in Ismailia Governorate and merely 3% out of 840 potable water samples in Dakahlia Governorate (el-Shazly et al. 2002)**.**The low percentage of parasite recorded by previous studies was attributed, by the investigators; to the low sensitivity of the immunofluorescent staining methods used. Oocysts contamination was covering all hospitals main water inlets supplied from the local water plant, reflecting the inadequate treatment process. But, the inlet contamination was in lesser oocysts intensity than hospital water tanks and outlets, indicating additional domestic contamination sources. Most hospital tanks were opened making them more liable to airborne *C. parvum* oocysts transportation from the nearby faculty of agriculture animal farm. The farm hosts numerous animals (neonatal calves, cows and sheep), which are considered by many authors as an important source of environment contamination and as a reservoir of the *C. parvum* (Castro-Hermida et al. 2007; Castro-Hermida et al. 2009).The detected oocysts were proved to be *C. parvum* enhancing our prediction that the source of infection of local water is mostly of zoonotic origin. Moreover, opened tanks were also more liable to be contaminated by rodent fecesshown to be reservoir host to *C. parvum* (Torres et al. 2000; Perec-Matysiak et al. 2015).The highest number of oocysts contamination was found in the main hospital building, which was built with metal water distribution pipes which are more prone to develop biofilms on their wall (Momba et al. 2000). The irregularly cleaned tanks and rusty old distribution pipes within the hospital buildings, have not been changed for decades, may be incriminated in escalating *Cryptosporidium* contamination. *Cryptosporidium* received from the supplying water plant accumulate in biofilms on the pipes and tanks interior surface. Consequently, *Cryptosporidium* get intensified inside hospital building water distribution system. Biofilms have the potential to hold large quantities of oocysts, which are liable to be released into drinking water.But ,also *Cryptosporidium* parasite has the ability to multiply within biofilms independently, in a “host-free biofilm environment “, potentially allowing them to complete an extracellular life cycle (Wolyniak et al. 2010; Hijjawi et al. 2010; Koh et al. 2014). In contrast, the recently opened El-Rajhi Liver Hospital, whose pipes are made of propylene, had the lowest prevalence of *Cryptosporidium spp*; 5 out of 6 water samples were negative and the positive one had the lowest number of oocysts.

As reviewed by Putignani and Menichella (2010), the difference in prevalence between developed and developing countries can reflect the possible causes of this difference.In developing countries there are: lack of efficient methods adapted by the water treatment facilities and poor controlling quality of drinking water. While developed countries are routinely monitoring water every year and establishing techniques to identify these parasites, in an attempt to control their existence. Also,these different results might not only reflect the actual difference in *Cryptosporidium spp.* contamination levels from various locations, but also the difference in techniques used.Thus in the present study authors adapted steps recommended by the USEPA method 1623 to collect, filtrate and concentrate surface water sample (EPA 2011).

The worldwide occurrence of *Cryptosporidium spp* raises a question about its seasonal variation in terms of prevalence, intensity and viability of the parasite. According to the Köppen climate classification system; Egypt is located in the dry climate zone. Assiut governorate is in dry arid one, which is characterized by little rain and a huge daily temperature range in winter (Kottek et al. 2006). Significant seasonal differences were found regarding the oocysts prevalence in winter (87.5%) versus 70.8% in summer. In contrast to the water intensity contamination, summer had higher intensity (4744 oocysts/liter) than winter (3698 oocysts/liter). A possible explanation of this seasonal variation is the higher turbidity of summer samples (naked eye observation) than winter samples; such turbidity might increase the specific gravity of the *Cryptosporidium* oocysts, leading to its adhesion to water particles, affecting the purification processes, finally leading to less floatation, thereby decreasing the recovery rate. This comes in agreement with the theory proposed byDumètre et al. (2012) assuming that turbidity might decrease *Cryptosporidium* oocysts recovery rate byincreasing the specific gravity of the parasite and decreasing its floatation. In addition, seasonal changes in temperature, precipitation, and water quality (including nutrient availability) may have a significant impact on the microbial composition and functional structure of a biofilm (Moss et al. 2006). According to Wolyniak et al. (2010) a seasonal pattern in the oocysts relative with biofilmwas reported.

Obviously, the seasonal patterns have a tendency to vary with location. In Egypt, the seasonality of the parasite in water has been merely investigated in a few studies, with diverse results. In contrast to the current study results, in the Egyptian Governorate of El Minia, showed a 22.6% prevalence in winter while 66.7% in summer. This variance could be attributed to the use of 10 ml water and the lone dependence on conventional staining technique for oocysts recognition (Khalifa et al. 2014). In another Arabian country, seasonality was found in the rate of human infection which could reflect the availability of the parasite in the environment according to season and community practice as reviewed by Areeshi et al.(2007). According to the meta-analysis carried out by Jagai et al. (2009), climate and weather influence cryptosporidiosis transmission as well as different seasonal agricultural practices.A seasonal incidence of infection in man and animals is sometimes present, possibly corresponding to rainfall peaks, increased pollution from farm waste, or calving and lambing activities increase surface water contamination (Zintl et al. 2009; Wilkes et al. 2009). In Spain, Castro-Hermida et al. (2009) found that C. parvum was more prevalent in summer in river taken sample. Whereas Koompapong and Sukthana (2012) from Thailand, C. parvum prevalence was 29% in cool seasons while negative results were recorded in summer. Muchiri et al.(2009) reported a substantial seasonal pattern, with a significant peak in the rainy season. Herein, the parasite viability was not affected by season either in winter or summer, as well as no correlations was found between the percent of viability and atmospheric temperature at the time of sample collection. Thus, no variation was found in the ability of the parasite to cause disease in either winter or summer, but this does not obliterate the effect that a greater number of oocysts were identified in summer, so a cup of local water is more dangerous in summer than in winter.

Cryptosporidium spp seasonal pattern infection in cattle were observed in many parts of the world as reviewed by Cacciò and Widmer (2014), this rise a question: Does the seasonal pattern of our hospital water infection is related to the possible seasonal infection of the surrounding cattle community?

It is concluded that waterborne cryptosporidiosis is a major problem as the present data revealed the presence of *Cryptosporidium parvum* in most of the distribution water system of the university hospitals. It represents an unacceptable health risk, particularly for vulnerablepopulations. This subtype of *Cryptosporidium parvum* was proved to be highly virulent when compared with reference Iowa isolate (unpublished data). The seasonal pattern had been proven which could be implied as a strategic indicator to design prevention platforms for waterborne cryptosporidiosis control in our locality. Such evidence suggests that focus must be placed on prevention of human and animal waste contamination as well as getting more attention to the distribution pipes enclosing biofilms recognized as the environmental reservoir for *Cryptosporidium spp*.

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