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Assessment of Parasitological Water Quality from House Kitchens and Desalination Plants Filters in Gaza Strip

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Abstract:

This study was conducted to assess parasitological water quality from house kitchens and desalination plants filters in Gaza Strip. A total of 420 samples were collected from the five Governorates of Gaza Strip; 300 samples were collected from 100 houses and 120 samples were collected from 40 desalination plants. All samples were examined using direct wet mount, acid fast stain and Polymerase Chain Reaction. The randomlly distributed questionnaire included questions regarding economic and social factors, water sources, reported symptoms and public health. Results revealed that only Cryptosporidium spp. oocysts were detected in eight of drinking water samples 1.9% (8/420). Eight samples were positive when using acid-fast stain (Cryptosporidium spp.) in Reverse osmosis (RO) house filters. Using PCR to identify Cryptosporidium spp. (C. parvum and C. hominis), only one sample (0.24%) was positive for C. parvum while there are no positive samples for C. hominis. The occurrence of Cryptosporidium oocysts in the investigated water supplies may require the water utilities and water authorities in Gaza Strip to apply additional monitoring, treatment and/or watershed controls for safe drinking water.

Keywords:

Cryptosporidium,
Filters, Gaza Strip, PCR,
Water

Highlights:

-This is the first study dealing with such water-borne disease.
-Using sensitive techniques like PCR.
-Tackling a health issue related to the public health.
-The efficacy of the filtration devises in Gaza.
-Monitoring safe drinking water.

Introduction:

Among waterborne outbreak agents, bacterial, viral, parasitic, and chemical agents can cause waterborne illness. Waterborne illnesses can be caused by ingestion or consuming water, by dermal contact, i.e. contact of the water with skin or mucous membranes, or by inhalation, i.e. by breathing in a mist or aerosolized water particles (Groen 2015). Cryptosporidiosis and giardiasis are waterborne diseases spread all over the world (Zakai and Barnawi 2014). In Gaza Strip, it was found that 14.9% of children attending Al-Nasser Children Hospital were infected by *Cryptosporidium*. Another study reported that 14.6% of children younger than 5 years were infected by *Cryptosporidium* spp (Al-Hindi et al. 2007, Sallon et al. 1994). In developing countries, waterborne gastrointestinal parasite pathogens such as *Giardia lamblia* and *Cryptosporidium parvum* are frequently associated with morbidity especially in children (Bakir et al. 2003). The most frequent symptoms caused by Cryptosporidiosis are weight loss, diarrhea, gas, malaise, watery diarrhea, nausea, abdominal cramps and headaches. These symptoms occur within two to 25 days of infection and usually last one or two weeks. Because of the poor quality of water in Gaza Strip, people tend to use water desalination by reverse osmosis (Ismail 2003). Desalination processes are used commercially to

provide fresh water for many communities and industrial sectors around the word. Several desalination techniques are widely used in the world either thermal processes or membrane processes (Rauly et al., 2004). One desalination treatment process with expended use in Gaza Strip is membrane-based Reverse Osmosis. Reverse Osmosis is a technology used to remove a large majority of contaminants from water by pushing the water under pressure through a semi-permeable membrane. Reverse osmosis works by using a high-pressure pump to increase the pressure on the salt side of the RO and force the water across the semi-permeable RO membrane, leaving almost all (around 95% to 99%) of dissolved salts behind in the reject stream (Puretec 2016). Groundwater is the only source of drinking water in Gaza Strip. However, the quantity and quality of drinking water have deteriorated over the past two decades. The aquifer is continuously overexploited to meet the demand of the rapidly growing population (Al-Jamal et al. 2001). The aim of the present study was to assess the parasitological drinking water quality of house kitchen and desalination plant filters in Gaza Strip.

METHODS

Data collection: During the period from May to December 2015, a total of 420 water and filter samples were collected for parasitological examination at the Medical laboratory Sciences Department, Islamic University of Gaza.

Sampling: A total of 300 samples of RO filters, tap water and filtered water were collected randomly from 100 houses. In addition, 120 random samples of cartridge filters, inlet water and outlet water were collected from 40 desalination plants in all Gaza Strip as shown in Table 1.

Table 1 Distribution of House samples and Desalination samples in the five governorates

	Sample source/item	Gaza	Khan Yunis	Mid Zone	Northern	Rafah	Total
House samples	Reverse Osmosis samples	25	23	10	18	24	100
	Kitchen tap water samples	25	23	10	18	24	100
	Reverse Osmosis filtered water samples	25	23	10	18	24	100
Desalination samples	Cartridge filters	13	8	8	6	5	40
	Inlet water	13	8	8	6	5	40
	Outlet water	13	8	8	6	5	40

Steps of sample processing before the parasitological examination

Reverse Osmosis units processing (houses and desalination plants)

Each RO unit was collected in a sterile bag and was cut with a sterile knife, then the plastic cover was removed, the filter paper was washed with 50 ml 0.85% normal saline, each sample was placed in 50 ml sterile tube and centrifuged for 10 minutes at 3000 rpm as shown in Figures (1.1. to 1.4). After centrifugation, the supernatant was discarded and then the sediment was divided into two microtubes. One tube was stored at -20°C for DNA examination, and the other tube content was mixed with 1-2 drops 4% formalin for microscopic examination.

Reverse Osmosis filtered (outlet) and tap (inlet) water samples processing (houses and desalinations)

Each water sample (500ml) was filtered using membrane filter (0.045 mm)which was placed into 10 ml 0.85% normal saline and was centrifuged for 10 minutes at 3000 rpm. After centrifugation, the supernatant obtained was discarded and then the sediment was divided into two eppendrof tubes as in Figures (1.4 to 1.5). One tube was stored in -20°C for DNA examination and 1-2 drops 4% formaline were added to the other tube for microscopic examination.

Parasitological analysis for samples (houses and desalination plants)

Microscopic examination

Four techniques were employed; direct smear technique using saline and iodine where one drop of saline mixed with one drop of water for egg and cyst stages detection. Ziehl-Neelsen acid fast stain technique as modified by Henriksen specific for *Cryptosporidium* spp. oocyst stage, in Figure (1.6). Iron hematoxylin stain technique to detect *Giardia lamblia* cyst according to WHO protocols (1994).

Molecular methods

DNA extraction: DNA was extracted from approximately 100µl of RO sediment samples using standard method of the ISOLATE II Genomic DNA Kit (Bioline).

Polymerase Chain Reaction (PCR) (Amplification and Primers used)

PCR amplified a 655 to 667 bp fragment, depending on the species of *C. parvum* genotype and using the forward primer N-DIAGF2 (5′-CAA TTG GAG GGC AAG TCT GGT GCC AGC-3′) and the reverse primer NDIAGR2 (5′-CCT TCC TAT GTC TGG ACC TGG TGA GT-3′). The cycling condition was as follows; hot start at 95 °C for 5 minutes, followed by 35 cycles of denaturing for 30 seconds at 94 °C , annealing for 1 minutes at 68 °C and extension for 30 seconds at 72°C, followed by a final extension at 72 °C for 10 minutes (Quah et al. 2011).

PCR was carried out using the amplification protocol for *C. hominis*. BCOWPF (ACC GCT TCT CAA CAA CCA TCT TGT CCT C) and BCOWPR (CGC ACC TGT TCC CAC TCA ATG TAA ACC C) were used to produce a fragment of 769-bp (Yu et al., 2009). Cycling conditions used: initial denaturation cycle of 94 ºC for 5 minutes, followed by 30 cycles of 65 ºC for one minute, 72 ºC for one minute and 94 ºC for one minute; and a final extension at 72 ºC for 10 minutes, which were carried out in a Master cycle Gradient (Figure 1.7).

Figure (1.1). Cutting the filter | Figure (1.2). Washing with normal saline | Figure (1.3). Putting the sample into 50ml tube

Figure (1.4). Centrifugation | Figure (1.5). Bukhner funnel | Figure (1.6). Ziehl Neelsen stain as modified by Henriksen | Figure (1.7). PCR

Agarose gel electrophoresis:

The PCR products were electrophoresed on 2% agarose gels at 100V and 1 ul ethidium promide and 100-bp DNA ladder were used .

Questionnaire:

Four hundred questionnaires were distributed and collected as simple random method from the five governorates of Gaza Strip. The questionnaires were as follows: 100 questionnaires were distributed to households owning R/O units and 300 questionnaires were randomly distributed to houses buying drinking water from desalination plants. The questionnaire was in Arabic to be easy to understand by the public. The questionnaire included several main themes: economic and social factors of water sources, symptoms of infection and public health. The questions evaluated the relation between the expected parasitic contamination of water and human health (source of water, changing of filters, water tank's location, and place of residence).

Statistical analysis

Data obtained were computerized, simple distribution of study variables, the cross-tabulation and Chi square test were used to identify the significance of the relationships between variables by SPSS software.

RESULTS

The prevalence and type of parasitic protozoa and helminths contaminating house kitchen filters, desalination filters, tap water and filtered water

A total of 420 samples (100 RO filters, 100 tap water, 100 filtered water, 40 cartridge filters, 40 inlet water and 40 outlet water) were collected randomly from 100 house kitchens and 40 desalination plants from the five Governorate of Gaza Strip to assess parasitological water quality. It was found that 1.9% (8/420) of water from different sources were contaminated with *Cryptosporidium* spp. Only one sample out of 420 (0.24%) was contaminated by *C. parvum* and all samples were negative for *C. hominis*.

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Detection of parasites contaminating kitchen RO filters

The results of examining 100 kitchen RO filters are described as follows: Eight samples 8% (8/100) containing *Cryptosporidium* spp. Figure (2). Four samples 14% (4/24) were found in Rafah, 1 (10%) in Mid zone and 3 (16.7%) in North Gaza using acid fast stain technique while the search for other parasites was negative in all samples using direct smear and iron hematoxylin stain techniques. This map shows the distribution of the positive results of acid-fast stain technique in Gaza Strip and describes some of the desalination plants figure (3). Kitchen tap water, filtered water, cartridge filters from desalination plants were negative for protozoa using direct smear, Iron hematoxyline stain and Acid-fast stain figure (2).In Rafah, Khan Yunis, Mid Zone, Gaza and Northern the results showed no parasites in all samples of outlet water from desalination plants using the performed tests. In this research no helminth parasites were found in different sources of drinking water and low level of *Cryptosporidium* were detected only in RO filters when using several techniques.

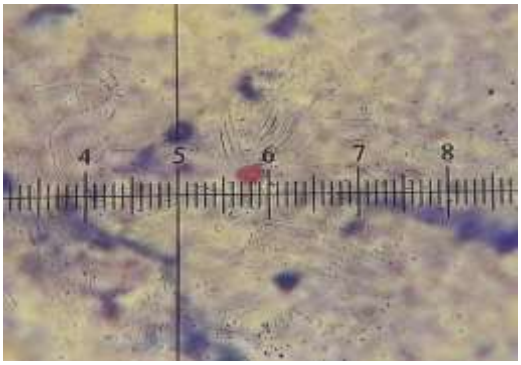

Figure (2): Positive acid-fast stain (*Cryptosporidium* oocyst 4.5 µm)

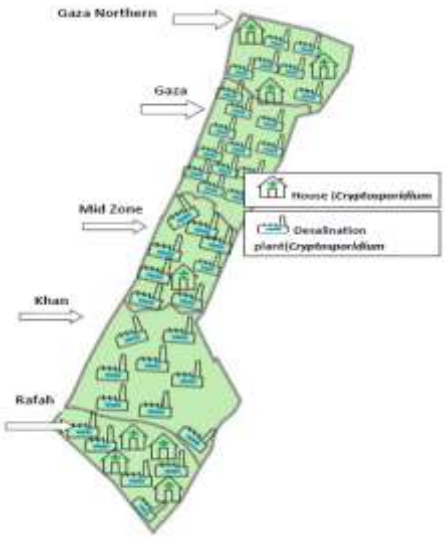

Figure (3): The representation of the results on Gaza Strip map

The Molecular results using PCR

One sample out of 420 (0.24%) was positive for *C. parvum* when PCR was used (Figure 4). One out of eight positive sample 12.5% (1/8) was identified as *C. parvum* and no detection for *C. hominis*. All samples were screened for *Cryptosporidium* using a MZN staining technique.

The relation of these protozoa and helminth parasites to human health through self-reported clinical symptoms of house residents

Economic and social factors:

Over the study period, information collected randomly through a pre-tested standard questionnaire including socio demographic information such as gender, place of resident and standard of sanitation among population measured by the educational level of father and mother as shown in Table (2).

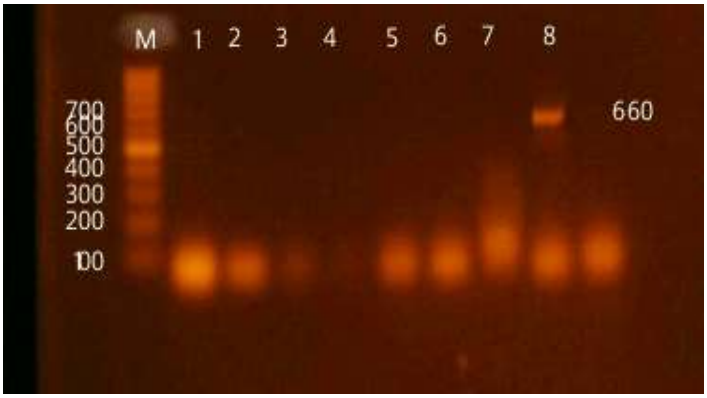

Figure (4): Fragment sizes of the PCR-amplified of NDIAG region for *C. parvum* as obtained by gel electrophoresis. In the periphery of the photograph, bands from a 100-bp DNA ladder scale (M) are shown. 1: Negative control, 2-7&9: Negative sample for NDIAG gene, 8: NDIAG gene for *C. parvum* (660 bp).

Water sources

A majority of participants (58%) were using desalination plants as a source of drinking water while 26.8% were using kitchen filter as a second source of drinking water in Gaza Strip. It has been shown that 12.3% of people did not wash drinking water tanks while 33.8% of tanks were at house roof, 42% of tanks were exposed directly to sun, 7.0% of tanks were uncovered, 61.3% did not analyze water and 77.5% did not use water disinfection materials (Table 3).

Symptoms of infection

Diarrhea was the most important gastrointestinal symptom: as reported by the patients, 17.5% of people showed diarrheal symptom and 15.8% of them have reported that the cause of diarrhea was parasites.

Public health

It was found that of 68.3% of people were aware that there is a relationship between human health and the contamination of drinking water while 70.3% of them thought that drinking water sources are close to the sewage plants (Table 4).

Table 2 Sociodemographic characters of the participants

Economic and social factors	Frequency (No)	Percentage %
Gender		
Male	237	59.2
Female	163	40.8
Father's educational level		
< high school		
High School	110	27.5
Diploma	89	22.2
Bachelor's	33	8.3
	168	42.0
Mother's educational level		
< high school	118	29.5
High School	130	32.5
Diploma	46	11.5
Bachelor's	106	26.5
Place of residence		
Village	89	22.2
Camp	140	35.0
City	171	42.8

Table 3 Participants drinking water sources

Water sources	Frequency (N=400)	Percentage %
Source of water used in the house		
Kitchen water filter	107	26.8
Municipal water	51	12.8
Desalination plants water	233	58.2
Artesian well water	9	2.2
Do you wash drinking water tank?		
Yes	251	62.7
No	49	12.3
How many times do you wash drinking water tank?		
At every filling	147	36.8
Two times per month	114	28.5
Never	39	9.5
Drinking water tank place		
House Roof	135	33.8
House Stairs	47	11.8
Inside the House	118	29.5
Is the drinking water tank covered?		
Yes	272	68.0
No	28	7.0
Is the drinking water tank exposed to the sun?		
Yes	168	42.0
No	132	33.0
Was the drinking water tested?		
Yes	155	38.8
No	245	61.3
Do you change the filter?		
Yes	90	22.5
No	10	3
Do you use water sterilization materials?		
Yes	90	22.5

No	310	77.5
Was the drinking water tested in the last six months?		
Yes	140	35
No	260	65.0

Table 4 Public health characters of the participants

Public health	Frequency (N=400)	Percentage %
Is there a relationship between health and contamination of drinking water?		
Yes	273	68.3
No	127	31.8
The distance between drinking water source and sewage plant		
Very close	27	6.8
Far away	92	23.0
close	281	70.3
Do you think that the cause of diarrhea is water contamination?		
Yes	191	47.8
No	64	16.0
I don't know	145	36.3
Are the garbage scattered in front of your home?		
Yes		
No	101	25.3
	299	74.8

4. DISCUSSION

Water is an essential substance for the development of life. It represents approximately 70% of the body weight of a human being (Assessment 2005). The protozoan parasite *Cryptosporidium* spp. has caused many outbreaks of gastrointestinal illness through contaminated water (Mercado et al. 2007). Source water monitoring for *Cryptosporidium* is applied in several countries, although usually in research rather than in routine monitoring (Figueras and Borrego 2010). The protozoan parasite *Cryptosporidium* have been described as important waterborne-disease pathogens, and are associated with severe gastrointestinal diseases (Razzolini et al. 2010) In Gaza Strip, Cryptosporidiosis is related with their positivity in diarrheal patients (Geurden et al. 2009).

In our one-year study, a total of 8 out of 420 samples (1.9%) of various sources of drinking water were infected by *Cryptosporidium* oocysts. This percentage was found when Acid fast stain were used to detect

Cryptosporidium oocysts in different sources from houses and desalination plants. Several reports from the neighboring countries showed that the prevalence rate varies between these countries and our findings. However, some other reports results were similar to our study. For instance, the prevalence of *Cryptosporidium* in filtered water in North Jordan was 2% (Abo-Shehada et al. 2004).

Another similar study was in Saudi Arabia which showed that 6.8% were positive for *Cryptosporidium* in Water from filling stations (Zakai and Barnawi 2014). Several studies have examined the presence of *Cryptosporidium* oocysts in the treated drinking water. The highest oocyst concentration were found in systems using poor source water quality with high oocyst counts. On the one hand, the highest presence of *Cryptosporidium* were found in many countries: It was found 35% in filtered water in Japan (Hashimoto et al. 2002), 32% in water supply in UK (Sturdee et al., 2007), 30.8% in treated water in Spain (Carmena et al. 2007), 29.8% in drinking water in Germany (Panagiotis Karanis et al. 1998). On the other hand, less oocyst concentrations rate of prevalence were found in various countries: 3.5% in treated water in Canada (Wallis et al. 1996), 5.2% in water sample in Colombia (Triviño-Valencia et al. 2016), 9% in Norway in raw water (Robertson and Gjerde 2001), 10.1% in South western Finland in surface water (Hörman et al. 2004), 10.2% in Portugal in drinking water resources (Almeida et al. 2012), 12.1% in Russia and Bulgaria in drinking water (Karanis et al. 2006), 13.3% in Hungarian in drinking water (Plutzer et al. 2007) and 0% in Philippines in drinking water (Onichandran et al. 2014).

In our research, the eight positive samples were detected in the RO filter samples. The source of drinking water is coming from the municipalities and is delivered to the houses roof or under the tower's tanks.

Then this water is connected directly to the home filter for more guarantee to be valid for drinking. This means that the source of water that feeds the filter is stored in these tanks. This poses a big question about the cleanliness of water supplied to the public. In our research, *Cryptosporidium* oocysts were found in RO filtered samples and no contamination found in tap and filtered water which was taken from house kitchen. Moreover, the samples of Cartridge filter, inlet and outlet water taken from desalination plants showed no parasitic contamination. According to our research, we can say that there are two main causes of pollution in RO filters in house kitchens: there is no chlorinating system enough at municipality level and the level uncleanliness of storage tanks at homes either monitored and followed or not. In our research, water specimens were examined by a concentration technique followed by a modified Ziehl-Neelsen (MZN), which is a routine and standard stain for *Cryptosporidium*. MZN was proved to be a simple and fast method (Awadalla et al. 1998). Many countries are routinely monitoring water using other techniques to identify these parasites. In Addis Ababa city, Brazil, Hungary, Portugal, Taiwan and China, they used the EPA Method 1623 which requires filtration, immunomagnetic separation of the oocysts and cysts from the material captured and enumeration of the target organisms based on the results of immunofluorescence assay, 4',6-diamidino-2-phenylindole (DAPI) staining results, and differential interference contrast microscopy, which is more specific than MZN (Almeida et al. 2012, Feng et al. 2011, , Razzolini et al. 2010, Fikrie et al. 2008). In Egypt, they used the flow cytometry technique,

which is the best because it is 100% sensitive. However, it is more expensive than other techniques. This technique is used to detect the viability of Cryptosporidium. The development of sensitive and specific molecular detection methods such as the PCR has greatly increased the knowledge about *Cryptosporidium* in the environment. Many PCR assays for detecting *Cryptosporidium* oocyst have been described. However, these methods required a large number of oocysts and were useful mainly as research tools (Khalifa et al. 2011). In our research, the PCR technique was able to detect *Cryptosporidium* DNA in only one sample 0.24% (1/420) samples in different sources of examined drinking water but negative for *C. hominis*. Positive PCR results were only achieved in a sample that came from RO and it was also positive when MZN were used. *C. parvum* (zoonotic) and *C. hominis* (anthroponotic) are the most common human-infecting species reported in river water samples in Europe (Xiao and Fayer 2008).

In Spain, it was found that a seeded water sample with 30 *Cryptosporidium* oocysts or more gave positive PCR results. The results of this research seem to indicate that the risk assessment for cryptosporidiosis (parasites) for humans is low in Gaza strip. However, for this, the condition that the whole population has access to the network system for drinking water needs to be fulfilled (Dellundé et al. 2002). The distribution of contamination among the various regions of Gaza strip was high in Northern 16.7% (3/18) then in Rafah 14% (4/24). However, the lowest level of contamination was in Mid Zone 10% (1/10), while Gaza and Khan Yunis were free of contamination. Sewage could be the main reason of contamination of drinking water by *Cryptosporidium* spp. in Rafah, Mid Zone and Northern.

Whereas in some countries such as Spain, they found a reasonable correlation between the rainfall and the presence of *Cryptosporidium* oocyst (Carmena et al. 2007). In Brazil, the causes of water contamination are the disposable waste and sewage discharges. These kinds of contaminators are responsible for carrying pathogenic organisms into water bodies (Razzolini et al. 2010). It seems that the contamination in Jeddah and Makkah would have occurred during transportation through damaged pipes by sewage. The majority of such tap water are usually transported to houses, schools and mosques using pipes and then stored in water reservoirs underground till used. Many pipes are broken and their water content might be contaminated by seepage because there is no efficient sewage system available in both the cities (Zakai and Barnawi 2014).

In the present study A total of 42% of the participants answered that the drinking water tank exposed to the sun. It was reported that solar disinfection of drinking water can be an effective household intervention against *Cryptosporidium* contamination (Fernando et al., 2007). The high attitude among participants towards the washing of drinking water tanks 62.7% indicating that they are aware from hygiene of these tanks.

Most Gaza Strip residents are depending on street water vendors to obtain the required drinking water where this issue should be tackled in the future.

In the present study small volumes of water were examined, and this might lead to a false negative bias. If the water is treated by RO, samples of 100 - 1000 L may be needed to detect Cryptosporidium, and methods such as United States Environmental Protection Agency Method 1622 for Cryptosporidium in Water may be more appropriate. As a limited resource area we are lacking sophisticated devised to filter high volumes of drinking water as happened in this study.

CONCLUSIONS

The presence of Cryptosporidium spp. in the examined filters are dangerous indicator but the filter used in houses and even in desalination plants are safe. This research could serve as a base line epidemiological surveillance of waterborne parasites in Gaza Strip and could further help to create more awareness among public and policy makers. Presence of *Cryptosporidium* in drinking water supplies of the examined sites Rafah, Mid Zone and Northern make people more exposed to the risk.

1. RECOMMENDATIONS

Studies with high volume of drinking water samples for analysis should be taken into consideration. Future studies should be carried out to provide an extensive platform for risk assessment with the occurrence of parasites in the water environment, in addition desalination plants should be monitored regularly for the safety of drinking water.

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