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

تقييم جودة المياه طفيليا المأخوذة من فلاتر مطابخ المنازل ومحطات التحلية في قطاع غزة

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إقـــــــــرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

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

تقييم جودة المياه طفيليا المأخوذة من فلاتر المطابخ ومحطات التحلية في قطاع غزة

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نتيجة الحكم على أطروحة ماجستير

بناءً على موافقة شئون البحث العلمي والدراسات العليا بالجامعة الإسلامية بغزة على تشكيل لجنة الحكم على أطروحة الباحثة/ رانية عدنان محمود غنيم لنيل درجة الماجستير في كلية *العلوم* قسم العلوم الحياتية- تحاليل طبية وموضوعها:

تقييم جودة المياه طفيلياً المأخوذة من فلاتر مطابخ المنازل ومحطات التحلية في قطاع غزة

Assessment of Parasitological Water Quality from Houses Kitchen and Desalination Plants Filters in Gaza Strip

وبعد المناقشة التي تمت اليوم الأحد 29 محرم 1438هـ، الموافق 2016/10/30 الساعة العاشرة صباحاً في قاعة مؤتمرات مبنى اللحيدان، اجتمعت لجنة الحكم على الأطروحة والمكونة من:

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وبعد المداولة أوصت اللجنة بمنح الباحث درجة الماجستير في كلية *العلوم*/ قسم العلوم الحياتية – تحاليل طبية.

واللجنة إذ تمنحه هذه الدرجة فإنها توصيه بتقوى الله ولزوم طاعته وأن يسخر علمه في خدمة دينه ووطنه.

والله ولي التوفيق،،،

نائب الرئيس لشئون البحث العلمي والدراسات العليا

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Abstract

Background:

Safe drinking water is a top priority in preventing disease outbreaks and is of general concern to everyone. Despite the amount of awareness created, waterborne disease still poses threat, especially in developing countries. Due to the scarcity of reported data on waterborne parasites, the consumption of unsafe water prolongs. Thus, the occurrences of waterborne parasites from various filter and water samples were investigated from Gaza Strip.

Objective:

This study has been conducted, to assess of parasitological water quality from house kitchens and desalination plants filters in Gaza Strip.

Methods:

A total 420 samples were collected from the five Governorates of Gaza Strip (Rafah, Khan Yunis, Mid Zone, Gaza and Northern); 300 samples (100 Reverse Osmosis filters, 100 tap water and 100 filtered water) were collected from 100 houses and 120 samples were collected from 40 desalination plants (40 cartridge filter, 40 inlet water and 40 outlet water). All Samples were examined using direct wet mount smear, acid fast stain, iron hematoxyline stain and Polymerase Chain Reaction. The random distributed questionnaire included a questions regarding economic and social factor, water sources, reported symptoms and public health.

Results:

Results revealed that only *Cryptosporidium* species oocyst were detected in eight of drinking water samples 1.9% (8/420). No positive samples were found when we used both direct wet mount smear and hematoxyline stain. Eight samples were positive when using acid fast stain (*Cryptosporidium* spp.) in RO house filters, 4 samples 14% (4/24) containing *Cryptosporidium* spp. in Rafah, 3 samples 16.7% (3/18) containing *Cryptosporidium* spp. in Northern, one samples 10% (1/10) containing *Cryptosporidium* spp. in Mid Zone, no *Cryptosporidium* spp. were detected in samples collected from KhanYonis and Gaza. By using PCR to identify *Cryptosporidium* spp. (*C. parvum* and *C. hominis*) which is the most common species responsible for human infections, only one sample 0.24% (1/420) was positive for *C. parvum* while there is no positive samples for *C. hominis*. The questionnaire analysis showed hight awareness among people.

Conclusion:

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: Gaza Strip, Water quality, Filters, *Cryptosporidium*, PCR.

الملخص

خلفية البحث:

تمثل المياه الصالحة للشرب أولوية قصوى في منع تفشي الأمراض وهي تثير قلق الجميع. وعلى الرغم من الوعي الموجود، فإن الأمراض المنقولة بالمياه لا تزال تشكل تهديدا، خصوصا في البلدان النامية. نظرا لندرة البيانات المبلّغة عن الطفيليات التي تنقلها المياه، فإن استهلاك المياه غير الصالحة للشرب في ازدياد. لذلك تم في هذا البحث الكشف عن الطفيليات التي تنقلها المياه من عينات مرشحات ومياه مختلفة من قطاع غزة.

أهداف البحث:

أجريت هذه الدراسة لتقييم جودة المياه طفيليا في مطابخ المنازل ومرشحات محطات تحلية المياه في قطاع غزة.

منهجية البحث:

تم جمع 420 عينة من المحافظات الخمسة لقطاع غزة (رفح، خان يونس، المنطقة الوسطى، غزة والشمال)؛ تم جمع 300 عينة (100 عينة من مرشحات التناضح العكسي، و 100 عينة من مياه الصنابير و 100 عينة من الماء المرشح) من 100 منزل وتم جمع 120 عينة من 40 محطة لتحلية المياه (40 خرطوشة مرشح، 40 عينة من الماء الداخل للمرشح و 40 عينة من الماء الخارج من المرشح). تم فحص جميع العينات باستخدام مسحة رطبة مباشرة، ومسحة سريعة الحمضية، ومسحة هيماتوكسلين الحديد و مسحة تفاعل البلمرة المتسلسل. اشتملت الاستبانة على مصادر المياه ومعلومات تخص العوامل الاجتماعية والاقتصادية والصحة العامة وأعراض ممكنة.

نتائج البحث:

كشفت النتائج أن الكريبتوسبوريديوم موجودة في ثمانية من عينات مياه الشرب 1.9٪ (420/8). لم يتم العثور على عينة إيجابية عندما استخدمنا كلا من المسحة الرطبة المباشرة ومسحة الهيماتوكسلين .كان هناك ثمان عينات إيجابية لطفيل الكريبتوسبوريديوم عند استخدام المسحة سريعة الحمضية في مرشحات التناضح العكسي للمنزل، 4 عينات 14٪ (04/24) تحتوي على الكربتوسبوريديوم في رفح، و 3 عينات 16.7٪ (18/3) تحتوي على الكربتوسبوريديوم في الشمال ، وعينة واحدة 10٪ (10/1) تحتوي على الكربتوسبوريديوم في المنطقة الوسطى، ولم يتم العثور على الكريبتوسبوريديوم في العينات التي تم جمعها من خانيونس وغزة. باستخدام تفاعل البلمرة المتسلسل لتحديد الأنواع الكربتوسبوريديوم *C. parvum* و*C. Hominis* وهي أكثر الأنواع شيوعا و المسؤولة عن الاصابات البشرية، وكانت عينة واحدة فقط إيجابية 0.24% (420/1)بـ*C. parvum* ، في حين ليس هناك عينة إيجابية ل*C. hominis*. تم من خلال الاستبانة قياس الظروف الصحية العامة.

خلاصة البحث:

ظهور الكريبتوسبوريديوم في امدادات المياه قيد الدراسة يتطلب من مؤسسات وسلطات المياه في قطاع غزة المزيد من الرصد والعلاج و / أو مراقبة مناطق تجمع المياه لضمان وصول ماء خالي من التلوث للمستهلك.

كلمات مفتاحية:

قطاع غزة، جودة المياه، المرشحات، الكريبتوسبوريديوم ، تفاعل البلمرة المتسلسل.

Dedication

This thesis is dedicated to

The sake of Allah, my Creator and my Master

My great teacher and messenger, Mohammed (May Allah bless and grant him), who taught us the purpose of life

My homeland Palestine, the warmest womb

My great parents for their endless love, support and encouragement

My husband and beloved kids: Omar, Ala'a, Mais, and Mera, whom I can't force myself to stop loving

My beloved brothers and sisters

To all my family, the symbol of love and giving

My friends who encourage and support me

All the people in my life who touch my heart

I dedicate this research

Acknowledgment

"رب أوزعني أن أشكر نعمتك التي أنعمت علي وعلى والدي أن أعمل صالحاً ترضاه وأدخلني برحمتك في عبادك الصالحين"

- My sincere thanks and great gratitude to Prof. Dr. Adnan Al-Hindi who graciously supervised this research.
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List of Abbreviations

C. hominis	*Cryptosporidium hominis*
C. parvum	*Cryptosporidium parvum*
CDC	Centers for Disease Control and Prevention
DNA	Deoxyribonucleic Acid
G. lamblia	*Giardia lamblia*
HIV	Human immunodeficiency virus
MZN	Modified Ziehl-Neelsen
PCBS	Palestinian Central Bureau of Statistics
PCR	Polymerase chain reaction
PWA	Palestinian Water Authority
RO	Reverse Osmosis
Spp	Species
TDS	Total Dissolved Solids
UN	The United Nations
UV	Ultraviolet
WHO	World Health Organization

Chapter 1
Introduction

Chapter 1
Introduction

Gaza Strip is an elongated zone located at southeastern coast of Palestine with coordination of Latitude N 31° 26' 25" and Longitude E 34° 23' 34". This area is surrounded by the Mediterranean Sea from the west, the 1948 cease-fire line from the north and east and by Egypt from the south. The total area of Gaza strip is 365 km^2 with width varies from 8 km in the north to 14 km in the south and approximately 40 km long (UNEP, 2003).

Gaza Strip is a small, densely populated area in the Middle East were the main water source is the groundwater. Gaza strip has many water problems such that inefficient water use in the agriculture, high water demand and limited fresh water supply, groundwater contamination, seawater intrusion and improper wastewater disposal. In Gaza Strip, there is a large gap between water demand and water resources. Groundwater is also diminished by pollution, increasing demands, local people misuse and control by neighbouring countries of Palestinian water resources. The citizens of Gaza Strip have pursued several alternatives to increase water supply; water desalination (house units), use of bottled water and imported water (Bashitialshaaer, Persson, & Aljaradin, 2011).

Long-term overexploitation in Gaza Strip has resulted in a decreasing groundwater accompanied by the degradation of its water quality. Due to high levels of salinity and nitrate and boron pollution, most of the groundwater is inadequate for both agricultural and domestic use. The rapid rate of population growth in Gaza Strip and dependence upon groundwater as a single water source present a serious challenge for future political stability and economic development (Weinthal, Vengosh, Marei, Gutierrez, & Kloppmann, 2005).

Waterborne diseases occur worldwide and outbreaks caused by the pollution of community water systems have the potential to cause disease for a big number of consumers. Waterborne outbreaks have economic consequences beyond the cost of health care for affected patients their families and contacts and the economic costs of disease and illness. Moreover, they create a lack of confidence in potable water quality and in the water industry in general. In addition to outbreaks caused by polluted potable

water, there are outbreaks caused following the accidental ingestion of recreational (or other) waters (Panagiotis Karanis, Kourenti, & Smith, 2007).

Pollution of groundwater with pathogenic microorganisms is generally believed to be a consequence of migration or introduction of fecal material either from humans or animals into the subsurface. Fecal pollution can reach groundwater from many concentrated pond sources such as cesspools, landfills, leaking sewer lines and filled septic systems (Sadallah & Al-Najar, 2014).

Cryptosporidiosis and Giardiasis are waterborne diseases spread all over the world (Zakai & Barnawi, 2014).

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 popular symptoms caused by Giardia are weight loss, diarrhea, gas, abdominal cramps and malaise. Moreover, vomiting, chills, headache, and fever may also occur. These symptoms usually appear 6 to 16 days after the initial contact and can continue as long as one month. The symptoms of cryptosporidiosis are similar; the most common include watery diarrhea, nausea, abdominal cramps and headaches. These symptoms occur within two to 25 days of infection and usually last one or two weeks. In some cases they stick around for up to a month (Health Canada, 2009).

Disinfection through inactivation usually involves the use of disinfectants such as chlorine, ozone and chlorine dioxide or a combination of chlorine and ammonia (chloramines) which can render many pathogenic organisms harmless. Other substances that can act as disinfectants include potassium permanganate, iodine, bromine, hydrogen peroxide, ferrate, silver and ultraviolet (UV) light (Cohn, Cox, & Berger, 1999).

According to the bad quality of water in Gaza Strip people tend to use water desalination by reverse osmosis (Ismail, 2003).

1.1 The General objectives

The aim of the present study is to assess the parasitological drinking water quality of house kitchen and desalination plant filters in Gaza Strip.

1.2 The Specific objectives

The objectives of this research were to answer the following study questions:

1. To determine the prevalence and type of parasitic protozoa and helminths contaminating house kitchen filters, desalination filters, tap water and filtered water.

2. What is the contamination level for such protozoan parasites and helminths?

3. What is the relation of these parasitic protozoa and helminth to human health through self-reported symptoms of house residents?

4. Efficiency of the used devices in filtration.

1.3 Significance

The over populated Gaza Strip may put high risk for more demands of safe drinking water which may be suspected for contamination. Also the poor living conditions may enforce peoples to use un-safe alternatives for drinking water as water vendors who sell water by potable tanks on cars. No such parasitological studies were carried out on the quality of drinking water in Gaza Strip.

Chapter 2
Literature review

Chapter 2
Literature review

2.1 Waterborne diseases

Water borne diseases are viral, bacterial and parasitic diseases which use water as a common mean of transmission. Waterborne diseases are major causes of morbidity and mortality worldwide. Developing countries carry a heavy burden of the water borne disease, the heaviest being the diarrheal diseases. Diarrheal episodes occur in all countries, but they are 5 to 6 times more common in developing countries. The problem of water borne diseases is especially prevalent where general hygiene and environmental sanitation are poor and where there is a shortage of protected water supply. It is believed that 80% of all diseases in the world are caused by inadequate sanitation, polluted water or unavailability of water (Demena, Workie, Tadesse, Mohammed, & Gebru, 2003)**.**

2.1.1 Etiologies of common water borne diseases

Waterborne outbreak agents, there are bacterial, viral, parasitic, and chemical agents which cause waterborne illness. Waterborne illnesses can be caused by ingestion or consuming water, by dermal contact, which is contact of the water with skin or mucous membranes, or by inhalation, which is by breathing in a mist or aerosolized water particles (Groen, 2015).

Table (2.1) Some parasitic protozoa and the waterborne route of transmission

(Karanis, Kourenti and Smith, 2007)

Organism	**Disease/symptoms**	**Transmissive stage (size range) and route of infection**
Entamoeba histolytica	Dysentery, liver abscess	Cyst (9–14.5 µm) ingestion
Giardia duodenalis	Diarrhea, malabsorption	Cyst (8–12µm) ingestion
Cryptosporidium spp.	Diarrhea	Oocyst (4–6 µm) ingestion
Balantidium coli	Diarrhea, dysentery	Cyst (50–60 µm) ingestion
Sarcocystis sp.	Diarrhea, muscle weakness	Oocyst (7.5–17 µm)
Toxoplasma gondii	Lymphadenopathy, fever,	Oocyst (10–12 µm) ingestion
Cyclospora sp.	congenital infections Protracted diarrhea	Oocyst (8–10µm) ingestion
Microsporidia	Enteritis, hepatitis, peritonitis, kerato-conjunctivitis	Spore (1.8–5.0 µm) ingestion/contact eye

2.2 Parasitic protozoa related to contamination of drinking water

Both *Cryptosporidium* and *Giardia* are currently considered of major importance for drinking water safety, both because of their high infectivity and because of their resistance to chemical disinfection (Teunis, Medema, Kruidenier & Havelaar, 1997).

2.2.1 *Cryptosporidium* species

Cryptosporidium is a microscopic parasite that causes the diarrheal disease cryptosporidiosis. Both the parasite and the disease are commonly known as "Crypto".There are many species of *Cryptosporidium* that infect humans and animals. The parasite is protected by an outer shell that allows it to survive outside the body for long periods of time and makes it very tolerant to chlorine disinfection. While this parasite can be spread in several different ways, water (drinking water and recreational water) is the most common method of transmission. *Cryptosporidium* is one of the most frequent causes of waterborne disease among humans in the United States (Groen, 2015).

A - Morphology of *Cryptosporidium*

The oocyst of *Cryptosporidium parvum* is small, measuring approximately in a range of 4 to 6 µm and when mature the oocyst contains four sporzoites that may not be visible. A thick double-layered wall protects the oocyst from environmental stresses and no sporocysts are visible but darkly stained granules may be present (Roberts, Janovy, Gerald and Schmidt, 1996).

B - Symptoms of Cryptosporidiosis

Cryptosporidiosis appear within 2 to 10 days after ingestion of infective stage. Symptoms include watery diarrhea, headache, abdominal cramps, nausea, vomiting, and fever. Both the respiratory and gastrointestinal systems may become involved. These symptoms lead to weight loss and dehydration. In healthy individual, symptoms such as diarrhea are self-limiting and last form one to two weeks, at which time the immune system eliminate the infection. However, in the immunocompromised persons and infants, the infection may continue, progressing to life- threatening condition (John, petri, Markell & Voge, 2006).

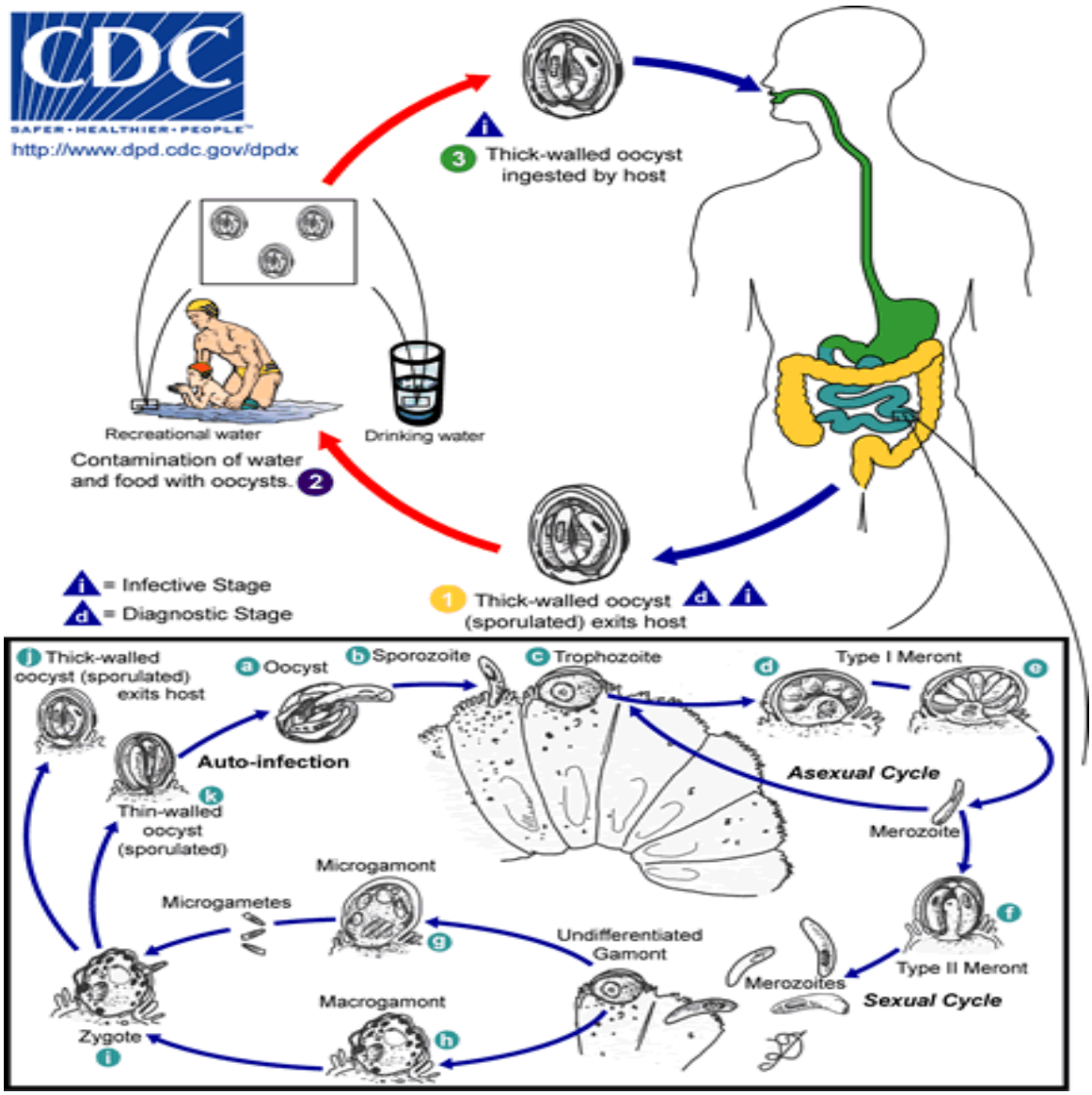

Figure (2.1): Life cycle of *Cryptosporidium*

(CDC, 2010)

D -Epidemiology of *Cryptosporidium*

Fecal-oral contamination is the route of infection with *Cryptosporidium*. Cryptosporidiosis should be considered as zoonosis and common cause of diarrhea in the population. The zoonotic potential of *Cryptosporidium* is illustrated by surveys on cattle. In one study Anderson examined nearly a hundred thousand cattle and discovered that 65% of the diaries and 80% of the feedlots had infected animals, and although the overall prevalence was low (less than 5% by state), and 31% of the cattle

were passing oocysts. In other studies swimming pools have been implicated as periodical source of infection (Roberts, Janovy, Gerald & Schmidt, 1996).

2.2.2 *Giardia lamblia*

The life cycle consists of two stages the trophozoite and cyst. The trophozoite is 10-20 μm long and 5-15μm wide anteriorly. It is bilaterally symmetrical pear-shaped with two nuclei (large central karyosom) four pairs of flagella, two axonemes, and a suction disc with which it attaches to the intestinal wall. The oval cyst (round or oval) is 11-14 μm long and 7-10μm wide, thick-walled with four nuclei and median bodies Each cyst gives rise to two trophozoites during excystation in the intestinal tract (Garcia & Shimizu, 1997)**.**

A -Pathogenesis of *Giardia lamblia*

Less common symptoms include itchy skin, hives, and swelling of the eye and joints. Sometimes, the symptoms of giardiasis might seem to resolve, only to come back again after several days or weeks. Giardiasis can cause weight loss and failure to absorb fat, lactose, vitamin A and vitamin B12. In children, severe giardiasis might delay physical and mental growth, slow development, and cause malnutrition (Groen, 2015).

B-Epidemiology and prevention of *Giardia lamblia*

Transmission is by ingestion of viable cysts and contaminated food or drink may be the source, intimate contact with an infected individual may also provide the infection mechanism. This organism tends to be found more frequently in children or in groups that live in close quarters. Often, there are outbreaks due to poor sanitation facilities or breakdowns as evidenced by infections of travels and camps. There is also an increase in the prevalence of giardiasis in the male homosexual population, probably because of anal and/or oral sexual practice. Although seasonal patterns have been identified for some infection diseases, limited information is available for giardiasis. Some data suggest an association with the cooler, wetter months of the year; this is not surprising if one considers the issue of environmental condition advantageous to cyst survival (Garcia & Shimizu, 1997).

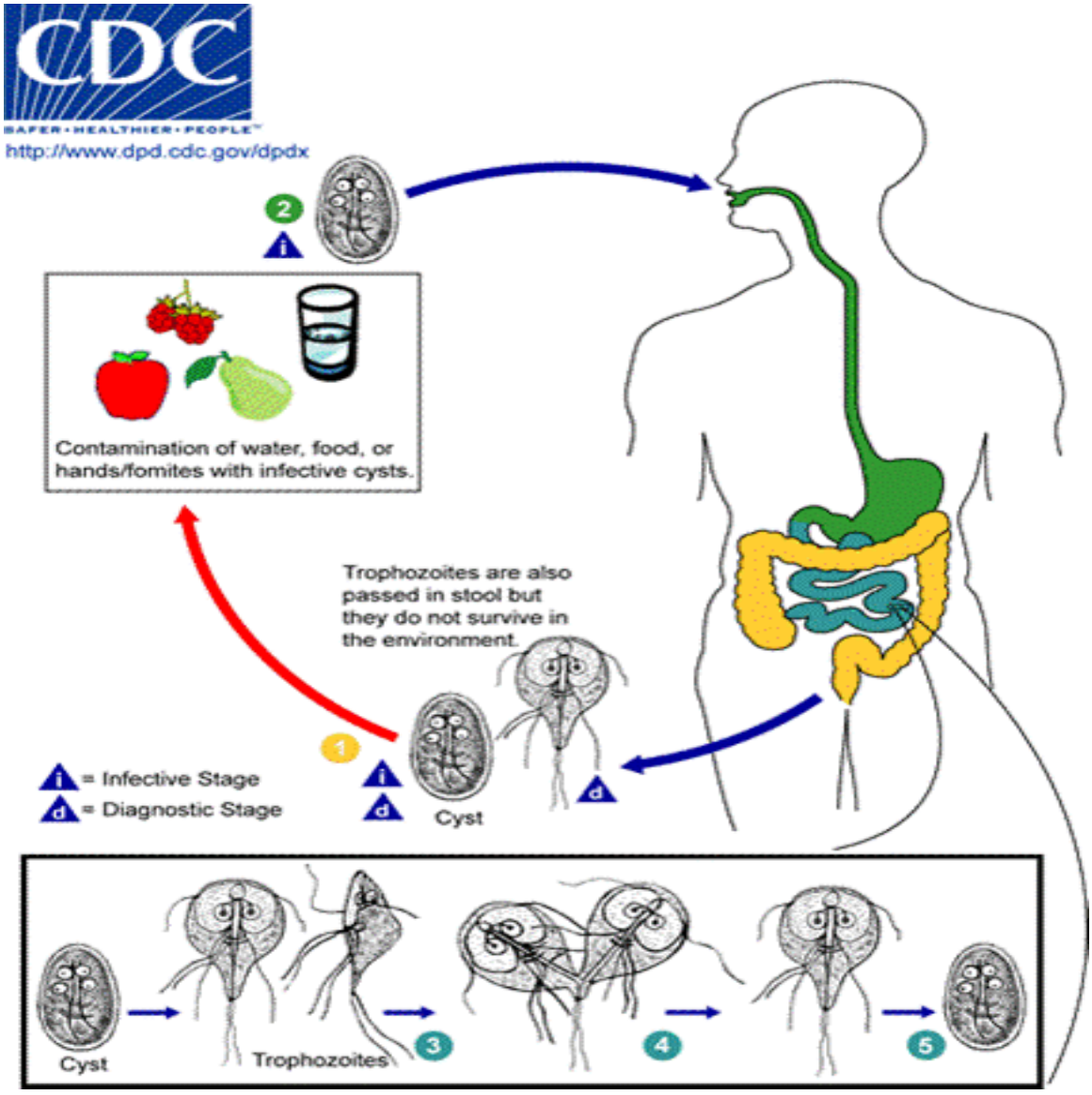

Figure (2.2): Life cycles of *Giardia lamblia*

(CDC, 2010)

2.3 *Cryptosporidium* and *Giardia* are particularly suited for waterborne transmission

Both (Oo)cysts of *Cryptosporidium* and *Giardia* have a common biochemical and physical features that make them especially robust to a wide range of ecological stressors and favour their successful dispersal in the aquatic environment as (Oo)cysts are shedding in high numbers, persistence in the aquatic environment, small size, resistance to chemical disinfection (Carmena, Aguinagalde, Zigorraga, Fernández-Crespo, & Ocio, 2007).

2.4 Diarrhea

Acute infectious diarrheal diseases and acute respiratory diseases are the two most frequent causes of childhood deaths in the developing countries. Diarrheal diseases represent 25% of all deaths in children lower than 5 years of age in these areas (Sack, Rahman, Yunus, & Khan, 1997).

Diarrhea can remain several days, and can leave the body without water and salts which are necessary for survive. Most people who die from diarrhea actually die from severe dehydration and fluid loss. Children who are malnourished or have impaired immunity as well as people living with HIV are most at risk of life-threatening diarrhea (Mahor, 2013).

Diarrhea is the passage of 3 or more loose or liquid stool per day, or more frequently than is normal for the individual. It is usually a symptom of gastrointestinal infection, which can be caused by a variety of bacterial, viral and parasitic organisms. Infection is spread through contaminated food or drinking-water, or from person to person as a result of poor hygiene (Velázquez, Calzada, Gayosso, & Bautista, 2012)**.**

2.5 Reverse Osmosis

Desalination processes are used commercially to provide fresh water for many communities and industrial sectors around the word. Many desalination processes are widely used in the world (thermal processes, membrane processes…). One desalination treatment process with expended use in Gaza Strip is membrane-based Reverse Osmosis. Reverse Osmosis is a technology that is 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).

RO membranes are capable of rejecting practically all particles, bacteria, and viruses. In water purification systems, a pump with 14 bars will provide enough pressure for RO application; pressure will be applied to the concentrated solution to counteract the osmotic pressure. Pure water is driven from the concentrated solution and collected

downstream of the membrane. Another membrane could be used to increase the amount of water in order to increases the capacity of the system. Water pressure also affects the quantity and the quality of the water produced. In the second path water flows to two series of activated carbon filters. These filters remove chlorine, sulfur, volatile organics and the remaining bad taste and odors from water. Water from the first path is mixed with the second path in tank B. This mixture will increase TDS to give the water adequate taste. A post treatment is performed to ensure a better quality of water. A pump of 6 bars pushes the water to 3 series filters. The first one is 5-micron cellulose filter, the second one is an activated carbon filter, and the third one is 1-micron cellulose filter. These 3 filters are installed to ensure the quality of water. They perform another treatment to remove the last remaining traces of resin fragments, carbon fines, colloidal and microorganisms. Finally, water flows to an ultraviolet unit (UV) where radiation is used as a germicidal treatment for water; few of the RO companies use UV light. Later, water flows to 1 micron cellulose filter. Finally, water is stored in tank C for domestic use (Aish, 2011).

2.6 Drinking water sources in Gaza strip

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, Al-Yaqubi, Eng, & Authority, 2001).

It is reported that groundwater from the coastal aquifer is the main source of water in Gaza Strip and provides about 98% of all water supplies, while the remaining 2% is provided through purchasing from the ‴ Israeli ‴ water company (MEKOROT). Domestic Water based on the total water production records received from the Coastal municipalities' water utility and the different municipalities in Gaza Strip. The following can be concluded: Total water supplied to Gaza people for domestic and drinking use is 103.34 MCM/y, categorized as follows; 94.1 MCM from municipal groundwater wells, 2.44 MCM from UN groundwater wells, 2.8 MCM from private groundwater desalination vendors resulting from 4.80 MCM abstracted from the aquifer, 4.00 MCM from mekorot (PWA, 2013).

Table (2.2) The cited literature for water research in Gaza Strip

Researcher Name	Results
PWA report, 2015	Despite that 66% of the plants stated that they use disinfection, the lab tests results for TC showed that 45 % of the plants were infected, and only 2.7% of the results of free residual chlorine showed use of chlorination in acceptable manner. These results are unacceptable comparing with any guidelines for quality of water used for drinking purposes.
PWA report, 2014	More than half of the available groundwater is used for irrigation (52%), while the remaining is used for domestic water supply and industry. The water situation in Gaza is very bad in terms of quantity and quality, where the Coastal Aquifer in the Gaza Strip receives an annual average recharge of 55 -60 MCM/y mainly from rainfall, while the annual extraction rates from the aquifer is about 200 MCM.
El Ramlawi, 2013	Show that a high percentage of microbiological contamination in the outlet water (21.6%) and the inlet water (16.6%), which exceeded the WHO guidelines. The study concluded that large scale sea water desalination plants should be established to overcome the current water quality problems and the quantity shortage.
PWA report, 2013	Chloride and nitrate, it's clear that 3.8% of the domestic water is only matching with WHO drinking limit, while the remaining 96.2% is out of limit.
Haneya, 2012	About 11.8% of Gaza city students noticed that there is no cover for the drinking tank, 28.6% of these students noticed birds standing on tank's upper hole, which may lead to a serious cause of animal source contamination.
Muhammad, Al-Khatib & Al-Najar, 2011	In Gaza Strip, The mean nitrate concentration is high at 199 mg/l and is attributed due to intensified agriculture activities and excess use of fertilizer. About 86% of the examined samples exceed the maximum permissible concentration of 50 mg/l set by WHO.
Aish, 20111	The study showed that 25% of product water sample were contaminated by Total Coliform, and about 15% of water sample were contaminated by fecal coliform.

Researcher Name	Results
Al-Khatib &Arafat, 2009	Indicated a contamination of the domestic water supply in the water network in Gaza Strip by wastewater. This could be due to leakage from the wastewater sewage system, openly flowing sewage, and seepage pits into the water pipelines, as some of the pipes in the water networks are old and cracked.
El-Naeem, Heen& Tubail, 2009	Nitrate concentration has also been detected at a high level, up to 500 mg/l at north Gaza.
Yassin, Amr, & Al-Najar, 2006	Revealed that total and fecal coliform contamination exceeded the WHO's limits for water wells and networks in Gaza governorate, the contamination percentages were higher in networks than in wells.
Baalousha, 2006	High chloride concentrations have been detected in Gaza City and the southern area.
Elmanama, Fahd, Afifi, Abdallah &Bahr, 2005	Salmonella and Vibrio isolation was also higher in sand than in water despite the fact that only 10 g of sand were used while 1L of seawater was collected. Statistically significant correlations between FC and streptococci and between Salmonella and Vibrio were found. Similar correlation was also detected between Pseudomonas and Salmonella in sand samples.
Sharif, 2003	Found various concentrations of total and fecal coliforms in water samples from 20 groundwater wells located in the surrounds of the wastewater treatment facility of Beit Lahia, Gaza Strip.
Melad, 2002	Showed that 68% of the sampling points in the water supply network have residual chlorine concentrations lower than the recommended values given by The WHO.
AL-Jamal & AL-Yaqubi, 2000	High levels of chloride and TDS in the groundwater cause high salinity in the water supply.
El-Mahallawi, 1999	The contamination level of total and faecal coliforms exceeded that of the World Health Organization (WHO) limit for water wells and networks. However, the contamination percentages in networks were higher than that in wells.

Table (2.3): The cited literature for *Giardia lamblia* and *Cryptosporidium* spp. in worldwide

Country	Year	Type of sample	Number of Samples	*G. lamblia*	*Cryptosporidium*
Colombia (Triviño-Valencia Lora, Zuluaga & Gomez-Marin, 2016)	2015	water samples	38	21%	5.2%
Saudi Arabia (Zakai & Barnawi, 2014)	2014	Water from filling stations	161	17.4%	6.8%
Philippines(Onichandran et al., 2014)	2014	Drinking water	16	0%	0%
Alexandria(Khalifa, Ibrahim, Said Abdel Aleem & Nabil, 2011)	2011	Water tank	30	16%	63%
North of Portugal (Almeida et al., 2012)	2010	Drinking water	167	8.4%	10.2%
Brazil (Razzolini, da Silva Santos & Bastos, 2010)	2010	Watersheds and drinking water sources	25	46.1%	7.6%
Legedadi (Addis Ababa) (Fikrie Hailu & Blete ,2008)	2008	Drinking water	22	73%	100%
Spain (Carmen et al., 2007)	2007	Treated water	284	19.2%	30.8%
UK (Sturdee Foster, Bodley-Tickell & Archer, 2007)	2007	Water supply	188	/	32%
Hungarian(Plutzer Tako, Marialigeti Törökné & Karanis, 2007)	2007	Drinking water	45	26.7%	13.3%

Country	Year	Type of sample	Number of Samples	*G. lamblia*	*Cryptosporidium*
Russia and Bulgaria (Karanis Sotiriadou, Kartashev, 2007)	2006	Drinking water resources	166	5.2%	12.1%
North Jordan (Abo-Shehada, Hindyia & Saiah et al., 2004)	2004	Filtered water	14	/	2%
Japan (Hashimoto, Kunikane& Hirata 2002)	2002	Filtered water	26	12%	35%
Norway (Robertson & Gjerde Gjerde, 2001)	2001	Raw water		/	9%
South western Finland (Hörman et al., 2001)	2001	Surface water	139	13.7%	10.1%
Taiwan (Hsu Huang & Hsu et al., 2001)	2000	Water sample	26	46.2%	46.2%
Germany (Karanis Schoenen & Seitz, 1998)	1998	Drinking water	47	14.9%	29.8%
Canada (Wallis Erlandsen et al.,1996)	1996	Treated water sample (chlorine)	58	18.2%	3.5%

Chapter 3
Materials and Methods

Chapter 3
Materials and Methods

3.1 Data collection

During the period from May to December 2015, a total of 420 samples of water and filters were collected for parasitological examination.

3.1.1 Sampling

A total of 300 samples of RO filters, tap water and filtered water were collected randomly from 100 houses in all Gaza Strip as follows:

Table (3.1) House sample

Governorate of Gaza Strip	Gaza	Khan Yunis	Mid Zone	Northern	Rafah	Total
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
Questionnaires	25	23	10	18	24	100

A total of 120 random of samples, cartridge filters, inlet water and outlet water were collected from 40 desalination plants in all Gaza Strip as follows:

Table (3.2) Desalination samples

Governorate of Gaza Strip	Gaza	Khan Yunis	Mid Zone	Northern	Rafah	Total
Cartridge filters	13	8	8	6	5	40
Inlet water	13	8	8	6	5	40
Outlet water	13	8	8	6	5	40
Questionnaires	80	34	76	51	59	300

3.1.2 Questionnaire

A total of 400 questionnaires were distributed and collected randomly from the five governorates of Gaza Strip (appendix 1). A total of 100 questionnaires were distributed to households owning reverse osmosis 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. Moreover, the questionnaire

included several main themes: economic and social factors of water sources, symptoms of infection and public health. The questionnaire questions evaluated the relation between the expected parasitic contamination of water and human health (source of water, changing of filters water tanks place, place of residence …).

3.2 Steps of samples processing before the parasitological examination

3.2.1 Reverse Osmosis units processing (houses and desalination plants)

Each RO unit was collected in a sterile bag and was cut with a sterile knife as shown in Figures (3.1) and (3.2). Then the plastic cover was removed and the filter paper was washed with 50 ml 0.85% normal saline as shown in Figure (3.3). Each sample was put in 50 ml sterile tube Figure (3.4) and was centrifuged for 10 minutes at 3000 rpm Figure (3.5). After centrifugation the supernatant was discarded and then the sediment was divided into two eppendrof tubes. One of the tubes was stored in -20°C for DNA examination, and the other tube contents were mixed with 1-2 drops 4% formaline for microscopic examination.

Figure (3.1): Cutting the filter

Figure (3.2): Cutting the filter

Figure (3.3): Washing with normal saline

Figure (3.4): Putting the sample into 50ml tube

Figure (3.5): Centrifugation

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

Each water sample was filtered using Bukhner funnel as shown in Figures (3.6) and (3.7). Then the filter paper (0.045 mm) was removed and was put into 10 ml 0.85% normal saline and was centrifuged for 10 minutes at 3000 rpm Figure (3.8). After centrifugation, the supernatant was discarded and then the sediment was divided into

two eppendrof tubes. One of the tubes was stored in -20°C for DNA examination and 1-2 drops 4% formaline were added to the other tube for microscopic examination.

Figure (3.6): Bukhner funnel

Figure (3.7): Using Bukhner funnel

Figure (3.8): Centrifugation

3.3 Parasitological analysis for samples (houses and desalination plants)

3.3.1 Parasitological methods

During our study four techniques were employed:

a- Direct smear technique using saline for egg and cyst stages detection.

b- Acid fast stain technique specific for *Cryptosporidium* spp. oocyst stage.

c- Iron hematoxylin stain technique to detect *Giardia lamblia* cyst.

d- Polymerase chain reaction (PCR) technique to detect *Cryptosporidium sp.*

a- Direct smear microscopy (wet mount)

One drop of diluted sediment was put on a slide and was covered with coverslip then it was examined at (10x), (40x) and if needed at (100x) (Organization, 1994).

b- Acid fast stain (Ziehl–Neelsen)

A thin smear was prepared. The smear should be thin enough. Then the smear was fixed with methanol and was stained with carbol-fuchsin solution for five minutes Figure (3.9). The slide was washed gently in the running water until no more stain appears in the washing water Figure (3.10). The smear was decolorized by acid 3% alcohol for three minutes. Methylene blue was added for one minute Figure (3.11),

then it was rinsed with the running water. The slide was dried. Each slide was examined using (40x) Figure (3.12). *Cryptosporidium* oocyst will appear as small red dots against a blue background as shown in Figures (3.13) and (3.14). Yeasts will not stain (Organization, 1994).

Figure 3.9): Acid fast stain kit

Figure (3.10): Stain with carbol fachsin

Figure (3.11): Washing with tap water

Figure (3.12): Stain with Methylene blue

Figure (3.13): Microscopic examination

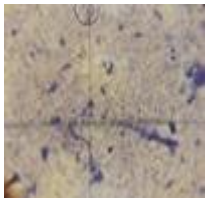

Figure (3.14): examined using (40x)

c- Iron hematoxylin stain Materials needed:

Stock solution A: One gm of hematoxyline crystals was dissolved in 100ml of 95% alcohol and the solution was allowed to stand in light for 1 week and filtered.

Stock solution B: One gm of ferrous ammonium sulfate and 1 ml of hydrochloric acid in 95 ml of distilled water were mixed.

A working solution by combining 25 ml each of stock solutions A and B was prepared at least 3-4 hours prior of staining. Picric acid solution for destination was prepared by adding 25 ml of saturated aqueous picric acid to 25 ml of distilled water.

Staining procedure

Each slide was placed into 70% alcohol for 5 minutes then the slides were replaced into another jar contains 50% alcohol for 2 minutes. Then they were removed into another jar contained tap water for 5 minutes, then into a jar containing working haematoxylin stain solution for 10 minutes. After that, the slides were removed into a jar contained distilled water for 1 minute, then into a jar of picric acid solution for 1 minute, then running tapeworm for 10 minutes, then to a jar containing 70% alcohol and 1 drop of ammonia for 5 minutes. Then the slides were dehydrated by placing them into a jar of 95% then 100% alcohol for 5 minutes. Used resinous mounting medium, and was examined under the high dry objective (40x) Figures (3.15) and (3.16) (Organization, 1994).

Figure (3.15): Iron hematoxyline kit

Figure (3.16): Staining technique by using glass jars

d- Molecular methods

Polymerase Chain Reaction (PCR)

DNA extraction

DNA was extracted from approximately 100µl of RO sediment samples using standard method of the Bioline kit (appendix 2) (Figures 3.17 and 3.18).

PCR reaction (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, Ambu, Lim, Mahdy, & Mak, 2011).

PCR was carried out using the protocol amplification depending on the species of *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; Cycling conditions used were: 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 Figures (3.19) and (3.20).

Agarose gel electrophoresis:

The PCR products were electrophoresed on 2% agarose gels at 100V Figures (3.21), (3.22) and (3.23) (Araújo et al., 2008).

Figure (3.17): Bioline kit

Figure (3.18): Incubation for DNA extraction

Figure (3.19): PCR

Figure (3.20): Diagnostic Laboratory

Figure (3.21): Agarose gel electrophoresis

Figure (3.22): Reading under UV

Chapter 4
Results

Chapter 4
Results

4.1 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 kitchen 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. and identified by using PCR. Only just one sample 0.24% (1/420) was contaminated by *C. parvum*. However, all of the samples were negative for *C. hominis***.** These results were described as follows Table (4.1):

Table (4.1) Detection of parasites contaminating kitchen RO filters

	Number of samples	Direct Smear Positive (%)	Acid Fast Stain Positive (%)	Iron Hematoxylin stain Positive (%)
Rafah	24	0	4 (16.7%)	0
Khan Yunis	23	0	0	0
Mid Zone	10	0	1 (10%)	0
Gaza	25	0	0	0
Northern	18	0	3(16.7%)	0

4.1.1 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.. Four samples 14% (4/24) were found in Rafah using acid fast stain technique while all samples were negative using direct smear and Iron hematoxyline stain techniques Figure (4.1).

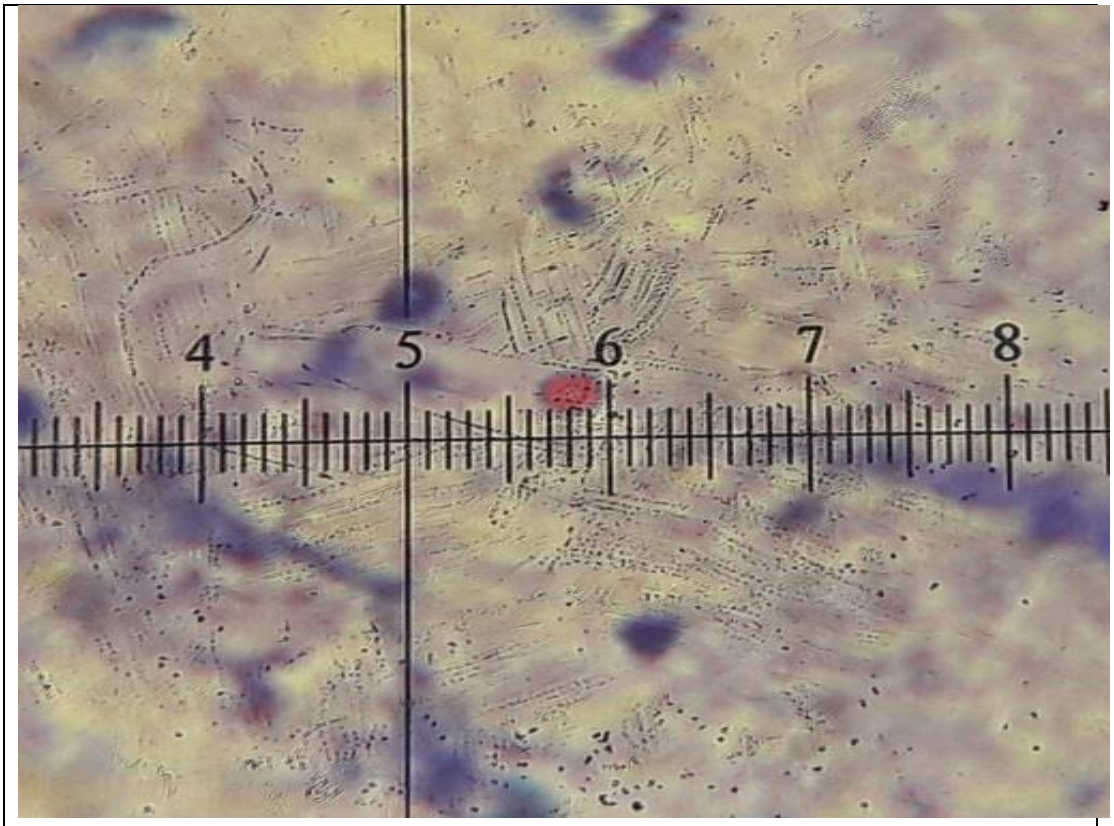

Figure (4.1): Positive acid fast stain (*Cryptosporidium* 4.5 µm)

All samples were negative for parasites in Khan Yunis when all techniques are used. The prevalence of infection with *Cryptosporidium* was 10% (1/10) in Mid Zone using acid fast stain technique while the other samples were negative using the other techniques. In Gaza the results of direct smear, Iron hematoxyline stain and Acid fast stain techniques were negative for parasites in all samples. Three samples 16.7% (3/18) containing *Cryptosporidium* were found in Northern by acid fast stain technique and the results were negative in other examinations Figure (4.2).

This map shows the distribution of the positive results of acid fast stain technique in Gaza Strip and also describes some of the desalination plants.

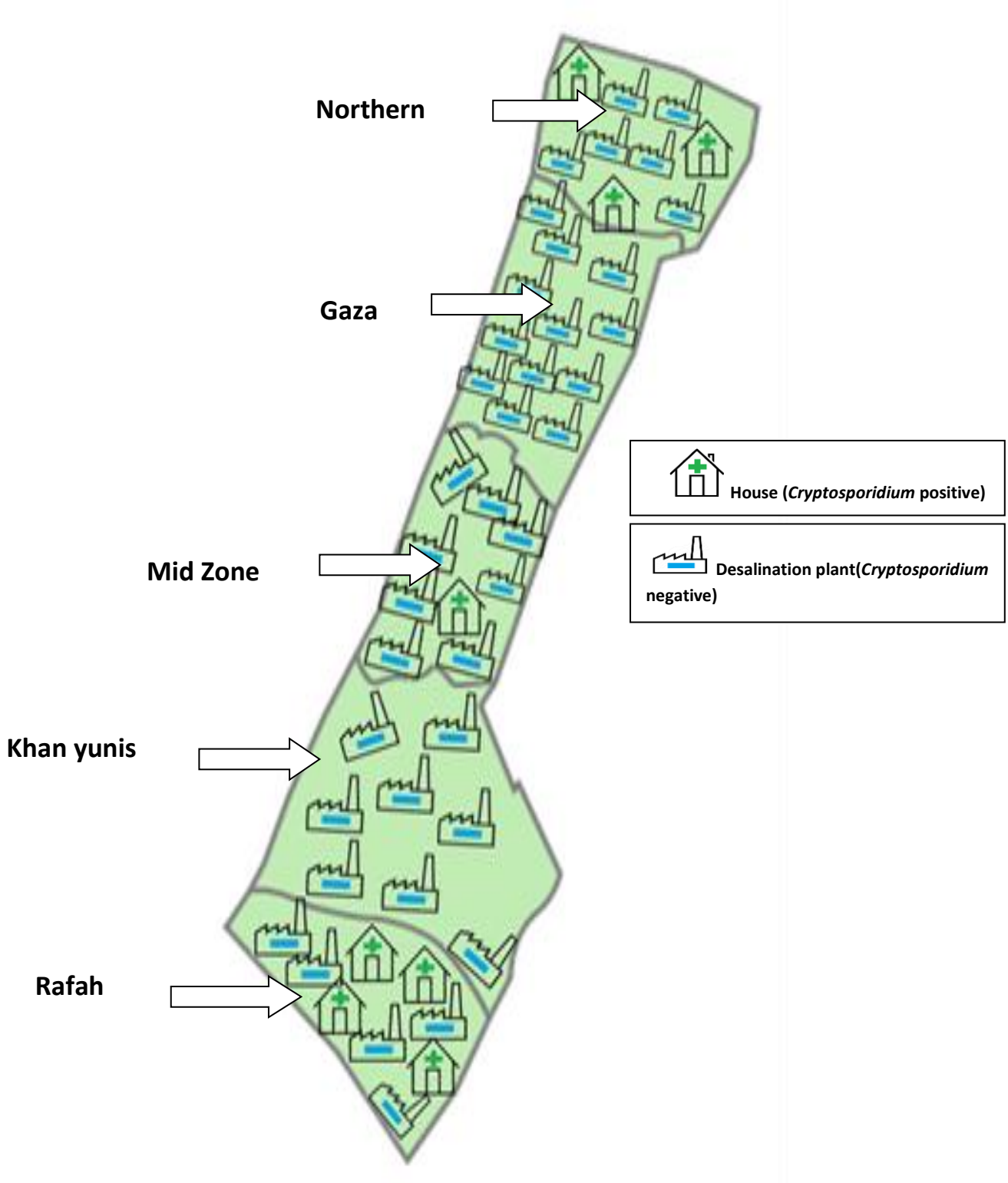

Figure (4.2): The representation of the results on Gaza Strip map

4.1.2 Detection of parasites contaminating kitchen tap water

The results of examining 100 kitchen tap water are described as follows:

No protozoa were detected in the examined samples in Gaza Strip by using all techniques Table (4.2).

Table (4.2): Detection of parasites contaminating kitchen tap water

	Number of samples	Direct Smear Positive (%)	Acid Fast Stain Positive (%)	Iron Hematoxylin stain Positive (%)
Rafah	24	0	0	0
Khan Yunis	23	0	0	0
Mid Zone	10	0	0	0
Gaza	25	0	0	0
Northern	18	0	0	0

4.1.3 Detection of parasites contaminating kitchen filtered water

The results of examining of kitchen filtered water are described as follows:

The result were negative using all examination techniques Table (4.3).

Table 4.3): Detection of parasites contaminating kitchen filtered water

	Number of samples	Direct Smear Positive (%)	Acid Fast Stain Positive (%)	Iron Hematoxylin stain Positive (%)
Rafah	24	0	0	0
Khan Yunis	23	0	0	0
Mid Zone	10	0	0	0
Gaza	25	0	0	0
Northern	18	0	0	0

4.1.4 Detection of parasites contaminating cartridge filters from desalination plants

The results of all kinds of samples were negative for parasites using direct smear, Iron hematoxyline stain and Acid fast stain Table (4.4).

Table (4.4): Detection of parasites contaminating cartridge filters from desalination plants

	Number of samples	Direct Smear Positive (%)	Acid Fast Stain Positive (%)	Iron Hematoxylin stain Positive (%)
Rafah	5	0	0	0
Khan Yunis	8	0	0	0
Mid Zone	8	0	0	0
Gaza	13	0	0	0
Northern	6	0	0	0

4.1.5 Detection of parasites contaminating inlet water from desalination plants

Water in Gaza Strip were negative for parasites when using direct smear, Iron hematoxyline stain and Acid fast stain Table (4.5).

Table (4.5) Detection of parasites contaminating inlet water from desalination plants

	Number of samples	Direct Smear Positive (%)	Acid Fast Stain Positive (%)	Iron Hematoxylin stain Positive (%)
Rafah	5	0	0	0
Khan Yunis	8	0	0	0
Mid Zone	8	0	0	0
Gaza	13	0	0	0
Northern	6	0	0	0

4.1.6 Detection of parasites contaminating outlet water from desalination plants

The results of examining the outlet water from desalination plants are described as follows:

In Rafah, Khan Yunis, Mid Zone, Gaza and Northern the results showed no parasites in all samples when using the required tests Table (4.6).

Table (4.6): Detection of parasites contaminating outlet water from desalination plants

	Number of samples	Direct Smear Positive (%)	Acid Fast Stain Positive (%)	Iron Hematoxylin stain Positive (%)
Rafah	5	0	0	0
Khan Yunis	8	0	0	0
Mid Zone	8	0	0	0
Gaza	13	0	0	0
Northern	6	0	0	0

4.1.7 The Molecular results using PCR

One sample 0.24% (1/420) was positive for *C. parvum* when PCR used Figure (4.3). One out of eight positive sample 12.5% (1/8) was identified *C. parvum* and no detection for *C. hominis* Table (4.7). All samples were screened for *Cryptosporidium* using a MZN staining technique. All negative acid fast stain samples were pooled and

every ten samples were re-examined as a one sample. This pooling showed negative PCR results for the two species used.

Table (4.7): The molecular results using PCR

	Positive acid fast stain	PCR result	
		C. parvum	***C. hominis***
Rafah	4	1	0
Mid Zone	1	0	0
Northern	3	0	0

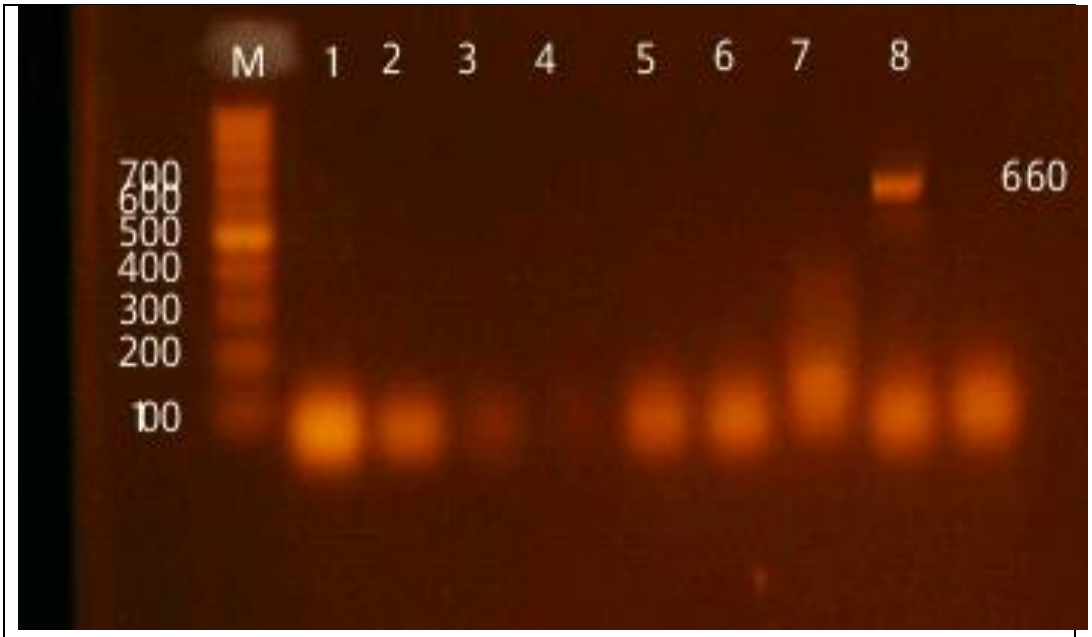

Figure (4.3): Fragment sizes of the PCR-amplified of NDIAG region for *C. parvum*. As obtained by gel electrophoresis. In the peripheral of the photograph, bands from a DNA ladder 100 bp scale (M) are shown. 1: Negative control, 2-7&9: Negative sample for NDIAG gene, 8: NDIAG gene for *C. parvum* 660 bp.

4.2 The contamination level for such protozoan and helminth parasites

In this research no helminths parasites were found in different sources of drinking water and low level of *Cryptosporidium* were detected only in RO filters when using several techniques.

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

4.3.1 Economic and social factors:

Over the study period, information were 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. All these information are represented in Table (4.8).

Table (4.8): Economic and social factors

Economic and social factors		Frequency	%
Gender	**Male**	237	59.2
	Female	163	40.8
Father's educational level	**Less than high school**	110	27.5
	High School	89	22.2
	Diploma	33	8.3
	Bachelor's Degree	168	42.0
Mother's educational level	**Less than high school**	118	29.5
	High School	130	32.5
	Diploma	46	11.5
	Bachelor's Degree	106	26.5
Place of residence	**Village**	89	22.2
	Camp	140	35.0
	City	171	42.8

4.3.2 Water sources

A majority 58% of people 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 washed 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% do not analyze water and 77.5% do not use water disinfection materials.

Table (4.9): Water sources

Water sources		Frequency	%
Source of water used in the house	**Kitchen water filter** **Municipal water** **desalination plants water** **Artesian well water**	107 51 233 9	26.8 12.8 58.2 2.2
Do you wash drinking water tank?	**Yes** **No**	251 49	62.7 12.3
How many times do you wash drinking water tank?	**At every filling** **Two times per month** **Never**	147 114 39	36.8 28.5 9.5
Drinking water tank place	**House Roof** **House Stairs** **Inside the House**	135 47 118	33.8 11.8 29.5
Is the drinking water tank covered?	**Yes** **No**	272 28	68.0 7.0
Is the drinking water tank exposed to the sun?	**Yes** **No**	168 132	42.0 33.0
Was the drinking water tested?	**Yes** **No**	155 245	38.8 61.3
Do you change the filter?	**Yes** **No**	90 10	22.5 3
Do you use water sterilization materials?	**Yes** **No**	90 310	22.5 77.5
Was the drinking water tested in the last six months?	**Yes** **No**	140 260	35 65.0

4.3.3 Symptoms of infection

Diarrhea was the most important gastrointestinal symptom as reported by the patients, Table (4.10) indicates that 17.5% of people showed diarrheal symptom and 15.8% of them have reported that the cause of diarrhea was parasites.

Table (4.10): Symptoms of infection

Symptoms of infection		Frequency	%
Does a family member suffering from diarrhea?	**Yes** **No**	70 330	17.5 82.5
Diarrhea Cause	**Unknown** **Parasites**	337 63	84.2 15.8
Type of parasites	***Ascaris lumbricoides*** ***Entamoeba hislolytica*** ***Enterobius vermicularis*** ***Giardia lamblia*** **Others**	4 16 13 5 4	1.0 4.0 3.3 1.3 1.0

4.3.4 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 nearby to the sanitation Table (4.11).

Table 4.11) Public health

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

4.4. Efficiency of the used device in filtration

RO filter of house kitchen is efficient in removing parasites from water such as *Cryptosporidium.* These protozoa (*Cryptosporidium*) were trapped at RO filter. So it can’t pass forward.

Chapter 5
Discussion

Chapter 5
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, Buck, Manque, & Ozaki, 2007). Source water monitoring for *Cryptosporidium* is applied in several countries, although usually in research rather than in routine monitoring (Figueras & Borrego, 2010). The protozoan parasite *Cryptosporidium* have been described as important waterborne-disease pathogens, and are associated with severe gastrointestinal diseases (Razzolini, da Silva Santos, & Bastos, 2010) In Gaza Strip, Cryptosporidiosis is related with their positivity in diarrheal patients (Geurden et al., 2009).

Al-Hindi, Elmanama & Elnabris, 2007, found that 14.9% of children in El-Nasser Children Hospital were infected by *Cryptosporidium*. Sallon et al., 1994, found that in Gaza Strip 14.6% of children less than 5 years were infected by *Cryptosporidium* spp. In our research , a total of 8 (1.9%) out of 420 samples of various sources of drinking water were infected by *Cryptosporidium* oocysts that collected during one year study period. 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, Hindyia, & Saiah, 2004). Another similar study was in Saudi Arabia which showed that 6.8% were positive for *Cryptosporidium* in Water from filling stations (Zakai & Barnawi, 2014). However, it was different from that study for (Khalifa, Ibrahim, Said, Abdel Aleem, & Nabil, 2011). In Alexandria in water tank which was 63%. 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 100% in Addis Ababa (Fikrie, Hailu, & Blete, 2008), 46.2% in Taiwan water sample (Hsu, Huang, & Hsu, 2001), 35% in filtered water in Japan (Hashimoto, Kunikane, & Hirata, 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, Schoenen, & Seitz, 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, Lora, Zuluaga, & Gomez-Marin, 2016), 9% in Norway in raw water (Robertson & 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 (P Karanis et al., 2006), 13.3% in Hungarian in drinking water (Plutzer, Tako, Marialigeti, Törökné, & Karanis, 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 towers 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 people. In our research, *Cryptosporidium* oocysts were found in RO filtered samples and no contamination found in tap and filtered water which 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, El Naga, El-Temsahi, & Negm, 1998). MZN is the most sensitive in detecting these

parasites comparing with other stains. Several studies confirmed MZN method safety, sensitivity, accuracy and simplicity (Khalifa et al., 2011).

Many countries are routinely monitoring water using other techniques to identify these parasite. 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; Fikrie, Hailu, & Blete, 2008; Razzolini et al., 2010).

In Egypt, they used the flow cytometry technique which is the best since it is 100% sensitive. However, it is more expensive than other techniques. This technique is used to detect the viability of *Cryptosporidium* (Khalifa et al., 2011).

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. Positive PCR results were only achieved in a sample that came from RO and it was also positive when MZN were used.

Morgan et al., 1998 found that microscopy (MZN) therefore exhibited 83.7% sensitivity and 98.9% specificity compared to PCR. An important benefit of the PCR test is its ability to directly differentiate between different *Cryptosporidium* spp. (Morgan et al., 1998).

Cryptosporidium species are protozoan parasites that infect humans and a wide variety of animals. *C. hominis* and *C. parvum* are the most frequently observed in intestinal infections in humans (Mercado et al., 2007).

C. parvum (zoonotic) and *C. hominis* (anthroponotic) are the most common human-infecting species reported in river water samples in Europe (Xiao & Fayer, 2008).

In this research, specific primers were used to identify the most two common *Cryptosporidium* species, *C. parvum* and *C. hominis*. The PCR results were positive just for *C.parvm.* In our research just one sample 0.24% (1/420) was *C. parvum* confirmed using PCR but negative for *C. hominis*.

Sensitivity tests were set in the laboratory for the PCR reactions and the number of 50 cysts or oocysts were our limit of sensitivity. For this reason, the great majority of drinking water samples with lower parasite load were out of selection for the PCR amplification. Furthermore, in the remaining samples subject to PCR, subjected to DNA extraction and PCR, the amplification did not occur(Jiang, Alderisio, Singh, & Xiao, 2005). This means that the possibility of the presence of *C. parvum* and *C. hominis* in drinking water exists but in few numbers.

In Spain, (Dellundé, Pina, Jofre, & Lucena, 2002) 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.

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 were found a reasonable correlation between the rainfall and the presence of *Cryptosporidium* oocyst (Carmena et al., 2007). In Addis Ababa city, they were observed that increments of the oocysts might be due to urban input such as contamination with human feces (Fikrie, Hailu, & Blete, 2008). 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) In Portugal, the surface water collected from the rivers is used as drinking water for the animals or is used for agricultural purposes, by the majority of farmers, the feces are directly released into the rivers or reach it by runoff waters. In Norway, the cause of *Cryptosporidium* water contamination presence of high numbers of domestic animals within the catchment

area (Robertson & Gjerde, 2001). 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 since there is no efficient sewage system available in both the cities (Zakai & Barnawi, 2014).

Chapter 6
Conclusions (and/or Recommendations)

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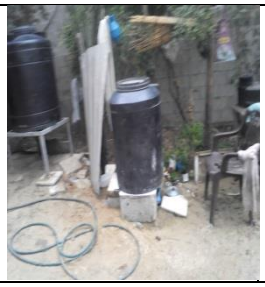
Conclusions (and/or Recommendations)

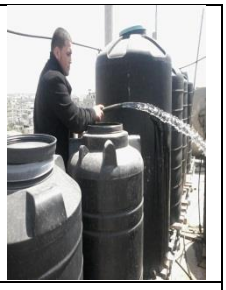
1. *Cryptosporidium* oocysts in water sources is a public health concern. Even the occurrence though concentrations of *Cryptosporidium* oocysts was not high in the analyzed samples, the results brought by this research highlight the need to monitor the presence of these organisms in drinking water.
2. This research could serve as a base line epidemiological surveillance of waterborne parasites in Gaza Strip. Moreover, this study could further help to create more awareness among public in general and policy makers in particular as water contamination is being a key health issue in the region. Studies with high volume of drinking water samples for analysis should be taken into consideration.
3. Presence of *Cryptosporidium* in drinking water supplies of the examined sites Rafah, Mid Zone and Northern make people more exposed to the risk. Future studies should be carried out in order to provide an extensive platform for risk assessment with the occurrence of parasites contamination in the water environment.
4. Sanitary survey should be done because it is the basis for effective strategies for the prevention and control of risk. Assessment of risk events includes understanding the characteristics of the drinking water system. What hazards may arise and from which sources? How these hazards create risks? And what is the efficacy of processes and practices that affect drinking-water quality? The complete system from catchment to tap should be described and analyzed for events/conditions that could lead to contamination of the water supply.
5. Research is required to understand the effect of repeated sampling of both source and stored water that results from variability over time (e.g., seasonality) and replicate sampling (sequential testing). Given their potential to inform assessments of safety of drinking water.
6. There is also a need to a better understanding of the role of water collection and storage on parasitology contamination and the associated risk to health.
7. The detection and enumeration methods for *Cryptosporidium* in environmental samples still need a considerable improvement. The high specificity of genotyping

methods are evolving rapidly and should be implemented in environmental surveys to increase our understanding of the environmental transmission of human cryptosporidiosis.

8. The public should be aware towards drinking water (clean + safe).
9. Awarness programs should be delivered to the public.

Future research

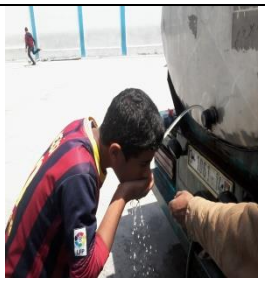
Monitoring the distribution and selling of drinking water in Gaza Strip





Establishment of legistic, guidelines and law for drinking water selling to homes.






Health education sessions to drinking water vendors in Gaza Strip.

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Desalination Plants Filters in Gaza Strip

3- Gender	2- Age	1- Name

Northern	Gaza city	Mid Zone	Khan Younes	Rafah	5	Father's Educational Level				4	**Economic and social factors**
						Bachelor's Degree	Diploma	High School	Less than high school		
Place of residence					7	Mother's Educational Level				6	
city	camp	village				Bachelor's Degree	Diploma	High School	Less than high school		
Name of station from which you buy drinking water					9	Source of water used in the house				8	**Water Sources (8-18)**
						Artesian well water	water stations water	Municipal water	Kitchen water filter		
How many times do you wash drinking water barrel?					11	Do you wash drinking water barrel?				10	
Never	Two times per month	At every filling				no	yes				
Does the drinking water barrel covered?					13	Drinking water barrel place				12	
no	yes					Inside the House	House Stairs	House Roof			
Was the drinking water tested?					15	Does the drinking water barrel exposed to the sun?				14	
no	yes					no	yes				
When did you change the filter					17	Do you change the filter?				16	
						no	yes				
Was the drinking water tested in the last six months?					18	Do you use water sterilization materials?				17	
no	yes					no	yes				

	Diarrhea Cause parasites / Unknown	20	Does a family member suffering from diarrhea? No / Yes, mention the number of patients	19	**Symptoms of**
Patient age in years 21 and above / 20-10 / 10-5 / Less than 5		22	Types of parasites Ascaris Entameob Giardia Enterobius OTHERS	21	
The distance between drinking water and sanitation Far away / Nearby / Very close		24	Is there a relationship between health and contamination of drinking water? I don't know / no / yes	23	**Public Health**
Are the garbage scattered in front of your home? no / yes		26	Do you think that the cause of diarrhea is water contamination? I don't know / no / yes	25	

Appendix 2: DNA extraction method
ISOLATE II Genomic DNA Kit BIOLINE

1 - Pre Lysis

Add 25µl proteinase k solution and 200µl Lysis Buffer G3 to 200µl sample then incubate at 70°C for 1-3 hours.(Incubate 10 min at 95°C to Lyse parasite).

2- Lyse sample

Vortex briefly and add 200µl Lysis Buffer G3.

Vortex vigorously and incubate at 70°C for 10 minutes.

3- Adjust DNA binding condition

Vortex briefly and added 200 ml lyses buffer G3.

Vortex vigorously and incubate at 70c for 10 minutes.

4- Bind DNA

Place ISOLAT II Genomic DNA spin column (green) in 2ml collection tube.

Load sample to column and centrifuge 1 min at 11,000 X g.

Discard flow thought reuse collection tube.

5- Wash silica membrane

Added 500 Ml wash buffer GW1.

Centrifuge 1 minute at 11,000 X g.

Added 600 Ml wash buffer GW1.

Discard flow thought reuse collection tube.

6- Dry silica membrane

Centrifuge 1 minute at 11,000 X g, to remove ethanol.

Place ISOLATE II Genomic DNA Spin Column in a 1.5 ml micro centrifuge tube (not supplied).

7- Elute DNA

Add 100µl preheated Elution Buffer G (70°C) onto center of silica membrane at room temperature for 1 minute.