Toxoplasma gondii detection and genotyping in the Environmental matrices: soil and water in Gaza, Palestine

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Abstract

The aim of the current study was to determine the prevalence and genotypes of *T. gondii* oocysts in soil and water samples from Gaza, Palestine. For this purpose290 environmental samples, including 200 from soil and 90 from water, were collected. Soil samples were collected from Wadi-Gaza, vicinity of trash dumpsters, residential home yards, open-air marketplaces, public squares and gardens. Water samples were collected from Wadi-Gaza, wells used for drinking and irrigation. The samples were examined utilizing PCR and Sheather's solution techniques and genotyping of 4 samples was performed by PCR-RFLP. The results revealed that out of the 290 investigated samples, 31 (10.68%) proved contaminated by Sheather's solution, whereas 22 (7.58%) were positive by PCR. Genotyping results showed that all of the four tested samples belonged to *T. gondii* Type I. The highest rate of contamination was detected in the samples collected from residential house yards, while public squares and gardens represented the lowest rate. Regarding the water samples, *T. gondii* oocysts were detected only in the samples collected from Wadi-Gaza. This work confirms the presence of Type I *T. gondii* in the soil and water of the study area, with soil contamination being heavier than that of water.

Introduction

T. gondii is an obligate intracellular protozoan replicating with a three-stage life cycle that is infectious to all warm-blooded animals(Robert-Gangneux and Dardé 2012; Maenz et al. 2014).Sexual reproduction occurs exclusively in the feline definitive host, leading to the formation of oocysts that are excreted in their feces. After sporulation in the environment, oocysts (the environmental stage) become infective. *T. gondii* oocysts are resistant to extreme environmental, physical, and chemical conditions and can survive for a long time in the environment (Robert-Gangneux and Dardé 2012; Dumètre and Dardé 2003). Ingestion of sporulated oocysts by intermediate hosts results in the release of sporozoites, which pass through the intestinal epithelium and develop into tachyzoites. This is followed by tachyzoite spread through body fluids, including enormous and rapid asexual reproduction in nucleated cells of the host (Robert-Gangneux and Dardé 2012; Maenz et al. 2014). This stage of infection is distinguished by a significant inflammatory response and host tissue damage, which can lead to clinical signs of toxoplasmosis. After the host's immunological response has progressed, replication slows, and the now-called bradyzoites settle in host cells of the retina, brain, skeletal, and cardiac muscles (Blanchard et al. 2015). The parasite may avoid the immune system and lives a lifespan within the host by forming tissue cysts. Humans are susceptible to *T. gondii*infection, which may be acquired horizontally by ingestion of oocysts via infected food (vegetables, fruits, and water), infection after gardening, or direct contact with cat feces. Consumption of raw or undercooked meat harboring highly infectious tissue cysts, can also result in oral-alimentary transmission)Tenter et al. 2000; Dias et al. 2005). A Research conducted in 1999 showed that drinking water functioned as a vehicle for oocyst transmission among Jains, a vegetarian group in India (Hall et al. 1999). Besides, a large outbreak of toxoplasmosis was recorded in Brazil in 2001 and 2002 as a result of consuming water from municipal sources (De Moura et al. 2006). Certainly, a variety of environmental conditions contribute to the spread of *T. gondii*. These serve as a vector for the

parasite's transmission to surface water sources, some of which include heavy rains and winds(Mac Kenzie et al. 1994). Toxoplasmosis is the third most common food-borne disease, behind salmonellosis and listeriosis (Jones et al. 2001; Dubey and Jones 2008), as well as being one of the most widespread zoonotic diseases. It is believed that about 30% of the human population is infected with *T. gondii* (Maenz et al. 2014). While the seroprevalence of this parasite is expected to be greater than 50% in regions where raw or undercooked meat is often consumed. Furthermore, high seroprevalence is prevalent in tropical parts of Latin America and Africa, where cat populations are considerable and climatic conditions are favorable for oocyst survival (Jones et al. 2001b; Di Carlo et al. 2008; Dubey et al. 2009). It is estimated that 15% of childbearing women aged 25 to 44 years in the United States are infected with *T. gondii*. Meanwhile, it is reported that between 400 and 4,000 cases of congenital toxoplasmosis occur annually (Jones et al. 2001b). *T. gondii* was also ranked fourth in an assessment of the worldwide significance of 24 food-borne parasites based on socioeconomic and trade impacts(FAO/WHO 2014). Furthermore, occasional outbreaks of waterborne toxoplasmosis have been observed in various places of the world e.g., Panama, British Columbia, and Brazil (Benenson et al. 1982; Bowie et al. 1997; Lass et al. 2009). Despite the fact that contaminated drinks and foods constitute the significant risk factors, a variety of other factors, including poor socioeconomic and educational levels, do also contribute to the occurrence of toxoplasma outbreaks (Ferreira et al. 2018). Numerous studies have been conducted to investigate the seroprevalence of *T. gondii* infection in pregnant and abortion-experiencing women in Palestine. According to Al-Hindi and Lubbad (2009), the occurrence of *T. gondii* specific antibodies was 12.8% for IgM among 312 women who had abortions in Gaza-Palestine (Al-Hindi and Lubbad 2009).While among 204 pregnant women in Hebron-Palestine, a high seroprevalence of 27.9% was recorded with women from rural areas being more affected than those from urban areas (Nijem and Al-Amleh 2009). Furthermore, in Gaza, Al-Jarousha (2012), reported 30.9% seropositivity among 255 pregnant women (Al-Jarousha 2012). The subject study's goal was to detect *T. gondii* oocysts in environmental samples, such as soil and water, in Gaza, Palestine, using conventional (Sheather's solution) and molecular (PCR) techniques, as well as to determine the genotype of some of the isolates.

Materials And Methods

Study area and samples collection

A total of 200 soil samples were randomly collected, with 40 samples taken from each of the following locations: Feras market, garbage dumpster premises, Wadi-Gaza, public squares and gardens, and private house yards. The 90 water samples were collected from Wadi-Gaza stream, drinking water wells, and the city’s waste water treatment plant as shown in table (1), below which outlines the number of samples and the corresponding locations from which they were collected.

Table 1. Type, source, and number of samples collected in the current investigation.

Locality	Type of sample	Number of samples
Wadi – Gaza	Soil	40
	Water (Running)	30
Trash dumpster premises	Soil	40
Houses yards	Soil	40
Public squares and gardens	Soil	40
Feras market	Soil	40
Sewage treatment plant	Water (Sewage)	30
Wells	Water (Household)	30
Total soil samples		200
Total water samples		90
Total environmental samples		290

Wadi Gaza is a water stream that originates in the mountains of Hebron (a Palestinian city located in the east of Gaza), flows through Gaza for about 8 kilometers, and then empties into the Mediterranean Sea (Madi 2005). Soil samples from Wadi Gaza were obtained precisely from the location where Wadi Gaza crosses the Salah El Deen bridge to roughly 1 km east, this is illustrated in Figure 1 below. The Feras Market, a significant local vegetable and other commodities market in downtown Gaza, was the second location where soil samples were collected. This market is regarded as the most popular and largest in the city. Furthermore, samples were obtained from soil near garbage bins in various active regions of the city. Following a rapid examination of certain residences where domestic and stray cats appeared to be in plenty, selected houses were picked for the purpose of collecting soil samples from their yards. Lastly, soil samples were collected from numerous public gardens and sites after monitoring them and taking into account their high cat population. Water samples were also obtained from various areas across the city. First, water samples were collected from Wadi-Gaza, which included a combination of sewage and rainfall. Water samples were also taken straight from a sewage treatment plant west of Gaza. Ultimately, they were collected from wells used for irrigation and household use, which are dispersed across the city and its neighboring suburbs.

Detection of *T. gondii* oocysts in soil and water:

From July through December of 2019, 290 soil and water samples were collected from the aforementioned locations. The methods used for analysis of soil samples were as described by Lass et al. (2009) and Colli et al. (2010). With minor modifications. Specifically, each soil sample was 300g in weight, taken at a depth of 2 to 5 cm below the surface layer, and dried for two days at room temperature.

A total of 40 grams of each sample were mixed with 250 ml of distilled water before being filtered through a sieve with 75 µm pores. After straining, the liquid was placed into a 250 ml cylinder tube and left overnight. The next morning, 4 ml of liquid from the surface layer, (supernatant layer), was poured into 8 ml of sediment layer collected at the cylinder's base. This 12 ml mixture was then transferred to a centrifuge tube and centrifuged at 2500 rpm for 10 minutes. Following that, 2 ml of the mixture was collected from the tube's surface layer and mixed with the sediment in order to be microscopically examined using the Sheather's solution flotation technique (Lass et al. 2009; Colli et al. 2010). Regarding water samples, 90 samples containing 500 ml of water (30nfrom each of the locations mentioned previously) were collected. Samples were filtered using a Büchner funnel with a 0.45 µm nylon filter membrane. Using saline, the membrane was washed and then examined through the light microscope employing Sheather's solution. Contaminated samples were put into Eppendorf tubes and stored at -20 °C for further PCR work. Meanwhile, samples showing no contamination were confirmed as negative using the PCR approach as reported by Papaiakovou et al (2019). (Papaiakovou et al. 2019)

Molecular Identification and Genotyping of *T. gondii*

DNA Extraction

The Wizard Genomic DNA extraction kit A1120 (Promega, Madisson, Wisconsin, USA) was used as directed by the manufacturer to extract DNA from the resuspended pellets. To easily detect the specified gene from the purified DNA, the final DNA pellets were resuspended in a 30 µL TE buffer (10 mMTris, 1 mM EDTA, pH 7.2) and preserved at -20 °C until the PCR procedure was completed (Amairia et al. 2016).

PCR Primers and Conditions

The PCR reaction was performed using a set of primers-TOXO1 (5' GGA ACT GCA TCC GTT CAT GAG 3') and Toxo2 (5' TCT TTA AAG GGT TGG TGG TC 3') reported by Lass *et al*. (2009). These premiers were particularly designed to target the 194 bp region of the 35-fold-repetitive B1 gene. To validate positive results, all positive samples were reexamined using TOXO-F (5' AGG GGA GGG TGA GGA TGA 3') and TOXO-R (5' TGG TCT CGT CTG GAT CGC AT 3') primers. These are especially unique to a 200 to 300-fold-repetitive element (REP) sequence segment (AF146527). Following that, all components were placed in the AccuPower PCR PreMix tube (Bioneer Corporation-Hylabs). For each isolate, PCR amplification of TOXO genes was carried out in a thermal cycler (Biometra, Germany). The following conditions were considered: a 5-minute initial denaturation phase at 95 °C, thermocycling for 30 cycles, with each cycle consisting of 30 seconds at 94 °C followed by 30 seconds at 55 °C for annealing, 45 seconds at 72 °C for extension, and a final extension cycle of 10 minutes at 72 °C (Lass et al. 2009; Hassanain et al. 2013; Dardona et al. 2021). Distilled water was used as a negative control, while a previously identified and well-defined *T. gondii* sample was used as a positive control. The PCR work was conducted in the molecular biology laboratory of the health sciences department at the Islamic University of Gaza.

Genotyping

The PCR-RFLP method described by Norouzi et al. (2016) was implemented for genotyping four of the PCR-positive samples. Briefly, a 791 bp DNA fragment was amplified using the GRA6 primers (5 -$GTAGCGTGC$ T G T $GGCGAC$ - 3) and (5TACAAGACATAGAGTGCCCC-3′). The fragment was then digested by the restriction enzyme *MseI*. The digestion products distinguish the different types of Toxoplasma gondii. Products of 168 and 544 bp, 75 and 623 bp, and 97 and 544 bp fragments identify type I, type II and type III, respectively (Norouzi et al. 2016).

Statistical Analysis

Analysis performed on the results drawn from the subject study was carried out through using the SPSS (USA, Il, Chicago, SPSS Inc) software package v. 15.0. Chi-square tests were performed to highlight the comparison between contamination of *T. gondii* oocysts and two other variables, the environmental samples from which they were obtained, and the months in which they were collected (July to December of 2019).A p-value less than 0.05 was considered significant.

Results

The present study results revealed that *T. gondii* oocysts had a conspicuous prevalence in some of the examined environmental samples. More precisely, 22 (7.58%) samples out of the 290 tested water and soil samples were found to be contaminated with oocysts according to the PCR technique, while 31 (10.68%) were positive according to Sheather's solution. Of the PCR-positive samples 18 were soil samples and four water samples. In the case of Sheather's solution, 26 of the 31 contaminated samples were from soil and 5 were from water samples. The distribution and comparison of the data obtained from the two techniques employed in the study are shown in Table (2) below. The current investigation concluded that water samples collected from Wadi Gaza were the only source of oocyst contaminated water, despite the fact that PCR and Sheather's solution revealed 4 and 5 contaminated samples, respectively. In addition, *T. gondii* oocysts were not detected in samples collected from wells or the sewage treatment facility. On the other hand, contamination has been documented by every source of soil samples. The greatest rate of contamination was seen in samples acquired from residential home yards, with 9 and 7 samples detected by Sheather's and PCR, respectively. Feras market and Wadi Gaza ranked second and third in terms of the level of oocyst contamination, with 5 samples per Sheather's solution for both of them, and 5 and 3 samples per PCR, respectively. Following that, soil samples were collected from the premises of garbage dumpsters, with 4 and 2 samples, respectively, according to Sheather's and PCR. Lastly, samples collected from public gardens and squares had the lowest contamination rate, with two samples per Sheather's solution and one sample per PCR (Table2). As shown in Table (2), there is a significant difference in the incidence of *T. gondii* oocysts in the various environmental samples studied by both techniques and their associated location sources. In terms of the month in which the samples were gathered, the subject study's findings showed an interesting trend. The highest number of contaminated samples was found in December, followed by November, October, September, August, and July (Table 3). The month of July had the lowest rate of contaminated samples.

Table 2

Prevalence of *T. gondii* oocysts in the environmental samples, based on the source of sample using both PCR and Sheather's techniques.

Environmental Sample (Type)	Sample (No)	Positive (Sheather's)	Positive (PCR)
Wadi –Gaza (Running water)	30	5	4
Sewage treatment plant (sewage water)	30	0	0
Wells (drinking and irrigation)	30	0	0
Total water samples	90	5 (5.55%)	4 (4.44%)
Wadi – Gaza (Soil)	40	5	3
Trash dumpster premises(Soil)	40	4	**2**
House yards (Soil)	40	9	7
Public gardens (Soil)	40	3	1
Feras market (Soil)	40	5	5
Total soil samples	200	26 (13.00%)	18 (9.00%)
Total (Water and Soil)	290	31	22
% positive		10.68%	7.58%
$\chi 2$ (PCR) = 16.79 P = 0.01 P < 0.05. Statistically significant			
$\chi 2$ (Sheather's) = 14.86 P = 00.038 P < 0.05. Statistically significant			

The relationship between sample contamination with oocysts by both techniques and the months in which samples were collected had demonstrated no statistical significance, as indicated by the findings in table (3) below. Figures (2) below presents a photograph of ethidium bromide stained agarose gel illustrating some of the PCR-positive *T. gondii* oocyst samples.

Table 3
Monthly prevalence of *Toxoplasma gondii* oocyst in the environmental samples in Gaza city during the period July-December 2019.

Month	Samples (No)	Soil (No)	Water (No)	(+ Ve) Sheather's	(+ Ve) PCR
July	43	28	15	3	1
August	67	41	26	2	2
September	20	12	8	4	2
October	57	50	7	7	4
November	60	42	18	8	6
December	43	27	16	7	7
Total	290	200	90	31 (10.68%)	22 (7.58%)
$\chi 2$ (PCR) = 9.047 P = 00.107 P > 0.05. Not Statistically significant					
$\chi 2$ (Sheather's) = 8.600 P = 00.126 P > 0.05. Not Statistically significant					

Regarding the PCR-RFLP genotyping, figure (3) below shows that the *MseI* digestion products are 544 and 168 bp thus indicating that the four samples tested are of Toxo-type I.

Discussion

The study's main purpose was to detect *T. gondii* oocysts in environmental samples, particularly soil and water. Given that the majority of *T. gondii* infections in pregnant women occur as a result of soil contact, as soil is assumed to be an important environmental factor in human infections (Ajmal et al. 2013). According to a European research, 17% of *T. gondii* infections are caused by contact with contaminated soil, as demonstrated by a direct survey of women infected with the parasite. Because oocysts can remain infectious in soil for more than a year, contaminated soil is seen as a serious risk factor in the parasite's spread (Lass et al. 2009). Indeed, soil is an important environmental source of *T. gondii* infection in humans and animals (Cook et al. 2000; dos Santos et al. 2010). This is corroborated by the findings of the present investigation, which revealed the existence of oocysts in all of the locations where soil samples were taken. Because oocysts are exclusively excreted through cat feces, and every site where samples were taken included cats. Because stray cats are found in almost every area of Gaza, particularly in Wadi Gaza and the surrounding agricultural lands, where there are abundant food sources and this explains the highest rate of contamination detected in Wadi-Gaza and residential home yards. Many of the cats found in those areas tend to be infected with *T. gondii e.g.*, Al-Hindi et al (2019). documented the occurrence of *T. gondii* oocysts in the feces of some stray cats in a study conducted in Khanyounis Governorate, located in the south of Gaza City (Al-Hindi et al. 2019). Since they are stray cats, they may easily move from one region to another, making them significant risk factors for *T. gondii* oocyst transmission and contamination of soil and flowing water. This is understandable considering

that cats, as previously stated, play a role in the dissemination of oocyst. The results of the current study are consistent with those of a comparable study conducted in Poland, which showed the existence of oocysts in 18 (17.8%) of 101 soil samples using the PCR technique. Furthermore, another study conducted in China reported that 34 (12.69%) of 286 soil samples were contaminated with *T. gondii* oocysts (Lass et al. 2009; Du et al. 2012). The present study's findings revealed that the surface water stream, Wadi Gaza, was contaminated with oocyst. This water stream is a mix of rain and sewage water that flows from east to west across Gaza and into the Mediterranean Sea. The presence of cats beside the water stream can be blamed for the contamination of this water. This creek provides nutritional resources to cats due to people's terrible habit of dumping waste along the stream, as well as the existence of local and immigrant birds, rats, and decaying animal corpses. Besides, other environmental elements that impact the spread of oocysts in surface water sources include rain, wind, soil, and mechanical vectors such as insects, flies, and earthworms (Dumètre and Dardé 2003). Other water samples collected from other locations were found to be free of oocyst contamination. This can be attributed to the absence of infected cats and other variables previously identified as being important for oocyst dissemination. The wells and the water treatment plant were correctly closed to avoid the existence of cats or the effect of other factors capable of spreading these oocysts. Another study conducted in the study area regarding the detection of parasitic contamination in the water used for kitchen purposes and carried out by AL-Hindi et al. (2021) confirms the results of the current study, as their study did not record the occurrence of *T. gondii* oocysts in this water and only recorded the occurrence of *cryptosporidium* (AL-Hindi et al. 2021). It is also commonly known that the water utilized for household purposes in that area is sourced from wells distributed around the area. On the other hand, *T. gondii* was not regarded as a serious aquatic pathogen, in contrast to *cryptosporidium* (Mac Kenzie et al. 1995). In view of current toxoplasmosis outbreaks in people, the topic study emphasized the significance of oocyst contamination in environmental samples. The findings of the current study were comparable to those of a study conducted in France, where oocysts were found in 37 (7.7%) of 482 environmental water samples (Aubert and Villena 2009). According to the interpretations formed by the author of the French study based on the study's data, the major cause of this contaminated water was contaminated soil rinsed by rain water. This view, in fact, confirms the conclusion obtained from the subject study's findings. Rainwater combined with dirt and sewage may have played a significant part in the oocyst contamination of Wadi Gaza, the primary surface water stream that runs for a long distance before emptying into the Mediterranean. To investigate the authenticity of this phenomenon, water samples from Wadi Gaza were obtained just one kilometer before its endpoint, the Mediterranean Sea. Given the length of the stream, this collection point enabled the subject researchers to recognize and quickly investigate the potential impact of soil and other environmental elements on water contamination. Not to mention, the topic investigation indicated a congruent conclusion based on comparable results obtained from a study conducted in Brazil, which discovered 3 (7.7%) of 39 environmental water samples collected from surface water streams were utilized largely for drinking water (Galvani et al. 2019). This research, as well as the previous ones, corroborates the existence of oocyst contamination in surface water sources, which is regarded as a high contamination rate, particularly when utilized for irrigation. The current investigation is one of the few that have been

conducted to detect oocyst contamination or even parasites in the Gaza environment. In their study, Dardona et al. (2021) recorded the occurrence of *T. gondii* oocysts in raw leafy vegetables, which are commonly consumed raw in Gaza. One of their interpretations of their findings is that soil or water contamination, as well as some environmental factors, play a significant role in oocyst spread to vegetables (Dardona et al. 2021). Another study conducted in the study region by Hilles et al. (2013), recorded the contamination of the Gaza shoreline with certain parasites without mentioning *T. gondii* among the detected parasites (Hilles et al. 2013). Nonetheless, the small differences in the results obtained by different research conducted in different cities and countries might be attributable to a variety of factors. First, there are the various detection procedures used; second, there are the various environmental elements that aid in the transmission of oocysts; and finally, there is a variation in cat population rates and presence in the neighborhood of where the samples were gathered. Even before 1970, when the *T. gondii* life cycle was established, there was a disagreement over the relevance of the epidemiological components of *T. gondii* infection, whether by ingestion of oocysts or tissue cysts (Jackson and Hutchison 1989). Although the relationship between the contamination of soil and water samples and the month in which the samples were collected was not found to be statistically significant, it can be noted that the highest contamination rates were during the months of November and December, and the lowest levels of contamination were during the months of July and August. This is easily explained by the fact that the quantity of rain that falls during the winter raises the average water level in the valley's stream, increasing the speed of the valley's run-off, which transports possible sources of contamination. Furthermore, the high level of the stream causes flooding, and the water floods the agricultural lands located on the valley's boundaries. This further increases the rate of contamination. Furthermore, the possibility of the previously stated environmental factors, such as rain water and wind, in the transmission and dissemination of *Toxoplasma* oocysts rises during the winter (Dumètre and Dardé. 2003). The first observation made upon discovery was that both vegetarians and non-vegetarians are susceptible to *T. gondii* infections, suggesting that carnivorism was not the only source of *T. gondii* infections (Dubey 2009; Shapiro et al. 2019). The degree of *T. gondii* infection in cats is determined in conjunction with the degree of infection in birds, rodents, and other sources of cat food. Just as the incidence of oocysts in the environment is increasing, contamination of surface water leading to sea water constitutes a concerning issue (Sibley and Boothroyd 1992; Afonso et al. 2007). Infected cats defecate millions of oocysts in soil after swallowing only one tissue cyst or bradyzoite, and the sporulated oocysts stay infectious in soil for up to 18 months, independent of temperature exposure. Despite this, finding oocysts with a light microscope was sufficient for significantly contaminated samples. However, the molecular technique is more tempting because of its high sensitivity and rapidity in detecting *T. gondii* by PCR amplification of the B1 gene (Dumètre and Dardé, 2003; Dubey, 2009).The current study, on the other hand, employed a PCR-RFLP assay on the GRA6 gene to genotype four DNA isolates. It is worth noting that this is the first study in the research area that tackled the genotyping of *T. gondii* isolates, and GRA6 was used in the parasite genotyping process because it is a coding region with a larger and more diverse polymorphism than other regions such as SAG1, SAG2, and GRA4 (Norouzi et al. 2016). Furthermore, the current study's findings revealed that all of the analyzed oocyst DNA isolates belonged to the Toxo-Type I. The genotyping of the parasite is regarded as a critical component in

generating the disease, as demonstrated by several mouse model trials, and it has been documented in greater detail that genotype I strains are very virulent, but the other kinds are not harmful to a considerable extent (Sibley and Boothroyd 1992.). It is crucial to note that *T. gondii* DNA isolates were categorized based on their virulence in outbred mice, but the first phylogenetic investigations of *T. gondii* strains revealed that their complexity was significantly lower than predicted (Dardé et al. 1992; Sibley et al. 1992). One of the most essential aspects of genotyping is its application in tracking epidemics (Ajzenberg et al. 2004). It has been demonstrated that there are large geographical variations in the distribution of *T. gondii* genotypes. For example, certain investigations performed in Spain and Portugal have reported the existence of types I and III in these locations (Fuentes et al. 2001; de Sousa et al. 2006). On the other hand, the findings of investigations conducted to assess the genotyping of *T. gondii* for isolates in both Crete and Cyprus reported the existence of type III in both areas (Messaritakis et al. 2008). To round out the findings, French research revealed that only four of 86 *T. gondii* isolates belonged to genotype I. On the contrary, genotype I was shown to be extremely dominant in one of the investigations done on the isolated strains of CSF of eight patients with acquired immunodeficiency in the United States, as the findings of this study revealed that the majority of them were infected with *T. gondii* genotype I (Ajzenberg et al. 2004; Khan et al. 2005). On the other hand, in a study conducted in Brazil to identify the genotyping of *T. gondii* isolates reported in water supply samples, after genotyping them based on the SAG2 locus, it was reported that genotype I is very pathogenic (De Moura et al. 2006).

Conclusion

The current study concluded that *T. gondii* oocyst contamination in soil is greater than in water. The highest rate of soil contamination was detected in samples obtained from private home yards, whereas the lowest rate was reported in public squares and gardens. The contaminated water samples, on the other hand, are solely from flowing water samples gathered from Wadi Gaza, whereas water collected from other sources was confirmed to be clear of contamination. Finally, this investigation revealed that all genotyped samples belonged to *T. gondii* type I.

Declarations

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Competing interests

Not applicable

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Not applicable

Availability of data and material

The authors affirm that the data supporting this study are available within the article and that raw data supporting the study's findings are available from the first author, Zuhair Dardona, upon reasonable request.

Authors' contributions

1- Zuhair Dardona (Practical work– writing – statically analysis).

2-Adnan Al-Hindi (writing – review)

3- Samia boussaa (writing- review)

4- Mohamed Hafidi (Review)

5- Ali boumezzough (Reviw)

6- Fadel Sharif (Genotyping – review)

Ethics approval

On August 5, 2019, the ethical research committee of the Islamic University of Gaza approved an ethical clearance for this study, which planned to gather water and soil samples from the study area.

Consent to participate

All of the authors have willingly agreed to take part in this research project.

Consent for publication

All authors consent to the publishing of identifying facts, which may include photographs, details within the text ("Material") data, and everything inside this paper, in a parasitology research journal.

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Figures

Figure 1

The location of Wadi Gaza (El-Hallaq 2019), the start of the arrow indicates the site of sample collection, whereas the arrow head points to the sea.

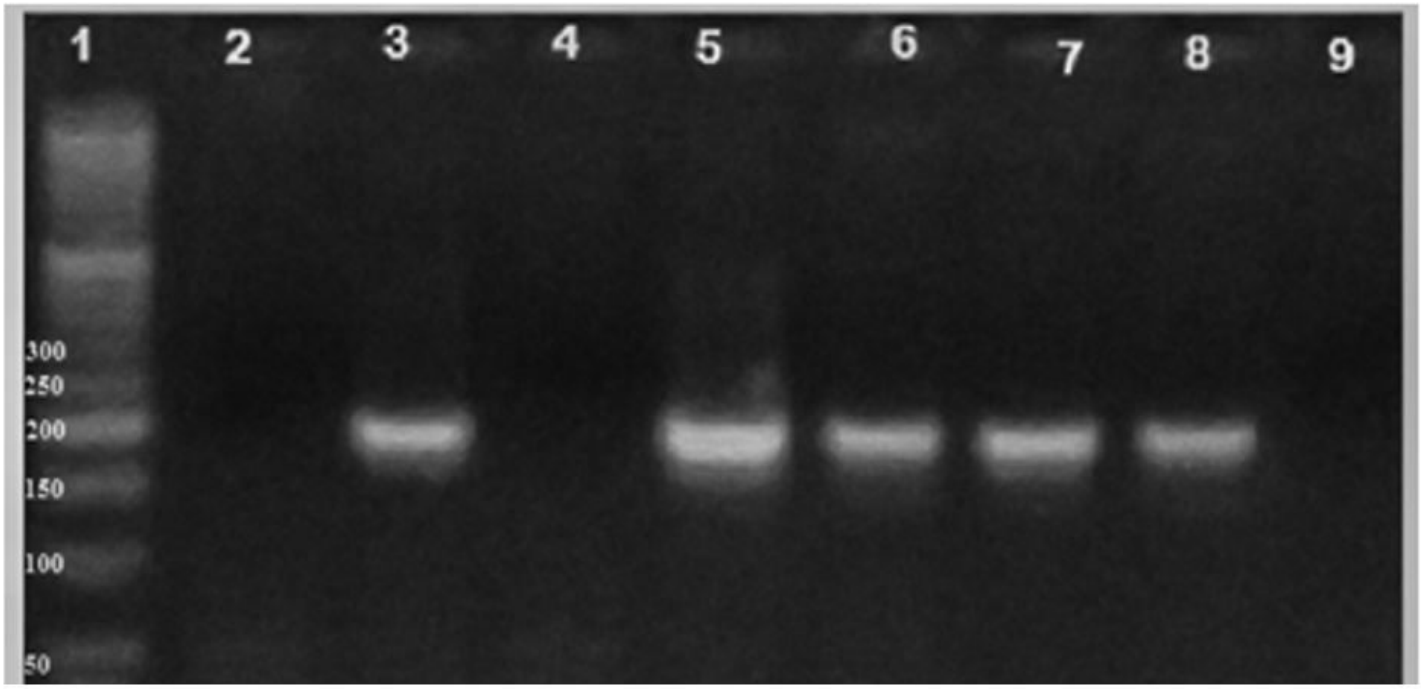

Figure 2

The recovery of *T. gondii* oocysts from experimentally contaminated water and soil samples. The B1 amplification products were placed on a 2% gel agarose. Lane 1, molecular weight size marker (50 bp, Bioline, Italy). Lane 2 is the negative control. Lane three is a positive control. Negative control (lane 4) (no template control). Lanes 5-8 positive samples contaminated with *T. gondii*. Lane 9 indicates a negative sample

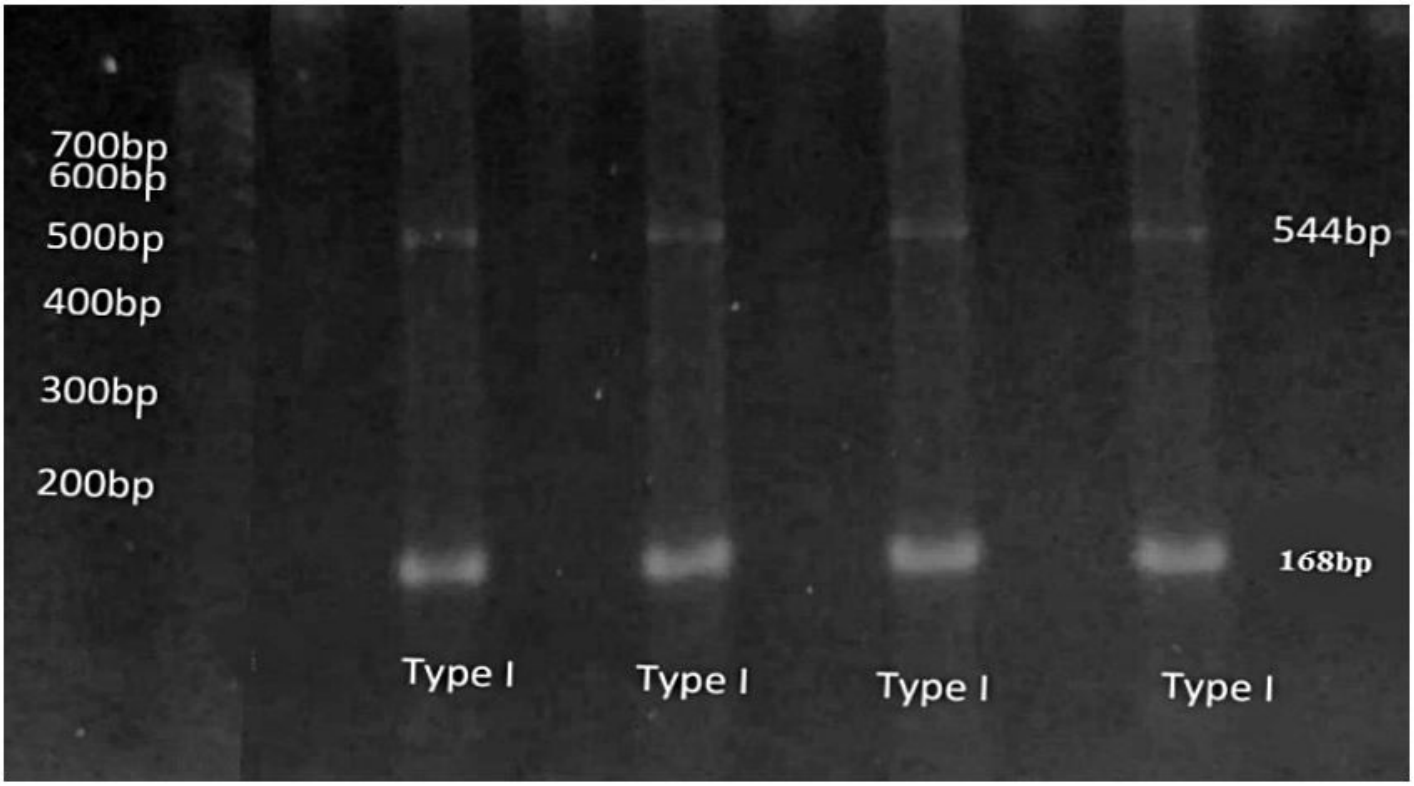

Figure 3

A photograph of ethidium bromide stained agarose gel showing the PCR-RFLP pattern of the GRA6 amplified fragment after *MseI* endonuclease digestion (168 and 544 bp bands). This pattern corresponds to type I *T. gondii*.