Diagnostic And Survey Study of *Toxoplasma Gondii* in The Soil in Mosul City-Iraq

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ABSTRACT

The present study deals to diagnose *Toxoplasma gondii* cysts in the soil of Mosul city, with a total of 50 samples were collected from each side of the city and three replications using flotation methods and microscopic methods. The study aims to detect contamination Toxoplasmosis oocysts, in the soil with making a comparison between the right and left side of the city and determine the effect of environmental factors such as humidity on the presence of the parasite through making a comparison between wet and dry soil samples. Molecular methods confirmed the presence of infection where the microscopic examination explained the presence of 20 positive samples out of 100 samples for both sides of the city where 11 positive cases found in the right side and 9 positive samples in the left side. The difference was clear between the wet samples with a rate of (14/48) positive samples, while the number of positive dry samples was (6/52) that confirmed infected 8 of the positive samples were subjected to a molecular examination using the polymerase chain reaction (PCR). Furthermore, the results showed the presence of 3 positive samples for B1 gene of the Toxoplasma parasite. The study proved the presence of soil contamination with parasite oocysts, which gives a measure of the high incidence of toxoplasmosis in Mosul city and the possibility of transmitting the infection to the rest of the intermediate hosts including humans.

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1. Introduction

Toxoplasma gondii is a primary parasite that causes infection with toxoplasmosis, which belongs to the Apicomplexa group, and is one of the parasites widespread in most countries of the world [1]. Toxoplasmosis has particular importance to human health, as it is one of the common diseases between humans and their domestic animals. it can cause infection in a large number of animals, such as mammals and birds [2]. *Toxoplasma gondii* has three phases; the oocyst phase, the tachyzoite phase, and the tissue cyst phase. Infection in the intermediate hosts usually occurs by ingestion of oocyst through water and fruits and vegetables contaminated with these oocysts, it is also transmitted by eating raw meat containing tissue cysts, as well as the possibility of transmission of the infection through the placenta from mother to fetus or through blood transfusions or organ transplants, and the final host represented by cat family, which infected by eating the meat of mammals or birds containing tissue cysts of Toxoplasma gondii [3], and cats are the first source of infection as the final host of the parasite [4]. The soil is the main source for the spread of the parasite, as cats throw their feces containing oocysts in the soil in large numbers that reach millions of oocysts [5], and they are not forming spores.

Usually, with dimensions of (10*12) µm, sporulation occurs in the soil after the availability of appropriate conditions of temperature, humidity, and proper ventilation, as these oocysts become capable of causing infection and transmission of infection [6,7,8] indicated that the soil factor constitutes a dangerous source of infection through direct contact in gardens or agricultural fields. Hamid and Abd, 2017 mentioned during a study conducted in the city of Nasiriyah to investigate toxoplasmosis among women of child-bearing age, they found an increase in the rate of infection among women who own gardens or cultivated areas in their homes, because these areas are subjected to contamination with infected cat feces. The properties and the nature of the soil, the presence of ions and organic matter in it, as well as the surface tension phenomenon, are factors that affect the survival and spread of the toxoplasma gondii parasite. One of the studies conducted the presence of *Toxoplasma gondii* at higher rates in sandy soils with high rates of lime content near the sea while the presence of the parasite was at higher rates in the surface layers compared to the deeper ones [9].

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2. Material and methods

2.1 Sampling

100 samples of soil were randomly collected from the surface layer of the soil from areas close to the presence of poultry for different areas of both sides of Mosul city from September to the end of November, with a total of (50) samples for each side of the city and three replicates, using a small shovel, at a depth of (2-5) cm, and a weight of about (300) gm. Samples were kept in plastic bags and transferred to the laboratory for examination, see Fig 1.

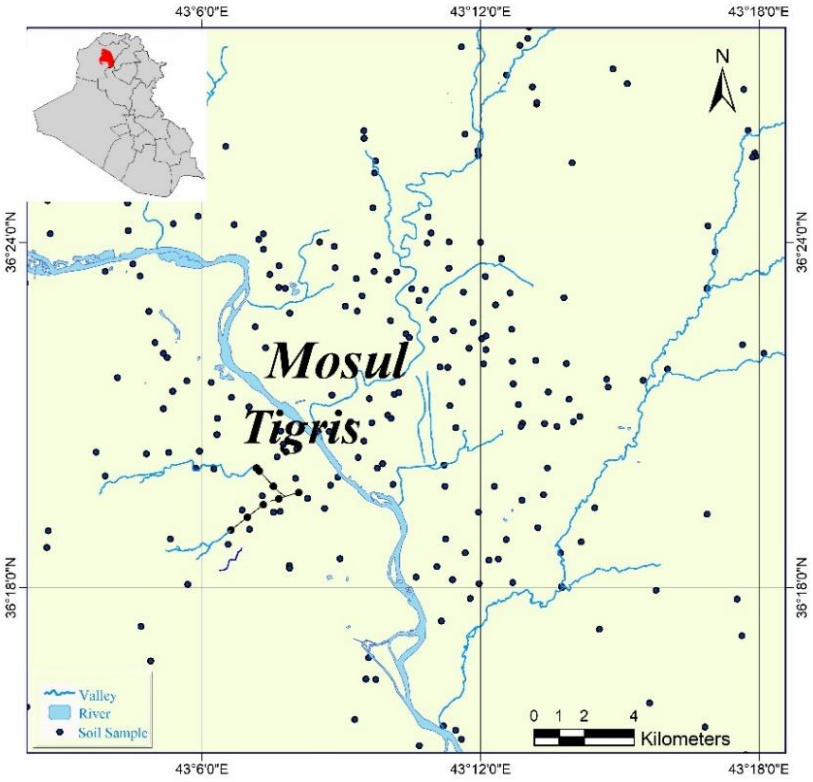

Figure 1: Mosul city/ Iraq map shows sample sites

2.2 Preparation of samples in the lab for diagnosis

The samples were kept for two days in the lab, before they were sieved, about (40) gm of each sample was placed in a clean sterile bag until the examination

2.3 Diagnostics by flotation methods

Two materials were used in the flotation method to examine soil samples as follows:

2.3.1. Flotation using sodium nitrate: A quantity of 40 gm of soil was mixed with 100 ml of (5%) sodium hydroxide solution and shaking for 20 minutes, and then the sample was

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deposited by centrifugation at 1500 rpm for 3 minutes. After the suspension was disposed of, distilled water was added to the sediment till a volume of 100 ml, and centrifuged at the same speed and time. The suspension was removed and a saturated solution of sodium nitrate was added to the precipitate and centrifuged, the slide cover was placed on the surface of the tube containing a saturated solution of nitrate and left for 15 minutes, then the cover was taken and stained with Giemsa stain and placed on the slide for microscopic examination [10].

2.3.2. Flotation using sugar solution: One gram of soil was mixed with 15 ml of physiological saline (0.85%), then the suspension was filtered through gauze to remove large particles and the mix was centrifuged at a speed of 1000 rpm for 10 minutes, 1 gm of the precipitate was taken. 9 ml of the sugar solution consisting of 454 gm of sucrose in 355 ml of distilled water, 6.7 ml of liquefied phenol was added to it. The mixture was expelled at a rate of 2500 rpm for 10 minutes, then a drop of supernatants was taken and placed on the slide and stained with Giemsa stain and the slide cover was placed for microscopic examination [11].

2.4. Microscopic study

To investigate the presence of Oocysts, a drop of the supernatant was taken or the cover of the glass slide was placed on the surface of the tube and left for 15 minutes, then the slide was stained by applying a drop of Giemsa stain and left for 15 minutes. The cover was placed on the glass slide quietly and examined using optical microscope under the power of 4x, 40 x, 100 x.

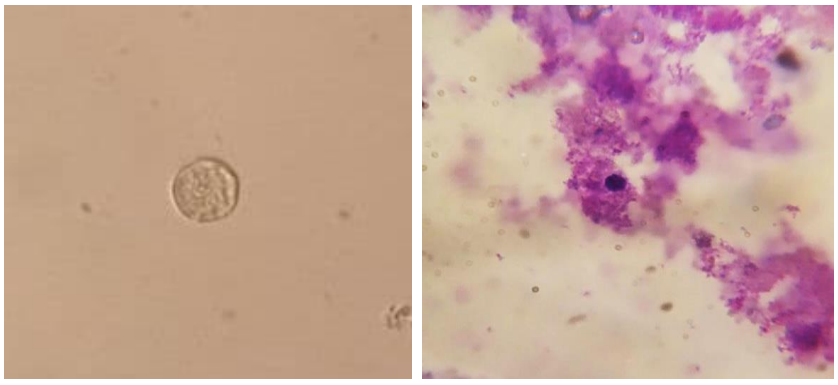

Figure 2: Optical microscope images of Oocysts

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The oocysts were observed and diagnosed using a novel type optical microscope and the dimensions of the oocysts were measured using a digital camera prepared for measurement of type HD Ce-5013 in a veterinary medicine college lab.

2.5. Molecular diagnosis for the detection of Toxoplasma gondii in soil samples

Parasite DNA was isolated from 8 soil samples according to the manufacturer instructions for the used kit Favorgen, Taiwan.

2.5.1. Preparation of agarose gel and electrophoresis

For DNA migration and detection, agarose gel was prepared at a concentration of 1%, and to obtain this concentration, 0.5 g of agarose powder was dissolved in 50 ml of tris-boric acid-EDTA, which is a buffer solution containing tri base, boric acid and EDTA (Ethylene Diamine Tetraacetic Acid) With concentration X1 and the addition of 3 microliters of safe red dye, the process was carried out using a heat source with continuous stirring until boiling and then left to cool to a temperature of 60-50 ºC. After that, the gel solution was poured into the basin of the relay device after fixing the special comb Tray, to form wells at the edges of the gel, taking into account that the pouring should be done quietly to avoid the formation of bubbles. Then the tray was placed in an electric relay tank containing an appropriate amount of loading solution X1 TBE, after which the comb was quietly lifted.Migration samples were prepared by mixing 5 µL of the DNA sample with 3 µL of the loading solution. After that, the relay was started bypassing the electric current at a voltage of (5) volts/cm, and the process took 2-1.5 hours. The gel was photographed under ultraviolet rays using a UV Transillumination gel imager to be able to see the DNA bands as well as the PCR reaction product.

2.5.2. Polymerase chain reaction (PCR)

The DNA concentration in all study samples was adjusted by dilution with Trace boric acid EDTA buffer solution to obtain the concentration required for PCR reactions of 50 ng/microliter for each sample. The master mix reaction was prepared for each PCR reaction by mixing the DNA sample and the special primers for each sample with the components of the master-mix inside the 0.2 ml Eppendorf tube supplied by the English company Biolabs, the reaction volume was fixed to 20 microliters. with distilled water, then discard the mixture in Microcentrifuge for (3-5) seconds to ensure the mixing of the reaction components, and to detect the presence of the gene in the parasite Oocysts in the soil, 4 microliters were added at a concentration of (50)

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ng/microliter of template DNA and 1 microliter of primer of B1 gene, to the contents of the master mix, as the primers were selected according to [12] and as shown in Table1. Then the reaction tubes were inserted into the thermocycler to conduct the multiplication reaction using the special program for the reaction as shown in Table 2.

Table 1: The primer sequence used to detect the parasite B1gene

Primer	Sequence
Forward	CGCTGCAGGGAGGAAGACGAAAGTTG
Revers	CGCTGCAGACACAGTGCATCTGGATT

Table 2: Special program using in polymerase chain reaction test

No.	Stage	Temperature ºC	Time	Cycle number
1.	Initial denaturation	95	6 min.	1
2.	Denaturation	95	45 sec.	35
3.	Annealing	55	1 min.	
4.	Extension	72	1 min.	
5.	Final extension	72	5 min.	1

3. Results

Depending on of the microscopic examination of soil samples, Toxoplasma was diagnosed and isolated from 20 positive samples out of 100 samples as shown in Table 3 isolated from different and random areas including 11 positive samples distributed in different areas from the right side of al-Mosul and 9 positive samples on the left side of the city, as shown in Table 4. From following up on the nature of the samples, examining them, and obtaining the results, it was noted that most of the positive samples were mostly wet soil samples, with 14 samples, while 6 samples were positive from dry soil and as shown in Table 5. Pictures 1 and 2 show the presence of Oocysts of Toxoplasma in soil samples.

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Table 3: Oocyst of *Toxoplasma gondii* isolation results from soil samples for different areas of Mosul city

Total samples for isolated soils in Mosul city	Positive samples	Negative samples
100	20 (20%)	80 (80%)

Statistical analysis that listed in Table 4 appeared significant differences between the percentage of each of the positive and negative samples, in terms of the probability value, which appeared equal to zero and is less than 0.05. When comparing the positive samples that shown in Table 4 for soil samples taken from the right and left sides of the city, found that the p-value is 0.803 and this value is greater than 0.05, which indicates that there is no significant difference between the positive samples for both sides of the city. There was no significant difference in using two flotation methods with two different solutions, a saturated solution of sodium nitrate and a sucrose solution as shown in Table 5.

Table 4: The result of the examination of soil samples collected from Mosul city

Site	Total number	Positive samples	Negative samples	Fisher's exact (P-value)
Right side of the city	50	11 (22%)	39 (78%)	0.000
Left side of the city	50	9 (18%)	41 (82%)	0.000
Fisher's exact (P-value)		0.803		

Table 5: Comparison of the two flotation methods

Test method	Total number	Positive samples	Negative samples
Sucrose flotation method	50	10 (%20)	40 (%80)
Nitrate flotation method	50	10 (%20)	40 (%80)

Table 6 shows a significant difference between the wet and dry soil samples in terms of the probability value of 0.044, which is less than 0.05 and this indicates that the positive samples in wet soils are higher than the number of positive samples in dry soils.

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Table 6: The nature of soil samples related to moisture and drought

Soil samples	Total number	Positive samples	Negative samples	Fisher's exact (P-value)
Wet samples	48 (%48)	14 (%29.17)	34 (%70.83)	0.000
Dry samples	52 (%52)	6 (%11.54)	46 (%88.46)	0.000
Fisher's exact (P-value)		0.044	0.044	

The results of the molecular examination using the PCR test for the eight soil samples that gave a positive result by microscopy showed the appearance of five negative samples and three positive samples with a percentage of 37.5% and Fig. 2 shows the appearance of the B1 gene bands with a molecular weight of 412 bp of the parasite by PCR assay.

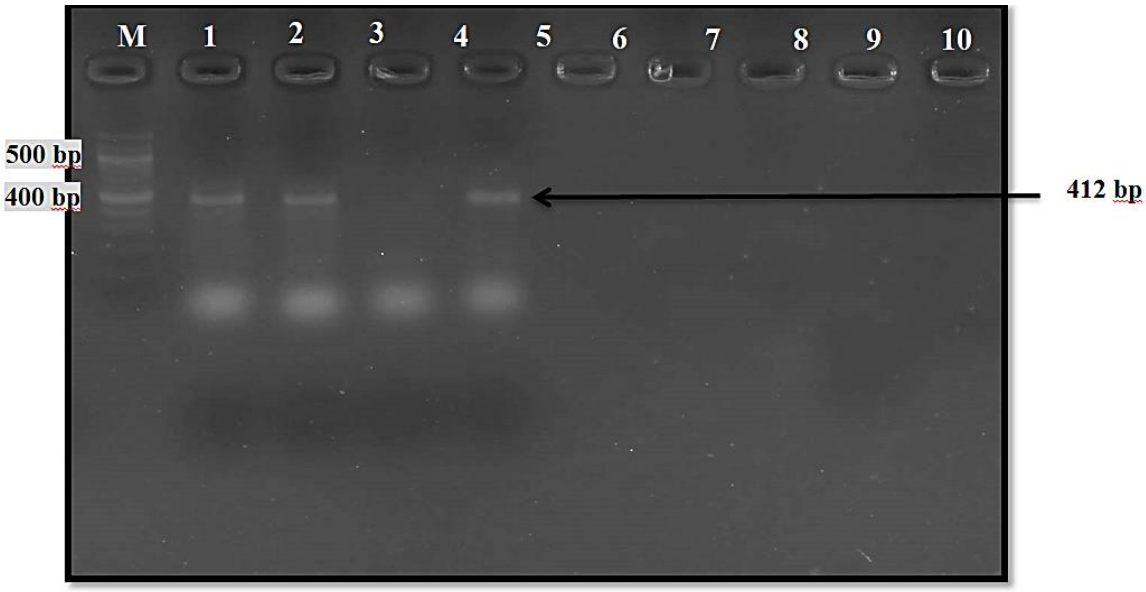

Figure 3: Product reaction of PCR for gene B1 subordinate for *Toxoplasma gondii* in the soil sample byproduct reaction (412) bp, represent ladder and (1, 2, 4) represent positive of soil samples while (3,5-8) represent negative samples, the deported had done using agarose gel at a concentration of 2%.

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4. Discussion

The results of the microscopic examination of (100) soil samples taken from the right and left sides of the city of Mosul, near the places where poultry are located, showed the appearance of (20) positive samples at a rate of (20%) for both sides of the city, as shown in Table (3). This percentage is higher than the percentage recorded by [13]. It is also less than the study by [14]. The final host of the parasite, which presents the Oocyst sac phase, in addition to the presence and multiplicity of intermediate hosts, as well as environmental factors such as temperature and humidity [15], and the nature of the place from which samples are taken has a significant impact on the presence and survival of Oocysts, as it was shown to us through our study. Currently, most of the positive soil samples return to places frequented by stray cats and shed their feces in these places, thus increasing the chances of exposure to infection through contact with feces containing the environmentally resistant Oocysts phase. It was observed during the collection of samples that humidity also had a significant impact on the chances of survival and the presence of cysts. It was shown to us through Table (5) that most of the positive samples were wet and by 14 samples, while only (6) positive samples were taken from dry soil, and the statistical analysis showed a significant difference between the wet and dry soil samples at the value (0.05), which indicates that moisture and drought play a major role in the vitality and permanence of the Oocysts, and this is consistent with what was indicated by Muhammad & Garedaghi, [16]. Microscopic examination of soil samples showed a difference between positive soil samples on the right and left sides of the city, (11) samples on the right side, and (9) on the left side, as shown in Table (4). The statistical analysis did not show any significant difference between the soil samples taken from both sides of the city. This study is the first molecular study about the *toxoplasma* oocyst phase in the soil of Mosul city and comparison between the two sides of the city. The two flotation methods used in the study did not show any difference between them, and the statistical analysis also showed that there was no significant difference between both methods, as they are effective in retrieving Oocysts. The sugar solution is somewhat confusing due to its high viscosity and being attractive to insects. Various complexes as well as the impurities and sediments present in the soil can cause interference in flotation diagnostic methods [17]. Also, the parasite phases a real difficulty in laboratory diagnosis during its presence in the ecological stage, due to the presence of many environmental pollutants that increase the difficulty of diagnosis, and this was indicated by Lélu *et al*., [18], in addition to the interference that occurs in diagnosis between *Toxoplasma* parasite and other microorganisms

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such as *Hammondia spp*. and *Neospora spp*. and this was indicated by dos Santos *et al*., [19]. To confirm the diagnosis, the molecular diagnosis was adopted to detect Oocysts of *Toxoplasma gondii* in the soil samples, as the molecular methods provide strong evidence for research and observation of the risk factors associated with the spread of epidemics and knowledge of the sources of infection and the extent of the parasite's spread in the environment [7]. The current study adopted the diagnostic method using the polymerase chain reaction (PCR), as it is characterized by speed and low percentage of sample contamination [20], and it is considered one of the methods with high sensitivity and specificity in diagnosis [21]. The current study showed the presence of *Toxoplasma* parasite in the soil with (3) samples out of (8) and at a rate of (37.5%). This percentage is higher than the percentages recorded by Lass *et al*., [10], Du *et al*., [22], which amounted to (17.8%), (17.5%), (12.69%), (9%), (7%) respectively, and they were Close to the percentage recorded by de Wit *et al*., [23], as it reached (44.3%) This convergence is probably due to the similarity of the conditions of experimenting, such as the season of sample collection, as the samples of the current study were collected during the fall season, as well as sites nature of the samples, most of them were working-class and slum neighborhoods, as well as the nature of the soil and the geographical location of the place of sampling, and this is what he indicated Cong *et al*., [8].

5. **Conclusions**

The presence of contamination with Oocysts in the soil, noting that the contamination in wet soils is higher than in dry soils. Further, molecular diagnosis is one of the most sensitive methods for detecting parasites, as specific genes are targeted, according to the parasite to be detected.

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دراسة تشخيصية ومسحية لطفيلي المقوسة الكوندية *Toxoplasma gondii* للتربة في مدينة الموصل

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المستخلص

استهدفت الدراسة الحالية تشخيص أكياس طفيلي المقوسات الكوندية *Toxoplasma gondii* في عينات التربة المأخوذة من جانبي مدينة الموصل الايمن والايسر وبواقع (50) عينة من كل جانب وبثلاثة مكررات، بإستخدام طرق التطويف بإضافة محلول النترات المشبع ومحلول السكروز وباستخدام الطرق المجهرية للكشف عن التلوث الحاصل في التربة بالاكياس البيضية لطفيلي المقوسات الكوندية وإجراء مقارنة ما بين جانبي المدينة الايمن والايسر ومعرفة تأثير العوامل البيئية مثل الرطوبة على تواجد الطفيلي عن طريق إجراء مقارنة ما بين عينات التربة الرطبة والجافة وتعتبر هذه الدراسة من أولى الدراسات في مدينة الموصل التي تطرقت للكشف عن الطفيلي واجراء مقارنة ما بين الجانبين عن طريق تأكيد وجود الخمج بالطرق الجزيئية وقد أظهرت نتائج الفحص المجهري وجود (20) عينة موجبة من بين (100) عينة لكلا جانبي المدينة الايمن والايسر في حين أظهرت المقارنة بين الجانبين وجود (11) عينة موجبة من الجانب الايمن و (9) عينات من أيسر المدينة، بينما كان الفرق واضحاً مابين العينات الرطبة وبواقع (48/14) عينة موجبة في حين كان عدد العينات الجافة الموجبة (52/6) ومن أجل تأكيد الخمج تم إخضاع (8) من العينات الموجبة للفحص الجزيئي باستخدام تقنية تفاعل البلمرة المتسلسل، إذ أظهرت النتائج وجود (3) عينات موجبة لتواجد الجين B1 الخاص بطفيلي المقوسات، وقد أظهر التحليل الاحصائي باستخدام اختبار Fisher's exact وجود فرق معنوي بين عينات التربة الرطبة والجافة بدلالة قيمة P Value الاحتمالية عند القيمة (0.05) بينما لم يظهر التحليل الاحصائي فرقاً معنوياً ما بين جانبي المدينة الايمن والايسر. أثبتت الدراسة وجود تلوث للتربة بأكياس بيض الطفيلي مما يعطي مقياساً لارتفاع نسب الاصابة بداء المقوسات في مدينة الموصل وإمكانية نقل الخمج الى باقي المضائف الوسطية بما في ذلك الانسان.

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