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Traditional and Phylogenetic Analysis of *Toxoplasma gondii* from Cats' Feces in Mosul City

Article Info.

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Abstract

Two hundred cat fecal samples were collected from various types of rearing, sexes, ages, sources, and health statuses for conventional and molecular investigations of the Toxoplasma gondii parasite. Microscopic fecal examination revealed that 32 of the 200 cats examined were infected with Toxoplasma gondii oocysts, with an overall infection rate of 16%. Stray cats, females, cats older than one year, and those suffering from diarrhea recorded the highest rates of infection with the parasite. Infection with the parasite was confirmed using a rapid immunochromatography test to detect the parasite antigen. Molecular identification of Toxoplasma gondii isolates was performed. A 32 positive samples were examined using conventional tests. DNA was extracted from fecal samples, and molecular identification of Toxoplasma gondii was done using specific target genes and a specific primer. To detect the B1 gene, polymerase chain reaction results showed that 29 samples were positive for the parasite. PCR results revealed that stray, local female cats, older cats, and diarrheic cats had high infection rates. Genetic sequencing results for the B1 gene of the Toxoplasma gondii parasite showed that the samples were sequenced. Only five isolates were found out of the total samples sent for sequencing, and they were registered in GenBank under the serial numbers (LC858431, LC858432, LC858433, LC858434, LC858435). Genetic analysis results for these isolates showed a high similarity between the local isolates and the isolates' registration numbers in the global database, with the highest similarity rate of 100%.

Keywords: *Toxoplasma gondii*, fecal, cat, oocyst, polymerase chain reaction.

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Introduction

Toxoplasma gondii is an obligate intracellular parasite capable of infecting various tissues in many mammals and birds. It is of great public health importance due to its wide distribution across a wide range of hosts. Cats serve as the parasite's definitive host, and the parasite's sexual phase is completed in the intestinal epithelial cells of the definitive host. *Toxoplasma gondii* distinguishes itself from other parasitic protozoa in its ability to parasitize a wide variety of hosts and attack most of their organs and tissues. Its infection is concentrated in the central nervous system and the reticuloendothelial system (1,2). The life cycle of *Toxoplasma gondii* includes two phases: the sexual phase and the asexual phase. The asexual phase occurs in hosts between humans and warm-blooded animals (3), such as birds. However, the sexual (intestinal epithelial) phase occurs in true hosts, such as cats (4,5). The pathogenicity of the *Toxoplasma gondii* parasite depends on several factors, including parasite virulence, parasite strain, pathogenic dose, host susceptibility, host age and sex, and the degree of host-acquired immunity (6,7). Genetic information also plays an important role in increasing susceptibility to *Toxoplasma gondii* (8). The clinical symptoms of toxoplasmosis in cats vary. It can cause disturbances in the digestive, respiratory, and ocular systems, leading to inflammation of the retina, choroid, and uvea, asymmetry of the pupils, blindness, and neurological symptoms. Nonspecific signs such as anorexia, lethargy, depression, fever, and weight loss also appear. Neurological signs can be observed alone or in combination with gastrointestinal, pulmonary, or ocular symptoms. Neurological signs are common in cats, typically characterized by personality and behavioural changes, circling, and ataxia. Ocular toxoplasmosis in cats is often detected without any systemic clinical symptoms (9). Toxoplasmosis is diagnosed using non-serological tests, such as microscopic examination of the parasite. It is also diagnosed using serological tests to detect antibodies specific to the *Toxoplasma gondii* parasite, the most important of which are IgG and IgM (10), and to detect the DNA of the *Toxoplasma gondii* parasite using molecular methods such as PCR (11).

Materials and Methods

Ethical approval

The scientific board of the College of Veterinary Medicine at Mosul University in Mosul, Iraq, approved this study; the approval number for this study is UM.VET.2023.037.

Animals

Cats of various rearing conditions, sexes, ages, sources and health statuses.

Sample collections

Two hundred fecal samples were collected from cats and placed in clean, sterile plastic containers. They were then labelled and transported to the Parasitology Laboratory/Department of Microbiology/College of Veterinary Medicine/University of Mosul as quickly as possible. Samples were refrigerated at 4°C in 10% formalin (12).

Traditional methods

Each fecal sample was examined immediately upon arrival at the laboratory, visually and using conventional examination methods, such as direct fecal examination (13) and Sheather's sugar flotation (14, 15). Stool samples were also examined by using ether-formalin sedimentation, according to García *et al.* (16). The dimensions of the parasite oocysts identified in the study were measured using an ocular micrometer. A rapid immunochromatography assay was used to detect *Toxoplasma gondii* antigens. Samples positive for the parasite were subjected to a rapid test to confirm the diagnosis in the feces, according to the manufacturer's instructions (Sunlong-China).

Molecular methods

Diagnosis of *Toxoplasma gondii* was made using conventional polymerase chain reaction (PCR) for samples positive for microscopy and a rapid test. DNA extraction was performed using the FavorGen FavorPrep™ Fecal DNA Isolation Mini Kit-FASTI 001-1, which extracted DNA from the fecal samples under study. The DNA concentration was adjusted in all study samples by dilution with TE buffer solution to obtain the required concentration for PCR reactions, which was 50 ng/µL for each sample. The master reaction mixture was prepared for each reaction by mixing the DNA sample and the primer specific to the B1 gene (Table 1) with the reaction mixture components inside a 0.2 ml Eppendorf tube prepared by the British company Biolabs. The reaction volume was fixed to 20 µL with distilled water, and the mixture was centrifuged in a Microfuge device for a period of 3-5 seconds to ensure that the reaction components were mixed. The reaction tubes were then inserted into the thermocycler device for the purpose of performing DNA amplification using the special program (Table 2) (17).

Table 1. The primer sequence of the B1gene of the *Toxoplasma gondii*

Primer	Gene	Size	Sequence	Reference
Forward	B1	400bp	TTTTGACTCGGGCCCAGC	17
Reverse			GTCCAAGCCTCCGACTCT	

Table 2. Steps of Amplification

Steps	Temperature ^{0}C	Time	Cycle number
Initial denaturation	95	6 min.	1
Denaturation	95	45 sec.	
Annealing	55	1 min.	35
Extension	72	1 min.	
Final extension	72	5 min.	1

A gel purification kit was used to purify DNA from agarose gel in TBE solution, allowing the DNA to be used for base sequence analysis. When DNA bands appeared, several DNA samples positive for PCR, identified during the study, were sent to the Macrogen Laboratory in Korea for genetic sequencing.

The similarity of the gene sequences in the database was also determined. The gene sequence of the B1 gene of the *Toxoplasma gondii* parasite was used in BLAST format to search the database using the Basic Local Alignment Search program available on the National Center for Biotechnology (NCBI) website (www.ncbi.nlm.nih.gor). The nucleotide sequence of the B1 gene of the Toxoplasma gondii parasite was used to create a sequence alignment for the isolates using the Cluster Omega program to identify developments and changes among isolates worldwide. A phylogenetic tree was constructed using the DNA sequences extracted from the samples by comparing these sequences with similar sequences in the NCBI GenBank database using the BLAST tool. The MEGA 4 program was used to construct the tree, using the Neighbor-Joining method with a 1000-iteration bootstrapped test to assess the confidence level of the branches (18).

Statistical analysis

The results were analyzed using the IBM-SPSS statistical program. Issue 22, using a two-sided chi-square test and a Fischer test, was used to determine differences in the incidence of infectious protozoa and their relationship to the nature of breeding, sex, age, animal source, and health status (19).

Results

This study, through microscopic examination of 200 stool samples and using traditional methods, identified Toxoplasma gondii oocysts based on morphological and morphometric characteristics. Small, spherical or sub-spherical sporocysts were identified, ranging in size from 10 to 12 micrometres, characterized by a thick wall and containing two sporocysts. Non-sporocysts, on the other hand, contained an undifferentiated mass (Figure 1).

Figure 1: Sporulated and unsporulated oocyst of Toxoplasma gondii, by the floating method, at 40X magnification.

The study showed that the overall infection rate with *Toxoplasma gondii* reached 16% (32 positive samples out of a total of 200 samples examined), and that the infection rate of stray cats was 27.2%, which was higher than the infection rate of domestic cats. The infection rate of females was 21.9%, which was higher than that of males, with a significant difference ($P<0.05$). Older cats recorded an infection rate 27.14% higher than young cats. Local cats also recorded an infection rate of 20.5% higher than imported cats, with a significant difference ($P<0.05$). Cats suffering from diarrhea also showed a high infection rate of 22.4% compared to apparently healthy cats, with a

significant difference (P<0.05). The rapid test also showed that the number of samples positive for the parasite was 29 out of a total of 32 samples positive by conventional microscopic examination. The test also recorded the highest infection rates in stray cats, females, old cats, local cats, and cats that were suffering from diarrhea and there were significant differences at P<0.05 (Table 3).

Table 3: The incidence of *Toxoplasma gondii* infection diagnosed by microscopic examination and rapid test in cats and its relationship to the nature of breeding, sex, age, source and health status.

Factor	Number of animals examined	Number of animals positive by microscopic examination	percentage %	Number of positive animals Confirmed by rapid test	Percentage %
Nature of rearing					
Domestic	108	7	6.5a	4	57.14a
Stray	92	25	27.2b	25	100b
Sexes					
Male	104	11	10.6a	9	81.8a
Female	96	21	21.9b	20	95.23b
Ages					
Less than six months	67	3	4.5a	2	66.7 a
Six months - one year	63	10	15b	8	80b
More than one year	70	19	27.14, c	19	100c
Sources					
Imported	88	9	10.22a	7	77.7a
Local	112	23	20.5b	22	95. 7b
Health status					
Clinically health	93	8	8.6a	6	75a
Suffering from diarrhea	107	24	22.4b	24	100b

Different letters indicate a significant difference at the probability level P<0.05.

A molecular detection study of the *Toxoplasma gondii* parasite in 32 cats previously examined by traditional methods, which had positive results for the parasite, showed through the polymerase chain reaction the presence of 29 positive samples (90.6%), as the highest infection rates were recorded in stray cats 96% compared to domestic cats, in females 95.2% compared to males, and in cats of old ages 100% with a significant difference at P<0.05. The results also showed that the local cats had a 95.6% higher infection rate than the imported cats, with significant differences at P<0.05. The study also showed that the examined cats that were suffering from diarrhea had a 96.8% higher infection rate with a homosporous parasite than the apparently healthy cats, with a significant difference at P<0.05 (Table 4) (Figure 2).

Table 4: The infection rate of *Toxoplasma gondii* infection in cats diagnosed by molecular method and its relationship to the nature of breeding, sex, age, source and health status.

Factor	Number of animals Examined	Number of animals positive	Percentage %
Nature of rearing			
Stray	25	24	96a
Domestic	7	5	71.4b
Sexes			
Male	11	9	81.8a
Female	21	20	95.2b
Age			
Less than six months	3	2	66.7a
Six months -one year	10	8	80b
More than one year	19	19	100c
Source			
Local	23	22	95.6a
Imported	9	7	66.7b
Health status			
Clinically health	8	6	75a
Suffering from diarrhea	24	23	96.8b

Different letters indicate a significant difference at the probability level P<0.05

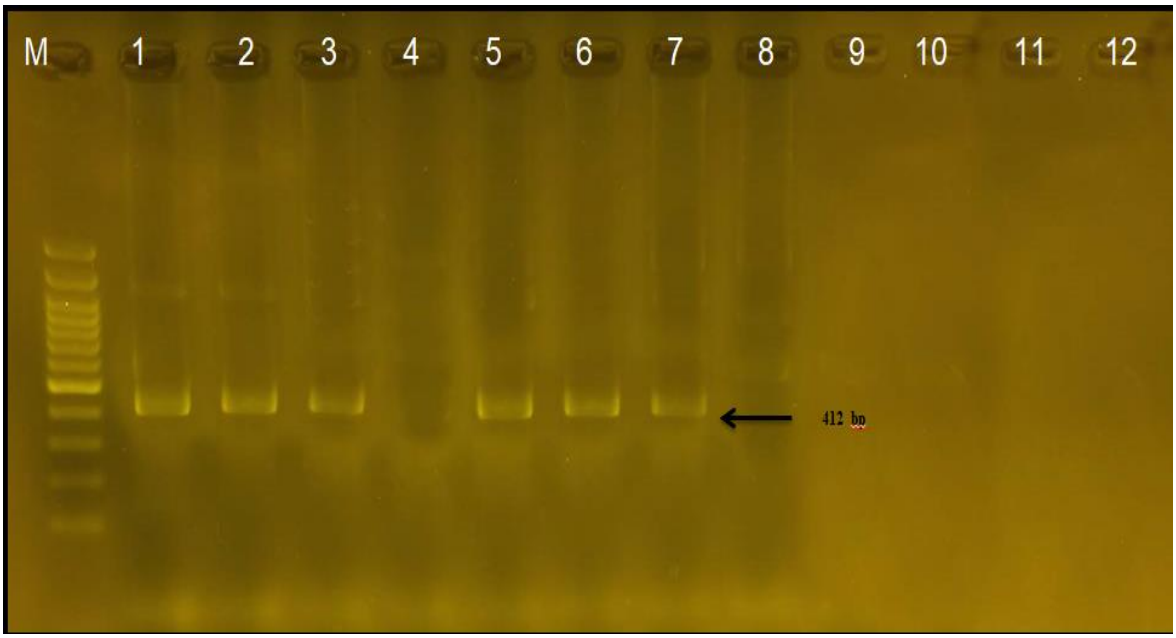

Figure 2: Electrophoresis results of the final reaction products of the *Toxoplasma gondii* parasite DNA at a reaction volume of 412, with samples 4, 8-12 being negative samples, and 1-3, 5-7 being positive samples, using the polymerase chain reaction.

The results of the genetic sequencing of the B1 gene of the *Toxoplasma gondii* parasite showed that the samples sent from cats in Mosul city carried a genetic sequence. Five isolates were discovered, and these isolates were registered in the GenBank with the serial numbers LC858431, LC858432, LC858433, LC858434, and LC858435 (Table 5,6). The results showed the presence of gene sequences with a percentage of similarity to the nucleotide sequence of the gene for each of the strains diagnosed in the study when compared in the database, compared to the nucleotide sequences of the international strains identified during this study, which were used to construct the genetic phylogenetic tree. The results showed a high degree of similarity between the local isolates bearing the registration number in the global database. The highest similarity rate of 100% was found with the strain registered in the World GenBank under the names KX008029.1, KX008031.1, and KX008004.1, isolated in China; LC749847.1, PV596303.1, and PV596297.1, isolated from Iraq; isolate AF252408.1, registered in the United States; isolate KP895868.1, registered in Thailand; isolate isolated in Norway, registered under the name KM657806.1; isolates registered in Japan, registered under the name AK318397.1; and isolate registered under the name M63161.1, isolated in Canada (Table 7).

Table 5: Local genetic sequences of the B-1gene of *Toxoplasma gondii*. Diagnosed in different samples of the cat with their serial numbers in GenBank.

Local isolate	Gene	Isolation source	Sequences 5'----------3'	Accession No.
Toxoplasma gondii	B1	Cat feces	CTGTCGGTCATACTGCGTCGAATACACCGGATCCCTTCAGACCTCCGAAGTTAAGCG GCGCAAGGCCCGGTTAGTACTTGGGTGGGGGACTCCCAGGGAATACCCTCGGGTGCT GACAGCTTTTTTCTTCCATCTGCCTGTGTTTTGTACTGGTGGTTGCAAACCATACTTTT TCTACCGATCTTACCACGAACAGTAATGAAACACTTGCGTCTCACAGTATGTGGCGT ATATGCATGAACAGTTCGCCCTATATGCGAGGAAACCTGTCAGCATGAATGAACGG AAGACGCCTATGTACTTACACTCCCCCGTCTTGATCATGAAAGATCTGTTCATTGTAT ATGGCTCTTCTGAAACTATGAAAGGAAAATATAGCTTGTTGTTGTTATTCCTTATCGT ACCAGCGTCCCTTTTACACAAACAAAATGTCTGCTGCCATTTCGAGAGTCTACAGAC AACGTGGAGAATGCGTGTATTTGTGCTGCTAGTGACTTTGATGCGTTTCACCAGACT CTTTACAAGAAAGCAAGTGATGTTAGTCCTTCATTAGTCAGTGAAATGTGTATTTTTT TTTGTGCTCGTGACTTGATGTGTTTCCCTAGACTGGTTACCAGAAAGCCAGTGGAAT GCTAGTTCAAAGTGGAATATGGTGTGTTTGTATTTGTGAAACTGTTGGCTTTCTGCTT TTCATTGGAGTGTTTACGAGACCATGAGAAAATTAGTGTTTTTCACCGCACGATGGA ATATATGATAAGGGCACTGGAGAAGAACTAGCTCTGTATTGTGCACCTGAGCTTGTC TCTACTGTTTGGGGTGGTGGATGGGGACGGGCGCTCGTACGCGTGGCGCTCGTTGTG CCGTATGGGAACACATGCATCATCGAAAAAAACAGACGGTTGCTCAGCGGTGCTAT AGGATTGTCGTGTGGTTGTGGTGCGCGTCACGAACAAGTGGTGTTGCGCCGGGTTGG CTGCACAGAAGTAATATCGTGCGCCAGATGACTGATCAGTGCACGATATAACAGAC ATTTGACTACGTGAGGTTATGAATTTGTTTTACCTCTACGCGAGAAAAACAAAATCA AATCTCACCGCTTGTTTTCCCATCGCTGGCTTGTTGTTTTTCGGCAAGCCAATTGGGG AGACCCCGTAAACGTGCTAAACCTTTGATTGGTTATGGCAGCGGGGTCAAGGAACG GGCATTGTCTCCGTCGGTAAATCAAGGTGTTGTATGGTTATGACCTTCAAAAAAGTT GAAGAAGATCTCTTGTTCTCCTTCGGGGGTTTTGAGAGATTTGGAAGTTGTTTAAGTG TGCGACTGTTTGAA	LC858431.1
			TCATATGCTTGTCTTAAAGATTAAGCCATGCATGTCTAAGTATAAGCTTTTATACGGC TAAACTGCGAATGGCTCATTAAAACAGTTATAGTTTATTTGATGGTCTTTACTACATG GATAACCGTGGTAATTCTATGGCTAATACATGCGCACATGCCTCTTCCCCTGGAAGG GCAGTGTTTATTAGATACAGAACCAACCCACCTTCCGGTGGTCCTCAGGTGATTCAT AGTAACCGAACGGATCGCGTTGACTTCGGTCTGCGACGGATCATTCAAGTTTCTGAC CTATCAGCTTTCGACGGTACTGTATTGGACTACCGTGGCAGTGACGGGTAACGGGGA ATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCTAAGGAAG GCAGCAGGCGCGCAAATTACCCAATCCTGATTCAGGGAGGTAGTGACAAGAAATAA CAACACTGGAAATTTCATTTCTAGTGATTGGAATGATAGGAATCCAAACCCCTTTCA GAGTAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAA TAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCTGCTGGAAG CAGCCAGTCCGCCCTCAGGGGTGTGCACTTGGTGAATTCTAGCATCCTTCTGGATTTC TCCACACTTCATTGTGTGGAGTTTTTTCCAGGACTTTTAC	LC858432.1
			CAAGAAATAACAACACTGGAAATTTCATTTCTAGTGATTGGAATGATAGGAATCCAA ACCCCTTTCAGAGTAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTC CAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTC TGCTGGAAGCAGCCAGTCCGCCCTCAGGGGTGTGCACTTGGTGAATTCTAGCATCCT TCTGGATTTCTCCACACTTCATTGTGTGGAGTTTTTTCCAGGACTTTTACTTTGAGAA AATTAGAGTGTTTCAAGCAGGCTTGTCGCCTTGAATACTGCAGCATGGAATAATAAG ATAGGATTTCGGCCCTATTTTGTTGGTTTCTAGGACTGAAGTAATGATTAATAGGGA CGGTTGGGGGCATTCGTATTTAACTGTCAGAGGTGAAATTCTTAGATTTGTTAAAGA CGAACTACTGCGAAAGCATTTGCCAAAGATGTTTTCATTAATCAAGAACGAAAGTTA GGGGCTCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAG AGATAGGAAAACGTCATGCTTGACTTCTCCTGCACCTTATGAGAAAT	LC858433.1
			AGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCT GCTGGAAGCAGCCAGTCCGCCCTCAGGGGTGTGCACTTGGTGAATTCTAGCATCCTT CTGGATTTCTCCACACTTCATTGTGTGGAGTTTTTTCCAGGACTTTTACTTTGAGAAA ATTAGAGTGTTTCAAGCAGGCTTGTCGCCTTGAATACTGCAGCATGGAATAATAAGA TAGGATTTCGGCCCTATTTTGTTGGTTTCTAGGACTGAAGTAATGATTAATAGGGAC GGTTGGGGGCATTCGTATTTAACTGTCAGAGGTGAAATTCTTAGATTTGTTAAAGAC GAACTACTGCGAAAGCATTTGCCAAAGATGTTTTCA	LC858434.1
			GAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTCACACGTCCTTA TTCTTTATTAACCATCAACCTTTGAATCCCAAGCAAAACATGAGTTTGCATCTCTCTC CATTGGAGAGATTTGCATTCAAGAAGCGTGATAGTATCGAAAGGTATTATTGCCTTC TTCATGTTGGATATCCTGCGCTGCTTCCAATATTGGAAGCCAGTGCAGGTATCCGGG GGTGCACAGCGAAGGGGCTCAATTTCT	LC858435.1

Table 6:Nucleotide sequence similarity results for the *Toxoplasma gondii* gene compared to global isolates registered in the World GenBank.

Local sequences Accession No.	Pathogen Identified	Name of gene	Identic Number %	GenBank Accession Number	Country Identification
LC858431.1	***Toxoplasma gondii***	**18S rRNA**	100	KX008004.1	China: Shenzhen
			100	KX008029.1	China: Qinghai
LC858432.1			100	KX008031.1	China: Shanghai
			100	LC749847.1	Iraq: Mosul
LC858433.1			100	PV596303.1	Iraq: Mosul
			100	PV596297.1	Iraq: Mosul
LC858434.1			100	AF252408.1	USA: California
			100	KP895868.1	Thailand
LC858435.1			100	KM657806.1	Norway
			100	AK318397.1	Tokyo
			100	M63161.1	Canada
			95.40	M97809.1	Canada

Table 7: The percentage of similarity between the isolates that were diagnosed during the study.

Sequences Accession No.	Alignment score
LC858431.1 LC858432.1 LC858433.1 LC858434.1 LC858435.1	20.44 – 100

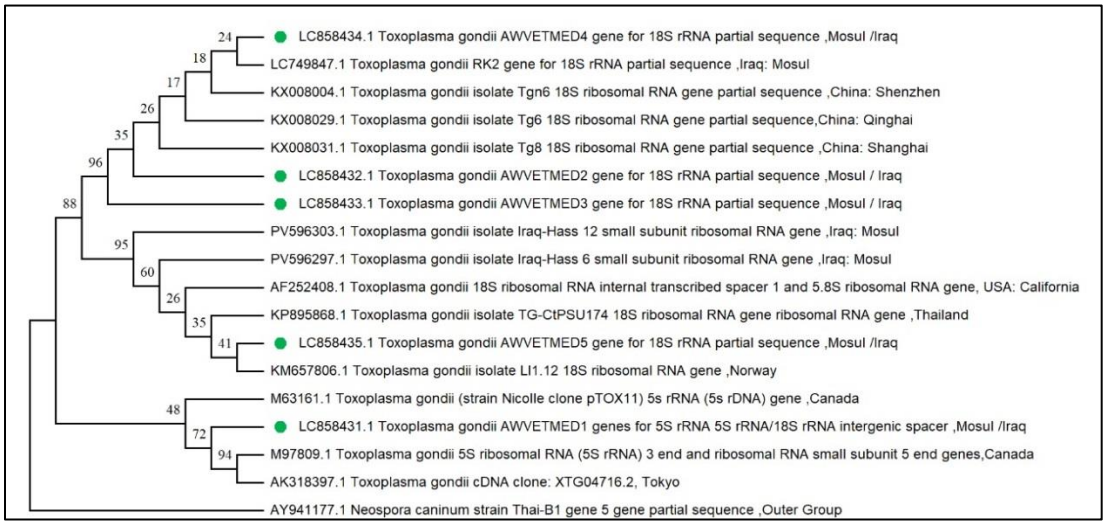

Figure 3: Phylogenetic tree of the genetic sequence of *Toxoplasma gondii* for local isolates with global isolates.

Discussion

Toxoplasma gondii is one of the most important parasites affecting cats. This importance stems from the pathological symptoms it causes in infected animals, as well as the risk of transmission due to the presence of infected animals without showing symptoms (20).

During the study, sporulated and unsporulated oocysts of *Toxoplasma gondii* were identified. Based on morphological and morphological characteristics, the oocysts were small, semi-spherical, measuring 10-12 micrometres. The sporocysts contained two sporangia. The oocyst wall is thick and consists of lipid and protein layers. This explains the resistance of the oocyst to the traditional stains used in the study, which is consistent with what was stated by (21). In this study, a *Toxoplasma gondii* infection rate of 16% was recorded, while researchers (22) in Mosul recorded a rate of 22%. In Erbil, researchers (23) recorded an infection rate of 11%. A 30.4% infection rate was recorded in Baghdad (24), and a 20.6% infection rate was recorded in Duhok (25). *Toxoplasma gondii* infection was also recorded in countries neighboring Iraq. In Syria (26), the overall infection rate was 36%. In Turkey, the infection rate was 14.3% (27). In Qatar, an infection rate of 3.4% was recorded (28). In Egypt, a study recorded a 67.2% infection rate with the parasite (29). In Iran, an infection rate of 72.7% was recorded, while in China, an infection rate of 72.7% was recorded (30). An infection rate ranging from 16-80% was recorded in Los Angeles County and rural areas in the United States (31).

The variation in infection rates of *Toxoplasma gondii* in cats between the studies or regions above is due to several factors, including epidemiological, environmental, and research factors. These factors include differences in sample type, parasite detection method, study size, number of samples, diagnostic methods, and genetic strains of the *Toxoplasma gondii* parasite. Some strains are more capable of infecting or sporulating. Moreover, human behaviour plays a major role in causing infection. Interaction with cats. In some countries with good health awareness, people are careful to keep cats clean and prevent them from coming into contact with sources of infection. In some regions, cat feces are not disposed of in a healthy manner, which increases contamination and transmission of the parasite (32). The infection rates of *Toxoplasma gondii* varied, with stray cats recording a higher infection rate than domestic cats. This is consistent with what (22) stated. This is explained by the fact that stray cats may feed on rodents and birds, which are potential carriers of the parasite, while domestic cats often feed on canned or cooked food that does not

contain the parasite. Furthermore, stray cats may be exposed to continuous contamination with parasite egg sacs in contaminated soil. Domestic cats also receive veterinary care and periodic examinations, which stray cats lack. This is consistent with (29, 21). Females recorded a higher infection rate than males, which is consistent with (33). This is attributed to immunological, hormonal, and behavioural factors, as well as the primary role of females in transmitting congenital vertical infection of the parasite. Older cats showed a higher incidence of *Toxoplasma gondii* infection compared to younger cats. This is consistent with (30). Researchers found that cats older than a year and a half were more susceptible to infection than cats under six months old. Researchers (29) recorded that cats older than one year were more susceptible to infection than kittens under six months old. This may be attributed to the fact that cumulative exposure increases with age, meaning that cats are more likely to be exposed to sources of infection such as raw meat, contaminated soil, or contact with other cats carrying the parasite. It may also be attributed to the fact that the infection may be old, and the parasite's tissue cysts are present within the tissues. Sometimes, with age, this can lead to a weakened immune system, which increases the likelihood of reactivating the latent infection. This is consistent with what was stated by (34). Another reason that provides evidence for the low infection rate in young cats is the low rate of congenital transmission of *Toxoplasma gondii*. This means that vertical transmission is low compared to horizontal transmission. This indicates the role of environmental contamination with the parasite, which increases the infection rate in adult cats and decreases it in young cats. The study recorded a higher infection rate in local cats compared to imported cats. This is consistent with what was reported by (35). This is attributed to the fact that imported cats are examined and treated before shipment, which reduces the likelihood of them carrying the parasite, while local cats are not subject to regular examination. It is also attributed to the fact that imported cats are often fed safe artificial foods (36).

The current study showed that the infection rate of cats with *Toxoplasma gondii* in cats suffering from diarrhea is higher than in cats with apparently healthy symptoms. Some studies support this (33), but this is not always the case, as most cats infected with *Toxoplasma gondii* are asymptomatic. If symptoms such as diarrhea appear, this may be due to the parasite multiplying in the intestinal cells, causing intestinal inflammation and diarrhea (29). In the study, samples positive for Toxoplasma gondii were examined by microscopic tests using a rapid

immunochromatography test to detect the parasite antigen to confirm infection. It was found that the ability to detect infection or the number of positive samples was lower compared to traditional microscopic examination.

Conclusion

Toxoplasma gondii is one of the Intestinal protozoan infections are prevalent among cats, with a significantly higher infection rate reported in Mosul city relative to other areas.

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Conflict of interest

The authors declare that there is no conflict of interest regarding this study.

Ethical Clearance

This work is approved by The Research Ethical Committee

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التحليل التقليدي والتطوري لطفيلي المقوسة الكوندية من براز القطط في مدينة الموصل

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الخلاصة

جُمعت مئتا عينة براز قطط مختلف أنواع التربية والأجناس والأعمار والمصادر والحالات الصحية، وذلك لإجراء فحوصات تقليدية وجزيئية على طفيلي المقوسة الكوندية , كشف الفحص المجهري للبراز أن 32 من أصل 200 قطة كانت ايجابية للطفيلي، بمعدل إصابة إجمالي بلغت 16%. وسجلت القطط الضالة، والإناث، والقطط التي يزيد عمرها عن عام واحد، ، والقطط التي تعاني من الإسهال، أعلى نسبة اصابة بالطفيلي. وتم تأكيد الإصابة بالطفيلي باستخدام اختبار الاستشراب المناعي السريع للكشف عن مستضد الطفيلي. تم التشخيص الجزيئي لعزلات طفيلي المقوسة الكوندية التي اعطت نتائج إيجابية باستخدام الاختبارات التقليدية (32 عينة)، واستُخلص الحمض النووي من عينات البراز، وتم تحديد طفيلي المقوسة الكوندية جزيئيًا باستخدام بادئات متخصصة للجينات الطفيلي، أظهرت نتائج تفاعل البوليميراز المتسلسل وجود 29 عينة إيجابية للطفيلي. أظهرت النتائج ارتفاع معدل الإصابة بالقطط المحلية الضالة، والقطط الكبيرة في السن، والقطط المصابة بالإسهال. أظهرت نتائج التسلسل الجيني لطفيلي المقوسة الكوندية أن العينات قد تم تسلسلها. اذ تم العثور على خمس عزلات فقط من إجمالي العينات المرسلة للتسلسل، وسُجلت في بنك الجينات تحت الأرقام التسلسلية (LC858431, LC858432, LC858433, LC858434, LC858435). أظهرت نتائج التحليل الجيني لهذه العزلات تشابهًا كبيرًا بين العزلات المحلية مع العزلات التي تحمل رقم التسجيل في قاعدة البيانات العالمية وبنسبة تشابه 100%.

الكلمات المفتاحية: المقوسة الكوندية، براز القطط، اكياس الطفيلي، تفاعل البلمرة المتسلسل.