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Detection of Toxoplasmosis in Cats by Traditional and PCR Techniques in Baghdad

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Abstract

This study was conducted to reveal the rate of infection with *Toxoplasma* parasite in 100 domestic cats by traditional method (floatation technique) and conventional PCR, in Baghdad province during October 2020 to March 2021. The total infection rate of *Toxoplasma* by floatation method with sheathes solution was 14%, According to study regions, the rate of infection with *Toxoplasma* was 23.8% in Abu-Ghraib, 15.38% in Al-Amriyah, 12% in Al-Saydiya and 7.14% in Hay Al-Jamia. According to months, the higher infection rate was reported in October 29.41%, while the lower rate in January (0%). Regarding age of study animals, cats older than one year of age were revealed a higher rate of infection (17.85%), whereas the lower rate recorded in cats of less than one year (9.09%). Concerning the sex of study cats, female and male cats were showed, respectively, 22.22% and 9.37% of infection with significant increases in females. Conventional polymerase chain reaction using B1 gene recoded 20% rate of infection with *Toxoplasma* in domestic cats.

Keywords: *Toxoplasma gondii*, Cats, Molecular assay, Iraq

Introduction

Toxoplasma gondii is an obligate intracellular parasite with worldwide distribution inducing toxoplasmosis and infecting humans via warm-blooded animals. Wild and domestic felids are the only known definitive hosts with the ability to shed oocysts in their feces. Common pathways of infection include oocyst-contaminated water, soil, and food, tissue cysts in undercooked or raw meat, and congenital transmission [1, 2, 3, 4]. Oocysts are the environmentally resistant form of the parasite and play a key role in transmission to new hosts and ecosystems, generating the need to study humans alongside domestic and wild animal populations [4]. The large number of oocysts shed during primary-infection by felids could lead to extensive environmental contamination, which can infect a high number of intermediate species, such as humans, mice or birds [5]. High rainfall rates can facilitate survival of oocysts for months, explaining why regions with higher precipitation show higher prevalence compared to arid regions, which show far lower rates of infection in the population living in these areas [6, 7, 8].

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In Baghdad, the prevalence in the human population varies between 30% and 60% [9], and this high prevalence has been linked to the existence of a high density of urban stray cats, exposing people to an elevated density of oocysts [10]. This high density of free-ranging domestic cats can explain why cat ownership in homes does not increase the risk of *T. gondii* in surveys in some cities in Baghdad [11]. A study in Turkey city in 2021 found 4.35% seroprevalence in 29 domestic cats and detected a 6.31% shedding prevalence of *T. gondii*-like oocysts by microscopy in fecal samples [12]. Although training helps with identification of *T. gondii*-like oocysts, morphological structure along cannot confirm the oocysts visualized are actually *T. gondii*, as Hammondia oocysts for example look identical [13]. Consequently, molecular detection-based methods, like PCR, can be an alternative and complementary method to microscopy to identify cats infected with *T. gondii*. As consequence, the objective of this study was to determine the prevalence of T. *gondii* DNA in cat fecal samples by conventional PCR from positive samples.

Materials and methods

A total of One hundred fecal sample of both sex cats, with less and more than one-year age from four regions in Baghdad /Al-Karkh during the period from 1/10/ 2020 – 1/4/2021 were collected end.
Floatation technique by sheathes sugar solution using as traditional diagnosis for detection *Toxoplasma* oocysts according to [14].
Molecular diagnosis using conventional PCR was performed for detection and confirm the diagnosis of *Toxoplasma* based on B1 gene from cat's fecal samples. This method was carried out according to method described by [15].That include DNA extraction from fecal samples using Monarch® Genomic DNA extraction kit (bio laps, Canada)And PCR master mix preparation by using primer

Table 1: The primer used for diagnosis of toxoplasmosis

Gene	Primer sequence 5’ to 3’		Amplicon size	References
B1	Forward	5-GGAACTGCATCCGTTCATGAG-3	194	**16**
	Reverse	5-TCTTTAAAGCGTTCGTGGTC-3		

DNA sequencing method

DNA sequencing method was performed for species typing of some positive local *Toxoplasma* isolates and constructed a phylogenetic tree for our *Toxoplasma* versus NCBI-Blast-Gene Bank. Positive PCR B1 gene were analyzed for DNA sequencing (Molecular Evolutionary Genetic Analysis Version 11)

Statistical analysis

Chi-square test was used to assess relationships with variables, p value <0.05 for the significant level was employed with using the Geneious computer program (version 11) to estimate the genetic statistical [17].

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Results

Traditional diagnosis of *Toxoplasma* in cats

Floatation technique using sheathes sugar solution was used for traditional detection of *Toxoplasma* parasite in cat's feces. The total infection rate of *Toxoplasma* in 100 cats using flotation technique was 14% (14/100). Abu-Graib recoded higher rate of infection 23.8% (5/21) followed by Al-Amriyah 15.38% (4/26) then Al-Saydiya 12% (3/25) and finally Hay Al-Jamia 7.14% (2/28) with significant differences between regions of study (Table 2).

Table 2: infection rate with *Toxoplasma* in cats in relation to regions of study

Region	Total No. of cats	Positive	
		No.	%
Abu-Ghraib	21	5	23.8
Al-Aamiriya	26	4	15.38
Al-Saydiya	25	3	12
Hay Al-Jami'a	28	2	7.14
Total	100	14	14
P-value	0.0082 **		

Association of infection to months of study

Significant differences were recorded between month of study as shown in (Table 3).Higher rate of infection with *Toxoplasma* recorded in October 29.4% (5 /17) followed by March with rate of 25% (4/16), and the lower rate 0% was recorded in January 0 % (0/17) (Table 3).

Table 3: Infection in cats with *Toxoplasma* in cats in relation to months of study

Months	Total No. of cats	Positive	
		No.	%
October	17	5	29.41
November	17	3	17.64
December	17	1	5.88
January	17	0	0
February	16	1	6.25
March	16	4	25
Total	100	14	14
P-value	0.0001 **		

Association of infection to age

The result showed that cats older than one year of age recorded higher rate of infection 17.85 % (10/56) with cats more than 1 year of age recorded 9.09% (4/44) (Table 4).

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Table 4: Infection rate with *Toxoplasma* in cat in relation to age

Age	Total No. of cats	Positive	
		No.	%
< 1 year	44	4	9.09 %
≥1 year	56	10	17.85 %
Total	100	14	14 %
	0.0401 *		

Association of infection to sex

Female and male cats recorded 22.22% (8/36) and 9.37% (6/64) respectively with significant difference (Table 5).

Table 5: Infection rate with *Toxoplasma* in cat in relation to sex

Sex	Total No. of cats	Positive	
		No.	%
Female	36	8	22.22 %
Male	64	6	9.37 %
Total	**100**	14	**14 %**
P-value	0.0084 **		

Molecular detection of *Toxoplasma* by conventional PCR

Molecular conventional PCR analysis identified *Toxoplasma* infection in cat fecal samples 20% (20/100). Genomic DNA samples obtained from cat's fecal samples were subjected to molecular analysis by conventional PCR using B1 gene specific primer to identify *Toxoplasma* parasite. PCR of all (100) samples employed in the study exhibited distinct band of 194 bp on agarose gel confirming the presence of *Toxoplasma* parasite (Figure 1).

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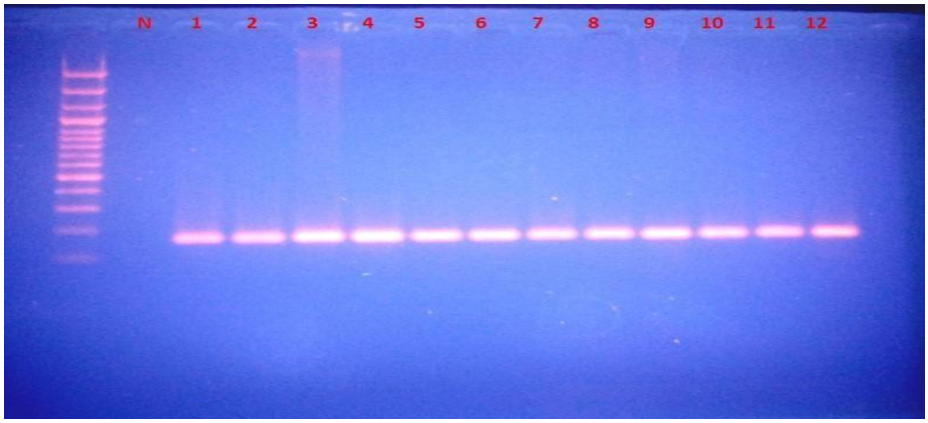

Figure 1: Electrophoresis of B1 Gene amplification products on 2% agarose gel and 70 volts for 1:30 h using DNA Ladder (100-1500 bp)

All the samples which were positive by conventional PCR amplification of B1 gene of *Toxoplasma* were successfully sequenced, and the result revealed presence of *Toxoplasma gondii*, that have identity of 100% and 99% (Table 6, Figure 2).

Table 6: Sequencing analysis of B1 gene

Gene: glycerol-3-phosphate dehydrogenase (B1) gene					
Type of substitution	**Location**	**Nucleotide**	**Sequence ID with compare**	**Source**	**Identities**
------	------	-----	ID: MK521885.1	*Toxoplasma gondii*	100%
Transition	91	G\A	ID: MK521885.1	*Toxoplasma gondii*	99%
-------	-------	------	ID: MK521885.1	*Toxoplasma gondii*	100%
------	------	------	ID: MK521885.1	*Toxoplasma gondii*	100%
-------	-------	-----	ID: MK521885.1	*Toxoplasma gondii*	100%
-------	------	-------	ID: MK521885.1	*Toxoplasma gondii*	100%
------	------	------	ID: MK521885.1	*Toxoplasma gondii*	100%
Transvertion	118	G\C	ID: MK521885.1	*Toxoplasma gondii*	99%
Transition	167	T\C	ID: MK521885.1	*Toxoplasma gondii*	99%
------	------	-----	ID: MK521885.1	*Toxoplasma gondii*	100%

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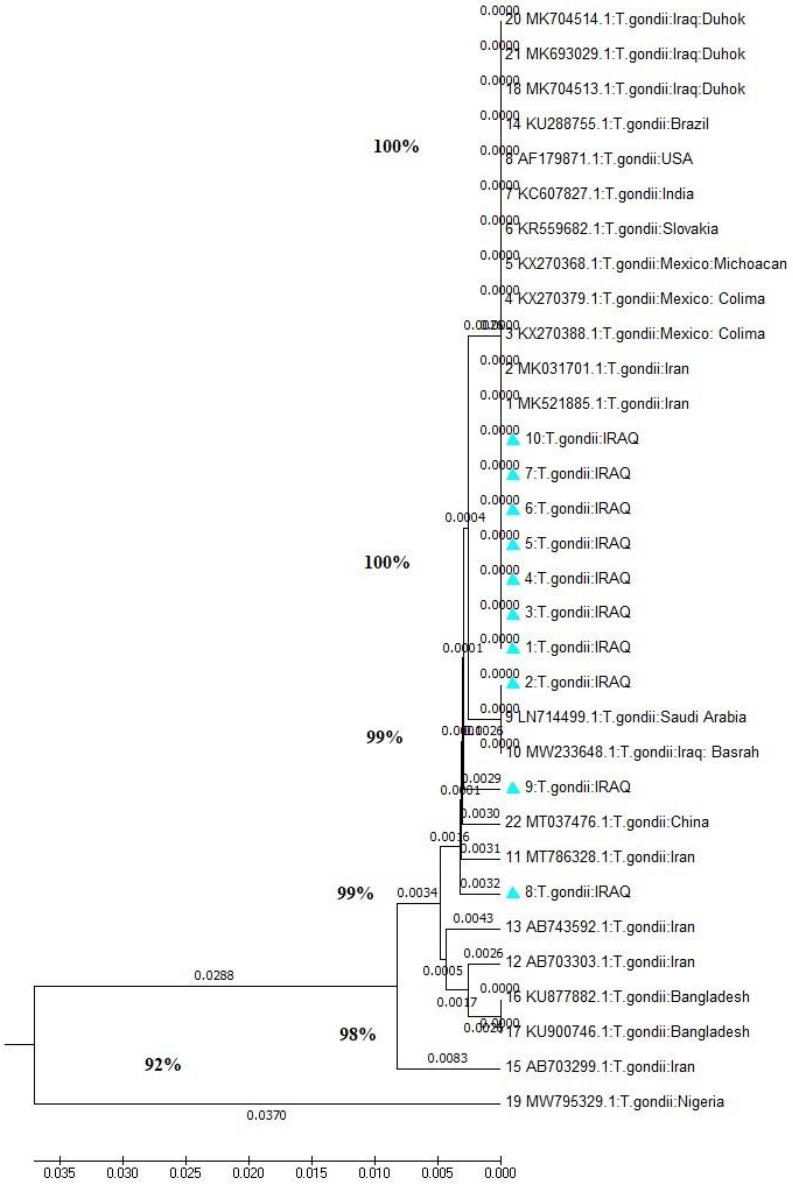

Figure 2: Phylogenetic relationships for *Toxoplasma* isolated from cat of B1 gene in the NCBI and Mega 7 (Neighbor joining)

Discussion

Floatation technique revealed the total rate of infection with *Toxoplasma* parasite in (100) domestic cats 14% (14/100) this result was in accordance with some studies showed significant association between regions such as [18] but not agree with [23] and [24].

Abu Ghraib recorded higher rate of infection 23.8% (5/21) while the lower rate recorded in Hay Al-Jamia 7.14% (2/28) with significant differences between all regions of study, these variations were attributed to the differences in socioeconomic habits between regions of study, which include the level of contact between human and cats. Existence of rodents with cats may increase the level of contamination, as well as breeding cats in areas of higher infection rate. These differences might belong to climatic conditions and increase opportunities of susceptible to severe source of toxoplasmosis, breeding conditions, level of contact feline with waters and food. The highest rate in Abu-Ghraib and Al-Ameria could be due to rural area the household, where high density of

domestication cat's feces was existence [21] and both cats and rodents increase in rural area led to increase chance of infection for both final host and intermediate host [26]. Differences were recorded between months of study where October recorded higher infection rate 29.41% (5/17) followed by March 25% (4/16). This result was compatible with [27] who pointed that rainfall cause difference in prevalence and remaining of oocysts in cool and moist climate and with [23] who recorded higher rates in Spring and lowest in Summer; whereas, [20,24] pointed that non-significant impact between months of study. Existence of cats are principle important in epidemiology of cats older than 1 year of age recoded higher rate of infection 17.85% (10/56) and lower rate 9.09% (4/44) recorded in cats less than 1 year of age. This result was attributed to the immunity of older animals that elevated and remain for life time due that elevated and remain for life time due to repetitive infection and maturation of immune system in contrast with small animals [25].

Differences recorded between female cats that recorded 22.22% (8/36) rate of infection with *Toxoplasma* and male with 9.37% (6/64) rate. There is no obvious explanation for this finding additional So you should be there studies with a larger sample size and random sampling would be needed to further investigate this finding. Although significant association was found between infection and age, it is difficult to make a strong conclusion regarding this association as a risk factor [26].

The results of molecular study recorded 20% rate of infection using conventional PCR compared to floatation technique that recorded of clinical toxoplasmosis [38]. Also the result is similar to that recorded by [14] in Portugal who found that the prevalence of *Toxoplasma* in domestic cats feces samples by PCR using B1 gene was 20.5%, while incomparable with rate recorded in Korean 4.5% by (20), and in Switzerland 0.4% by (4), and lower than 66.6% that recorded by [27].

Phylogenetic tree results showed that there is a large similarity in gene B1 and identical between isolates used in the study that isolated from the Baghdad regions, although there are different mutations and variations indicated by the results of the sequence between isolates as show in figure (1). These differences might be attributed to methodology, life style, ecology of geographical area and cats samples, The previous studies used the B1 gene as reliable molecular markers for different parasites in cats [15, 16, 18, 20]. This is the first molecular study in Baghdad city detecting *T. gondii* DNA from feces samples. However, the lack of data within the geographic locations renders the comparison with the corresponding references insufficient to address genetic variation. Therefore, future studies with similar or different genetic markers such as mitochondrial DNA are needed.

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