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PREVALENCE OF *TOXOCARA CATI* INFECTION AND MOLECULAR CHARACTERIZATION IN DOMESTIC AND STRAY CATS IN THE KARBALA PROVINCE, IRAQ.

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ABSTRACT

Toxocara (T.) cati is the feline parasite nematode a member of the Ascarididae family In the fourth week of their life, kittens excrete eggs along with their feces. This first study in Karbala province performed to determine the prevalence and identify precisely *Toxocara cati*, which infects cats based on molecular approaches. One hundred fecal samples of cats were gathered for the molecular detection of infection in stray and domestic cats and extended from January to October 2023.Detection of *T. cati* was conducted by utilizing the conventional polymerase chain reaction (PCR) technique with the impact of the risk factor. The results showed that 36% of cats were positive for the internal transcribed spacer 2 (ITS2) ribosomal RNA in cats. A significant correlation was found between the lifestyle, age and infection rates, and the infection rate between the stray cats was higher (58%) than domestic cats which was (14%). Moreover, and regarding the sex-related differences, no significant *T. cati* infection rates have been observed. Even though, seasonal differences in the infection rates were detected as highest in January.The PCR based molecular analysis of *T. cati* isolates indicated high percentage of similarities with strains from different geographical regions. Therefore, these results indicated a diverse and wildly spreads genetic lineage of *T. cati* and the similarities percentage were between 97.59% to 100% in different countries like Turkey, Iran, France, Germany, and New Zealand.

***Key words*:** Cats, polymerase chain reaction, Karbala, Toxocariasis, *Toxocara cati* .

INTRODUCTION

The parasite *Toxocara cati* is the source of the illness Toxocariasis, Domestic animals are suffering from intestinal parasite infections due to *T. cati* (1).The name of an ascarid nematode that belongs to the families Toxocaridae, Ascaridoidea, and Ascarididia. The mature parasite's' last host in felines is the small intestine. The diseased kittens typically exhibit acute clinical Toxocariasis symptoms. Clinical indicators include inadequate development, loss of condition, and occasionally a potbelly (2).Up to 200,000 eggs which have thick; pitted shells that are dark brown in color can be laid by female Toxocara worms every day in their excrement (3). Defecation eggs are immature and require a month to hatch in the earth, depending on the temperature. Under the right conditions, third-stage larvae-containing eggs can remain attractive for months or even years (4). After being eaten by the cat, the eggs hatch in the small intestine, pierce the stomach wall, and travel via the circulatory system to the liver and lungs. The larvae enter the trachea via swallowing, and this allows them to pass from the lungs into the digestive tract. The larvae mature into adults in the

small intestine, where they lay eggs that are expelled with feces (5).A second mechanism of *T. cati* transmission, apart from fecal-oral transmission, is trans-mammary transmission (6).Vertical transmission is absent in *T.cati*. Not having reached the adult stage, irresistible larvae in paratenic have stayed in tissues at a formatively imprisoned stage (7). Ingestion of partially cooked meat containing larvae or unintentional ingestion of embryonated eggs accidentally discovered in contaminated soil or food can lead to human infection (8).Understanding the epidemiology and controlling parasitic diseases is crucial for both medical and veterinary purposes (9). Cat helminthes parasite diagnosis is accomplished using microscopic inspection techniques such as sedimentation and flotation. Due to their affordability and ease of use, these techniques are still useful for identifying gastrointestinal parasites through inspection of adults, larvae, and other stages that are excreted in feces. For molecular systematic studies of numerous parasite groups, polymerase chain reaction techniques utilizing the ITS1 and ITS2 regions of rDNA offer genetic markers (10).To distinguish the eggs of *Toxocara spp*., a polymerase chain reaction (PCR)-based molecular method utilizing the ITS sequence was utilized in multiple investigations (11). This study was designed since there are no epidemiological data on *T. cati* in cats and no research on sequence variants of *T. cati* in cats in Karbala city.

MATERIALS AND METHODS

Samples collection:

Fife gram from fecal sample of 100 cats (50 domestic and 50 stray cats) was collected from Karbala. The samples of fecal were put in the container with date including age, date, and sex and transferred to the University of Al-Qadisiyah's College of Veterinary Medicine's Microbiology Laboratory, for investigation. The study was carried out from January 2023 to October 2023.

Molecular study:

Molecular study was conducted for detection of *T.cati* in cats and construction phylogenetic relationship. This study was including:

PCR and isolation of DNA :

The following changes were made to the manufacturer's instructions for the Geneaid, Korea, in order to extract Toxocara egg DNA from feces for the PCR: The samples were heated in buffer ASL to 95°C for 30 minutes, then proteinase K digestion was done for 30 minutes at 70°C. DNA was diluted to a final concentration in 80 µl of elution buffer and kept cold until needed. Using a spectrophotometer (Thermoscientific Nanodrop-ND 2000), the positive control sample consisted of the *T. cati* egg and adult isolates that were prepared in Oguz *et al* ,(12) . As a negative control, distilled water was also employed. A 300-bp section of Target Internal Transcribed Spacer 2 (ITS2) was amplified using a forward (GTAAGATCGTGGCACGCGTACGTA) and reverse (TCTTTGATGTCAAGACTTCAGCGC) primer set. DNA polymerase (Fermentas, Waltham, MA, USA), PCR water (6 \ 18 mM/cm, AppliChem, Darmstadt, Germany), master mix 10, forward primer (0.5 pmol/20 µl), reverse primer (0.5 pmol/20 µl) 1, and 2 µl of template DNA were added

to a 20 µl total volume mix for the PCR. The parameters of the reaction were as follows: Three minutes at 95 °C, 41 cycles of 35 s denaturation at 95 °C, 35 s annealing at 52 °C, and 35 s extensions at 72 °C. Final extension utilizing a heat cycler (PX2 Thermo, USA) at 72°C in 5 minutes. On a 3% agarose TAE gel stained with ethidium bromide, amplicons were found. It was visualized under UV light with a gel imaging system (NDR Bio-Imaging Systems Mini Bis Pro).

Amplicon sequencing and analysis :

From the positive PCR results, ten samples were chosen for Macrogen DNA Sanger sequencing, South Korea. Once the generated sequences were obtained, and they were trimmed from noisy signals and then deposited in the gene bank. After getting the relevant accession numbers, they were being analyzed for phylogeny and compared with other global strains as follows: The DNA sequencing study (phylogenetic tree analysis) was carried out using Molecular Evolutionary Genetics study version 10 (Mega X) and multiple sequence alignment analysis based on Clustal W alignment analysis. These were contrasted with susceptible strains and global resistance to find similarities and differences. The construction of the phylogenetic tree study involved a comparison with known sequences from NCBI-Blast. The software from the following reference (13) was used to conduct this analysis.

Statistical analysis:

The Statistical Analysis System (SAS 2018) was program used to detect the effect of various factors on parameters of study.A chi-square test was used to significantly compare between percentages (0.05 and 0.01 probability) in this study (14).

RESULTS

The outcomes revealed that out of 100 fecal samples of cat were examined by PCR, 36 (36 \ 100) tested positive for Toxocara DNA.

Infection rate of *Toxocara cati* according to Lifestyle by PCR :

There was a higher risk of infection among the stray cats *T. cati* was detected in 58% of cats as opposed to only 14% of domestic cats, The lifestyle and the rate of infection in cats showed a significantly substantial positive association.

Table 1. Prevalence of infection among domestic and stray cats :

Lifestyle	No. of samples	No. Positive	Percentage %	χ^2	P-value
Stray	50	29	58	15.902 **	0.0001
Domestic	50	7	14		
Total	100	36	36		
** (P≤0.01).					

** (P≤0.01)= highly significant

Infection rate of *Toxocara cati* according to age by PCR:

In Table 2 The highly significant association between the age and the infection rate of cats , There

was a higher infection rate in kitten cats (62.1%) than in adult cats (14.2%).

Table 2. Effect of age on the infection rate in cats:

Age	No. samples	Positive	Percentage %	χ^2	P-value
Kitten	37	23	62.1	8.315 **	0.0071
Adult	63	13	14.2		
Total	100	36	20.6		
	** (P≤0.01).				

** (P≤0.01)= highly significant

Infection rate of *Toxocara cati* according to Sex by PCR:

Using conventional PCR, Toxocara infection was detection in 35.2% (18\51) examined male cats, whereas in female cats, it was found in 34.6% (17\44) of examined. No significant difference (p > 0.05) between males and females cats.

Table 3. Effect of Sex on the infection rate in cats:

Sex	No. of examined samples	Positive	Percentage %	χ^2	P-value
Male	51	18	35.2	0.257 NS	0.902
Female	49	17	34.6		
Total	100	36	36		
	NS: Non-Significant.				

Infection rate of *Toxocara cati* according to months by PCR :

Monthly examination of 10 fecal samples for each month from January to October 2023 was shown in table (4). The highest infection rate was observed in January (60 % in cats), while lowest infection rate was observed in July and August months (20 % in cats) with non-significant (p > 0.05) differences was found between prevalence of infections according to months.

Table 4. Effect of monthly on the infection rate by PCR :

Month	No. of Samples cat	positive	Percentage %
January	10	6	60
February	10	5	50
March	10	4	40
April	10	4	40
May	10	3	30
June	10	4	40
July	10	2	20
August	10	2	20
September	10	3	30

October	10	3	30
Total	100	36	36
χ^2	1.208 NS		
P-value	0.251		
NS: Non-Significant.			

Results obtained by PCR technique:

Figure (1) shows positive amplicons (1-10) of *Toxocara cati* targeting partial region of ITS2 (size= 300 bp). NC is negative control in which H2O was added instead of DNA. M is molecular marker (100- 1500 bp) from Genedirx (South Korea).

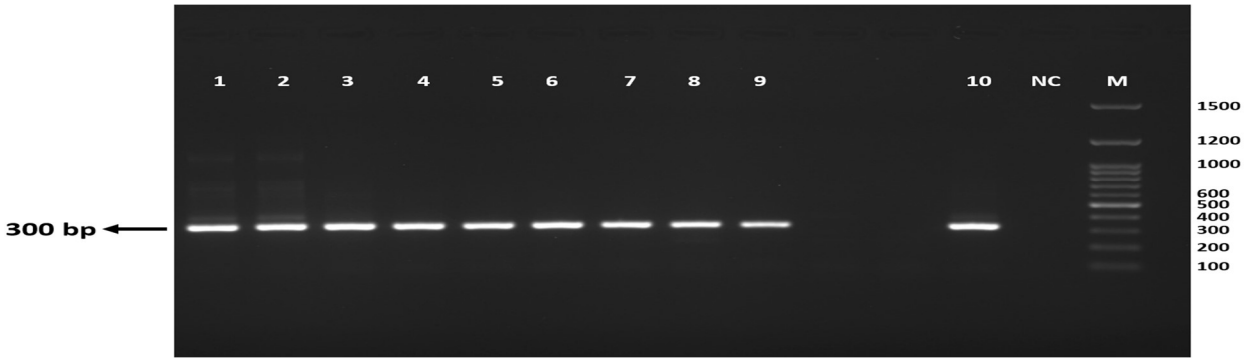

Figure 1.Agarose gel electrophoresis image (1.5 % agarose) for ITS2 (size= 300 bp) .

Result of sequencing DNA and phylogenetic tree :

Using data from the local *T. cati* nucleotide sets used in the current investigation, the National Center for Biotechnology Information (NCBI) evaluated and verified the findings. Local isolates of *T. cati* were entered into the NCBI-Genbank database and assigned Genbank accession numbers. Sequences of local strains were aligned with reference strains for *T. cati* that had previously been documented in GenBank using the MEGA 6.0 Clustal W alignment tool.Results showed three *T.cati* isolates (OR625131, OR625137, and OR625139) had a close relationship with NCBI-Blast *T.cati* of Iran (LC700099) with 99.60%, 97.59%, and 100% identity, whereas other one isolates (OR625137) were related with 97.59% identity with (AB743605) in Iran . Two *T.cati* isolates (OR625130 and OR625138) had a close relationship with the NCBI-Blast *T.cati* of Turkey (MH043958) with 100% identity. Isolate (OR625132) a close relation with NCBI-Blast *T.cati* of China (KY003092) with 99.60% identity, Isolate (OR625133) a close relation with NCBI-Blast *T.cati* of Germany (LC762620) with 99.60% identity, Isolate (OR625134) a close relation with NCBI-Blast *Toxocara cati* of France (MW144961) with 99.60% identity, Isolate (OR625135) a close relation with NCBI-Blast *T.cati* of New Zealand (MN585770) with 98.80% identity.

DNA sequencing and phylogenetic tree of *Toxocara cati*:

The branch lengths in the tree are expressed as the number of substitutions per site (below the branches), and the tree is depicted to scale. Beside each internal node in the tree is the percentage

of places where at least one clear base appears in at least one sequence for every descendent clade. There were 25 nucleotide sequences in this investigation. The final dataset contained 259 locations in total. The tree was rooted using Ascaridoidea sequence and the evolutionary analyses were conducted in MEGA11.

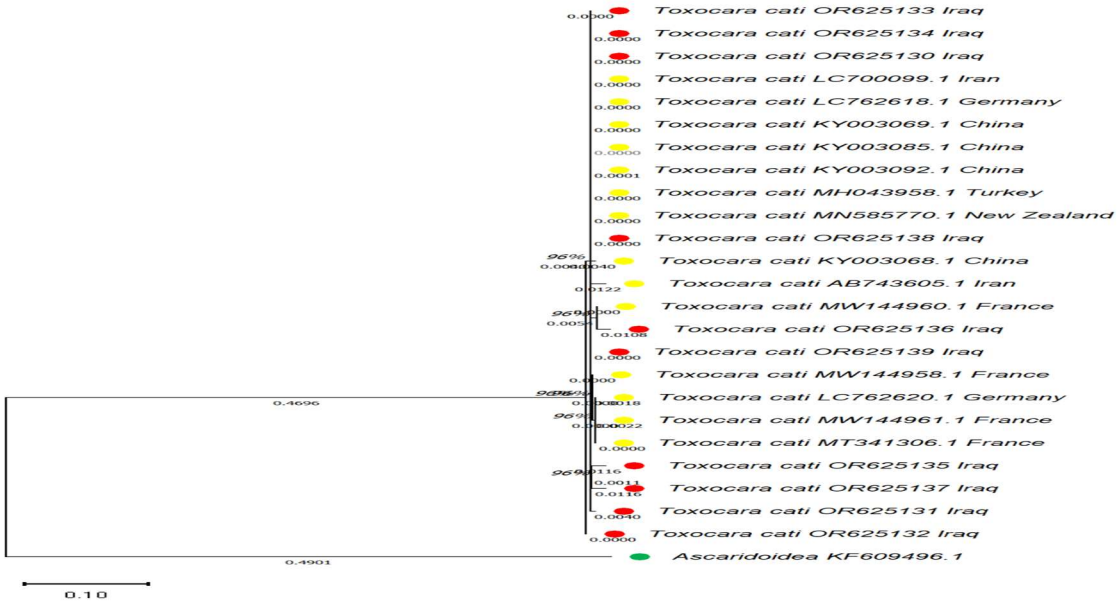

Figure 2.Evolutionary analysis by Maximum Likelihood method *of T. cati*. (red circles referred to the currently identified sequences while yellow circles referred to other global sequences).

Table 5. The NCBI-BLAST Homology Sequence identity (%) in local *Toxocara cati*.

The following accession codes (OR625130, OR625131, OR625132, OR625133, OR625134, OR625135, OR625136, OR625137, OR625138, and OR625139) were used to deposit these sequences in the gene bank, where they were compared to other global sequences.

Sequence number	Obtained Accession number	NCBI-BLAST Homology Sequence identity (%)			
		Identical to	Genbank Accession number	Country	Identity (%)
1	OR625130	*Toxocara cati*	MH043958	Turkey	100
2	OR625131	*Toxocara cati*	LC700099	Iran	99.60
3	OR625132	*Toxocara cati*	KY003092	China	99.60
4	OR625133	*Toxocara cati*	LC762620	Germany	99.60
5	OR625134	*Toxocara cati*	MW144961	France	99.60
6	OR625135	*Toxocara cati*	MN585770	New Zealand	98.80
7	OR625136	*Toxocara cati*	LC700099	Iran	98.93

8	OR625137	*Toxocara cati*	AB743605	Iran	97.59
9	OR625138	*Toxocara cati*	MH043958	Turkey	100
10	OR625139	*Toxocara cati*	LC700099	Iran	100

DISCUSSION

Approximately 36% of cats in the current study had *T. cati* infection. With varying frequency rates, reports of these illnesses have been made in Iraq (15) and other nations. For instance, a prevalence of 26.7 percent (31%) of *T. cati* was found from Iran (16). The prevalence percentage of *T. cati* was found to be 17.4% in Hungary (17) and 27.8% in Turkey (18). *T. cati* was found in Egypt at a rate of 8–9%, which was substantially lower than that of the current investigation (19, 20). Geographical variance and the type of detection technique may be to blame for the variations in *T. cati* prevalence seen in these investigations. When addressing the term "prevalence," it is crucial to remember that different study results may vary depending on the size of the sample, sampling techniques, kind of sample (necropsy or corroscopic), and other epidemiological and statistical aspects. Therefore, when comparing or debating the findings of different studies, caution should be used. To illustrate the widespread distribution of this virus worldwide, we have included here the varying prevalence rates of *T. cati* in several nations and areas. According to Faraj et al. (21), the PCR methodology and phylogenic tree analysis are the ideal molecular approaches for identifying and detecting genetic variants of parasites.Despite the fact that some researchers estimated the genetic parameter and evaluated the genetic impact using molecular techniques (22 and 23). The infection rate was higher in younger animals than in older age groups, according to the results. According to research, cats under the age of one year old in Canada (24) and the US, Mexico, the Caribbean, and Central America (25) had greater infection rates than older dogs and cats. However, this outcome made more sense given the intricate *T.cati* life cycle, which infects kittens through a variety of routes (i.e., ingesting embryonated eggs and obtaining infective larvae from contaminated mother milk). In the current investigation, there was no significant difference in the prevalence of a particular parasite in males and females. This result is consistent with earlier research by (26). The current study reveals non-significant differences in infection rates throughout the study's months. The highest infection rates were seen in the chilly months of February and January, while the lowest rates were seen in July and August. The greater temperature during the summer season may inhibit the survival of the parasites; the colder months extend the survival of the eggs in the soil due to humidity for the growth of parasite eggs and subsequently the formation of infective larvae. By causing water logging and the dissemination of infectious eggs and larvae, heavy or early rainfall, contributes to greater parasite infections (27).Results showed three *T.cati* isolates (OR625131, OR625137, and OR625139) had a close relationship with NCBI-Blast *T.cati* of Iran (LC700099) with 99.60%, 97.59%, and 100% identity, whereas other one isolates (OR625137) were related with 97.59% identity with (AB743605) in Iran .Two *T.cati isolates* (OR625130 and OR625138) had a close relationship with the NCBI-Blast *T.cati* of Turkey (MH043958) with 100% identity. Isolate (OR625132) a close relation with NCBI-Blast *T.cati* of China (KY003092) with 99.60% identity; Isolate (OR625133) a close relation with NCBI-Blast

T.cati of Germany (LC762620) with 99.60% identity; Isolate (OR625134) a close relation with NCBI-Blast *T.cati* of France (MW144961) with 99.60% identity; Isolate (OR625135) a close relation with NCBI-Blast *Toxocara cati* of New Zealand (MN585770) with 98.80% identity. The highly identity can be due to it clustering in the same nodule, which explains a tight relationship between them, and this may be because these countries are adjacent to one another and situated along a single geographic line, allowing for migration of the paratenic hosts like rodents and birds between these countries.

Conclusion: According to the current study, *T. cati* is somewhat common in cats living in the study region. This is the first genetic investigation that verifies the presence of cats infection with *T. cati* living in Karbala. Furthermore, the primary risk factor for the disease's transmission is stray of cats.

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Conflict of interest:
There is no conflict of interest.

References

1. Bowman DD. Georgi's parasitology for veterinarians. 9th ed. Washington: Library of Congress Cataloging; 2009.202 p. https://epdf.pub/georgis-parasitology-for-veterinarians-9thedition .
2. Holland C.V.Knowledge gaps in the epidemiology of Toxocara: the enigma remains. *Parasitology*, 2017, *144*(1), 81–94. doi:10.1017/S0031182015001407
3. Okulewicz A, Perec-Matysiak A, Bu´nkowska K and Hildebrand J. *Toxocara canis, Toxocara cati* and *Toxascaris leonina* in wild and domestic carnivores. Helminthologia 2012, 49, 3–10.https://doi.org/10.2478/s11687-012-0001-6
4. Strube C, Heuer L and Janacek E..*Toxocara spp*. Infections in paratenic hosts. Vet. Parasitol.J., 2013, 193(4) : 375– 389.
5. Despommier D. Toxocariasis: Clinical aspects, epidemiology, medical ecology, and molecular aspects. Clin Microbiol Rev. 2003,16(2):265-72. DOI: 10.1128/cmr.16.2.265-272.2003
6. Azam D, Ukpai O.M, Said A, Abd-Allah G.A. and Morgan E.R. Temperature and the development and survival of infective Toxocara larvae. Parasitol. Res. J., 2012 110(2):649–656.
7. Barutzki, D., & Schaper, R. 2013. Age-dependant prevalence of endoparasites in young dogs and cats up to one year of age. Parasitology Research, 112, S119-S119.
8. Hade B. F. Molecular sequancing and phylogenic analysis to virulence nmuc-1 gene in visceral larvae migrance. Iraqi Journal of Agricultural Sciences,2020; 51(3), 894-902.
9. Avcioglu H, Burgu A. Seasonal prevalence of toxocara ova in soil samples from public parks in Ankara, Turkey. Vect Borne Zoonot Dis. 2008;8(3):345-50. DOI: 10.1089/vbz.2007.0212
10.Rostami A, Sepidarkish M, Ma G, Wang T, Ebrahimi M, Fakhri Y., *et al* . Global prevalence of Toxocara infection in cats. Advances in Parasitology,2020; 109, 615-639.

11. De Van N, Trung N.V, Duyet L.V and Chai, J-Y.Molecular diagnosis of an ocular toxocariasis patient in Vietnam. Kor. J. Parasitol. 2013 , 51(5): 563-567.
12. Oguz B,Ozdal N & Serdar Deger M.Genetic analysis of Toxocara spp. in stray cats and dogs in Van province, Eastern Turkey. Journal of Veterinary Research, 2018,62(3) 291-295. https://doi.org/10.2478/jvetres-2018-0042
13. Fahrion A.S, Schnyder M, Wichert B and Deplazes P.Toxocara eggs shed by dogs and cats and their molecular and morphometric species specific identification. Vet. Parasitol.J. 2011,177(1): 186–189.
14. SAS. 2018. Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA.
15. Hassan HF, Barzinji AKRA. Epidemiological survey on stray dogs and cats gastro-intestinal parasites in Kirkuk province, Iraq. Kirkuk Uni J Sci Stud. 2018;13(1):228-38. DOI: 10.32894/kujss.2018.143035
16. Eslahi A.V.M, Badri A, Khorsidi H, Majidiani E, Hooshmand H, Hosseini M.Prevalence of Toxocara and Toxascaris infection among human and animals in Iran with meta-analysis approach. BMC infectious diseases, 2020, 20(1), 1-17.
17. Capári B, Hamelc D, Visserc M, et al. Parasitic infections of domestic cats "Felis catus" in west-ern Hungary. Vet Parasitol. 2013; 192(1-3):33-42.
18. Gürler At, Bölükbaş Cs, Pekmezci Gz, et al. Nematode and cestode eggs scattered with cats-dogs feces and significance of public health in Samsun, Turkey. Ankara Üniv Vet Fak Derg. 2015; 62:23-26.
19. El-Seify MA, Aggour MG, Sultan K, Marey NM. Gastrointestinal helminthes of stray cats in Alexandria, Egypt: A fecal examination survey study. Vet Parasitol Reg Stud Reports. 2017; 8:104-106.
20. Khalafalla RE. A survey study on gastrointesti-nal parasites of stray cats in northern region of Nile delta, Egypt. PLoS One. 2011; 6(7):e20283.
21.Faraj A. A, Hade B. F, Al-Amery A. M. Conventional and molecular study of Babesia spp. of natural infection in dragging horses at some areas of Baghdad city, Iraq. Iraqi Journal of Agricultural Sciences, 2019; 50(3), 909-915.
22. Lucio A, Mizhquiri JS, Mohammed HO, Kornreich BG, Bowman DD. Comparison of the prevalence of Toxocara egg shedding by pet cats and dogs in the USA, 2011-2014. Vet Parasitol. 2016;5:1-13.DOI: https://doi.org/10.32894/kujss.2018.143035
23.Wuhaib K. M, Hadi B. H, Hassan W.A. Genotypic and Phenotypic Correlation in Maize and Path Coefficient I Agronomic Traits, Iraqi Journal of Agricultural Sciences:2018; 49(2):179-187.
24. Jenkins EJ. *Toxocara spp*. in dogs and cats in Canada. 1st ed. NY: Elsevier Ltd; 2020. 641-653 p. DOI: 10.1016/bs.apar.2020.01.026.
25. Ketzis JK, Lucio-Forster A. *Toxocara canis* and *Toxocara cati* in domestic dogs and cats in the United States, Mexico, Central America, and the Caribbean: A review. 1st ed. NY: Elsevier Ltd.; 2020. 655-714 p.
26. Baneth G, Thamsborg S.M, Otranto D, Guillot J, Blaga R, Deplazes P & Solano-Gallego L.

Major Parasitic Zoonoses Associated with Dogs and Cats in Europe. *Journal of comparative pathology*, 2016, *155*(1 Suppl 1), S54–S74. https://doi.org/10.1016/j.jcpa.2015.10.179
27. Overgaauw PA, Van Knapen F. Veterinary and public health aspects of *toxocara spp*. Vet Parasitol. 2013;193:398-403.DOI: 10.1016/j.vetpar.2012.12.035.

