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# Molecular and serological detection of *Toxoplasma gondii* in three species of wild birds of Babylon province, middle Iraq

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Article information	Abstract
Article history: Received March 29, 2022 Accepted June 11, 2022 Available online June 12, 2022	Birds are intermediate hosts and important reservoirs that play a significant role in <i>Toxoplasma gondii</i> (Apicomplexa, Sarcocystidae) epidemiology and infection transfer to humans by eating their raw or undercooked meat. The aim of this study is to diagnose the <i>Toxoplasma gondii</i> infection in three species of wild birds ( <i>Columba livia, Streptopelia</i> )
<b>Keywords:</b> Columba livia Nested PCR Passer domesticus Streptopelia senegalensis Toxoplasma gondii	<i>senegalensis</i> and <i>Passer domesticus</i> ) in the province of Babylon from May 2021 to August 2021, using a latex agglutination test and molecular diagnosis with nested PCR for SAG1 gene identification. Results showed that antibodies were detected in 56/144 (38.88%) samples. Furthermore, results of the nested PCR technique for detection of SAG1 gene revealed that 41 (73.21%) of the samples positive for the latex agglutination test were only
Correspondence: B.H. Abdullah drbasimabdulah@gmail.com	<i>gondii</i> with possible transmission to human beings. For the first time, a <i>S. senegalensis</i> , was infected with the <i>T. gondii</i> in Iraq.

DOI: <u>10.33899/ijvs.2022.133394.2219</u>, ©Authors, 2023, College of Veterinary Medicine, University of Mosul. This is an open access article under the CC BY 4.0 license (<u>http://creativecommons.org/licenses/by/4.0/</u>).

#### Introduction

Toxoplasmosis is very important zoonotic parasitic disease caused by a parasite belonging to the group Coccidia, the class of Sporozoa known as the parasite Toxoplasma gondii (1,2). T. gondii is a major public health concern because it affects a large number of people around the world (3). T. gondii (Apicomplexa, Sarcocystidae) is one of the most common parasitic infections of humans and other warm-blooded animals, including birds (4,5). Sexual reproduction of this parasite develops in the intestines of cats, while asexual reproduction occurs in the tissues of mammals and birds (6). Toxoplasmosis in bird populations can be a substantial concern from the wildlife conservation perspective, as a result of many species are acutely sensitive to T. gondii (7). Toxoplasma was considered a cause of death for birds of various species (8). The parasite goes through three stages that are contagious: Oocysts, which are introduced into the external environment with the feces of infected cats, which later form spores. Tachyzoite divides rapidly into all host cells and intermediate host cells, sometimes surrounded by an irregularly shaped cyst. The thin layer of the wall is known as the pseudocyst, and the bradyzoite, which reproduces slowly, spreads into a thick wall sac known as the tissue cyst, which forms within various organs of the host's body (9-12). Cats are the only definitive host for the T. gondii and thus the only source of infective oocysts, however different mammals and birds can develop tissue cysts (13). Cats can spill millions of oocysts after ingesting just one bradyzoite (14). Ground-feeding birds are essentially important in the epidemiology of T. gondii because they act as indicators of soil oocyst contamination and can be deemed important reservoirs of the parasite as they are often eaten by felids (15,16). T. gondii oocysts undergoes environmentally- resistant stages that can survive in soil for years. When climatic factors such as humidity and temperature are favorable; they lead to the infection of birds (17,18). Infection of birds with T. gondii is a good indicator

of soil contamination by the parasite oocysts (19). Clinical signs of infection in birds are often indefinite and vary in severity depending on the age of the host, dose of the infective agent, species of the host, rate of infection, and virulence of the Toxoplasma strain (20). Symptoms in birds include loss of appetite, pallor of the mucous membranes, emaciation, diarrhea, and central nervous system dysfunction. The parasite spreads easily through the vascular system and lymphoid tissues, parasitizing and killing individual cells. The characteristic tissue lesion is severe necrosis surrounded by lymphocytes, macrophages and heterotrophic cells (21). Toxoplasmosis may cause diarrhea in infected birds, and this indication is worth research because it harms the animal's nutrition and thus its growth. A possible explanation may be that T. gondii, unknowingly so far, alters the functioning of the enteric nervous system responsible for coordinating intestinal motility (22). The determination of T. gondii spread in indigenous birds, as well as other indications for detecting T. gondii oocyst in the environment, is of significant interest (23). T. gondii serological and molecular diagnosis are critical because microscopic detection is difficult and can be confounded with other species, particularly Sarcocystis spp. (24). The use of agglutination latex test for diagnosis of T. gondii is common nowadays, because of its ease of use and its reduction of money, time and effort required to perform it (25). Polymerase chain reaction (PCR) technique is used to confirm the results of serological tests because this technique is highly sensitive and specific when used to detect T. gondii parasite in different biological samples (26).

The current study aims at evaluating the incidence rates of *T. gondii* in three species of birds in the province of Babylon to detect antibodies (Abs) of the parasite in the birds' sera using Latex Agglutination Test (LAT) as a primary test, and to confirm the presence of the parasite *T. gonodii* through a polymerase chain reaction technique (nested PCR) by confirming the presence of the parasite's SAG1 gene in tissue samples due to the economic importance of birds and their proximity to humans, specifically *Columba livia, Streptopelia senegalensis* and *Passer domesticus* that act as intermediate hosts for the parasite.

# **Materials and Methods**

## **Ethical approve**

The approval was given to conduct this scientific work by the University of Basra, College of Education for Pure Sciences in their book No. 3/7/3061 on 14/12/2020.

## **Collection of bird specimens**

A total of 144 birds (*Columba livia*, *Streptopelia* senegalensis and *Passer domesticus*) were hunted at different locations of the province of Babylon.

# Collection of blood samples and serum isolated

Blood samples were collected from the brachial vein. Serum was isolated by centrifuging blood samples for 5-10 minutes at 3500 rpm. Then the serum was transferred to Eppendorf tubes that were kept frozen until the day of the experiment.

#### Latex agglutination test (LAT)

The latex agglutination test (Toxo-latex, spinreact, Spain) was performed according to the method described by Campbell (27). On the day of the latex agglutination test experiment, the serum was left at room temperature, and then 50  $\mu$ l of serum was used, mixed with 25  $\mu$ l of reagent and left for 5 min with good mixing by plastic sticks supplied with the test kit. Positive samples for the latex agglutination test showed clear agglutination, while negative samples did not adhere to the reagent. Avian tissues (liver, heart, and brain) positive for the latex test were kept frozen for use for molecular purposes.

# DNA isolation and Nested PCR amplification of T. gondii

The AddPrep Genomic DNA Extraction kit (Addbio, Korea) was used to extract genomic DNA from tissue samples from 56 wild birds that tested positive for the latex test. This was carried out in accordance with the protocol described by the manufacturer's instructions. Until the extracted DNA was analyzed, it was stored at -20°C. Molecular diagnosis was performed by the nested PCR method using primers targeting the SAG1 gene for T. gondii (First round: SAG1ExF (5-GTTCTAACCACGCACCCTGAG-3), SAG1ExR (5-AAGAGTGGGAGGCTCTGTGA-3) 430 bp, second round: (5-CAATGTGCACCTGTAGGAAGC-3), SAG1InF SAG1InR (5-GTGGTTCTCCGTCGGTGTGAG-3) 390 bp ) described by Grigg et al. (28) and Lass et al. (29) to detect possible infection with T. gondii and determination of the results of doubling on agarose gel. The positive samples fourteen were deposited in the NCBI database that provided us with the accession numbers explained in (Table 1).

# Results

# LAT and Nested PCR

Results showed that antibodies were detected in 56/144 (*Columba livia* 37.5%, *Streptopelia senegalensis* 28.57% and *Passer domesticus* 33.92%) samples three species, while the nested PCR technique for detecting the SAG1 gene revealed that 41 (*C. livia* 36.58%, *S. senegalensis* 29.26% and *P. domesticus* 34.14%) of the samples positive for the latex test from the three birds were only discovered to be positive. Furthermore, the SAG1 gene has a 390bp molecular weight (Figure 1). The results also showed significant differences in the infection with *T. gondii* parasite between males and females, where the infection in males 55.35% was higher than it was in females 44.64% using the latex

agglutination test, while the infection was in females 51.21%, which was higher than that of males 48.78% using the nested polymerase chain reaction technique.



Figure 1: Gel electrophoresis image (2%) shows the second round amplicons of SAG1 gene of *T. gondii* in positive samples (size= 390 bp). M is molecular marker (genedirex, Korea). C is control negative in which H<sub>2</sub>O was added instead of DNA.

# The sequences

From matching the sequences of the *Toxoplasma* parasite recorded in the current study with the sequences of the same parasite registered in the NCBI Gene bank, it was observed that there was a congruence of (97.41-99.71) between the parasite samples isolated in the current study with the species registered in the National Center for Biotechnology Information (NCBI) (Table 1). Importantly enough, three strains were closely related to the strains from Poland and Australia, including: OK138619, OK138624, OK138626.

# **Phylogenetic tree**

After reading the sequences of the SAG1 gene of the *T. gondii* parasite isolated in this current study, which was described as the red circles and deposited at the NCBI GenBank website, a phylogenetic tree analysis was drawn using Clustal Omega software (Figure 2). These are aligned and compared with other global strains indicated by the yellow circles.



Figure 2: Phylogenetic tree analysis of currently identified sequences of *T. gondii*.

Table 1: The NCBI-BLAST Homology sequence identity (%) between local *T. gondii* of bird's tissue isolates deposited in the NCBI under the accession numbers shown in the table below

Toxoplasma gondii	Accession	NCBI-BLAST Homology sequence identity (%)				
sequence No.1	number	Identical to	GenBank Accession number	Country	Identity (%)	
1	OK138613	Toxoplasma gondii	MH606165	Poland	99.43	
2	OK138614	Toxoplasma gondii	KT881323	Australia	99.43	
3	OK138615	Toxoplasma gondii	DQ872517	India	99.14	
4	OK138616	Toxoplasma gondii	MN958072	Italy	98.57	
5	OK138617	Toxoplasma gondii	LC414528	Iran	98.85	
6	OK138618	Toxoplasma gondii	LN714498	Saudi Arabia	99.71	
7	OK138619	Toxoplasma gondii	KT881352	Australia	99.70	
8	OK138620	Toxoplasma gondii	MG588014	Italy	98.28	
9	OK138621	Toxoplasma gondii	KY618706	China	99.68	
10	OK138622	Toxoplasma gondii	MH606164	Poland	97.90	
11	OK138623	Toxoplasma gondii	MH704654	Iran	97.41	
12	OK138624	Toxoplasma gondii	LC414529	Iran	99.71	
13	OK138625	Toxoplasma gondii	MG588013	Italy	98.28	
14	OK138626	Toxoplasma gondii	MH606155	Poland	99.43	

## Discussion

The nested PCR technique has been used to corroborate the results of serological tests represented by the latex agglutination test as a diagnostic method, being a more sensitive tool allowing specific amplification product to be extracted from an abundance of non-specific products. Even if PCR primers amplify non-specific sequences; making it impossible to identify the desired product, a second PCR, using nested primers, is designed to amplify an inner region of the original amplified product (30).

The results of immunological detection, using latex agglutination test and molecular detection, using nested PCR technique to search for the SAG1 gene showed that the incidence of *T. gondii* in *Columba livia* is higher than the percentage that was recorded by Alaraji *et al.* (31) in Babylon Province using the latex agglutination test which is 20% and in some other governorates of Iraq, including Hamza and Dakhel (32) in Al-Qadisiyah governorate using latex agglutination assay and Al-Abodi (33) in Al-Qadisiyah governorate using latex agglutination assay and PCR technique to search for B1 gene, which are 32.3%, 13.74 (5% and 5%) respectively.

Also, the incidence of infection was higher in percentage than what was recorded in several studies in the world, including Valian and Ebrahimi (34) in Iran using direct agglutination test (DAT), and Waap *et al.* (35), in Portugal using direct agglutination test (DAT), Alvarado-Esquivel *et al.* (36) in Mexico using modified agglutination test (MAT), and Khademvatan *et al.* (37) in southwestern Iran using PCR-RFLP technology to search for the GRA6 gene, which were: 2.8, 4.6, 1.3, and 6.9%, respectively. The number of positive cases in the current study was lower than the percentage reported by Tayyub *et al.* (38) in Pakistan using PCR technique which is 38.3%.

The percentage of positive cases recorded in the current study of Streptopelia senegalensis is higher than the percentage recorded by Valian and Ebrahimi (34) in Iran using direct agglutination test (DAT), which is 5.1%. As for the number of positive cases in *Passer domesticus*, it is higher than the percentage recorded by Valian and Ebrahimi (34) in Iran using the direct agglutination test (DAT) Literák et al. (39) in the Czech Republic using an indirect fluorescent antibody (IFA) assay and Cong et al. (40) in Northwest China using MAT and PCR technology to search for the B1 gene and Khademvatan et al. (37) in southwestern Iran using PCR-RFLP technology to search for the GRA6 gene, which are 17%, 12.3%, (12.46%, 28.20%) and 26.5%, respectively. However, the number of positive cases of *P. domesticus* is lower than the percentage recorded by Hussein et al. (10) in Iraq using the rapid cassette testing 71.25%. As for Streptopelia Senegalensis in Iraq, there are no studies conducted.

The cause of the disparity in infection rates of T. *gondii* in the above studies can be explained on the basis of the

difference in the number of samples examined, the sensitivity of the tests used in diagnosis, and the variation in the geographical and environmental location of those areas (32).

#### Conclusion

Three species of birds were found infected with *T. gondii* with possible transmission to human beings. It was the first time that *S. senegalensis* had been reported infected with the parasite.

## Acknowledgements

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### **Conflict of interest**

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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# الكشف الجزيئي والمصلي عن المقوسة الكوندية في ثلاثة أنواع من الطيور البرية في محافظة بابل، وسط العراق

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#### الخلاصة

الطيور هي مضائف وسيطة وخازنات مهمة تلعب دورا مهما في وبائية المقوسة الكوندية (معقدات القمة، عائلة Sarcocystidae) وتنتقل العدوى إلى البشر عن طريق تناول اللحوم النيئة أو غير المطبوخة جيدا. الهدف من هذه الدراسة هو تشخيص عدوى المقوسة الكوندية في ثلاثة أنواع من الطيور البرية (الحمام الطوراني وفاختة النخيل والعصفور الدورى) في محافظة بابل من مايو ٢٠٢١ إلى أغسوسطس ٢٠٢١،

باستخدام اختبار تلازن اللاتكس والتشخيص الجزيئي باستخدام تقنية تفاعل البلمرة المتسلسل المتداخل لتحديد الجين SAG1. أظهرت النتائج أن الأجسام المضادة وجدت في ١٤٤/٥٦ عينة (٣٨,٨٨٪). علاوة على ذلك، أظهرت نتائج تقنية تفاعل البلمرة المتسلسل المتداخل للكشف عن جين SAG1 في أن ٤١ (٧٣,٢١) من العينات الإيجابية لاختبار

تلازن اللاتكس وجدت إيجابية فقط في ثلاثة أنواع من الطيور. تم العثور على هذه الأنواع الثلاثة من الطيور مصابة بطفيلي المقوسة الكوندية مع احتمال انتقالها إلى البشر. ولأول مرة تم تسجيل إصابة طائر فاختة النخيل بطفيلي المقوسة الكوندية في محافظة بابل خصوصا وفي العراق عموما.