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Serological detection of *Toxoplasma gondii* in local duck birds, and *Gallus gallus* in Basrah Province, Iraq

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Abstract

Toxoplasma gondii was widely spread in humans and other animals, including domestic poultry, worldwide and depends on a unique collection of secretory and cytoskeletal organelles for host cell invasion. The meat of *T. gondii* infected poultry which includes chickens and ducks was widely consumed in most countries and was known as a primary source of human infection. In Iraq generally and in Basrah province especially, the prevalence of *T. gondii* in local ducks (*Anas platyrhynchos domesticus*) and domestic chicken (*Gallus gallus*) was scarcely known. In the current study, the Latex Agglutination Test (LAT) and (TOX) *Toxoplasma* IgM/IgG Antibody Rapid Test Kit (Immunochromatography) were used to examine antibodies to *T. gondii* in 40 local duck birds and 62 domestic chickens from different regions in Basrah Province. Antibodies to *T. gondii* (LAT) were found in 14 (35%) of 40 local duck birds, and 23 (37.09%) of 62 domestic chickens. While the *T. gondii* antibodies (Rapid Test) were detected in 10 (25%) of 40 local duck birds and 17 (27.41%) of 62 domestic chicken. The findings showed that the soil was contaminated by *T. gondii* oocysts represented a food source for domestic chicken and local duck birds due to the birds feeding on the ground. It suggested that the meat from domestic poultry may be an important source of human *T. gondii* infection in Iraq. This study was the first study in Basrah province to detect toxoplasmosis in domestic chicken and local duck birds.

Keywords: Ducks, *Gallus gallus*, Latex Agglutination Test, Rapid Test, *Toxoplasma gondii*.

Introduction

Toxoplasmosis is a widespread zoonosis illness brought on by the parasite *Toxoplasma gondii*, an obligate intracellular coccidian parasite that infects most avian and mammalian species and is thought to manipulate in intermediate hosts' behavior for easier transmission into feline hosts. Although intermediate hosts can retain infective tissue cysts, felines are the only animals that discharge oocysts in their feces as definitive hosts for this pathogen [1-5]. The parasite is more prevalent in warm and humid areas compared to dry areas [6]. The parasite alters the conduct of its intermediate hosts by decreasing their natural aversion to cat odors, potentially boosting the likelihood that the infected host will be eaten by the definitive host. The parasite depends on a unique set of cytoskeletal and secretory organelles for host cell invasion [7-9].

Birds including chickens are important *T. gondii* reservoirs because they are often hunted by felids; they reproduce out of control as they are not eclectic about food, eating food waste that may be polluted with *T. gondii*. Furthermore, because they can fly

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long areas and eat on the soil, they could be prospect hosts for this coccidian[10, 11]. Tissue cysts of *T. gondii* (bradyzoites) found in undercooked or raw meat from many sources, including birds, are thought to be important sources of infection in humans [12].

In the epidemiology of *T. gondii* infection, poultry is one of the most significant hosts because they are the effective source for infection cats that excrete the environmental factors resistant oocysts, also humans can contract the parasite by eating undercooked infected chicken mea[13].

Toxoplasmosis mostly affects the central nervous system, although it can also impact the skeletal muscles reproductive system, and visceral organs in mammals, birds, and reptiles [14]. Myositis in the skeletal and cardiac muscles, diarrhea, nonsuppurative meningoencephalitis, nephritis, and focal nonsuppurative hepatitis are some of the clinical symptoms of toxoplasmosis in chickens [15].

In Iraq, the consumption of chicken and duck meat has increased, so, this could be one of the sources of human infection[16]. The objective of the current study is to determine the prevalence of *T. gondii* in local duck species and *Gallus gallus* in the province of Basrah. *Toxoplasma* IgM/IgG Antibody Rapid Test Kit (Immunochromatography) and the latex agglutination (IgM and IgG) test (LAT) were used to detect antibodies (Abs) to the parasite in the serum of local duck species and domestic chickens, due to the economic significance of these species and their proximity the presence of a human, which acts as a carrier and intermediary for the parasite as well as cats.

Materials and Methods

Collection of samples:

Domestic chickens (*Gallus gallus*)

A total of 62 domestic chickens' samples were cluster screened randomly purchased from different locations in the province of Basrah from March to November 2022.

Local ducks (*Anas platyrhynchos domesticus*)

A total of 40 local duck birds were purchased from rural areas and Basrah bird sale places from August 2022 to February 2023.

Blood collecting and separation of serum

Brachial vein blood samples (between 2-4 mL) were taken. Blood samples were centrifuged for 6–10 minutes at 4000 rpm to separate the serum, which was then put into Eppendorf tubes and stored in the freezer at –20 °C until the experiment day.

Test for Latex Agglutination (LAT)

The idea behind this test was that when latex particles coated with soluble T. gondii antigen were mixed with anti-Toxoplasma antibody samples, they accumulated. The Campbell[17] method was followed when conducting the latex agglutination test (Toxo-latex, Spinreact, Spain). On the day of the latex agglutination test experiment, the serum was kept at room temperature. About 50 µl of the serum and 25 µl of the reagent were used. The mixture was mixed thoroughly with the plastic sticks included in the test kit and then left for 5 minutes. Positive samples showed clear agglutination in the latex agglutination test, whereas the reagent did not adhere to negative samples.

(TOX) *Toxoplasma* IgM/IgG Antibody Rapid Test Kit (Immunochromatography)

Toxoplasma IgM/IgG Antibody Rapid Test Kit (HIGHTOP, China) was carried out according to the manufacturer's instructions. The plastic dropper was filled with serum, the dropper was vertically, and three full drops 80-100 µL of serum, making sure that there were no air bubbles (bubbles may prevent the complete transfer of the sample and invalidate the test), reading the results within 15-30 minutes, and positive results may appear within one minute[18]. The use of the latex agglutination test (LAT) and the Rapid Test Kit for the diagnosis of *T. gondii* due to their ease of use and low cost in addition to saving the time and effort required to perform them. The test result appears after 5–15 minutes. It does not require expensive equipmen[19].

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Results and Discussion

Latex agglutination test (LAT)

Results showed that antibodies were detected in 14/40 local duck birds (35%), and 23/62 domestic chickens (37.09%).

(TOX) *Toxoplasma* IgM/IgG Antibody Rapid Test Kit (Immunochromatography)

According to the type of antibodies in local duck birds toxoplasmosis, samples using rapid test kit (Immunochromatography) results indicated that ten samples out of 40 (25%) were found positive, however IgG, IgM, and IgG plus IgM were found in 20%, 10%, and 70% respectively. While results of domestic chickens indicated that 17 samples out of 62 (27.41%) were found positive, however IgM, IgG, and IgG plus IgM were found in 17.64%, 11.76% , and 70.58 respectively (Table 1). It was clear from the results that the percentage of the presence of antibodies together (IgG & IgM) higher than the presence of one them, which indicates the presence of both recent (acute) and latent (chronic) infections, while the latent or chronic infections (IgG) were higher than the acute infections (IgM).

Table 1. Infection rate with *T.gondii* according to the type of antibodies using the rapid test kit.

samples		Total sample number	a positive number (%)	Antibody Type		
				IgG	IgM	IgG + IgM
				a positive number (%)	a positive number (%)	a positive number (%)
Local duck birds	No.	40	10	2	1	7
	%		25	20	10	70
Domestic chickens	No.	62	17	3	2	12
	%		27.41	17.64	11.76	70.58

The outcome of the latex agglutination test was for immunological detection, and *Toxoplasma* IgM/IgG. Antibody Rapid Test Kit (Immunochromatography) demonstrated that *T. gondii* incidence in local duck birds and domestic chickens were higher than the percentage that was recorded in Iraq by Mikaeel, and Al-Saeed [20] of local chickens in Duhok Province using the enzyme linked immunosorbent assay (ELISA) (IgG) is 22.8%, and Issa *et al*.[21] of chickens, turkey, geese, and ducks in Duhok Province using (ELISA) which is 21.1%.

The incidence rate recorded in the current study was higher than the percentage, which was recorded by Adhoi and Mahmood16 of chickens in Tikrit Province using the latex agglutination test, (LAT) which was 32.1%, and Mohammed[22] of domestic chickens in Sulaimani Province (LAT) was 25.64%.

Additionally, the rate of infection was higher than the percentage that was recorded in several studies in the world,, including Yan *et al.*[23] on chickens and ducks in China using the modified agglutination test (MAT) was 11.4%, 16%, and Puvanesuaran *et al.*[24] on ducks in Malaysia using (MAT) which was 14.63%. As well as Mahmood *et al.*[25] on *Gallus domesticus* in Pakistan using indirect hemagglutination antibody (IHAT) was 18.85%, and Zhao *et al.* [26] on chickens in China using the *Toxoplasma* circulating antibodies (TCAb) and (ELISA) were 16.97% and 5.88%.

Also, the percentage recorded in the current study was higher than the percentage recorded by Li *et al*. [27] on chickens, and ducks in Northeastern China using indirect hemagglutination antibody (IHA) 10.6%, 21.0%, and Saichua *et al*.[28] on chickens in Thailand using (LAT) was 10.1%, and Xu *et al*.[29] on Chickens in China using (MAT) which was 18.8%.

Infection incidences were lower than the percentages noted in several studies by Mohammed [21] on chickens in Sulaimani Province using (LAT) was 60%. In addition to the percentages recorded in some studies, including Namroodi *et al*.[30] on ducks in Iran using (MAT) was 63.3%, and Harfoush and Ael-N[31] on domestic ducks in Egypt using (IHAT) which was 55, 38%.

The variance in *T. gondii* infection rates in the studies mentioned above can be attributed to differences in number of the samples examined, the sensitivity of the diagnostic tests used, and variations in the

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geographical and environmental location of those locations[32].

Conclusion

This study can be a first step in Basrah province to determine the prevalence of toxoplasmosis in local duck birds and domestic chickens by (TOX) *Toxoplasma* IgM/IgG Antibody Rapid Test Kit (Immunochromatography). The results confirmed that toxoplasmosis was common in ducks and free_range poultry. To limit the danger of toxoplasmosis infection, efforts must be taken to obtain agreeable control over food safety and stray cats.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Basrah.

Authors' Contribution Statement

A. I. A., A. I. S., collected samples, performed the experiments, and wrote the manuscript, and A. I. A., A.T. F. designed the work, directed it, and corrected the paper.

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الكشف المصلي عن المقوسة الكوندية في البط المحلي والدجاج المحلي في محافظة البصرة, العراق

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الخلاصة

تنتشر المقوسة الكوندية على نطاق واسع في البشر والحيوانات الأخرى، بما في ذلك الدواجن، في جميع أنحاء العالم وتعتمد على مجموعة فريدة من العضيات الإفرازية والهيكلية الخلوية لغزو الخلايا المضيفة. يتم استهلاك لحوم الدواجن المصابة بالمقوسة الكوندية والتي تشمل الدجاج والبط على نطاق واسع في معظم البلدان وهي معروفة كمصدر رئيسي للعدوى البشرية. في العراق بشكل عام وفي محافظة البصرة على وجه الخصوص، نادرًا ما يُعرف عن انتشار المقوسة الكوندية في البط والدجاج المحلي. في الدراسة الحالية، تم استخدام اختبار تلازن اللاتكس (LAT) ومجموعة الاختبار السريع للاجسم المضادة IgM/IgG لفحص الأجسام المضادة لطفيلي المقوسة الكوندية في 40 طائر من البط محلي و 62 دجاج محلي من مناطق مختلفة في محافظة البصرة. تم العثور على الأجسام المضادة للطفيلي (LAT) في 14 (35٪) من 40 طائر بط محلي ، و 23 (37.09٪) من 62 من الدجاج المحلي. بينما تم الكشف عن الأجسام المضادة للطفيلي (الاختبار السريع) في 10 (25٪) من 40 طائر بط محلي و 17 (27.41٪) من 62 من الدجاج المحلي. أظهرت النتائج أن التربة ملوثة بالكيسات البيضية للطفيلي التي تمثل مصدراً غذائياً للدجاج والبط المحلي نظرا لتغذية الطيور على الأرض. وتشير الدراسة إلى أن لحوم الدواجن قد تكون مصدراً مهماً للعدوى البشرية بالطفيلي في العراق. تعتبر هذه الدراسة هي الدراسة الأولى في محافظة البصرة للكشف عن داء المقوسات في الدجاج والبط المحلي.

الكلمات المفتاحية: المقوسة الكوندية, الدجاج المحلي, البط المحلي, اختبار تلازن اللاتكس, الاختبار السريع للاجسام المضادة.