Research Article

BASJVETRES

BASRAH JOURNAL OF VETERINARY RESEARCH, 2024, 23(2): 11-18.
https://bjvr.uobasrah.edu.iq/

Detection of Toxoplasma gondii in domestic turkey (*Meleagris galbpavo*) in Basrah Province using Serological methods

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DOI: https://doi.org/10.23975/bjvr.2024.147309.1064
Received: 10 March 2024 Accepted: 25 April 2024.

Abstract

Toxoplasma gondii, a parasite found extensively in humans and various animals, including domestic poultry, is widely distributed across the globe. Its ability to invade host cells depends on a special combination of cytoskeletal and secretory organelles. Meat from infected poultry, such as turkey and chicken, is widely consumed worldwide and is the primary source of *T. gondii* infection in humans, however, scant information exists regarding the prevalence of *T. gondii* in domestic turkey (*Meleagris galbpavol*) in Iraq. In this study, antibodies against *T. gondii* were investigated in 38 *M. galbpavol* randomly from different areas of Basrah Province utilizing the latex agglutination test (LAT) and *T. gondii* (Toxo) IgM/IgG Antibody Rapid Test and Enzyme Linked Immunosorbent Assay (ELISA). *T. gondii* antibodies (LAT) were discovered in 14 (36.84%) of the 38 samples, whereas *T. gondii* antibodies (Rapid Test) were found in 8 (21.05%), As for the ELISA test were found 7(18.42%) infected. High rates of toxoplasmosis in Basrah Province indicate soil contamination with the Oocysts of parasite, which can be attributed to the presence of cats and turkeys in the same locations. Depending on the present results, the turkey meat may be representing a significant source of human *T. gondii* infection in the studied area. This is the first study in Basrah province to detect *Toxoplasmosis* in *M. galbpavol* by using serological methods.

Key words: ELISA, *Meleagris galbpavol*, *Toxoplasma gondii*.

Introduction

Toxoplasma gondii may reason fatalities and subclinical infections in Numerous warm-blooded animals, such as birds (1). One of the most well-known zoonotic infections, toxoplasmosis, is brought on by this protozoan parasite. The zoonosis has a widespread distribution. The parasite lives inside the cell and is obligatory in nature (2). The main reservoirs of the parasite are birds, which are frequently preyed upon by felids. The birds' protracted flight habits, eating on the ground, making them potential hosts of this coccidian (3). There have been reported of natural infections in 63 species of wild and domestic birds, including game birds, pigeons, turkeys, chickens, and ducks (4).

Birds are essential *T. gondii* (Apicomplexa, Sarcocystidae) reservoirs and intermediate hosts play a crucial role in the transmission of diseases to humans through the consumption of undercooked or raw meat (5), serving as pivotal conduits for infection. Herbivorous birds, as they search for food on the ground, and birds of prey, which annually consume substantial quantities of mice and other small animals, act as important intermediate hosts for *T. gondii*. Both wild and domestic avian species serve as valuable indicators of environmental contamination with *T. gondii* oocysts (6).

Turkeys are a substantial meat source worldwide, primarily raised in free-range systems, either through backyard operations or on a large commercial scale (7). It can be found alone, in couples, or in groups of up to five birds. It prefers dense ground cover, such as tall grass clumps or sugarcane fields, and departs for more open crops and grassland to feed (8). They fall within the Animalia kingdom, Chordata phylum, Aves class, Califormis order, Meleagridinae family, *Meleagris* genus, and *M.gallopavo* species, as classified by Linnaeus in 1758. You become afflicted with two types of parasites: ectoparasites and endoparasites. The risk of ectoparasites is nearly as large as the risk of endoparasites due to their vast distribution, as well as their high reproductive efficiency and capacity to endure improper circumstances and hide, which made them lethal avian parasites (9).

Clinical toxoplasmosis has not been reported in turkeys since 2009, and turkeys are thought to be resistant to the disease (10). Turkey flesh might be regarded as a source of infection for people since turkeys have a comparatively high level of *Toxoplasma* infection (11). Throughout the course of 12 weeks, *T. gondii* remained persistent in turkeys (12).

In mammals, birds, and reptiles, toxoplasmosis is a parasitic disorder that primarily affects the central nervous system, though it can occasionally also affect the skeletal muscles, reproductive system, and visceral organs (13). Toxoplasmosis in poultry manifests clinically as anorexia, emaciation, decreased egg production, blindness, ataxia, and even a 50% mortality rate (14). Turkeys and other birds can become infected with *T. gondii* as intermediate hosts by digesting infective oocysts shed by the feces of the definitive host. Because they are not permitted to come into touch with infective oocysts or felines, domestic breeding poultry and birds are less likely to contract the disease than free-ranging or industrial breeding animals (15). *T. gondii* infection in poultry, particularly in

free-range, is regarded as an important indicator of environmental contamination with *T. gondii* sporulated oocysts (16). The objective of the present study was to perform a serological diagnosis of *T. gondii* in *M. galbpavol* utilizing the Latex agglutination test and a rapid test kit within the Basrah province.

Materials and methods

Collection of bird specimens

From March to October 2023, 38 of *M. galbpavol* adult birds were purchased from various areas within the Basrah province.

Blood Sample:

Samples of blood (about 3-5 ml) were obtained from the Metatarsal vein because it is one of the clearest veins in large birds and the required amount of blood can be obtained from it without harming the bird.

Serum separation

Serum was separated from blood samples by centrifuge blood samples at 4500 rpm for 6-8 min. Then, the serum was transferred to Eppendorf tubes and stored at -20°C until the day of experimentation.

The Latex Agglutination Test (LAT)

Latex Agglutination Test assay was done as described by Campbell (17) . The serum was kept at room temperature. The experiment with the agglutination of latex was then conducted. Serum (50 µl) and reagent (25 µl) were mixed well with plastic sticks in the test set for 5 min. The positive sample revealed agglutination in the latex agglutination assay. However, the negative samples did not adhere to the detector.

***Toxoplasma gondii* (Toxo) IgM/IgG Antibody Rapid Test**

Toxoplasma gondii (Toxo) IgM/IgG Antibody Rapid Test (Healgen, USA) was applied to perform the test in accordance with the published methodology. To apply two full drops (approximately 50 µl) of venipuncture whole blood, the plastic dropper was filled with whole blood and held it upright. Then, one drop of buffer (approximately 40 µl) was added. Test results was read within 15 min, and sometimes positive results appeared a minute later.

Enzyme-linked immunosorbent assay (ELISA)

Chicken *Toxoplasma* IgG & Chicken *Toxoplasma* IgM ELISA Kit (Bioassay Technology Laboratory, China) were employed to assess *Toxoplasma gondii* infection in the serum, following the manufacturer's guidelines. The ELISA test was conducted in the Immunology Laboratory/ Department of Pathological analysis/ College of Science/ Thi-Qar University. A microplate reader with a 450 nm setting was quickly used to confirm the optical density (OD value) of every well. The average OD positive ≥1.0, while the average OD negative ≤0.10. The equation was applied (Cutoff Value = average Negative Control value + 0.15). If sample OD < Cutoff Value, it was determined as negative. If sample OD ≥ Cutoff, it was determined as positive.

Results

Latex agglutination test (LAT)

Latex agglutination test revealed that antibodies were detected in 14/38 (36.84%).

***Toxoplasma gondii* (Toxo) IgM/IgG Antibody Rapid Test**

Toxoplasma gondii (Toxo) IgM/IgG antibody rapid test revealed that there is 8 samples out of 38 (21.05%) were positive. However, IgG, IgM, and IgG plus IgM were (50%), (12.5%), and (37.5%), respectively (Table. 1).

Enzyme-linked immunosorbent assay (ELISA)

ELISA analysis gave the optimal cutoff OD value of 0.25 for marker and the table (2) showed descriptive analysis of marker OD, 7 out of 38 samples gave positive results at cutoff 0.25. According to the type of antibodies in *M. galbpavol* toxoplasmosis, samples using (Chicken *Toxoplasma* IgG and Chicken *Toxoplasma* IgM ELISA Kit) results indicated that the percentage of IgG and IgM were (85.71%) and (14.28%), respectively.

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

Samples		Total sample number	Positive Number (%)	Antibody Type IgG Positive number (%)	IgM Positive number (%)	IgG + IgM Positive number (%)
M. galbpavol	NO	38	8	4	1	3
	%		21.05	50	12.5	37.5

Table. 2: Infection rate with *T.gondii* according to type of antibodies using ELISA.

Samples		Total sample number	Positive Number (%)	Antibody Type IgG Positive number (%)	IgM Positive number (%)
M. galbpavol	NO	38	7	6	1
	%		18.42	85.71	14.28

Discussion

The findings from the antibody detection of the present work which using the latex agglutination test revealed that the prevalence of *T. gondii* in *M. galbpavol* exceeds the rates documented in numerous studies on avian toxoplasmosis globally, including Quist *et al*. (7) in West Virginia (USA) using avidin-biotin immunohistochemical analysis, and Özkan *et al*. (18) in Ankara, Turkey using indirect fluorescent antibody test (IFAT), and Mohamed & Abdel Naby (19) in Kafr EI-Sheikh, Egypt using indirect hemagglutination antibody test (IHAT), and Koethe *et al*., (20) in 5 states Germany using ELISA, and Asgari *et al*. (21) in Shiraz Iran utilizing modified agglutination test (MAT), and Sá *et al*. (22) in Northeastern Brazil using (MAT), and Ayinmode *et al*. (23) in Osun, Oyo Nigeria using (MAT), and Cerqueira-Cézar *et al*. (24) in Pennsylvania Iran using (MAT) were 10%, 34.3%, 29.4%, 20.2%, 11.1%, 11%, 4.1% and 30%, respectively. Although the count of positive cases is reduced when employing the latex agglutination test, rapid testing kit and Enzyme-Linked Immunosorbent Assay (ELISA) compared to the percentages reported by El-Massry *et al.* (25) in Giza, Egypt using the Modified Agglutination Assay (MAA), & by Sarkari *et al.* (11) in Fars, Iran using MAT, which were 59.5% and 89.8%, respectively. The reason for the variety in *T. gondii* infection rates observed in the aforementioned research is due to the quantity of samples that were analyzed in each case, the sensitivity of the diagnostic tests employed, and the geographic and environmental conditions of those places.

In comparison to other studies conducted in Iraq by Al-Mayali (26) in Diwaniya, Najaf, and Karbala using the Latex Agglutination Test (LAT), and in Diwaniya, Babylon, Najaf, and Karbala using a rapid test kit, as well as by Issa *et al.* (27) in Duhok using ELISA, which reported proportions of positive cases at (35%, 30%, 20%), (20%, 35%, 25%, 15%), and 23%, respectively. The current study revealed a higher incidence. Furthermore, when employing a rapid test kit, the prevalence was greater than that reported by Al-Mayali (26) in Diwaniya and Karbala, which were 20% and 15%, respectively.

Additionally, the prevalence of infection was lower than the percentage noted in other studies conducted in Iraq, including Butty (28) in Ninevah using (LAT), Al-Mayali (26) in Babylon using (LAT), and Issa *et al*. (27) in Duhok using (LAT), (MAT) were 76.63%, 55%, and (57.6%, 38.45), respectively. The incredible public health implications of the substantial prevalence of *T. gondii* infection in turkeys (*Meleagris galbpavol*) within Basrah province emphasized the significance of this study. High rates of toxoplasmosis in the environment indicate soil contamination with the Oocysts of parasite, which might be attributed to the presence of cats and turkeys in the same locations. Different methods are used for serological diagnosis in order to test the sensitivity of these methods in detecting the parasite and also to confirm infection, also, the results indicate the Enzyme-linked immunosorbent assay (ELISA) method, more accurate than the above tests in this study.

Conclusions

The current study might be viewed as a first step in the province of Basrah to confirm the prevalence of toxoplasmosis in turkey (*M. galbpavol*) using the Latex Agglutination Test (LAT), *Toxoplasma gondii* (Toxo) IgM/IgG Antibody Rapid Test, and Enzyme-linked immunosorbent assay (ELISA). According to current findings, toxoplasmosis is widespread in turkeys (*M. galbpavol*), and the town is home to a lot of stray cats, which can contaminate their surroundings with Oocysts. Achieving reasonable control over stray cats and food safety is necessary to reduce the risk of toxoplasmosis infection. Further study can be achieved to detect such parasites by PCR in turkeys and cats and make sequences for all parasite strains. Achieving reasonable control over stray cats and food safety is necessary to reduce the risk of toxoplasmosis infection.

Conflicts of interest

The authors weren't upfront about any conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee

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الكشف عن المقوسة الكوندية في الديك الرومي *Meleagris galbpavol* في محافظة البصرة باستخدام الطرق المصلية

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الخلاصة

المقوسة الكوندية، وهو طفيلي موجود على نطاق واسع في البشر والحيوانات المختلفة، بما في ذلك الدواجن المنزلية، منتشر على نطاق واسع في جميع أنحاء العالم. تعتمد قدرتها على غزو الخلايا المضيفة على مزيج خاص من العضيات الهيكلية والإفرازية. يتم استهلاك لحوم الدواجن المصابة، مثل الديك الرومي والدجاج، على نطاق واسع في جميع أنحاء العالم وهي المصدر الرئيسي لعدوى المقوسة الكوندية في البشر، ومع ذلك، توجد معلومات قليلة فيما يتعلق بانتشار المقوسة الكوندية في الديك الرومي (*Meleagris galbpavol*) في العراق. في هذه الدراسة، تم فحصت الأجسام المضادة في 38 من الديك الرومي *M. galbpavol* من مناطق مختلفة من محافظة البصرة باستخدام اختبار التراص اللاتكس (LAT) واختبار الأجسام المضادة IgM/IgG السريع ومقايسة الامتصاص المناعي المرتبط بالإنزيم (ELISA). تم اكتشاف الأجسام المضادة بواسطة اختبار (LAT) في 14 (36.84%) من الـ 38 عينة، في حين تم العثور على الأجسام المضادة للطفيلي باستخدام الاختبار السريع في 8 (21.05%)، أما اختبار ELISA فقد وجد 7 (18.42%) مُصاب. تشير معدلات الإصابة بداء المقوسات المرتفعة في البيئة إلى تلوث التربة بالاكياس البيضية الطفيلي، وهو ما يمكن أن يعزى إلى وجود القطط والديوك الرومية في نفس المواقع. ويشيرون إلى أن لحوم الدواجن من المحتمل أن تمثل مصدرًا مهمًا لعدوى المقوسة الكوندية البشرية في العراق. اعتمادا على النتائج الحالية، قد يكون لحم الديك الرومي مصدرا هاما لعدوى المقوسة الكوندية في منطقة الدراسة. هذه هي الدراسة الأولى في محافظة البصرة للكشف عن داء المقوسات في الديك الرومي باستخدام الطرق المصلية.

الكلمات المفتاحية: *المقوسة الكوندية, Meleagris galbpavol,* ELISA