Open Veterinary Journal, (2025), Vol. 15(11): 5948-5953
Research Article
DOI: *10.5455/OVJ.2025.v15.i11.49*

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Submitted:10/05/2025 Revised: 25/09/2025 Accepted: 08/10/2025 Published: 30/11/2025

Seroprevalence of *Toxoplasma* infection in free-range chickens in northeastern Libya

Khalil M. A. Akra[1], Hana A. Ali. Awad[2], Loeki Enggar Fitri[3]*, Teguh Wahju Sardjono[3] and Monier Sharif[4]

[1]*Department of Medical Laboratories, Faculty of Medical Technology, Zintan University, Zintan, Libya*
[2]*Department of Parasitology, Faculty of Veterinary Medicine, Omer Al-Mukhtar University, Al Bayda, Libya*
[3]*Department of Clinical Parasitology, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia*
[4]*Department of Biomedicine, School of Basic Sciences, Libyan Academy for Graduate Studies, Al Jabal Al Akhdar, Al Bayda, Libya*

ABSTRACT

Background: The poultry industry in Libya has recently experienced significant growth, which is critical for improving food security. However, *Toxoplasma gondii* poses a threat to this industry because it infects warm-blooded animals, including poultry. Chickens, particularly free-range chickens, may serve as an important reservoir for *T. gondii*, yet there are no reports regarding the prevalence of this disease in free-range chickens in Libya. Understanding the seroprevalence of *Toxoplasma* in poultry is crucial for mitigating these risks and ensuring the health of the poultry industry.

Aim: This study examines the occurrence of *T. gondii* infection in poultry and evaluates its impact on public health in Libya.

Methods: A total of 315 free-range chickens were procured, with blood serum samples collected from the Al-Marj (n = 123), El-Bayda (n = 104), and Derna (n = 88) districts. The sera were analyzed using the indirect enzyme-linked immunosorbent assay. The chi-square test was used to evaluate a significant correlation between the seroprevalence of *T. gondii* and area, sex, and age.

Results: The overall seroprevalence of *T. gondii* antibodies was 23.8%. Among the districts, the highest prevalence was recorded in Al-Marj (26.8%), followed by El-Bayda (25%) and Derna (18.2%). Male chickens had a higher prevalence rate (26%; 25/96) than females (22.8%; 50/219), although no statistical difference was found between the genders. Interestingly, older chickens had a significantly higher seroprevalence (39.2%; 31/79) than younger chickens (28.6%; 24/84).

Conclusion: In Libya, free-range chickens are a potential source of *Toxoplasma* infection, with older chickens having a significantly higher seroprevalence than younger chickens. These free-range chickens could pose an infection risk to humans.

Keywords: Age, ELISA, Free-range chicken, Libya, *Toxoplasma gondii*.

Introduction

Toxoplasma gondii is a protozoan parasite of global importance due to its role in medical and veterinary diseases (Attias *et al.*, 2020). It is the cause of toxoplasmosis, a condition that is commonly found in both humans and animals worldwide (Attias *et al.*, 2020; Dubey *et al.*, 2020; Salinas *et al.*, 2021). Human infections are primarily acquired by ingesting contaminated food, particularly undercooked meat or water. Toxoplasmosis is reported to affect about 30%–50% of the human population globally (Flegr *et al.*, 2014). Severe clinical outcomes are usually restricted to individuals with compromised immunity, including neonates and patients with immunodeficiency (Sanchez and Besteiro, 2021). Recent studies have indicated a significant prevalence of toxoplasmosis in Libya (Gamal and Jaroud, 2015; Gashout *et al.*, 2016).

The parasite leads to numerous health challenges and significant economic burdens on livestock sectors (Stelzer *et al.*, 2019). Chickens, particularly free-range chickens, are regarded as one of the most important hosts for *T. gondii* infection, and humans can become infected with this parasite by eating undercooked infected chicken meat. Free-range chickens are at a high risk of exposure to oocysts shed by cats (Salant *et al.*,

***Corresponding Author:** Loeki Enggar Fitri. Department of Clinical Parasitology, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia. Email: *lukief@ub.ac.id*

2016). In addition, chickens are considered sensitive indicators of environmental contamination with *T. gondii* oocysts and have been deployed as sentinel sources. Because they feed on the ground, they can be easily infected with *T. gondii* (Dubey *et al.*, 2020).

The poultry sector in Libya is increasingly becoming a significant part of the agricultural industry and economy. The commercial poultry sub-sector has become a significant protein source for consumers. This is part of Libya's continuous endeavor to realize self-sufficiency in animal-based foods, such as poultry meat and eggs. In addition to producing laying poultry and hatching eggs, poultry complexes supply more than 40% of Libya's chicken meat requirements (Guèye, 2004). However, inadequately cooked or improperly handled poultry products are believed to play a role in the spread of *T. gondii*.

Serological surveys remain the gold standard for assessing the infection rates and risk factors of poultry populations (Burrells, 2014). Several studies have highlighted substantial variation in *T. gondii* seroprevalence in free-range and backyard chickens, with rates ranging from 0% to over 100% (Dubey *et al.*, 2010; Burrells, 2014; Stelzer *et al.*, 2019; Salinas *et al.*, 2021). However, comparisons across studies are challenging due to inconsistencies in diagnostic tools and methodologies.

Molecular research has examined the occurrence of *T. gondii* infection in the internal organs of poultry in Libya (Awad *et al.*, 2023). However, there is a scarcity of serological studies regarding *T. gondii* in animals within Libya, especially in free-range chickens. Previous research conducted in Libya has indicated that *T. gondii* seroprevalence is present in livestock, including sheep (El-Gomati *et al.*, 2008; Al-mabruk *et al.*, 2013). This study aims to fill this gap by assessing the prevalence of *T. gondii* antibodies in chickens and examining possible risk factors that may contribute to its transmission in Libya.

Materials and Methods

Study design

A cross-sectional study was conducted in the Al Jabal AL Akhdar region. The sample size was calculated assuming a prevalence of 50% with a 95% confidence interval (CI) and a statistical error of 5%, according to a previous study on the prevalence of toxoplasmosis (71%) reported by Al-mabruk *et al.* (2013) in sheep from western, central, eastern, and southern Libya.

Study area

Previously, Libya was divided into 22 districts **(***shabiyat),* and in the newer administrative framework following reforms, Libya has been subdivided into 106 districts (*baladiyat*) **(**https://everything.explained.today/Subdivisions_of_Libya/#google_vignette**).** Certain districts are notably recognized for their concentration of poultry farming and distribution of poultry stores; however, comprehensive official data identifying which specific districts are involved in poultry production are lacking. The last previously reported data indicate six poultry complexes in Libya. The majority of production is concentrated in the western region of the country, accounting for 52% of broiler production and 58% of layer production, followed by the eastern region with 19% and 18%, respectively, and the northern region with 17% and 14%, respectively, while the southern region contributes only 12% of all broilers and 10% of layers (Guèye, 2004). The research was conducted in 3 districts of the Al Jabal Al Akhdar region of northeastern Libya: Al-Marj, El-Bayda, and Derna.

Blood collection

A total of 315 free-range chickens were obtained from the province of Al Jabal Al Akhdar in northeastern Libya. After slaughtering, blood samples were collected in sterile collection tubes. Samples were transported in a cold box to the Parasitology Laboratory at the Faculty of Veterinary Medicine, Omar Al-Mukhtar University, Libya. Blood samples were centrifuged at 3,000 rpm for 10 minutes in the laboratory to isolate the serum. The serum was then stored at −20°C until further analysis.

Serological examination

Sera were analyzed with a commercial enzyme-linked immunosorbent assay (ELISA) (ID Screen® Toxoplasmosis Indirect Multi-Species, IDVET, Montpellier, France) for the detection of anti-*T. gondii* antibodies, performed according to the manufacturer's instructions. The optical density (OD) value was measured at 450 nm using a microplate reader (Bio-Tek-USA). Positive and negative control sera were used as controls. For each sample, the resulting values were calculated 5 times by applying the following formula: S/P% = OD sample − OD-negative control/ OD-positive control – OD-negative control) × 100. Samples with SP% ≥ 50% were considered positive (Gazzonis *et al.*, 2020; Issa *et al.*, 2020).

Evaluation of risk factors

To examine the risk factors associated with the seroprevalence of *T. gondii*, a structured questionnaire was used to collect information regarding the age and sex of the chickens and the specific study area where the samples were collected. A total of 315 healthy free-range chickens of both sexes with a body weight average of 1–1,5 kg, were kept by farmers as a source of meat and eggs. Based on the information provided by the veterinarian, the chickens were grouped into four groups: 4 months − 6 months; ≥ 6 months − 1 year; ≥ 1 year − 2 year; and ≥2 years. Variables such as area, chicken age, and gender were analyzed to assess their potential association with *T. gondii* infection.

Statistical analysis

Descriptive statistics were performed with percentages and their corresponding 95% confidence intervals (95% CI) calculated. Chi-square and a *p*-value of 0.05 were applied to evaluate significant variations between

Table 1. Risk factors associated with seroprevalence in chickens infected with *T. gondii.*

Risk factors	Positive samples	Percentage	*p* and X^2 values
Chicken Area			
Al-Marj distract	33/123	26.8%	($p = 0.327$; $\chi^2 = 2.236$)
El-Bayda distract	26/104	25%	
Derna distracts	16/88	18.2%	
Chicken age			
4–6 month	24/84	28.6%	($p = 0.000$; $\chi^2 = 21.482$)
≥6 months -1 year	7/68	10.3%	
≥1year - 2year	13/84	15.5%	
≥2 years	31/79	39.2%	
Chicken sex			
Male	25/96	26%	($p = 0.538$; $\chi^2 = 0.379$)
Female	50/219	22.8%,	

the seroprevalence of *T. gondii* and the independent variables (area, sex, and age). All statistical analyses were conducted using IBM SPSS (ver. 28; Armonk, New York, USA).

Ethical approval

The study was initiated after obtaining approval from the Libyan National Committee for Biosafety and Bioethics in the Higher Education Ministry in Libya (Approval number: SH/3/2021).

Results

To the best of our knowledge, this is the first study to determine the seroprevalence of *T. gondii* infection in free-range chickens in Libya. The research was conducted in the Al Jabal Al Akhdar region of Northeast Libya, which spans approximately 37,000 km^2 and is situated at 32° 45’ 45” N and 21° 45’ 18” E latitudes. This area stretches along the Mediterranean coast for approximately 100 miles (160 km). It features a mountainous plateau that rises to an elevation of 900 m (3,000 ft), interspersed with various valleys and wadis. The region experiences an average annual rainfall of 400–600 mm, with an average annual temperature of 16°C, characterized by distinct summer and winter seasons.

The ELISA test was employed to identify antibodies against *T. gondii* in chicken blood. A total of 315 chicken samples were examined, of which 75 were positive, with a seroprevalence rate of 23.8% (95% CI = 1.59–1.95). Table 1 presents the results of the spatial distribution of *T. gondii* in the chicken based on area, sex, and age.

Discussion

In this current investigation, the ELISA test demonstrated notable specificity and sensitivity, identifying antibodies against *T. gondii* at a rate of 23.8% in free-range chickens in northeastern Libya. The seroprevalence of *T. gondii* in chickens reported in this study aligns with similar investigations carried out in Morocco, a middle-income nation where the seroprevalence of *T. gondii* in chickens was 30.65% (Hoummadi *et al.*, 2025). Additionally, other middle-income countries, such as Brazil, demonstrated a notable prevalence of toxoplasmosis at 30.8% in free-range chickens (Vieira *et al.*, 2018). In low-income and resource-limited settings, poultry farming is frequently semi-intensive or conducted in backyards, often lacking adequate biosecurity measures. In Ethiopia, serum samples were obtained from 384 backyard chickens, and *T. gondii* antibodies were tested using the Toxo-Latex slide agglutination method, yielding a positive result in 72.4% of the 384 birds (Chaklu *et al.*, 2020).

This result also aligns with findings from other regions using a similar method ELISA, including backyard chicken studies that reported rates of 26% in Palestine (Dardona *et al.*, 2022), 33.3% in Sumel district, Duhok province, Iraq (Issa *et al.*, 2020), and 38.4% in Egypt (Barakat *et al.*, 2012). Similar results were obtained from Indonesia (24.4%), Poland (30%), and Vietnam (24.2%), using the Modified Agglutination Test (MAT) for detection of IgG responses to *T. gondii* (Dubey *et al.*, 2008). Conversely, substantially higher seroprevalence levels were observed using this method, reaching 100% in Tunisia, 64% in Ethiopia, and 50% in Uganda (Tonouhewa *et al.*, 2017).

Libya is classified as a middle-income nation, characterized by extensive rural and agro-pastoral communities and a significant presence of free-ranging and backyard poultry. Research conducted in Egypt, Ethiopia, Morocco, and Brazil can facilitate cross-comparisons by demonstrating consistent patterns of elevated seroprevalence in free-range poultry. Biosecurity measures and husbandry practices play

a crucial role in exposure levels. This reinforces the need for public health initiatives in Libya, including consumer education, safe cooking methods, and effective cat management, especially since poultry meat is a vital part of the local diet.

Among the serological tests, ELISA and MAT have been widely used both in clinical and epidemiological surveys for screening of *T. gondii* infection (Zhu *et al.*, 2012). Serological assays, such as ELISA, are commonly used to identify both qualitative and quantitative antibodies against *T. gondii* (Issa *et al.*, 2020). ELISA has lower sensitivity and negative predictive values than MAT but higher specificity and positive predictive value (Gebremedhin EZ Abdurahaman and Hadush T Tessema, 2013).

The ID Screen® Toxoplasmosis Indirect Multi-species kit (IDvet, France) is the most widely used commercial kit. It employs native P30 (SAG1) antigen along with an anti-multi-species conjugate as the secondary antibody. Additionally, the use of the SAG1 antigen in this kit may offer enhanced specificity compared to tests that use whole tachyzoite antigen. (Liyanage *et al.*, 2021). The interpretation of ELISA results is significantly affected by the cutoff value used. The S/P% was determined using the formula provided with the ID Screen® kit, where samples with a percentage of ≥50% are deemed positive. This cutoff is established to enhance both sensitivity and specificity, as validated by the manufacturer across various species (iDvet., 2020). Nevertheless, these cutoffs have a direct effect on seroprevalence estimates and case predictions. A lower cutoff enhances sensitivity, allowing for the identification of more true positives; however, it may also increase prevalence due to false positives. Conversely, a higher cutoff improves specificity but may result in an underestimation of exposure due to false negatives. Therefore, it could be beneficial to validate this test across various wildlife and other animal species that are suspected of being infected with *T. gondii*.

Poultry meat is a valuable protein source; however, it is a potential indicator of *T. gondii* infection, which can pose a risk of human toxoplasmosis if consumed raw or inadequately cooked. The transmission of this parasite through contaminated chicken products underscores the importance of proper cooking practices to ensure food safety and prevent potential health hazards. Epidemiological data indicate that consuming raw or undercooked meat containing *T. gondii* poses a risk of infection for humans. Accurate, science-based information is essential for enhancing the understanding of the risks associated with *T. gondii* infection related to meat product intake (Guo *et al.*, 2015).

The ground-feeding nature of poultry makes them susceptible to infection through contact with contaminated sources, such as cat feces (Salant *et al.*, 2016). This study implies that *T. gondii* oocysts may have contributed significantly to environmental contamination across Libya. Comparable studies in different Libyan regions, including Western, Central, Eastern, and Southern areas, reported elevated *T. gondii* prevalence rates, such as 71% in sheep (Al-mabruk *et al.*, 2013). In addition, recent investigations have highlighted a low molecular prevalence (9.5%) of *T. gondii* in infected chickens (Awad *et al.*, 2023). The same samples were detected using the PCR method; however, the results indicated that the PCR data were lower than those obtained using ELISA. The false-negative results from PCR could be attributed to the uneven distribution of cysts in the digested tissues.

Our results indicated that the highest seroprevalence was observed in Al-Marj, followed by El-Bayda. In contrast, the Derna city had a lower rate. The differences in these rates may be attributed to variations in agricultural practices and poultry management systems, as livestock activities and exposure to contaminated environments seem to be more significant in both Al-Marj and El-Bayda. The *Toxoplasma* prevalence estimates are often not comparable among areas because of the different feed sources, presence or absence of cats, rodent or bird control, and water quality. Owing to the ground feeding behavior of poultry, the oral ingestion of material or water contaminated with *T. gondii* oocysts is most likely the main route of infection (Stelzer *et al.*, 2019). Poultry such as chickens, turkeys, ducks, and geese are omnivorous, i.e., they may also feed on earthworms, cockroaches, and other insects that may harbor or be contaminated with oocysts. In general, the economic impact of toxoplasmosis has different aspects that need to be considered. Direct costs of a disease, animal treatment, and disease prevention. There is a clear need for further assessment of the economic consequences of *T. gondii* infection in chickens (Stelzer *et al.*, 2019). Several studies have documented an increasing prevalence with age (Dubey *et al.*, 2020). This study showed that *the seroprevalence of T. gondii* increased significantly in older chickens, particularly those aged over 2 years (39.2%). This result is similar to the high seroprevalence observed in domesticated chickens aged over 2 years in Pakistan (Khan *et al.*, 2020). A high prevalence of toxoplasmosis in older chickens has also been reported in Kenya (Mose *et al.*, 2016). The susceptibility of poultry to experimentally induced toxoplasmosis depends on the infectious dose, parasite strain, stage, infection route, and animal age (Stelzer *et al.*, 2019).

Male chickens showed a higher prevalence rate (26%; 25/96) than females (22.8%; 50/219), although no statistical difference was found between the sexes. However, several reports indicated that females exhibit greater sensitivity to toxoplasmosis than their male counterparts (Akhtar *et al.*, 2014; Mose *et al.*, 2016). Variations in the hormonal profiles of males and females may significantly influence their susceptibility to parasitic infections (Mose *et al.*, 2016). The gender of livestock animals as a putative risk factor has been

studied only occasionally. Nevertheless, in a few studies, females were more frequently seropositive. Whether these apparent associations were in fact related to gender or to other underlying factors, such as the way animals of different genders are reared, needs to be questioned. Gender frequently appears as a confounder in epidemiological studies because "gender" may mask these underlying factors. (Stelzer *et al.*, 2019).

Conclusion

Our research identified antibodies against *T. gondii* in free-range chickens in Al-Marj, El-Bayda, and Dernaof Libya, Northeastern Libya. Free-range chickens are a potential source of *Toxoplasma* infection, with significantly higher seroprevalence in older chickens than in younger chickens. This poses a potential risk to human health, as free-range chickens could act as sources of infection if raw or improperly cooked.

Acknowledgments

None.

Conflict of interest

The authors declare no conflict of interest.

Funding

None.

Authors' contribution

All authors contributed to the completion of this manuscript: conceptualization, KMA, HAA, LEF, TWS, and MAMS. Collecting samples and conducting the research trials: KMA and HAH. Data curation: KMA, HAA, LEF, TWS, and MAMS. Writing the original draft: KMA, HAH. Critical revision of the manuscript: LEF

Data availability

All data were provided in the manuscript.

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