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Determination of *Toxoplasma gondii* in Beef, Chicken Meat and Offal Sold in Kayseri, Türkiye

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ABSTRACT

Toxoplasma gondii is a globally distributed protozoan capable of infecting humans as well as numerous warm-blooded hosts, such as poultry, and livestock. One of the primary ways it is transmitted to humans is through the consumption of raw or insufficiently cooked meat containing the parasite's tissue cysts. This study investigated the existence of *T. gondii* DNA in retail beef, chicken meat and offal sold in Kayseri, Türkiye. A total of 100 samples were collected from local butchers and delicatessens, comprising 25 samples each of chicken meat, chicken liver, cubed beef and beef liver. Genomic DNA was extracted from these samples and analyzed using TaqMan-based real-time PCR targeting the B1 gene, which is a highly conserved and sensitive marker for *T. gondii*. All samples tested negative, with no DNA amplification observed within the assay's threshold (Ct ≤ 35). These results indicate a low level of, or absence of, *T. gondii* contamination in the meat products tested in this region during the sampling period. However, when interpreting the results, factors such as limited sample size, seasonal variation, regional animal husbandry practices, and low parasitic load must be considered. Additionally, the heterogeneous distribution of tissue cysts and the possible degradation of parasitic DNA may influence detection. Given the public health significance of toxoplasmosis, further studies involving larger sample sizes, diverse geographical locations and complementary diagnostic techniques are recommended. Continuous surveillance, the implementation of good agricultural and hygienic practices, and raising public awareness of proper meat handling, preparation, freezing and cooking are crucial to minimising the risk of *T. gondii* transmission through foodborne pathways.

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INTRODUCTION

Toxoplasma gondii (*T. gondii*) is a foodborne protozoan parasite with a worldwide distribution and an obligatory intracellular lifestyle, exhibiting a broad host range that includes humans, domestic animals, and birds (FAO/WHO, 2014; Khan et al., 2025; Naeem et al., 2025). The parasite exhibits three infectious forms: tachyzoites (both intracellular and extracellular), bradyzoites that reside within tissue cysts, and sporozoites enclosed in oocysts. (Culbasan et al., 2023; Opsteegh et al., 2024). Cats, the definitive hosts, shed oocysts in their feces, which may contaminate soil, water, and food. These oocysts, once ingested, can infect numerous animal species. Additionally, the parasite invades the muscles and brain of intermediate hosts, and ingestion of these tissue cysts can cause human infection. (Pinto-Ferreira et al., 2019; Almuzaini, 2023; Ahmad et al., 2024; Naeem et al., 2025).

The majority of *T. gondii* infections are asymptomatic or present with mild influenza-like illness. In contrast, severe complications may occur when the parasite establishes tissue cysts in the brain, ocular structures, or other organs, particularly in pregnant women and immunocompromised individuals. (Wang et al., 2017; Bağcı et al., 2022; Goh et al., 2023). Maternal infection during pregnancy poses a major risk for congenital transmission, whereas reactivation of latent toxoplasmosis can lead to life-threatening disease in patients with HIV, organ transplant recipients, or other immunosuppressed populations (Weiss and Dubey, 2009; Bălălău et al., 2020). According to a World Health Organization (WHO) report, *T. gondii* ranked fourth among 24 important foodborne parasites worldwide and second in Europe (WHO, 2015; Bouwknegt et al., 2018; Opsteegh et al., 2024). Epidemiological data indicate that about one-third of the global population carries serological evidence of *T. gondii* infection (Tenter et al., 2000). Human toxoplasmosis can be transmitted through blood transfusion, organ transplantation, by ingestion via the oral route, or vertical transmission from mother to fetus. Numerous studies have identified foodborne transmission as a significant contributor to human toxoplasmosis (Almería and Dubey, 2021; Marín-García et al., 2022; Culbasan et al., 2023; Opsteegh et al., 2024). Oocysts excreted in feline faeces can contaminate fruit and vegetables, while unpasteurised milk products containing tachyzoites and undercooked meat harbouring tissue cysts or tachyzoites are considered major sources of oral transmission (Marín-García et al., 2022; Culbasan et al., 2023). In livestock, toxoplasmosis results in substantial economic losses due to mortality, abortion, neonatal deaths and decreased production (Ahmed et al., 2014; Culbasan et al., 2023). Food-producing animals pose a potential public health risk due to their ability to harbour varying concentrations of *T. gondii* tissue cysts in their edible tissues (Yang et al., 2024). Although chickens are susceptible to *T. gondii* infection, they generally remain asymptomatic. Viable tissue cysts have been detected in various organs and tissues (eg, brain, heart, and skeletal muscles), representing the primary sites of transmission risk (Opsteegh et al., 2016). The role of cattle in *T. gondii* epidemiology is unclear, but undercooked beef and raw milk are potential sources of transmission (Belluco et al., 2018). From a food safety perspective, beef, chicken and their internal organs are potential risk factors for *T. gondii* transmission (Ducrocq et al., 2021; Symeonidou et al., 2023). The objective of this study was therefore to determine the presence of *T. gondii* in beef, chicken and their internal organs consumed in Kayseri, Türkiye, and to assess the potential risks to public health.

MATERIAL and METHODS

Samples

For this study, samples of chicken meat, chicken liver, cubed beef meat, and beef liver were obtained from various butchers and delicatessens in Kayseri, Türkiye. Between November and December 2021, a total of 100 samples were collected from five different sales points to examine the

presence of *T. gondii*. From each of the 100 samples, five isolates were obtained, resulting in a total of 500 isolates for further analysis. This comprised 25 randomly selected samples each of chicken meat, chicken liver, cubed beef meat, and beef liver. All materials were transported under cold chain conditions and processed in the laboratory on the day of collection.

DNA Extraction

Tissues were minced, pooled, and homogenized in 90 mL distilled water using a Stomacher Lab Blender for 2 min. A 10 mL aliquot was centrifuged at 5,400 rpm for 20 min at 4 °C, and the resulting pellet was further centrifuged at 12,000 rpm for 3 min. Pellets were rinsed sequentially with 200 µL of buffer (1 mM EDTA, 10 mM Tris-HCl) and 300 µL of 0.5 M EDTA (pH 8.0), followed by centrifugation at 12,000 rpm for 10 min at 4 °C. The final pellet was resuspended in 200 µL PBS and mechanically disrupted with 0.5 mm glass beads in a TissueLyser LT for 5 min. DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN, Germany) according to the supplier's instructions. DNA quality was verified on a 1% agarose gel, and concentration was quantified with the Qubit dsDNA HS Assay Kit and Qubit Fluorometer 3.0 (Thermo Fisher Scientific, USA). Genomic DNA was stored at −20 °C until qPCR.

Real-Time PCR Analysis

Detection of *T. gondii* DNA was performed according to the procedure previously described by Culbasan et al. (2023). Real-time PCR analyses were performed on a CFX96 Touch Detection System (Bio-Rad, USA) using a TaqMan probe (carrying a 6-FAM reporter dye at the 5' end and a BHQ-1 quencher at the 3' end) assay designed to amplify a 529 bp region of the B1 gene. gDNA obtained from *T. gondii* tachyzoites was used as a positive control, while nuclease-free water served as a negative control. Each reaction mixture (total volume 25 µL) contained 1x Luminaris Color Probe High ROX qPCR Master Mix, 0.3 µM of each primer, 0.2 µM of the probe, and 2.5 µL (5-10 ng/µL) of template DNA. Samples with a threshold cycle (Ct) value of ≤35 were considered positive. Details of primer sequences and PCR cycle parameters are presented in Table 1.

Table 1. Primer and probe sequences and amplification conditions used for the detection of *T. gondii* by qPCR.

Primer/Probe Name	Sequence (5′–3′)	qPCR Amplification Conditions	Reference
ToxoRE_F	CACAGAAGGGACAGAAGTCGAA	50°C 2 min 95°C 10 min 95°C 15 s 60°C 30 s 72°C 30 s (40 cycle)	Kasper et al., 2009
ToxoRE_R	CAGTCCTGATATCTCTCCTCCAAGA		
TaqMan Probe	6-FAM-CTACAGACGCGATGCC-BHQ1		

Statistical analysis

The prevalence of *T. gondii* DNA among the analyzed samples was assessed using the chi-squared test implemented in PAST software (version 4.03). A significance level of $p \leq 0.05$ was adopted, corresponding to a 95% confidence interval. Descriptive statistics, including frequency distributions and percentage values, were employed to summarize the findings.

RESULTS

This study involved collecting a total of 500 tissue samples from 100 specimens, including chicken meat (n = 25), chicken liver (n = 25), cubed meat (n = 25) and beef liver (n = 25). The samples were analyzed using real-time PCR targeting the B1 gene of *T. gondii*, which is commonly used due to its high sensitivity and specificity. All samples yielded negative results for *T. gondii* as their threshold (Ct) values were above 35, indicating an absence of detectable parasitic DNA within the limits of sensitivity of the assay (Figure). As all of the samples tested negative for *T. gondii* DNA, it was not possible to conduct a statistical analysis to compare prevalence among the different sample types (Table 2).

Figure. Amplification plot showing *T. gondii* positive control (Ct = 28.85) and no amplification in tested samples

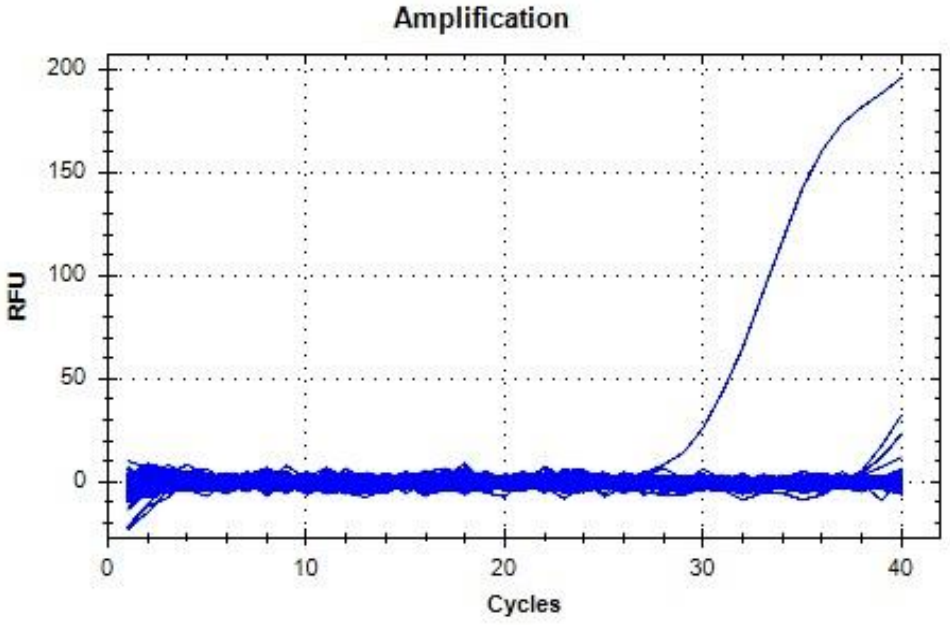

Table 2. Distribution of *T. gondii* in the samples analyzed in the study

Samples	Number of Samples	Positive Samples (n, %)	Negative Samples (n, %)
Chicken Meat	25	0	25 (100%)
Chicken Liver	25	0	25 (100%)
Cubed Beef	25	0	25 (100%)
Beef Liver	25	0	25 (100%)
Total	100	0	100 (100%)

DISCUSSION

The ingestion of raw or inadequately cooked meat continues to be recognized as a primary route of *T. gondii* transmission to humans. Therefore, epidemiological investigations through prevalence studies of food, evaluation of public health risks and development of targeted control measures are essential (Vilela et al., 2024). The prevalence of *T. gondii* in poultry changes significantly, linked to factors such as the serological techniques used, geographic location, sample size, animal species, and farming practices, particularly hygiene standards. In this study, qPCR analysis targeting the B1 gene revealed no detectable *T. gondii* DNA in any of the examined poultry or beef tissues, suggesting an absence of the parasite.

Although cattle are a major source of meat globally, the clinical signs of toxoplasmosis in this species tend to be mild and are less well recognized than in other livestock (Shariatzadeh et al., 2023). Tissue cysts are more susceptible to environmental conditions than oocysts; nevertheless, they can stay infectious for up to three weeks at standard refrigeration temperatures (1–4 °C), aligning with the typical shelf life of fresh meat. DNA of *T. gondii* has been identified in a range of meat products, including raw or smoked sausages, cured bacon, ham, and minced meat (dos Santos Silva et al., 2020; Marín-García et al., 2022). Previous studies on the detection of *T. gondii* in beef samples have reported prevalence rates ranging from 0.6% to 19.3% (Amdouni et al., 2017; Iqbal et al., 2018; Lafrance-Girard et al., 2018; Mahami-Oskouei et al., 2017; Rahdar et al., 2012; Mazuz et al., 2023). However, similar to our findings, Plaza et al. (2020) reported a prevalence of 0%. According to Burrells et al. (2018), the lower prevalence of *T. gondii* in cattle samples compared to chicken samples and its absence in our study may be due to cattle's greater biological resistance.

Chicken meat is widely consumed because it is inexpensive and quick to produce. As an alternative to red meat, consumers are turning to poultry, which is lower in fat and more affordable, to meet their protein needs (Yesilova, 2024). However, poor hygiene during production can result in poor poultry performance and meat quality (de Aquino et al., 2020; da Silva et al., 2024). Chickens are considered important intermediate hosts in the epidemiology of *T. gondii* due to their susceptibility to infection, ground-feeding behavior and access to outdoor environments (Chikweto et al., 2011; Rodrigues et al., 2019; Dubey et al., 2020). In our study, no *T. gondii* DNA was detected in chicken meat or offal samples. However, global studies report vast diversity in the prevalence of *T. gondii* in chicken meat (Mahami-Oskouei et al., 2017; Iqbal et al., 2018; Salinas et al., 2021; Zrelli et al., 2022a; 2022b). Meat from free-range chickens and pasture-raised livestock was found to pose a higher risk of *T. gondii* exposure than meat from captive-raised cattle and cage-raised chickens. These results indicate that rearing conditions and environmental exposure significantly impact the contamination of meat products with *T. gondii*. Free-range chickens and pasture-raised pigs are at a higher risk of infection due to their increased likelihood of meeting *T. gondii* oocysts in the environment (Guo et al., 2015). The absence of *T. gondii* DNA in all samples may be due to the animals being raised under closed or intensive farming conditions, which limits exposure to oocysts. Additionally, the discrepancy in prevalence observed in our study compared to previous findings may be attributed to variations in the quantity of oocysts shed by infected cats into the environment, differential susceptibility among breeds, distinct rearing conditions and climatic factors. Furthermore, *T. gondii* cysts are susceptible to low temperatures, with viability decreasing significantly below -12°C (Gencay et al., 2013). Furthermore, the absence of *T. gondii* detection in this study may be due to the small number of *T. gondii* tissue cysts and their uneven distribution within muscle tissue. To minimise this limitation, we pooled five different meat tissues per animal to increase the effective sample size, as suggested by Opsteegh et al. (2020). Despite this approach, no *T. gondii* B1 gene was detected in any of the pooled samples, suggesting a very low parasite burden or absence of infection.

CONCLUSION

Edible tissues of food animals may harbor latent *T. gondii* cysts, which represent an important reservoir for human infection, even though the animals themselves often show no clinical signs. In conclusion, the study's sampling does not encompass all meat products from naturally infected animals in Türkiye, highlighting the need for nationwide research to assess the presence and viability of *T. gondii* in dairy and meat, as well as to estimate the occurrence of foodborne toxoplasmosis in humans. To reduce the risk of infection, (i) undercooked meat and meat products should either be avoided or properly frozen, (ii) good agricultural and husbandry practices should be implemented at farm level, and (iii) health authorities should develop and enforce effective training programs focused on controlling and preventing *T. gondii* transmission.

Ethical Statement

This study has not been presented or published anywhere else before.

Ethics Committee Approval

This study does not require ethics committee approval.

Author Contributions

Research Design (CRediT 1) Author 2 (%40) – Author 6 (%60)
Data Collection (CRediT 2) Author 1 (%25) – Author 3 (%25) – Author 4 (%25) – Author 5 (%25)
Research - Data analysis - Validation (CRediT 3-4-6-11) Author 1 (%25) – Author 2 (%25) – Author 3 (%20) – Author 4 (%15) – Author 5 (%15)
Writing the Article (CRediT 12-13) Author 2 (%55) – Author 3 (%30) –Author 6 (%25)
Revision and Improvement of the Text (CRediT 14) Author 1 (%35) – Author 2 (%35) –Author 5 (%30)

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Conflict of Interest

The authors declare that there is no conflict of interest of interest associated with this manuscript.

Sustainable Development Goals (SDG)

3 Good Health and Well-Being
12 Responsible Consumption and Production

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