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Molecular detection of *Toxoplasma gondii* using Real-Time PCR in broiler chickens from Baghdad city, Iraq

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Abstract

Objective

Toxoplasma gondii is an intracellular protozoan parasite responsible for toxoplasmosis, an important zoonotic disease affecting humans and animals worldwide. Broiler chickens are considered potential sources of infection due to their exposure to contaminated environments and their role in food transmission. The present study aimed to detect *T. gondii* in broiler chickens in Baghdad city using conventional PCR and reverse transcription quantitative PCR (RT-qPCR) techniques.

Materials and methods

A total of 50 broiler chicken samples were collected from local live chicken selling and slaughtering markets in different areas of Baghdad city, including Abu Ghraib, Al-Ghazaliya, Al-Adl, Al-Shorja, and Jameela markets, during the period from January 2026 to June 2026. Blood samples were collected under aseptic conditions and transferred to the College of Veterinary Medicine, University of Baghdad, for molecular examination. Genomic DNA was extracted for conventional PCR analysis, while total RNA was extracted, reverse-transcribed into cDNA, and analyzed by RT-qPCR targeting the *B1* gene of *T. gondii*.

Results

Conventional PCR successfully amplified the *18S rRNA* gene, producing the expected 976 bp amplicon confirmed by agarose gel electrophoresis. *T. gondii* was identified in 20 of 50 (40.0%) blood samples from broiler chickens, whereas 30 (60.0%) were negative for T. gondii by RT-qPCR targeting of *B1* gene. Ct values ranged from 27.5 to 41.9 in the positive samples. The difference between positive and negative samples ($P = 0.0471$, $P \leq 0.05$) was proved by statistical analysis of the data.

Conclusion

The study concluded that broiler chickens in Baghdad city may play an important role in the transmission of toxoplasmosis and represent a potential public health risk. Real-Time

PCR was found to be a sensitive and reliable method for molecular detection of *T. gondii* infection in broiler chickens.

Keywords: broiler chickens, health, infection, Real-Time qPCR, *Toxoplasma gonidii*

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Introduction

Toxoplasma gondii is an obligate intracellular protozoan that causes toxoplasmosis, one of the most common zoonotic diseases globally (Kochanowsky & Koshy, 2018). The parasite has the ability to infect a wide range of warm-blooded animals, including humans, mammals, and birds (Hill et al., 2007). Chickens are regarded as important intermediate hosts and their infection reflects environmental contamination with *sporulated oocysts* shed by cats, the definitive hosts of the parasite. Birds usually acquire the infection through ingestion of contaminated feed, water, or soil containing infective oocysts (Lourido, 2019). Livestock production in general and domestic chicken production in particular plays a vital socio-economic role for people living in low-income countries of Africa and Asia. Domestic chickens are widely distributed avian species around the world, due to their short generation interval and adaptability in a wide range of agro ecologies. The domestic chickens provide high-quality protein and income for the poor rural households and are the most widely kept livestock species in the world. This is due to the presence of the valuable traits of chicken like disease resistance, adaptation to harsh environments and ability to utilize poor quality feeds. Broiler chickens may acquire the infection through ingestion of contaminated feed, water, or soil containing sporulated oocysts of *T. gondii* (Tainika et al., 2023). The presence of infection in broiler chickens is considered an important indicator of environmental contamination and may represent a potential source of transmission to humans through consumption of raw or undercooked chicken meat (Apalowo et al., 2024). Several studies have reported different prevalence rates of *T. gondii* infection in poultry depending on geographical location, management

system, hygiene conditions, and feeding patterns. Free-range and poorly managed broiler chickens are generally more exposed to infection because of their direct contact with contaminated surroundings. In addition, epidemiological factors such as age, sex, and environmental conditions may affect the distribution and prevalence of the parasite (Adaszyńska-Skwirzyńska et al., 2025; Liu et al., 2023). Recently, molecular techniques have become essential tools for the diagnosis of toxoplasmosis because of their high sensitivity and specificity. Polymerase Chain Reaction (PCR) is one of the most reliable molecular methods for detecting *T. gondii* DNA in biological samples, even when only small amounts of parasite DNA are present, providing greater diagnostic accuracy than conventional methods (Kim et al., 2024). Although several studies have been conducted on *T. gondii* infection in domestic animals in Iraq using serological and molecular methods, most have targeted livestock or poultry species other than broiler chickens, or have relied primarily on serological techniques. Additionally, very few molecular studies have been conducted on bird species in Baghdad, and data regarding the molecular prevalence of *T. gondii* in slaughtered broiler chickens remain unavailable (Alanad & Abdullah, 2023; Kowalczyk et al., 2026). PCR allows rapid identification of the parasite even in samples containing low quantities of DNA, making it more reliable than conventional diagnostic methods (Zeedan et al., 2022). Therefore, the prevalence of this zoonotic parasite in poultry should be investigated using the latest molecular epidemiological data. Therefore, this study was designed to focus on the molecular detection of *T. gondii* in broiler chickens in Baghdad using PCR to provide primary baseline epidemiological data that can contribute to future surveillance and control programs in Iraq.

Materials and methods

Sample collection: The present study was carried out on 50 broiler chicken samples collected from local live chicken selling and slaughtering markets in different areas of Baghdad city, including Abu Ghraib market, Al-Ghazaliya markets, Al-Adl district markets, Al-Shorja markets, and Jameela markets. The samples were collected during the period from 28 January 2026 to 30 June 2026.

Blood sample collection: Blood samples were collected from broiler chickens under aseptic conditions from different areas of Baghdad city. Approximately 2–3 ml of blood was obtained from the wing vein using sterile disposable syringes. The collected samples were transferred into sterile EDTA tubes to prevent coagulation and preserve the nucleic acids for molecular analysis. Peripheral blood was selected as the diagnostic sample because it can be collected from live chickens in a minimally invasive manner without sacrificing the birds. All samples were properly labeled and transported in an ice box to

the laboratory for further processing conventional PCR, and reverse transcription quantitative PCR (RT-qPCR) examination.

Molecular detection of *T. gondii* by conventional PCR

DNA extraction: Genomic DNA was extracted from EDTA blood samples using the FavorPrep Blood/Cultured Cells Genomic DNA Extraction Mini Kit (FABGK 001-1) (Favorgen Biotech Corp., Ping-Tung, Taiwan) according to the manufacturer's instructions. DNA quality was evaluated by electrophoresis on a 1% agarose gel prepared in 1× TBE buffer and stained with RedSafe nucleic acid stain. DNA samples mixed with loading dye were electrophoresed at 70 V for 1 h and visualized under a UV transilluminator. The primers used in this study were prepared from lyophilized stock solution and diluted with nuclease-free water to obtain a working concentration of 10 pmol/µL (Table 1). Conventional PCR reactions were performed in a final volume of 25 µL containing GoTaq® Green Master Mix, forward and reverse primers, DNA template, and distilled water. The thermal cycling conditions are presented in Table 2.

Table 1: Characteristics of used primers in the present study

Primer Sequence		Primer sequence	Tm (°C)	GC%	Size of Product (bp	Accession number
18s RNA gene	F	TCCGTAGGTGAACCTGCGG	61.64	63.16	976 bp	X75453.1
	R	TCCTCCGCTTATTGATATGC	55.09	45.00		

Table 2. Thermal cycling conditions of reaction

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	5 min	1
2-	Denaturation -2	95°C	Sec35	
3-	Annealing	52°C	Sec35	30
4-	Extension-1	72°C	1min	
5-	Extension -2	72°C	5 min.	1

Molecular detection *T. gondii* by RT-qPCR

RNA extraction: Total RNA was extracted from whole blood using the TransZol Up Plus RNA kit (TransGen Biotech, China) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from the extracted RNA using the Easy Script® One-Step gDNA Removal and cDNA Synthesis Super Mix, according to the manufacturer's instructions.

RT-qPCR Assay: Molecular verification of *T. gondii* detection through Real-Time PCR and in-house specific primers targeting the *B1* gene were performed (Table 3). Amplification reactions consisting of 25 µL final volume were performed with the qPCR Master Mix, forward and reserve primers, DNA template and nuclease-free water following manufacturer instructions. Fluorescence signals were automatically monitored in each amplification process using the Rotor-

Gene Q Real-Time PCR System (QIAGEN, Hilden, Germany), and Ct values were analyzed with the Real-Time qPCR instrument software. Table 4 provides the thermal cycling conditions, while the reaction mixture is shown in Table 5.

Table 3. Primers used in present study

Primer Sequence		Primer sequence	Tm (°C)	GC%	Size of Product (bp)
B1 gene	F	TCCCCTCTGCTGGCGAAAAGT	64.08	57.14	98
	R	AGCGTTCGTGGTCAACTATCGATTG	63.59	48.00	

Table 4. Real-Time PCR thermal cycling conditions

Step	Temperature	Time	Cycles
Initial denaturation	94°C	30 s	1
Denaturation	94°C	5 s	45
Annealing/Extension	60°C	35 s	45
Melting curve	90°C	15 s	1

Table 5. Components of the RT-qPCR reaction mixture.

Master Mix	10 µL
Forward Primer (20X)	1 µL
Reverse Primer (20X)	1 µL
cDNA	8 µL
Nuclease-Free Water	to a final volume of 25µl

Statistical Analysis: Statistical analysis was performed using SPSS version 26 (IBM Corp., Armonk, NY, USA). The Chi-square test was used to compare the prevalence of *T. gondii* infection among the studied groups. A value of $P < 0.05$ was considered statistically significant.

Results

Agarose gel electrophoresis was used to analyze the PC products to verify the amplification of the target gene. The electrophoresis results showed sharp DNA bands at 976 base pairs, which is the expected size for the PCR amplification portions of the *18s RNA* gene of *T. gondii*. Positive samples revealed distinct bands under UV illumination, demonstrating the successful molecular identification of the parasite. (Figure 1). Results of molecular detection of *T. gondii* in broiler chicken samples using RT-qPCR assay are summarized in Table 6. Of the 50 samples analyzed, 20 (40.00%) were positive and 30 (60.00%) were negative. Positive samples showed Ct (Cp) values ranging from 27.5 to 41.9, while no amplification was observed in negative samples. Statistical analysis revealed a significant difference between positive and negative samples ($P = 0.0471$, $P \leq 0.05$).

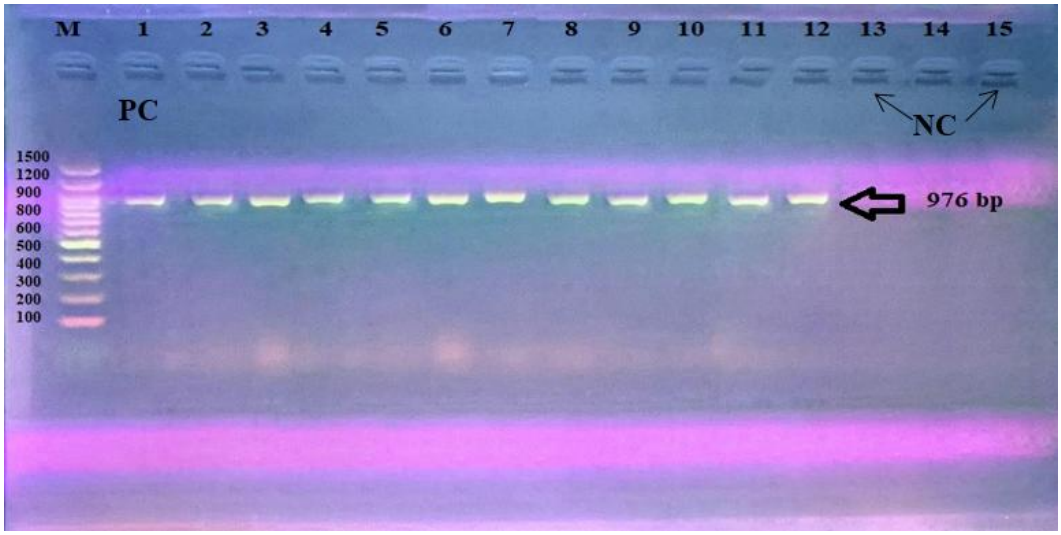

Figure 1. Agarose gel electrophoresis showing amplification of the 18S rRNA gene (976 bp) in representative *T. gondii* DNA samples. Lane M: DNA ladder; Lanes 1: Positive control (PC), Lanes 13-15: negative control, Lanes 1-12: representative amplified samples

Table 6. RT-qPCR results showing the corresponding Cp values for samples

Number of the well	ID of the tube	Cp, Fam	Result	Number of the well	ID of the tube	Cp, Fam	Result
A1	Sample_1 (qpcr)	34.7	+	C2	Sample_26 (qpcr)	29.5	+
A2	Sample_2 (qpcr)	33.6	+	C3	Sample_27 (qpcr)	27.5	+
A3	Sample_3 (qpcr)	34.5	+	C4	Sample_28 (qpcr)	28.5	+
A4	Sample_4 (qpcr)	35.1	+	C5	Sample_29 (qpcr)	28.9	+
A5	Sample_5 (qpcr)	33.5	+	C6	Sample_30 (qpcr)	30.4	+
A6	Sample_6 (qpcr)	34.9	+	C7	Sample_31 (qpcr)		-
A7	Sample_7 (qpcr)	34.4	+	C8	Sample_32 (qpcr)		-
A8	Sample_8 (qpcr)	35.3	+	C9	Sample_33 (qpcr)		-
A9	Sample_9 (qpcr)	34.2	+	C10	Sample_34 (qpcr)		-
A10	Sample_10 (qpcr)	33.9	+	C11	Sample_35 (qpcr)		-
A11	Sample_11 (qpcr)	35.5	+	C12	Sample_36 (qpcr)		-
A12	Sample_12 (qpcr)		-	D1	Sample_37 (qpcr)		-
B1	Sample_13 (qpcr)		-	D2	Sample_38 (qpcr)		-
B2	Sample_14 (qpcr)		-	D3	Sample_39 (qpcr)		-
B3	Sample_15 (qpcr)		-	D4	Sample_40 (qpcr)		-
B4	Sample_16 (qpcr)	41.9	+	D5	Sample_41 (qpcr)		-
B5	Sample_17 (qpcr)	38.6	+	D6	Sample_42 (qpcr)		-
B6	Sample_18 (qpcr)		-	D7	Sample_43 (qpcr)		-
B7	Sample_19 (qpcr)		-	D8	Sample_44 (qpcr)		-
B8	Sample_20 (qpcr)		-	D9	Sample_45 (qpcr)		-
B9	Sample_21 (qpcr)		-	D10	Sample_46 (qpcr)		-
B10	Sample_22 (qpcr)		-	D11	Sample_47 (qpcr)		-
B11	Sample_23 (qpcr)		-	D12	Sample_48 (qpcr)		-
B12	Sample_24 (qpcr)	28.7	+	E1	Sample_49 (qpcr)		-
C1	Sample_25 (qpcr)	29.7	+	E2	Sample_50 (qpcr)		-

Real-Time qPCR amplification: Representative RT-qPCR amplification plots showed successful detection of *T. gondii* in positive broiler chicken blood samples. Positive samples displayed characteristic amplification curves in the FAM fluorescence channel, with fluorescence intensity for each sample rising steadily over cycles during amplification and crossing the threshold within expected Ct values corresponding to successful amplification of target B1 gene. Negative samples did not exhibit any amplification signals at all during each reaction, confirming the absence of the target sequence (Figure 2).

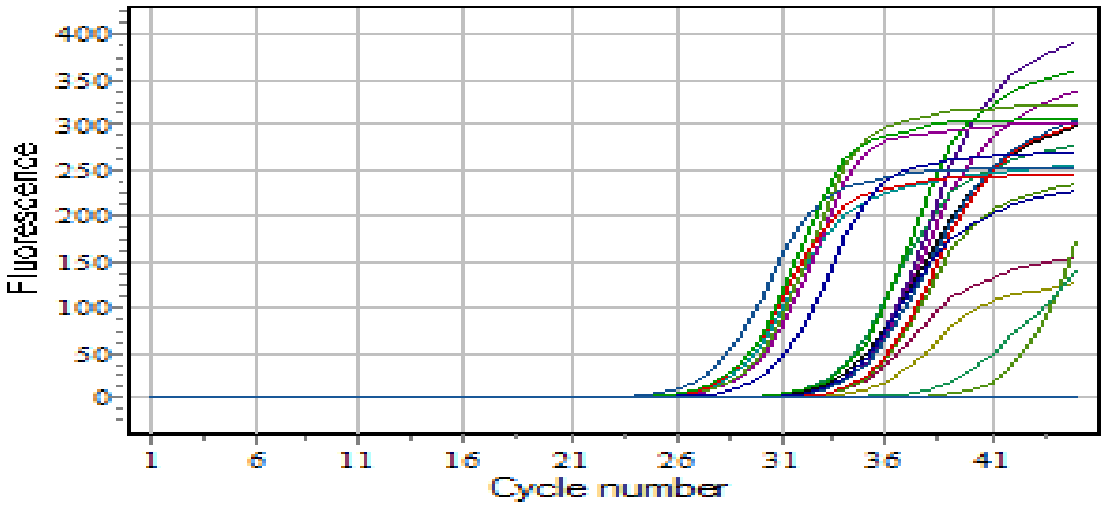

Figure 2. Fluorescence intensity of the FAM channel in Real-Time qPCR assay

Discussion

The current study showed that the prevalence of *T. gondii* infection in broiler chickens in Baghdad city was 40% using Real-Time qPCR assay targeting the *B1* gene, this finding suggested that broiler chickens should also be considered important in the epidemiology and transmission of toxoplasmosis in Iraq. The presence of *T. gondii* DNA in broiler chickens indicates environmental contamination via several routes, such as contaminated feed, contaminated drinking water, and farm environments with a high human and stray cat population, which are the definitive hosts of *T. gondii* and responsible for releasing oocysts (Mancusi et al., 2023). Furthermore, poor biosecurity on the farm, inadequate hygiene, and the activity of rodents or wild birds can increase the risk of infection. Transmission of the parasite typically occurs through the ingestion of oocysts, with entry routes being contaminated feed, water, or soil (Ahmad et al., 2024). In the poultry context, presence of infection is therefore regarded as an implicit epidemiological evidence of environmental contamination and a potential public health hazard (Naeem et al., 2025). The relatively high prevalence in the present study may be associated with insufficient hygienic conditions etc., at poultry markets, exposure of chickens to contaminated environment, presence of stray cats within and along the streets surrounding breeding and slaughtering areas, storage not well organized feed and water (Khan et al., 2020). Usually, live poultry markets in Baghdad city feature mixed animal environments with insufficient sanitary management that may spread oocysts transmission between animals and birds. Besides soil, dust

would be considered a common exposure route when broiler chickens are transported and marketed (Farhan et al., 2024). Real-Time PCR always performed as an effective and sensitive detection method for *T. gondii* DNA (Condoleo et al., 2024). Molecular techniques have greater sensitivity than serological methods since they detect the parasite genetic material itself at very low concentrations, like in PCR (Lin et al., 2000). Indeed, positive samples showed a characteristic fragment amplified at 976 bp by Agarose gel electrophoresis and amplification curves through FAM fluorescence channel. We confirm that PCR-based assays of molecular diagnosis of toxoplasmosis in poultry are reliable (Villar et al., 2023). Results in the present study are consistent with those reported by (Abdulzahra et al., 2025) in Basra Governorate, where serological testing revealed infection rates of 37.09% in domestic chickens. The current findings are consistent with those of (Abdulzahra & Abdullah, 2023), whose molecular study revealed the presence of the *T. gondii* parasite in several wild bird species using CRT, demonstrating the important role of birds in the epidemiology of toxoplasmosis. The current study also recorded the prevalence of *T. gondii* infection in broiler chickens using real-time PCR. The results in our study were higher than those recorded in neighboring countries such as India (6.06%) and Iran (8%), but within the range of infection rates recorded in Iraq and other countries. The current study yielded lower results than those reported by Hussein and Jasim (2022), according to PCR technique, the infection rates of 88.8% and 100% were recorded in industrial chickens and free living (wild) chickens Al-Qadisiyah province. The differences between the 2 studies are possibly due to the geographical location, environmental contamination, type of sample used, size of poultry populations sampled, type of poultry management systems subjected to assessment and/or diagnostic targets employed. In contrast, free-range chickens encounter contaminated environment and infested intermediate hosts more than commercial broiler chickens (Mancusi et al., 2023). The results of this study were also lower than those obtained by Hraija & Abdair (2025). They performed PCR assay and found *T. gondii* infection in 56.9% domestic chickens at the province of Wasit. In their study, high infection rates were shown in chicken tissues including liver, brain, and muscles. The elevated prevalence in their study could be explained by utilizing tissue samples, which typically have much higher loads of tissue cysts than blood samples. Moreover, the climatic and environmental conditions of Baghdad province differ from those of Wasit province which may affect oocysts survival and transmission(Al-khanak & Salman, 2021). The difference was significant in the present study at ($P \leq 0.05$), and this positive prevalence was not random but actually reflects a circulation of the parasite among broiler chickens in Baghdad city in the distribution of *T. gondii* infection. The oocyst survival varies in environmental conditions, such as temperature, humidity and by the presence of cats which could also influence the infectivity rates (Abdulwahed, 2025; Naeem et al., 2025). High humidity promotes sporulation and survival of oocysts in the environment for long periods, thus supporting transmission.

Conclusion: Present study showed the detection of *T. gondii* infection in broiler chickens from Baghdad city by using Real-Time PCR targeting the *B1* gene. The results obtained show a prevalence of 40% in the analyzed samples suggesting that broiler chickens are likely an important source of transmission of toxoplasmosis and environmental contamination with oocysts from *T. gondii*. Moreover, the study also revealed these experiments could make an overview that

Real-Time qPCR is a reliable rapid method for detection of *T. gondii* in broiler chicken samples with high level of specificity and sensitivity as compared to conventional methods. The high prevalence obtained in this study points to the continuous monitoring of poultry and maid hygiene measures at live bird markets and sexes with poultry producing facilities as a part policy to mitigate human- or animal-based avian influenza infection transmission. The present study has a limitation including a relatively small sample size (n = 50), which may not be entirely reflective for the prevalence of *T. gondii* infection in broiler chickens from all over Baghdad city. Also, lacking details on key demographic information surrounding the broiler chickens analyzed (age, body weight, and commercial breed/strain) at the time of sample collection. These factors could influence the vulnerability of broilers to *T. gondii* naturally and should be included as variables in future epidemiological studies. Future studies should also investigate poultry from varying regions of Iraq with larger sample sizes to shed more light on the molecular epidemiology of *T. gondii* infection in this important animal reservoir vector.

Data availability statement

Data sets are available from the author upon request.

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Ethical considerations

The current investigation was managed at the Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Iraq and was approved by the Institutional Animal Care and Use Committee Protocol based on the guidelines of the Iraqi Council of Animal Care.

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This study did not receive any external funding.

Conflict of Interest

The author declares that there are no conflicts of interest.

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تشخیص مولکولی *Toxoplasma gondii* با استفاده از PCR زمان واقعی در جوجه‌های گوشتی شهر بغداد، عراق

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چکیده

هدف: Toxoplasma gondii یک انگل تک‌یاخته‌ای داخل‌سلولی است که عامل بیماری توکسوپلاسموز، یک بیماری مهم مشترک بین انسان و حیوان، در سراسر جهان محسوب می‌شود. جوجه‌های گوشتی به دلیل قرار گرفتن در معرض محیط‌های آلوده و نقش آن‌ها در انتقال آلودگی از طریق مواد غذایی، به‌عنوان منابع بالقوه عفونت در نظر گرفته می‌شوند. مطالعه حاضر با هدف شناسایی T. gondii در جوجه‌های گوشتی شهر بغداد با استفاده از روش‌های PCR معمولی و PCR کمی زمان واقعی با رونویسی معکوس (RT-qPCR) انجام شد.

مواد و روش‌ها: در مجموع، ۵۰ نمونه از جوجه‌های گوشتی از بازارهای محلی فروش و کشتار مرغ زنده در مناطق مختلف شهر بغداد، شامل بازارهای ابوغریب، الغزالیه، العدل، الشورجه و جمیله، طی دوره زمانی ژانویه ۲۰۲۶ تا ژوئن ۲۰۲۶ جمع‌آوری شد. نمونه‌های خون در شرایط آسپتیک جمع‌آوری و برای بررسی‌های مولکولی به دانشکده دامپزشکی دانشگاه بغداد منتقل شدند. DNA ژنومی برای انجام PCR معمولی استخراج شد. سپس RNA تام استخراج شد و پس از رونویسی معکوس به cDNA، با استفاده از RT-qPCR و با هدف قرار دادن ژن B1 متعلق به T. gondii مورد بررسی قرار گرفت.

نتایج: PCR معمولی با موفقیت ژن 18S rRNA را تکثیر کرد و محصول مورد انتظار به اندازه ۹۷۶ جفت‌باز تولید شد که با استفاده از الکتروفورز روی ژل آگارز تأیید گردید. T. gondii در ۲۰ مورد از ۵۰ نمونه خون جوجه‌های گوشتی (۴۰.۰٪) شناسایی شد، درحالی‌که ۳۰ نمونه (۶۰.۰٪) در RT-qPCR با هدف قرار دادن ژن B1 برای T. gondii منفی بودند. مقادیر Ct در نمونه‌های مثبت از ۲۷/۵ تا ۴۱/۹ متغیر بود. تحلیل آماری داده‌ها اختلاف بین نمونه‌های مثبت و منفی را نشان داد ($P = 0.0471, P \leq 0.05$).

نتیجه‌گیری: این مطالعه نشان داد که جوجه‌های گوشتی در شهر بغداد ممکن است نقش مهمی در انتقال توکسوپلاسموز داشته باشند و به‌عنوان یک خطر بالقوه برای سلامت عمومی مطرح باشند. PCR در زمان واقعی (Real-Time PCR) به‌عنوان روشی حساس و قابل اعتماد برای تشخیص مولکولی عفونت T. gondii در جوجه‌های گوشتی شناخته شد.

کلمات کلیدی: جوجه‌های گوشتی، سلامت، عفونت، Real-Time qPCR، Toxoplasma gondii

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