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Research Article

Epidemiological investigation and phylogenetic analysis of *Toxoplasma gondii* strains from animal and human hosts as evidence of zoonotic spread in Duhok, Iraq

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Abstract

This study aimed to investigate the genetic diversity and transmission dynamics of *Toxoplasma gondii* strains isolated from animals and humans in Duhok Governorate, Iraq. A total of 15 strains originating from chickens, horses, sheep, and women with a history of abortion were analyzed using PCR amplification and sequencing of the ITS1 rDNA region. Phylogenetic analysis revealed all local strains clustered into a single, well-supported monophyletic clade, regardless of host species or geographic location, indicating a common ancestral origin. In contrast, global reference strains showed marked genetic divergence and did not cluster with the Duhok isolates. Although most Duhok strains appeared generalist, minor host-associated sub-clustering and unique branching patterns were observed. These findings underscore the importance of region-specific control strategies, including improved sanitation, safer animal management, and public education. The study advocates for a "One Health" approach, integrating human, veterinary, and environmental health efforts to monitor and mitigate the spread of *T. gondii* in endemic areas.

Keywords: *Toxoplasma gondii*, ITS1 rDNA sequencing, Phylogenetic clustering, Cross-species transmission, One Health approach

Introduction

Toxoplasma gondii is a globally distributed zoonotic protozoan parasite that infects a wide range of warm-blooded animals, including humans, and is the causative agent of toxoplasmosis (Robert-

Gangneux et al., 2022). Infection can occur through ingestion of tissue cysts in undercooked meat, oocysts from contaminated food or water, or congenitally from mother to fetus (Hill and Dubey, 2018). Although often asymptomatic in immunocompetent individuals, *T. gondii* poses a significant risk to immunocompromised individuals and during pregnancy, potentially leading to severe complications or congenital defects (Wang et al., 2017).

The genetic diversity of *T. gondii* has important implications for its virulence, transmission dynamics, and epidemiology (Shwab et al., 2018). Molecular markers, particularly those targeting ribosomal DNA regions such as the internal transcribed spacer 1 (ITS1), have been widely used in genotyping studies due to their high sensitivity and discriminatory power. The ITS1 region of the rDNA gene is located between the 18S and 5.8S rRNA genes and is considered a reliable target for molecular detection and phylogenetic analysis of *T. gondii* isolates (Taalay et al., 2022). Despite the significance of this approach, there is limited data on the phylogenetic characteristics of *T. gondii* in Iraq, particularly in the Duhok Governorate, a region with considerable human-animal interaction, including livestock rearing and domestic cat presence, which may facilitate parasite transmission. Given the scarcity of molecular epidemiological data from this region, the current study aims to analyze the phylogenetic relationships of *T. gondii* strains collected from Duhok Governorate using the ITS1 region of the rDNA gene. Understanding the genetic diversity of local strains can provide insights into regional transmission patterns and potential public health risks.

Material and methods

Toxoplasma gondii strains and study area description

A total of 15 *T. gondii* strains were previously isolated from various animal species and women with a history of abortion across different locations in Duhok Governorate. These included four strains from chickens (Mikaeel and Al-Saeed, 2020a), four from horses, three from sheep (Mikaeel and Al-Saeed, 2020b), and four from women (Mikaeel and Al-Saeed, 2019). This study was conducted in Duhok Governorate, situated in the Kurdistan Region of northern Iraq. The region is primarily agrarian, with domestic animals such as sheep, free-range chickens, and horses often raised in proximity to human households. Traditional farming practices, free-range animal husbandry, and a high density of domestic and stray cats further characterize the study area.

DNA extraction, DNA sequencing, and phylogenetic analysis

Genomic DNA was extracted from each isolate using a commercial DNA extraction kit (Addbio / Korea) according to the manufacturer’s instructions. The quality and concentration of the extracted

DNA were assessed using a Nanodrop spectrophotometer (Thermo Scientific, USA) and by agarose gel electrophoresis to ensure integrity before PCR amplification. These DNA samples were then used as templates for amplification of the internal transcribed spacer 1 (ITS1) region of the rDNA gene. For sequencing, the PCR products of the amplified ITS1 were sent to the Macrogen firm (Korea). The chromatograms of each sample were assessed using the BioEdit program (version 7.2). All sequences were submitted to GenBank. According to Tamura et al. (2011), nucleotide sequence homology searches were completed using the BLAST network server at the National Center for Biotechnology Information (NCBI). Multiple sequence alignments were performed using the MEGA X program, and molecular evolutionary genetic analysis software (MEGA X) was used to create a phylogenetic tree using distance matrices and the Maximum Composite Likelihood substitution approach. A bootstrap study using 1000× data sets was used to determine how reproducible the node was for tree topology. A total of 15 isolates were used in this study to construct the phylogenetic tree analysis.

Results

A total of 15 *T. gondii* strains originating from humans, chickens, horses, and sheep were successfully sequenced. The sequences were deposited in GenBank under these accession numbers: ON337179 to ON337193 (Table 1). Phylogenetic analysis based on ITS1 region sequences revealed that the 15 *Toxoplasma gondii* strains from Duhok, Iraq (accession numbers ON337185.1 to ON337199.1), clustered into a distinct and well-supported monophyletic clade, indicating a close genetic relationship among the local strains. Within this clade, the Duhok strains formed separate branches (e.g., ON337186.1, ON337190.1, ON337180.1, ON337184.1) that formed a closely related group. These strains were obtained from diverse hosts, including humans, horses, chickens, and sheep. Notably, strains from humans (e.g., ON337186.1, ON337180.1, ON337184.1) and animals (e.g., ON337190.1 from horses, ON337192.1 from sheep) shared high genetic similarity. While other Duhok strains (e.g., ON337181.1, ON337193.1) exhibit unique branching patterns (Fig. 1). In contrast, reference strains from other geographic regions—including China (JQ235642.1), the USA (KP996969.1), Germany (EU602505.1), Thailand (KP868068.1), Turkey (OL418220.1, KU726889.1), Pakistan (MW885251.1), Brazil (MH736504.1), Iran (ON337186.1), Tunisia (ON337200.1), and Japan (LC722483.1), were distributed across distinct branches of the phylogenetic tree. These international strains exhibited significant genetic divergence from the Duhok strains, as evidenced by longer branch lengths and lower bootstrap values supporting shared ancestry. Notably, none of the global reference strains clustered within the Duhok clade (Figure 1).

Table 1. Host species and assigned GenBank accession numbers of the isolated *T. gondii* strains.

Strain number	Accession number	Host species
1	ON337179	chicken
2	ON337180	chicken
3	ON337181	chicken
4	ON337182	chicken
5	ON337183	Homo sapiens
6	ON337184	Homo sapiens
7	ON337185	Homo sapiens
8	ON337186	Homo sapiens
9	ON337187	horse
10	ON337188	horse
11	ON337189	horse
12	ON337190	horse
13	ON337191	sheep
14	ON337192	sheep
15	ON337193	sheep

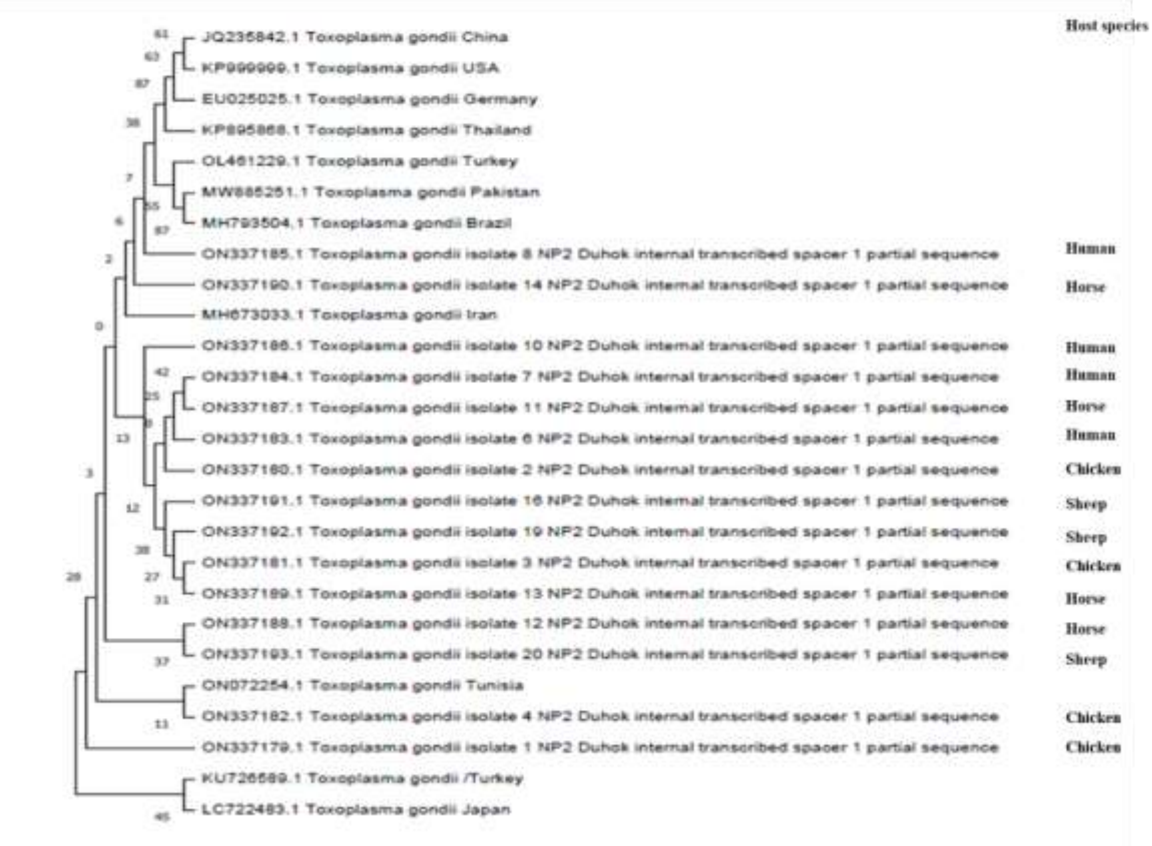

Figure 1. Maximum-likelihood phylogenetic tree of 15 *T. gondii* strains derived from animal and human hosts in the study region.

Discussion

This investigation was designed to address several critical questions that may guide veterinary and public health authorities in implementing effective biosecurity measures to prevent the cross-species transmission of *Toxoplasma gondii*, including its zoonotic spread to humans. The key questions explored in this study include: do *T. gondii* strains circulating in Duhok Governorate originate from a common source, or do they represent multiple, independent introductions, is there evidence of strain sharing between humans and animals in the region, how do the genetic profiles of *T. gondii* strains from Duhok compare with those of globally reported isolates, and finally, do certain strains exhibit host-specific clustering patterns in the phylogenetic tree, suggesting potential adaptation to particular animal hosts.

The clustering of all Duhok strains into a single monophyletic clade strongly indicates that these strains likely originate from a common ancestral source, rather than representing multiple, independent introductions. The close genetic proximity among strains from different hosts and geographical areas within Duhok supports a scenario of localized transmission within a relatively closed ecological system. This likely stems from widespread contamination of shared water and soil sources with oocysts shed by resident felids, enabling infection across livestock, wildlife, and humans without significant influx of outside genotypes (VanWormer et al., 2013; Mikaeel et al., 2025a, b). Traditional husbandry and feeding practices such as free-ranging poultry and grazing sheep facilitate cross-species exposure in neighboring villages (Vicente *et al.*, 2021). The close interaction between livestock animals in this region, along with widespread backyard livestock farming and the presence of free-roaming domestic cats, creates favorable conditions for the transmission of *T. gondii* (Hatam-Nahavandi et al., 2021). Cats, as the definitive hosts of the parasite, shed environmentally resistant oocysts in their feces, contaminating soil, water, and vegetation. These oocysts can be ingested by intermediate hosts, including livestock, which then develop tissue cysts in their muscles (Zhu et al., 2022).

All of the above factors may have collectively implicated the clustering of all Duhok strains into a single monophyletic clade. This has implications for biosecurity, highlighting the need to identify and control local reservoirs (domestic animals or contaminated environmental sources) that may serve as persistent sources of infection.

The high genetic similarity between strains isolated from humans and those from animals such as horses and sheep suggests cross-species transmission of *T. gondii* within this region and was likely driven by shared environmental exposures and husbandry practices (Suminda et al., 2022). Contaminated water sources and pasture lands where oocysts shed by local felid populations persist can simultaneously infect grazing livestock and people who use the same water for drinking or crop irrigation (Polley *et al.*, 2022). Consumption of undercooked meat from infected animals, which harbor tissue cysts, provides a direct zoonotic route to humans (Pradhan and Karanth, 2023). Furthermore, free-range grazing and mixed-species rearing systems increase the likelihood of interspecies contact and mechanical transport of oocysts via farm equipment, footwear, or wild birds that move between pastures and human settlements (Barron, 2017). This finding reinforces the zoonotic nature of the parasite and underscores the public health risk posed by shared environments, especially where close contact between animals and humans occurs. These results support the "One Health" approach, advocating for integrated surveillance across human and veterinary sectors.

When compared to *T. gondii* reference strains from diverse counties, the Duhok strains demonstrated marked genetic divergence. The international strains were widely distributed across distinct branches and did not cluster with the Duhok clade. This finding implies a localized evolutionary trajectory for the Duhok strains, possibly shaped by regional ecological and host-specific pressures (Boothroyd, 2009). The genetic distinctiveness of these strains further suggests limited recent gene flow between Duhok and other global populations of *T. gondii* (Galal et al., 2019). Although the Duhok isolates overall formed a single clade, some degree of host-associated sub-clustering was observed. For instance, strains from humans and animals often grouped closely, indicating low host-specific divergence. This suggests that *T. gondii* strains circulating in Duhok are generalist in nature, capable of infecting multiple host species without substantial genetic specialization (Hussein et al., 3023; Wilson et al., 2024). However, the unique branching of some strains (e.g., ON337181.1, ON337193.1) may reflect microevolutionary divergence or less commonly encountered transmission pathways (Galal et al., 2022), warranting further investigation.

Conclusion

This study provides molecular and epidemiological evidence that *Toxoplasma gondii* strains circulating in Duhok Governorate are genetically homogeneous, likely originating from a common ancestral source and spreading through localized transmission within a closed ecological system. The clustering of all isolates, regardless of host or location, into a single monophyletic clade highlights the role of shared environmental exposures, traditional husbandry, and free-roaming felids in sustaining cross-species transmission. Practices such as backyard farming, free-range animal rearing, and limited biosecurity further elevate zoonotic risk. Compared to global strains, Duhok isolates show distinct genetic divergence, suggesting a regionally confined lineage with limited external gene flow. While generally infecting multiple hosts, some strains displayed host-associated sub-clustering, indicating possible microevolution or rare transmission events. These findings underscore the need for targeted control strategies and support a "One Health" approach integrating veterinary, environmental, and public health efforts to reduce transmission and monitor the evolving epidemiology of *T. gondii* in the region.

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