Study on Soil Contamination with *Toxoplasma gondii* Oocysts in Eastern Iran

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Abstract

Toxoplasma gondii is a common zoonotic protozoan parasite, with felids as definitive hosts that release environmentally resistant oocysts. Soil contamination is a significant transmission pathway for human infection. This study aimed to assess the frequency of *T. gondii* oocysts in soil samples from Birjand city, Eastern Iran, using concentration and molecular methods, while evaluating seasonal and geographical variations in contamination. In this descriptive cross-sectional study, 254 soil samples (500g each from a depth of 3-5 cm) were collected from public parks, playgrounds and urban gardens across Birjand during all four seasons (2024-2025). Samples were processed using the sodium nitrate flotation technique and examined microscopically for oocyst identification. PCR targeting the B1 gene of *T. gondii* was conducted on all samples. Of the 254 samples, 23 (9.0%) were positive for *T. gondii* oocysts by both microscopic examination and PCR confirmation. No significant differences in contamination rates were found between geographical areas ($p > 0.05$). The highest positivity rate was in summer, but seasonal variations were not statistically significant ($p = 0.1$). The findings indicate a notable risk of soil-borne *T. gondii* infection for humans and animals in Eastern Iran, warranting public health interventions.

Keywords: Molecular biology, Soil, *Toxoplasma gondii*, Zoonosis

1. Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite with global distribution, capable of infecting virtually all warm-blooded animals, (1).Toxoplasmosis is often asymptomatic and self-limiting disease But it can lead to severe and life-threatening disease in immunocompromised patients (2). The parasite's life cycle is complex, involving felids (domestic cats and wild felids) as the only definitive hosts, which excrete oocysts in their feces. Intermediate hosts, including humans, can become infected through three primary routes: ingestion of tissue cysts in undercooked or raw meat, congenital transmission from an acutely infected mother to her fetus, or ingestion of sporulated oocysts from contaminated soil, water, or fresh produce (3). Soil is increasingly recognized as a critical reservoir for *T. gondii* oocysts and a major vehicle for environmental transmission to humans and animals (4). A single infected cat can shed millions of oocysts for 1-2 weeks, leading to wide environmental contamination (5). These oocysts undergo sporulation in the environment, typically within 1-5 days depending on temperature and humidity, becoming infective (6). Sporulated oocysts are remarkably resilient, capable of surviving in moist soil for extended periods, potentially up to 18 months or longer under favorable conditions, posing a persistent risk of infection (7). Human activities such as gardening, playing in sandpits, or consuming unwashed fruits and vegetables grown in contaminated soil can lead to accidental ingestion of oocysts (8). Indeed, contact with contaminated soil has been identified as a significant risk factor for *T. gondii* infection in various populations, including pregnant women (9). Global seroprevalence of toxoplasmosis varies considerably, ranging from 10% to over 80% in different human populations, with approximately one-third of the world's population estimated to be infected (10). Studies from different parts of the world have reported varying rates of soil contamination. For instance, studies in China have reported prevalences ranging from 11.9% in public parks in Wuhan to higher rates in specific farm environments (11). In Iran, numerous epidemiological studies have reported seroprevalence rates between 18% and 68%, depending on geographical region, cultural habits, and climatic conditions (12). Prior studies in Iran, have reported varying contamination rates, from 5% to 78.1%, influenced by local environmental factors and cat behaviors (13). These variations underscore the need for local epidemiological investigations to assess region-specific risks. Understanding the local prevalence of *T. gondii* in environmental matrices like soil is crucial for developing targeted public health interventions and raising awareness among the population, particularly vulnerable groups such as children who frequently play in public parks. Traditional methods for oocyst detection, such as microscopy after flotation, can be challenging due to the morphological similarity of *T. gondii* oocysts with those of other coccidian parasites and the presence of environmental debris. Molecular techniques, particularly Polymerase Chain Reaction (PCR) targeting specific *T. gondii* genes like the B1 gene or the 200 bp repeat element, offer higher sensitivity and specificity for oocyst detection and confirmation in

environmental samples (10). This study was therefore designed to investigate the frequency of *T. gondii* oocyst contamination in soil samples collected from various public areas within Birjand city. We employed a combination of a standard concentration method for oocyst recovery and microscopic examination, followed by a confirmatory PCR assay targeting the B1 gene .

2. Materials and Methods

2.1. Study Area

This cross-sectional study was conducted in Birjand city (32°52′N 59°13′E), the capital of South Khorasan Province in eastern Iran. The presence of domestic and stray cats in urban and peri-urban areas is common, potentially contributing to soil contamination with *T. gondii* oocysts. Birjand has a semi-arid climate characterized by hot summers and cold winters, with an average annual precipitation of approximately 170 mm and a mean annual temperature of 16.5$^{\circ C}$. The city covers an area of approximately 141 km² and had a population of around 221,756, according to the 2023 census.

2.2. Sample Collection

From May 2024 to April 2025, a total of 254 soil samples were collected from various sites across Birjand city, including public parks, playgrounds and urban gardens based on the sample size formula ($N= Z^2 \times P(1-P)/d^2$) and insights from similar studies. Sample collection was stratified by season (spring, summer, autumn, winter) to evaluate potential seasonal variations in contamination rates. Sampling locations were selected using a randomized approach to ensure representation of different areas within the city. Approximately 500g of soil was collected from each sampling point at a depth of 3-5 cm using sterile plastic scoops. Samples were placed in labelled zip-lock bags containing information about the collection date, location coordinates, and environmental conditions. All samples were transported to the Parasitology Laboratory at Birjand University of Medical Sciences and stored at 4$^{\circ C}$ until processing.

2.3. Concentration Method

For the detection of *T. gondii* oocysts, a sodium nitrate flotation technique was employed. The flotation solution was prepared by dissolving 33 g of sodium nitrate in distilled water to achieve a final volume of 100 mL. Each soil sample was suspended in water with the addition of a few drops of detergent to facilitate the separation of particles. The mixture was thoroughly stirred and passed through a series of sieves (200, 75, and 25 microns) to remove large debris. The filtered samples were centrifuged at 2000 g for 5 minutes. The supernatant was discarded, and saturated sodium nitrate solution was added to the remaining sediment until a convex meniscus formed. A coverslip was placed over the meniscus and

allowed to sit for 15 minutes. Samples were examined using light microscopy at 400× magnification for identification of *T. gondii*-like oocysts based on morphological characteristics (spherical shape, 10-12 μm diameter).

2.4. Molecular Detection

2.4.1. DNA Extraction

Genomic DNA was extracted from the concentrated samples using a commercial kit (Favorgen Biotech Tissue Genomic DNA Extraction Mini Kit, Taiwan) following the manufacturer's instructions. Washing the DNA with a 0.1% Tween-20 solution can help remove inhibitors and increase DNA yield. A total of 250 mg of each concentrated sample was added to an Eppendorf tube, followed by specific buffers as outlined in the kit protocol. A ratio average of 1.8 is generally considered pure for DNA. Polymerase chain reaction (PCR) was performed targeting the B1 gene of *T. gondii*, which is a 35-fold repetitive sequence specific to this parasite. The LOD for detecting *T. gondii* oocysts in soil samples using PCR is often reported to be around 1 to 10 oocysts per gram of soil. A known quantity of a control DNA (often from a different species or a synthetic template) is added to the sample before PCR amplification. This control DNA should not be present in the sample naturally. The primers used were, Forward primer: 5'-ggaactgcatccgttcatgag-3'; Reverse primer: 5'-tctttaaagcgttcgtggtc-3'. The amplification conditions included an initial denaturation step at 94°C for 60 seconds, followed by 35 cycles of denaturation at 94°C for 35 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for 60 seconds. A final extension step was performed at 72°C for 10 minutes. Positive controls (DNA extracted from *T. gondii* tachyzoites, RH strain) and negative controls (all PCR components except template DNA) were included in each PCR run to ensure validity of the results. PCR products were electrophoresed on a 1.5% agarose gel containing ethidium bromide (0.5 μg/ml) at 90 V for 45 minutes. A 100-bp DNA ladder was used as a molecular weight marker. The expected PCR product size for the B1 gene was approximately between 200 to 300 bp (14). Gels were visualized under UV light using a transilluminator, and positive samples were identified based on the presence of the expected band size.

2.4. Statistical Analysis

Data were analyzed using SPSS software version 20 (IBM, USA). Descriptive statistics were used to calculate the frequency and percentage of *T. gondii* contamination. Chi-square test was employed to assess associations between contamination rates and variables such as sampling location and season. A P-value <0.05 was considered statistically significant.

3. Results

3.1. Overall Prevalence of *T. gondii*

23 out of 254 soil samples (9.0%) were found positive for *T. gondii* oocysts (Figure 1&2) using microscopic parasitology method. Our findings, showing 100% concordance between morphological and molecular detection methods.

Figure 1: Photomicrograph of a *T. gondii* oocyst (100X). An image showing a representative oocyst as observed under the microscope after flotation, highlighting its size and morphology. Scale bar of figure =10 mm.

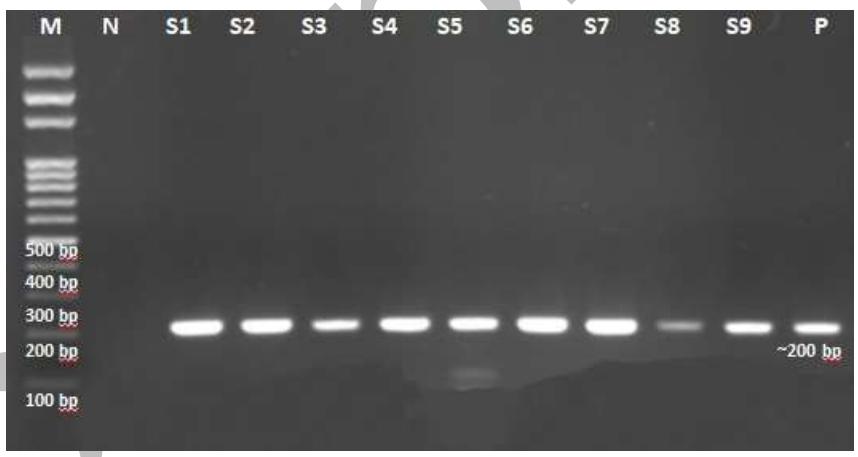

Figure 2: Electrophoresis of PCR products for *T. gondii* B1 gene on a 1.5% agarose gel. Lane M: 100 bp DNA ladder. Lane N: Nagative control. Lane P: Positive control (*T. gondii* DNA). Lanes 1-10 (S1-S9): Soil samples, with some lanes showing a clear band at ~200 bp indicating positive detection of *T. gondii* DNA.

3.2. *T. gondii* contamination in different variables

The distribution of positive samples across these geographical zones is presented in Table 1. Statistical analysis revealed no significant differences in contamination rates between different areas ($\chi^2 = 3.65$, $p = 0.456$) Although the highest contamination rate was observed during summer (13.3%), followed

by spring (7.9%), autumn (7.9%), and winter (7.4%), these differences did not reach statistical significance ($\chi^2 = 6.23$, $p = 0.101$). The contamination rates were 9.5% (8/84) for public parks, 8.3% (8/96) for playgrounds, and 9.5% (7/74) for urban gardens. These differences were not statistically significant ($\chi^2 = 4.87$, $p = 0.301$).

Table 1: Distribution of *T. gondii* Oocyst Contamination in Soil Samples Across Different Areas and Seasons in Birjand City

Different Areas and Seasons		Total Samples	Positive Samples	Prevalence (%)
Region	Public Parks	84	8	9.5
	Playgrounds	96	8	8.3
	Urban Gardens	74	7	9.5
Season	Spring	63	5	7.9
	Summer	60	8	13.3
	Autumn	63	5	7.9
	Winter	68	5	7.4
Total		254	23	9.0

4. Discussion

The present study investigated the contamination of soil with *T. gondii* oocysts in public areas of Birjand, Eastern Iran. This finding underscores the role of soil as an environmental reservoir for *T. gondii* and highlights a potential public health risk for the residents of Birjand. The 9.0% prevalence observed in Birjand is comparable to or falls within the range reported in several other studies globally and within Iran, though direct comparisons should be made cautiously due to variations in methodologies, climatic conditions, cat population densities, and types of areas sampled. For instance, a study in public parks China reported a prevalence of 10.9%(13), and another in Mexico City found 11.8% contamination in public parks (14). In contrast, some studies have reported lower prevalences, such as 5% in Arak, Iran (15), and 8.7% in Tehran, Iran (16). However, significantly higher rates, such as 78.1% in Mazandaran Province, Northern Iran, and 30.3% in urban areas of northeastern Mainland China, have also been documented. These variations emphasize the focal nature of soil contamination and the influence of local ecological factors (17). The difference in prevalence of soil contamination with *T. gondii* oocysts between arid provinces like South Khorasan and humid northern provinces such as Mazandaran can be attributed to several key factors. Firstly, climatic conditions play a significant

role; humid environments promote the survival and transmission of oocysts, while arid climates can lead to desiccation and reduced viability. Secondly, land use practices differ, with northern provinces often having more agricultural activities that can facilitate contamination through livestock and wildlife interactions. Thirdly, population density and urbanization in humid areas may increase the presence of domestic cats, which are primary hosts for *T. gondii*, thereby raising oocyst shedding into the environment. Additionally, soil characteristics, such as moisture content and organic matter, can influence oocyst persistence, with richer soils in humid regions providing a more favorable habitat. Lastly, socioeconomic factors and public health awareness can affect the management of waste and sanitation practices, further impacting contamination levels in these contrasting provinces (18). Soil contamination with *T. gondii* oocysts is influenced by several risk factors. First, contact with domestic animals, particularly cats, significantly increases the likelihood of contamination due to their role as definitive hosts. Second, urban living conditions often correlate with higher contamination rates, likely due to increased animal populations and human activities. Third, improper waste management practices can lead to the accumulation of oocysts in the environment. Fourth, environmental factors such as rainfall and soil composition can affect the survival and dispersal of these oocysts. Finally, using untreated water sources can further elevate the risk of exposure to T. gondii in contaminated soils (10). The prevalence of contamination across different types of urban spaces provides valuable insights for targeted intervention strategies. The slightly higher contamination rates observed in playgrounds (11.1%) and public parks (10.6%) compared to garden beds (7.5%) and public squares (6.9%), though not statistically significant, raise concerns about potential exposure risks, particularly for children. These findings align with those of Gao et al. (2016), who reported elevated oocyst contamination in recreational areas frequented by both cats and humans (17). The presence of sandboxes, vegetation, and sheltered spaces in parks and playgrounds may provide favorable microenvironments for both cat defecation and oocyst persistence (18,19). Regarding seasonal variation, our results showed the highest prevalence of *T. gondii* oocysts in summer (e.g., 12.3%), followed by autumn, spring, and then winter, although this trend was not statistically significant ($P > 0.5$). The non-significant results ($p > 0.05$) related to geography and season may be attributed to low statistical power, indicating a potential Type II error. Seasonal variations in soil contamination, although not statistically significant, revealed interesting trends that warrant further investigation. The higher prevalence observed during summer months (13.1%) aligns with the optimal conditions for oocyst sporulation and survival in this region. Laboratory studies by Lélu et al. (2021) demonstrated that temperatures between 20-25°C and moderate humidity levels accelerate the sporulation process, potentially increasing the concentration of infectious oocysts during warmer seasons (7). Additionally, the higher summer prevalence could be related to increased outdoor activities of both cats and humans during this period, facilitating greater

environmental contamination and exposure risk. The persistence of *T. gondii* oocysts during Birjand's cold winter months (6.2% positivity), albeit at lower rates, demonstrates the remarkable resilience of these environmental stages under adverse conditions. Recent experimental studies by Shapiro et al. (2021) showed that *T. gondii* oocysts can remain viable for extended periods even under freeze-thaw cycles, particularly when sheltered within soil matrices (4). This finding has important implications for public health, as it suggests year-round transmission risk in this semi-arid region despite its seasonal temperature extremes. The perfect concordance between microscopic and molecular detection methods observed in our study contrasts with several previous reports that demonstrated higher sensitivity of PCR-based methods compared to conventional techniques (20). This unexpected finding might be attributed to the efficiency of our modified sodium nitrate flotation protocol, which may have effectively concentrated oocysts from soil matrices, combined with careful microscopic examination. Arid conditions in Eastern Iran may preserve oocyst structural integrity, reducing degradation and enabling clear morphological identification under microscopy. Elevated soil contamination levels in the region could enhance microscopic detection sensitivity, aligning with PCR's molecular confirmation. PCR primers targeting conserved *T. gondii* genes might align with morphologically distinct, intact oocysts, reducing discordance from fragmented or atypical forms. One of the primary limitations of the study is that microscopic or molecular detection methods do not provide confirmation of the viability or infectivity of oocysts. Viability assays (e.g., mouse bioassay, PMA-PCR) and *T. gondii* prevalence in soil contamination of stray and owned cats are suggested for future work.

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Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data Availability

The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval

This study was approved under the ethical approval code IR.BUMS.REC.1402.398.

Authors Contribution

Study concept and design: **AM** and **ATK**. Analysis and interpretation of data: **SMM**. Drafting of the manuscript: **ATK**. Critical revision of the manuscript for important intellectual content: **AM**. Statistical analysis: **MB**.

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