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Research paper

# Prevalence and genetic diversity of *Toxoplasma gondii* oocysts in cats of southwest of Iran

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**KEYWORDS**
Prevalence;
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*Toxoplasma gondii*;
Oocyst;
Cat;
Iran

**Abstract** *Objective:* Contamination of soil and water with infected oocysts have an important role in both animals and humans toxoplasmosis. Cats shed oocytes by their feces that can survive in environment such as moist soil, warm and humid weather for months and even years. The present study designated to molecular detection of *Toxoplasma gondii* oocysts in stool samples obtained from cats in Ahvaz, southwest of Iran.
*Methods:* Initially, 486 stool samples were randomly collected from cats. After sucrose flotation method, the DNA was extracted and PCR was carried out using the amplification of the B1 gene and repeat element sequence (RE). For strain typing purpose, the genetic marker of SAG2 was used in nested-PCR. To perform RFLP procedure, the products of nested-PCR were digested using *Sau3A*I and *Hha*I enzymes.
*Results:* A total 486 stool samples were examined to the amplification of the 194 bp fragment of B1 gene that 35 samples (7.2%) were positive. All positive samples were confirmed by using another PCR that was amplified the 130 bp repeat element sequence (RE). The identified genotypes were type III (32 cases), mix of type I and III (2 cases) and one of the samples was type I.
*Conclusion:* Our findings revealed a relatively high prevalence of *T. gondii* oocysts in stool samples obtained from cats. It is essential that the high-risk people receive the information about the risk of direct and indirect contact with the animals.


## Highlights

- The present study designated to molecular detection of *T. gondii* oocysts in stool samples of cats that were collected from Ahvaz, southwest of Iran.

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- Identified genotypes were type III (32 cases), mix of type I and III (2 cases) and 1 of the samples was type I.
- Our findings revealed a relatively high prevalence of *T. gondii* Oocysts in stool samples of cats in Ahvaz, southwest of Iran.

## Introduction

*Toxoplasma gondii* is a cosmopolitan coccidian parasite, infecting almost all warm-blooded animals including humans [1,2]. *T. gondii* requires 2 hosts to complete its life cycle that includes the family of cats as the definitive host and vertebrates such as birds and mammals as an intermediate host [3]. In the life cycle of this ubiquitous intracellular parasite, cats can excrete millions of oocysts in the environment as definitive hosts [4–6]. Contamination of soil and water with infected oocysts (survive for a long time in the surrounding environment) have an important role in both animals and humans toxoplasmosis [1,4,7]. Humans can be infected via the ingestion of oocysts from contaminated water, soil, vegetables and fruits, intake of undercooked or raw meat of intermediate hosts such as sheep or birds with tissue cysts and the congenital transmission of tachyzoites from non-immune mother to her fetus [2,8,9].

In healthy people, most infections with *T. gondii* are asymptomatic but in immunocompetent individuals are mainly subclinical. Congenital toxoplasmosis may lead to serious disorders including lymphadenopathy, chorioretinitis, cerebral calcifications, microcephaly, hydrocephalus, and abortion in the fetus. In immunocompromised humans such as HIV positive individuals, the infection can lead to morbidity and mortality [1,10,11]. Symptoms in individuals with healthy immune system are mild and transient that include mild fever and swollen lymph glands, while in individuals with immune system dysfunction and hemodialysis, the latent form of the parasite can be activated and leading to the different clinical complicacies from swelling of lymph, damage to the central nervous system, epilepsy, cerebral calcifications, pneumonia and myocarditis [12]. It is important to be noted that this zoonotic protozoan parasite lead to economic losses since has implications for veterinary and public health worldwide [13–15].

The prevalence of *T. gondii* in Iran had been reported up to 50% that varied in the different parts of the country due to changing the weather conditions and geographical areas as well as toxoplasmosis occurs mostly in tropical and subtropical areas [3]. In addition, one of the risk factors of infection with the parasite is "contact with cat" and "place of residence". Cats shed oocytes by their feces that can survive in environment such as moist soil, warm and humid weather for months and even years [16,17]. Stray cats extensively exist in urban and rural areas of Iran (including farms, gardens and parks) and rate of infection with this obligatory parasite had been shown 32.1–86% [18,19]. *T. gondii* grouped into 3 main clonal lineages including types I, II and III and type 12 that was recently reported. Detection of oocysts in cat feces is an indicator of the risk transmission of toxoplasmosis to humans and other animals. Molecular characterization of *T. gondii* with various genetic markers (such as Surface Antigen two (SAG2) and B1) have important role for identifying the epidemiology, the different isolates, the patterns of transmission and the prevention strategies worldwide [14,15]. The present study designated to molecular detection of *T. gondii* oocysts in stool samples obtained from cats in Ahvaz, southwest of Iran, as well as B1 gene to identify isolates and genotypes in SAG2 locus using Nested-PCR and PCR-RFLP.

## Methods

### Study area

Ahvaz is a city in the center of Khuzestan province in Southwest of Iran. The city is 375 square kilometers and its population has been reported as 1,425,891 until 2006. The city has a desert climate with temperatures above 50 °C that is one of the warmest cities in the world. The average annual rainfall is about 230 mm. According to recent report by the World Health Organization (WHO), Ahvaz has the most polluted climate in the world [20].

### Collection and preparation of samples

A total of 486 stool samples of cats (20–30 g for each sample) were obtained from public places, parks, and children's playground that the specimens were collected between Januarys to May 2012 from 5 points of Ahvaz city (Central, South, North, East, and West), Khuzestan province, southwest of Iran. The regions of the samples are shown in Fig. 1 [21]. The collected samples were transferred to Department of Parasitology in Ahvaz Jundishapur University of Medical science. For evaluating the presence of oocysts in stool of cats, sucrose flotation method was performed as previously described [22].

### Standard reference strains of *T. gondii*

As control standard references three strains were obtained as described previously by Tavalla et al. [18].

### Screening of *T. gondii* oocysts by PCR

DNA was extracted from every sample with the commercial genomic mini kit (A & A Biotechnology, Gdynia, Poland)

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