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Toxoplasma gondii in Cattle, Camels and Sheep in Isfahan and Chaharmahal va Bakhtiary Provinces, Iran

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Background: *Toxoplasma gondii* is a zoonotic parasite, which is assumed to have cosmopolitan distribution.
Objectives: Adopting a cross-sectional study design the current research aimed to determine the occurrence of the parasite in cattle, camels and sheep in Isfahan and Chaharmahal va Bakhtiary provinces of Iran.
Materials and Methods: Animals in the field and those brought for slaughter at abattoirs were included. Blood samples were randomly collected from animals and investigated by polymerase chain reaction (PCR).
Results: *T. gondii* infections were detected in 0.00%, 6.60% and 17.9% of the sample cattle (n = 155), camels (n = 122) and sheep (n = 95) respectively. Sheep were more frequently affected in Chaharmahal va Bakhtiary (33.33%) compared to Isfahan (8.47%) (P = 0.005, 95%; CI = 6.88-43.35). No statistically significant difference was observed in infection prevalence between camels and sheep; and between the different sex categories in both camels and sheep.
Conclusions: Evidence of *T. gondii* occurrence in sheep and camels was provided in the provinces under study. There is a need to investigate the potential risk factors of zoonotic infections. Furthermore, animal health and production losses caused by the parasite; and associated zoonotic implications in the area under study need to be explored.

*Keywords:*Blood; Polymerase Chain Reaction; Ruminants; *Toxoplasma*; Iran

1. Background

Toxoplasma gondii is a multi-host obligate intracellular protozoan parasite, causing zoonotic infections throughout the world. Definitive hosts for this coccidian parasite are felids (both domestic and wild); and the intermediate hosts are mammals and birds (1, 2). The felids disseminate oocysts into the environment, where they can infect all types of warm-blooded animals (wildlife, companion animals, domestic livestock), including humans. The intermediate hosts can be infected by ingesting food or water contaminated with oocysts, eating undercooked meat with tissue cysts or by transplacental infection with tachyzoites (2, 3).

Humans acquire toxoplasmosis mainly through ingestion of tissue cysts present in undercooked meat or by accidentally ingesting oocyst (4). Infections involving immunocompetent humans usually result in asymptomatic cases, although cervical lymphadenopathy or ocular diseases occur in some of them (up to 10%). Occurrence of the parasite in immunocom-promised hosts and infants can however be an important cause of morbidity and mortality, causing serious disease symptoms (5, 6). In immunodeficient individuals such as HIV patients, the parasites can cause severe toxoplasmic encephalitis (7, 8). Infections in infants can cause mental retardation, loss of vision and jaundice.

In Iran about 50% of the human population has been exposed to *T. gondii*, which makes toxoplasmosis one of the major public health problems (9). Contact and interaction between domesticated animals and humans are known to be responsible for an increased risk of transmission of the parasite (10).

2. Objectives

There is a need for regular updates on the prevalence rates of this zoonotic pathogen in different animal species in order to provide useful epidemiological data to plan control strategies and eventually stem the consequences associated with the pathogens. The current study aimed to determine the presence of *T. gondii* in the blood samples of cattle, camels and sheep in Isfahan

Implication for health policy/practice/research/medical education:
There is a need to investigate the potential risk factors of zoonotic infections. Furthermore, animal health and production losses caused by the parasite; and associated zoonotic implications in the study area need to be explored.

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and Chaharmahal va Bakhtiary provinces by Polymerase Chain Reaction (PCR) method. The obtained information through this sensitive technique would add value to the available data most of which rely on serology.

3. Materials and Methods

3.1. Study Area

The present study was conducted in Isfahan and Chaharmahal va Bakhtiary provinces, Central and South-West of Iran respectively. Livestock production in Isfahan province is mostly traditional whereas in Chaharmahal va Bakhtiary province commercial livestock production is common. Samples were collected from randomly selected cattle, camels and sheep from slaughterhouses and peripheral farms.

3.2. Study Design, Sample Size Determination and Animal Sampling

It was a cross-sectional study conducted from January to March 2013. Blood samples were collected from the jugular veins of 155, 122 and 95 randomly selected cattle, camels and sheep, respectively. Respective numbers of samples from the abattoirs and farms were 100 and 55 for cattle, 90 and 32 for camels; and 65 and 30 for sheep, respectively. Five milliliters of blood was collected from each animal, stored in heparinized vacutainer tubes and conveyed on ice to the laboratory for analysis.

3.3. DNA Extraction

Genomic DNA was extracted from blood specimens using CinnaGen DNA extraction kit (Cinnagen, Tehran, Iran) according to the manufacturer's instructions. The extracted DNA was quantified by spectrophotometric measurement at a wavelength of 260 nm according to the method described by Sambrook and Russell (11). Extracted DNA samples were stored frozen at -20°C until used for molecular analysis using PCR at the Biotechnology Research Center of the Islamic Azad University of Shahrekord.

3.4. Gene Amplification

The PCR was performed on 50 μL total reaction volume including 5 μL of 10x PCR buffer [70 mM Tris-HCl (pH 8.8), 200 mM $(NH_4) So_4$, 0.1% Tween 20], 2 mM $MgCl_2$, 250 μM of each of the four deoxynucleotide triphosphate, 1.25 U Taq DNA polymerase (Fermentas, city, Country), 50 pmol of each Species-specific oligonucleotide primers sized 171 base pares (Toxo-F: 5'-CATTGGAGAGATTTGCATTC-3') and (Toxo-R: 5'-ATCAGTATCCCAACAGAGACAC-3') (Cinnagen, Tehran, Iran) designed from 18S ribosomal RNA gene of *Toxoplasma* (Accession number: JQ235842) and 5 μL of the extracted template DNA. Amplification of parasite DNA was done in (Eppendorf, Hamburg, Germany). DNA amplification was performed in a thermocycler apparatus for 33 cycles as follows: primary denaturation of the samples was performed at 94°C for 7 minutes, denaturation at 94°C for 1 minute, annealing at 56°C for 1 minute, extension at 72°C for 1 minute and final extension at 72°C for 5 minutes.

3.5. Analysis of PCR Products

Distilled water served as the negative control. Polymerase chain reaction products were run using 1.5% agarose gel in 1X TBE buffer at 80 V for 30 minutes, stained with ethidium bromide and the images were visualized in UVIdoc gel documentation systems (Uvitec, UK). The PCR products (171bp) were identified by 100 bp DNA size marker (Fermentas, Germany) (Figure 1).

3.6. Statistical Analysis

The obtained data were analyzed using SPSS (Statistical Package for the Social Sciences) software (Version 17. SPSS Inc, USA). Descriptive statistics were used to determine proportions of positive animals for *T. gondii*. The differences in proportions between animal species, different sexes and locations were determined by the Chi square test at $P \leq 0.05$.

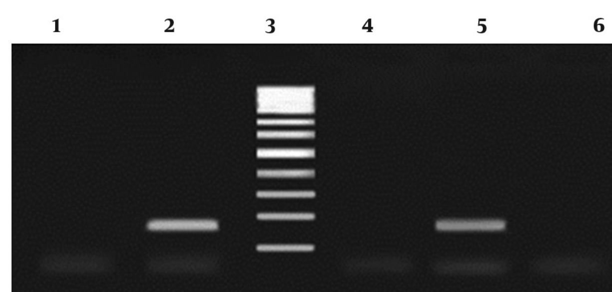
4. Results

In the present study a total of 155 cattle, 122 camels and 95 sheep were selected as samples from the two provinces.

Table 1. PCR Test Results For Toxoplasmosis in Camels and Sheep in Isfahan and Chaharmahal Va Bakhtiary Provinces, Iran

Attribute	Prevalence of *T. gondii*	
	Camel	Sheep
Province		
Chaharmahal va Bakhtiary	-	33.33% (12/36)
Isfahan	6.60% (8/122)	8.47% (5/59)
Sex		
Female	5.17% (3/58)	19.72% (14/71)
Male	6.76% (5/74)	12.5% (3/24)

Figure 1. Ethidium Bromide-Stained Agarose Gel Electrophoresis of PCR Products (171 bp) to detect *Toxoplasma gondii* in Cattle, Camel and Sheep Blood



Lane 1: Negative control; lanes 2 and 5: positive samples (171 bp); lane 3: 100 bp DNA ladder; and lanes 4 and 6: negative samples.

No evidence of contamination with *T. gondii* was detected in the sample cattle using PCR method. The overall prevalence of *T. gondii* in camels was 6.6% while the overall prevalence of the parasite in sheep was 17.9%. The distribution of *T. gondii* in camels and sheep in the two provinces is presented in Table 1. Table 1 also displays the infections in the different sex categories. No statistically significant differences were observed between camels and sheep, male and female camels, and male and female sheep. In sheep, however, the prevalence of *T. gondii* was significantly higher in Chaharmahal VA Bakhtiary (33.33%) compared to Isfahan (8.47%) (P = 0.005, 95% CI 6.88-43.35).

5. Discussion

The current study recorded a 0.00% prevalence of *T. gondii* infection in cattle (n = 155). This finding is consistent with previous reports in the country in which no evidence of *T. gondii* infection was noted in cattle (12, 13). Several other studies have recorded lower infection levels of *T. gondii* in these animal species (14-22). The obtained results, showing no or lower *T. gondii* infections in cattle, are in support of the hypothesis that cattle are not a favoured host for *T. gondii,* and that human infection is most likely associated with consumption of pig, lamb and goat meat (13, 20, 23-25). However, other studies (26-28) have obtained high levels of *T. gondii* infection among cattle thereby contradicting the hypothesis. Variations in the levels of infection with *T. gondii* among cattle in the different studies could probably reflect differences in exposure rates to the parasite, which is attributable to the contamination rate of the environment.

According to Dubey and Thulliez (29), cattle have a high natural resistance to *T. gondii* and infection in them does not usually cause clinical symptoms. *T. gondii* infections in sheep and goats are known to induce abortions, pre-term deliveries, weak newborns and neonatal mortality (30-33). This frustrates scientific endeavours of geneticists, nutritionists and livestock breeders working for the propagation of small stock. It is a drain of breeding animals and thus heightens the gap of animal proteins between an increasing human population. In the current study the prevalence of *T. gondii* infection in the sheep samples was at 17.90%. *T. gondii* infections in sheep have been also observed in a number of investigations worldwide (12, 14-16, 18, 20-22, 26-28, 34-37). Considering reproductive losses caused by the parasite, these results imply substantial economic losses to the sheep raising industry worldwide (38).

Among the sample camels (n = 122) 6.60% were infected with *T. gondii*. A more or less similar prevalence of the parasite in camels (4.2%) was obtained in a previous investigation conducted in the country (39). High levels of infection with *T. gondii* among camels have been found in Saudi Arabia (40), Sudan (41) and Egypt (42). It is noteworthy that in the current study no association was found between the prevalence of *T. gondii* and sex, both in camels and sheep. A similar observation was made in a study conducted in Nigeria (43). However, studies in Ghana (44), Pakistan (45), Brazil (46) and China (47) revealed higher prevalence of *T. gondii* in female than in male sheep.

Some authors have indicated that female animals are more susceptible to infections with protozoan parasites than males (48). According to Kittas et al. (49) innate immune responses are enhanced in males. A significantly higher prevalence of *T. gondii* was recorded in the sheep raised in Chaharmahal va Bakhtiary than the ones raised in Isfahan. This is in accordance with previous findings which also identified geographical differences in *T. gondii* infections among animals (18, 50-52). This observation could be an attribute of differences in levels of environmental contamination. Investigations elsewhere have attributed human population density to geographical differences in the prevalence of protozoan parasites among animals (53).

Overall, the results of the present study confirm that *T. gondii* is prevalent; and that the infection is widely distributed in camels and sheep in the two provinces. There is a need to investigate the potential risk factors for infection of humans with the zoonotic parasites. Zoonotic implication of the parasite associated animal health and production losses in the area under study still need to be explored.

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Authors' Contributions

All authors had an equal role in the design, work, statistical analysis and manuscript writing.

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The authors declare that they have no conflict of interest regarding the study design, study area and study findings.

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