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Use of PCR to determine *Toxoplasma gondii* in milk samples from camels (*Camelus dromedarius*), cattle (*Bos taurus*) and buffalos (*Bubalus bubalis*) in East Azarbaijan province, Iran

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Abstract

Background: Toxoplasmosis as a zoonotic condition is developed by an intracellular protozoan parasite *Toxoplasma gondii* from the Apicomplexa phylum, which imposes economic losses on herds of animals and severe complications in immunocompromised people and pregnant women. This infectious disease can be transmitted to human beings from the contaminated unpasteurized milk, uncooked meat, water and food contaminated with sporulated oocysts and transplacental transmission.

Objectives: This study amid to determine *T. gondii* DNA in camel, buffalo and cow milks in using the PCR method based on the B1 gene.

Methods: A total of 100 milk samples, including 55 cows, 30 buffalos and 15 camels, were collected from different regions of north-western using direct milking and then transferred to the Food and Aquatic Health Laboratory under refrigerated conditions.

Results: The results showed that out of 100 milk samples examined, 5 samples (5%) were contaminated, and *T. gondii* DNA was detected in the milk samples of 2 (3.63%) cows, 1 (3.33%) buffalos and 2 (13.33%) camels, respectively.

Conclusions: Our findings reveal that raw milk contaminated with *T. gondii* can be an important route of transmission of infection for human beings.

KEYWORDS
buffalo, cow, camel, milk, *Toxoplasma gondii*

1 | INTRODUCTION

Milk of herbivores, such as cattle, buffalo and camels, contains high amounts of proteins, minerals and vitamins essential for the growth of organisms. Higher levels of cholesterol are present in buffalo milk than in camel or cow milks, and higher levels of protein and lipid are found in cow milk than in human milk (Boughattas, 2017). The levels of vitamin C and iron in camel milk are 3 and 10 times higher than in cow milk, respectively. Consumption of camel milk can manage the type 1 diabetes owing to its insulin-like molecules, boost the cell-mediated immune response owing to great doses of lactoferrin with antimicrobial potential and alleviate the hypersensitivity in children (Boughattas, 2017; López-Soto et al., 2010). Based on statistical reports in 2012, the mean per capita intake of milk in Iranian urban and rural areas was 29.13 and 46.37 kg, respectively (Shokrvash et al., 2015). According to results of a study conducted on seventh-grade students (mean age was 12.9 years), in Tabriz, East Azerbaijan province, Iran, the average daily intake of milk and dairy products was 1.64 servings per day

(Shokrvash et al., 2015). However, the average consumption of milk among households in Tabriz is 15.34 L per month (Dashti et al., 2015). So far, no study has been conducted on the amount of milk consumed from different animals by households in Tabriz, but the evidence shows that people prefer camel, buffalo and cow milks to sheep and goat milks. Proponents of raw milk, especially nomads and local residents, claim that unpasteurized or unheated milk is more nutritive than pasteurized or heated dairy, so nearly 50% of people consume milk obtained from cow, buffalo and, especially, camel milks in raw and heated forms (Boughattas, 2017). It is supposed that any milk consumed raw is a potential *Toxoplasma gondii* infection route. Toxoplasmosis as a common zoonotic condition among humans, ruminants, birds and some other warm-blooded mammals as intermediate host and felids including cats as definitive host is developed by *T. gondii*, an obligatory intracellular protozoan parasite from the Apicomplexa phylum with cosmopolitan distribution (Dubey & Beattie, 1988). *T. gondii* infection is a leading reason for abortion and imposes enormous economic losses on breeders of ruminants, such as sheep, goats, cattle, buffaloes and camels (Sharif et al., 2015). One of the major consequences of pregnant women becoming infected by *T. gondii* is vertical transmission to the foetus that can cause severe neurological or ocular diseases (leading to blindness), as well as cardiac and cerebral anomalies in the newborn. The infection at the second or third gestational trimester can result in Sabin's tetrad, with microcephaly, retinochoroiditis, cerebral calcifications and mental disorder (Havelaar et al., 2007; Sakamoto et al., 2018). Besides its vertical transmission through *T. gondii* tachyzoites passed from placenta to foetus, a horizontal transmission can occur by taking drinking water, meat, milk, fruits or vegetables contaminated with bradyzoites or oocytes released from cats (Tavassoli et al., 2013). The parasite is detectable in the host milk samples, such as those from sheep, goats, cows, buffaloes and camels (Ahmed et al., 2014; Amroabadi, 2021; Camossi et al., 2011; Costa et al., 2020; Dehkordi et al., 2013; Fusco et al., 2007; Iacobucci et al., 2019; Rocha et al., 2015; Silva et al., 2015; Saad et al., 2018; Sadek et al., 2015; Tavassoli et al., 2013). Based on seroepidemiological findings, *T. gondii* infection is significantly associated with drinking raw cow milk (Alvarado-Esquivel et al., 2013; Silva et al., 2014). High *T. gondii* seropositivity in camels and buffaloes of China, Sudan, Iran and Egypt reveals a public health problem for nomads consuming raw milk of buffalos or camels (Hamidinejat et al., 2013, 2010; Sadrebazzaz et al., 2006; Wang et al., 2013). This study aimed to determine *T. gondii* DNA in camel, buffalo and cow milks in East Azarbaijan province, Iran based on the PCR method using *B1* gene.

2 | MATERIALS AND METHODS

2.1 | Sampling

One hundred animals, including 55 cows (*Bos taurus*), 30 buffaloes (*Bubalus bubalis*) and 15 camels (*Camelus dromedarius*), were chosen from different parts of randomly between April and November 2019, using the following formula, assuming a prevalence of 5%–10% of *T. gondii* in animal milk based on previous studies, as well as a 96% confidence interval and an accuracy of 5%.

$$n = \frac{z^2_{1-\alpha/2} \times p(1-p)}{d^2}$$

where P is the prevalence, α is the error rate and d is the accuracy.

Milk samples (200 ml of each animal) were collected by manually milking previously iodine alcohol-disinfected teats using gloves, which were refrigerated in sterile micro-tubes and sent to the laboratory of Parasitology, Faculty of Veterinary Medicine, University of Tabriz, for PCR assay.

2.2 | DNA extraction

The collected milk samples (50 ml) were concentrated via centrifugation for 5 min at 2500 g (Murphy et al., 2002). The obtained sediment (1 ml) was dispersed in 200 μl of TE (consisting of EDTA (1 mM) and Tris–HCl (10 mM) with pH value of 7.6) and 300 μl of 0.5 M EDTA (with a pH value of 8), followed by centrifugation for 10 min at 3000 g to eliminate interference with casein (Psifidi et al., 2010). Milk pellet dilution was performed in PBS (200 μl), and DNA extraction was done by a DNA extraction kit () based on the manufacturer's protocol. The DNA qualities were checked by electrophoresis on the 1% agarose gel.

2.3 | PCR Amplification

The *T. gondii* B1 gene was amplified via species-specific sensitive primers using a 529-bp fragment (Homan et al., 2000; Tavassoli et al., 2013). The primer sequences were TOX4 (CGCTGCAGGGAGGAAGACGAAAGTTG) and TOX5 (CGCTGCAGACACAGTGCATCTGGATT), PCR buffer, 2 mM $MgCl_2$, 250 μM of each four deoxynucleotide triphosphates, 1.25 U of Taq DNA polymerase (Fermentas, Germany), 5 μl of extracted DNA and 50 pmol of each primer. Dr. from, presented a positive control of *T. gondii*. Negative control was considered to be sterile water. Cycling profile was set at 94°C for 7 min and then 33 cycles set at 1 min at 94°C, 1 min at 55°C and 1 min at 72°C with a final step at 72°C for 10 min. The DNA samples (5 μl) were applied as the template. A 2% agarose gel electrophoresis was utilized to analyse the PCR products, followed by DNA-safe stain (Yekta Tajhiz Azma, Iran; Cat no: YT0001) staining. The gel was photographed under a gel Documentation system (Axygen Gel Documentation systems, German). The results have been analysed using SPSS statistical software version 21 and chi-square test.

3 | RESULTS

Out of 100 milk samples from different animals, the infection of 5 samples (5%) was performed by *T. gondii*. The results demonstrated that out of 55 specimens of cow milk, 2 samples (3.63%), out of 30 samples of buffalo milk, 1 sample (3.33%) and out of 15 samples of camel milk,

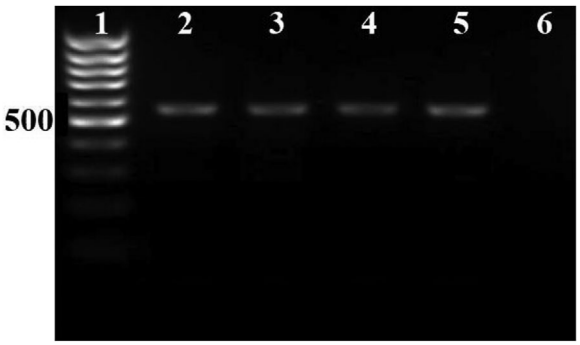

FIGURE 1 Products amplified with PCR via primers specific to *T. gondii*: lane 1, 100 bp Ladder (Fermentas, Germany); lane 2, positive control; lane 6, negative control, lanes 3–5, cow, buffalo and camel milk samples

2 samples (13.33%) were infected with *T. gondii* (Figure 1). Statistical analysis showed that the infection rate among different animals was not significant ($p < 0.05$).

4 | DISCUSSION

Cattle, buffaloes and camels are valuable assets in most of the countries economically in terms of milk and meat production. The unpasteurized milk of such animals is an infection source for human beings (Alimi et al., 2016; Boughattas, 2017; Dehkordi et al., 2013; Gebremedhin et al., 2014; Saad et al., 2018). Our results showed that cow, buffalo and camel milks can carry *T. gondii* tachyzoites. However, the camel milk had the greatest incidence rate of *T. gondii*, but cattle and buffaloes can also expel tachyzoites from their milk. Lower rate of infection in cows and buffalos when comparing with camels may be due to differences in susceptibility to *T. gondii* and the animal feed habits (Tavassoli et al., 2013). We found *T. gondii* DNA in 13.33%, 3.63% and 3.33% milk samples from camels, cows and buffalos, respectively. In a study by Dehkordi et al. (2013), 160 camel milk specimens exhibited a 2.5% by PCR. The conducted study by Wang et al. (2013) showed that the *T. gondii* seropositivity in Bactrian camels was variable between 2.13% and 3.57% (Wang et al., 2013). Medani and Mohamed (2016) confirmed the *T. gondii* tachyzoite in camel milk by ELISA test (Medani & Mohamed, 2016). The high contamination rate of camel milk with *T. gondii* in our studies compared to other studies may be due to the geographical location of the study area. These weather conditions affect the infecting of oocytes and also the extent of their contact with cat faeces. In a recent study, the contamination rate of the cow milk with *T. gondii* was 3.63% nearly similar to the studies of others (Dehkordi et al., 2013). They determined *T. gondii* in bovine milk using PCR in 3.5% of cases (Dehkordi et al., 2013). The *T. gondii* risk is less important with drinking cow milk as they are resistant to *T. gondii*. It was recently indicated that 14.1% of seropositive Brazilian pregnant women used to consume untreated goat or cow milk (Moura et al., 2013). Numerous reports in Brazil indicate a significant correlation of *T. gondii* infection with raw cow milk consumption.

Like cattle, buffaloes are toxoplasmic infection resistant. Recently, 87.79% of buffaloes were reported to have anti-*T. gondii* antibodies in Turkey (Beyhan et al., 2014). In a study by Dehkordi et al. (2013), the buffalo milk contamination with *T. gondii* had a rate of 3.65% by PCR which was consistent with our studies (Dehkordi et al., 2013). The overall incidence rate of *T. gondii* infection in buffaloes from Ahvaz, Khouzestan province, south-west of Iran was 14.33% by ELISA (Hamidinejat et al., 2010). Bărburaș et al. (2019) determined the rate of *T. gondii* infection with a prevalence of 2.7% in autochthonous Carpathian buffaloes, in north-western Romania (Bărburaș et al., 2019) which was somewhat in-line with our results. Serum samples from water buffaloes ($n = 104$) were examined for the presence of anti-*T. gondii* antibodies using a latex agglutination. Antibodies to *T. gondii* were found in 3.85% of water buffaloes, and the results of our study also showed that 3.33% of buffalo milk samples were contaminated. However, seropositive results of *T. gondii* in the studied buffaloes from different regions were higher than the amount of contamination that we reported in milk samples (Almería et al., 2007; Alvarado-Esquivel et al., 2014; Ciuca et al., 2020; de F Santos et al., 2013; Huong et al., 1998; Pita Gondim et al., 1999).

5 | CONCLUSION

According to the results attained from the current work, the *T. gondii* DNA was present in the cattle, buffalo and camel milk samples. Because cattle, buffalos and camels are the most critical sources of milk for human use in Iran, a high risk of parasitic contamination can occur via milk because of the high susceptibility of such livestock to the infection. So, health authorities must provide guidance to milk consumers in relation to boiling or pasteurization, which eliminate the risk of transmission of this parasite.

AUTHOR CONTRIBUTIONS

Data curation and methodology: Somayyeh Asiyabi Aghdam. *Conceptualization, investigation, methodology, supervision, writing–original draft, review, and editing*: Nasser Hajipour. *Conceptualization and supervision*: Mir-Hassan Moosavy.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

ETHICS STATEMENT

This study was approved by the University of Tabriz Ethical Committee.

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PEER REVIEW

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