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Detection of *Toxoplasma gondii* infection in buffaloes (*Bubalus bubalis*) and cattle (*Bos taurus*) at the Tabriz abattoir, Iran

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Abstract

Background: *Toxoplasma gondii* is a widely prevalent zoonotic protozoan parasite in humans and warm-blooded animals worldwide. Infection of humans by this parasite can result in severe clinical symptoms, particularly in individuals with congenital toxoplasmosis or immunocompromised patients. Contamination mainly occurs through foodborne routes, especially the consumption of raw or undercooked meat from animals.

Objectives: The aim of this study was to use PCR to detect *T. gondii* in tissues and organs of buffaloes and cattle slaughtered at Tabriz slaughterhouse, in Iran.

Methods: Fifty grams of heart, thigh, diaphragm and tongue from 50 buffaloes and 100 cattle slaughtered at the Tabriz industrial slaughterhouse were selected for sampling using a combination of convenience sampling. The samples were tested using a previously published PCR method.

Results: Out of the 150 animal samples, *T. gondii* was detected in 10 (6.7%, 95%CI: 3.2–11.9), including one buffalo (2%, 95%CI: 0.1–10.6) and nine cattle (9%, 95%CI: 4.2–16.4). There was no statistically significant difference in the rate of *T. gondii* infection among cattle based on age and sex ($p > 0.05$).

Conclusions: The results indicated a potential risk of *T. gondii* transmission to humans through the consumption of infected meat. Therefore, appropriate and effective preventive measures should be taken to limit the transmission of this parasite to humans, and the consumption of raw and undercooked meat should be discouraged.

KEYWORDS
buffalo, cattle, meat, PCR, *Toxoplasma gondii*

1 | INTRODUCTION

Foodborne diseases encompass a wide range of acute and chronic syndromes that vary in severity of infection, clinical symptoms and prevalence (Gérard et al., 2019). According to the World Health Organization (WHO), approximately 600 million foodborne illnesses occurred globally in 2010, caused by 31 bacterial, viral, parasitic and chemical hazards, with parasites accounting for 15% of the cases (World Health Organization [WHO], 2015). *Toxoplasma gondii*, a zoonotic parasite, is among these foodborne pathogens and has a worldwide distribution, infecting humans, ruminants and other warm-blooded animal species (Gérard et al., 2019; Soulsby, 1982). The final host of this protozoan is cats, and its intermediate hosts are warm-blooded animals (e.g. rodents) (Lyons et al., 2002). Humans become infected by ingesting uncooked or under cooked meat containing tissue cysts, by consuming raw milk contaminated with tachyzoites or ingestion

of soil or water contaminated with oocysts (Dubey, 2008; Weiss & Dubey, 2009).

It has been estimated that up to one third of the human population worldwide is infected with *T. gondii* (Daryani et al., 2014; Havelaar et al., 2007). The clinical signs of toxoplasmosis in humans include abortion, stillbirth, foetal death in utero or severe central nervous system involvement in newborns, such as cerebral calcifications and hydrocephalus (Havelaar et al., 2007; Tenter et al., 2000). In addition to the effects in pregnant women, toxoplasmosis can cause acute disease in immunocompromised individuals. In livestock, *T. gondii* is a major cause of abortion, with significant economic losses for sheep, goat, cattle, buffalo and camel breeders (Sharif et al., 2007, 2015).

Many studies have assessed the prevalence of *T. gondii* in the meat of different animals, with the importance of a particular animal as a source of infection to humans dependent on locality and culture (Azizi et al., 2014; Cong et al., 2018; Ergin et al., 2009; Guo et al., 2015; Laura et al., 2013; Leong et al., 2023; Medeiros et al., 2014; Rahdar et al., 2012a; Santos et al., 2013; Slany et al., 2016; Sroka et al., 2018, 2017; Wang et al., 2013). Studies show that although mutton, goat and pork infected with *T. gondii* play a major role in causing infection in humans, contamination of beef and buffalo meat is also a way of transmitting the infection and can be considered a serious risk to humans. In Iran, few studies have been conducted on the contamination of beef and buffalo meat with *T. gondii* (Azizi et al., 2014; Hamzavi et al., 2007; Mahami-Oskouei et al., 2017; Rahdar et al., 2012a). The aim of this study was to use PCR to detect *T. gondii* in buffaloes (*Bubalus bubalis*) and cattle (*Bos taurus*) slaughtered at the Tabriz abattoir, Iran.

2 | MATERIALS AND METHODS

2.1 | Sampling

This cross-sectional study was conducted from April to September 2020. The sample size for the study was calculated using the following formula, assuming an expected prevalence of 25% and 15% of *T. gondii* in buffalo and cattle meat, respectively, based on previous studies (Alipour Amroabadi et al., 2020), with a desired absolute confidence level of 95% and a percent error of 5% (Motamed & Zamani, 2016). Based on these parameters, it was estimated that a total of at least 47 buffaloes and 97 cattle needed to be sampled:

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

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

Animals selected for sampling were chosen based on convenience. The abattoir was visited on at least 8 different days per month, based on the number of animal slaughters. A random number generator (Microsoft Excel 2016) was then used to determine which animals to test. In the case of the cattle, it was estimated that 500 animals would be slaughtered over the days visited. The first 100 of the 500 numbers generated were then the target animals to sample. If an owner did not give permission, then the next number was used (unless already passed) until 100 animals were sampled. Before slaughter, the sex and age (<2 years old or ≥2 years old, based on dentition) were recorded for each animal. Fifty grams of heart, thigh, diaphragm and tongue were sampled from each animal, based on the availability of organs for sampling and the permission of the animal owner and sent, on ice, to the Food Hygiene Laboratory of Tabriz Veterinary School where they were stored at −20°C until analysis.

2.2 | DNA extraction

DNA was extracted using a commercial kit (MBST, Rapid DNA Isolation Kit from Meat), according to the manufacturer's instructions (Cinna Gen). Briefly, a piece of meat (50 mg) was homogenized with 300 µL lysis buffer (1 mM of EDTA, pH = 8.0; 50 mL of Tris–HCl, pH = 7.6; 1% of Tween 20). A volume of 20 µL proteinase k was added, mixed by vortexing and then incubated at 37°C for 24 h. Then 580 µL binding buffer was added, and the sample incubated for 10 min at 70°C, centrifuged for 1 min at 8000 × *g*, and the supernatant was transferred into a clean 1.5 mL Eppendorf tube with 440 µL ethanol (100%). Half of the mixture was placed into a spin column ^{MBST} and centrifuged at 8000 × *g* for 1 min. The filtrate was removed, and the remainder was centrifuged again for 1 min at 8000 × *g*. After placing the spin column ^{MBST} in a new Eppendorf tube, 500 µL of wash buffer (26 mL of ethanol 96%–100% was added to wash buffer before using) was added, centrifuged at 8000 × *g* for 1 min with the infiltrate discarded. This stage was repeated with 500 µL wash buffer at 20,000 × *g* for 2 min. After discarding the tube containing the filtrate and placing the spin column ^{MBST} in a clean Eppendorf tube, 100 µL elution buffer preheated to 70°C was added, incubated at room temperature for 30 min and then centrifuged at 8000 × *g* for 1 min. After repeating this step, the filtrate containing the tube was collected and stored at −20°C for analysis. DNA quality was checked by electrophoresis on the 1% agarose gel.

2.3 | PCR amplification

Amplification of the B1 gene of *T. gondii* was performed using previously reported species-specific primers to amplify a 529 bp fragment (Homan et al., 2000; Tavassoli et al., 2013). The sequences of primers were as follows: TOX4 (CGCTGCAGGGA GGAAGACGAAAGTTG)/TOX5 (CGCTGCAGACACAGTGCATCTGGATT). PCR buffer, 2 mM $MgCl_2$, 250 µM of each of the four deoxynucleotide triphosphates, 1.25 U Taq DNA polymerase (Fermentas), 50 pmol of each primer, and 5 µL of the extracted DNA (Homan et al., 2000). The positive control for *T. gondii* was kindly provided by Dr. Ehsan Ahmadpour from Tabriz University of Medical Sciences, Iran. Sterile water served as the negative control. The cycling conditions were 94°C for 7 min, followed by 33 cycles of 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. Five microliters of each DNA sample were used as the template. PCR products were analysed by 2% agarose gel electrophoresis followed by staining with DNA-safe stain (Yekta Tajhiz Azma; Cat no: YT0001). The gel was photographed under a Gel Documentation system (Axygen Gel Documentation systems).

TABLE 1 Detection of *Toxoplasma gondii* in cattle and buffaloes slaughtered at Tabriz slaughterhouse based on age, sex and organ.

			Age (yeas)		Sex		Organ			
Animal	Variation		≤2	>2	Male	F	Heart	D	To	Th
Buffalo ($n = 50$)	Examined no.	50	10	40	38	12	15	10	10	25
	Infected no. (%)	1 (2)	0	1 (2.5)	1 (2.6)	0	0	1 (10)	0	0
Cattle ($n = 100$)	Examined no.	100	55	45	65	35	10	45	10	35
	Infected no. (%)	9 (9)	3 (5.4)	6 (13.3)	7 (10.7)	2 (5.7)	1 (20)	7 (15.5)	0	1 (2.8)
	p-Value		0.292[a]		0.488[a]					

Abbreviations: D, diaphragm; F, female; Th, thigh; To, tongue.
[a]Fisher's exact test.

2.4 | Data analysis

Statistical analysis was performed using SPSS software version 21.0. The descriptive statistics are described by frequency and percentage. Difference in *T. gondii* positive animals by sex and age were evaluated using Fisher's exact test due to low numbers in some categories. No assessment of differences in organs infected was performed due to the low numbers of positive samples within each category, with several categories having no positive samples. A *p*-value less than 0.05 was considered statistically significant.

3 | RESULTS

Of the 150 animals studied (50 buffaloes and 100 cattle), the meat of 10 animals (6.7%, CI: 3.2–11.9) was infected with *T. gondii* (Table 1). The results showed that nine (9%, CI: 4.2–16.4) cattle and one (2%, CI: 0.1–10.6) buffalo meat samples were infected (Figure 1). Of 40 buffalo over 2 years old, the meat of one (2.5%, 95%CI: 0.1–13.2) was contaminated with *T. gondii*, and no positive cases were observed in buffalo less than 2 years old, although the tested number in this age group was low. Meat contamination with *T. gondii* also was higher in cattle >2 years old (13.3%, 95%CI: 5.1–26.8) than those <2 years old (5.5%: 95%CI: 01.1–15.1). There was no statistically significant relationship between *T. gondii* infection and animal age or sex. In both buffalo and cattle, *T. gondii* was detected most often in the diaphragm.

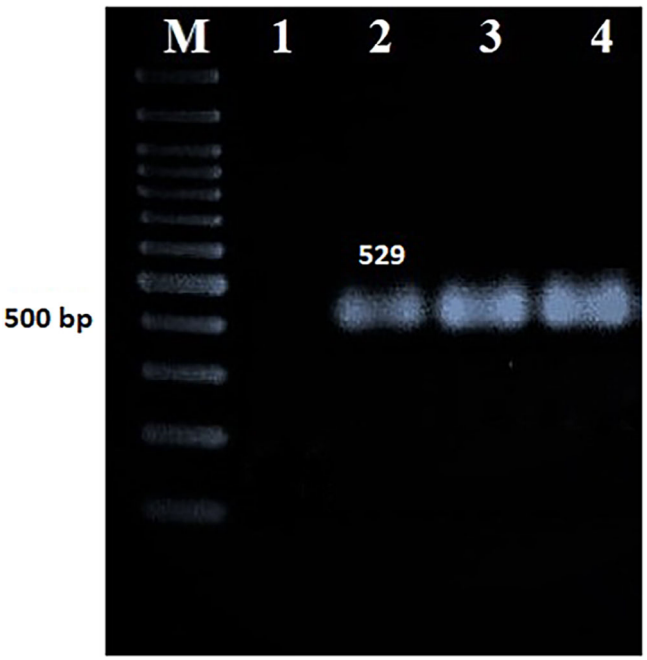

FIGURE 1 PCR-amplified products using *Toxoplasma gondii*-specific primers (fragment of 529 bp): M, 100 bp ladder (Fermentas); lane 1: negative control, lane 2: meat of cattle, lane 3: meat of buffalo, lane 4: positive control, lane.

4 | DISCUSSION

In the study presented herein, *T. gondii* was detected less frequently in buffalo meat (2%) compared to cattle meat (9%). The lower detection rate in buffalo compared to cattle may be attributed to differences in susceptibility to *T. gondii* and the feeding habits of the animals (Tavassoli et al., 2013). The higher rate in cattle vs. buffalo is in agreement with a study by Huong et al. (1998) who found *T. gondii* antibodies in 10.5% of the cattle sera, and 3% of the water buffalo sera tested in southern Vietnam. They stated that the differences may be due to management conditions or species susceptibility. Water buffalo in Vietnam is, in general, raised more extensively than dairy cattle, being out on pastures most of the time and thus less likely to be exposed to contaminants that may be present in feedstuffs (Huong et al., 1998).

In contrast, in studies conducted in Brazil, more buffaloes were found to be for *T. gondii* antibodies than cattle, with 27.2% of the buffaloes and 17.4% of the cattle testing positive (Laura et al., 2013). The overall high level of positive animals observed in the study by Laura et al. (2013) could potentially be explained by an increase in the number of abandoned cats in the vicinity. However, no explanation about the differences between buffaloes and cattle was provided, although the inclusion of older buffaloes in the study could provide some explanation for the higher prevalence in buffaloes.

In regards cattle, other studies have found prevalence rates of 21.1%, 8.57%, 16% and 4% (Ghaffari & Dalimi, 2019; Mahami-Oskouei et al., 2017; Rahdar et al., 2012b) in Iran, and as low as 0% in Scotland (Plaza et al., 2020). The findings of the study presented herein are

within the range found in other studies. Moreover, the number of cattle in which *T. gondii* was detected increased with age, which was consistent with the studies conducted by Azizi et al. (2014). Furthermore, the rate of infection of different tissue samples is in agreement with other studies (Azizi et al., 2014; Ergin et al., 2009).

Toxoplasmosis is considered one of the most important food-borne zoonotic parasites (Bigna et al., 2020; Milne et al., 2020; Truppel et al., 2010). Meta-analyses and reviews have shown a wide variation in the rate of meat contamination in animals, with results related to animal species, geographical location, type of meat assessed, detection test used and the sample size (Azizi et al., 2014; Hajimohammadi et al., 2022). Comparisons between studies and regions must be made with caution given these differences, and regional studies, such as the one presented herein, are needed to understand the risk for the human population consuming the meat. In the study presented here, PCR was used which has been shown to detect infection earlier than serum-based antibody tests and can be more sensitive than antibody and antigen tests. However, if no cysts are present in the tissue sample analysed, PCR can result in underestimating the prevalence (Liu et al., 2015; Yousefvand et al., 2021). Therefore, although the detection of *T. gondii* in buffalo and cattle slaughtered in the Tabriz slaughterhouse was low compared to the studies conducted in other parts of Iran (Ghaffari & Dalimi, 2019; Hamidinejat et al., 2010; Mahami-Oskouei et al., 2017), it could be an underestimate and still pose a risk for consumers. Although the number of positive samples found was lower in the buffalo, they still serve as a potential source of human infection with appropriate and effective preventive measures are needed to prevent the transmission of this parasite from buffalo and cattle to humans. Within the region, consuming raw and undercooked meat should be discouraged, and methods for the preparation of meat and meat products that kill tissue cysts should be encouraged.

4.1 | Limitations of the study

This study has limitations that need to be taken into consideration. For instance, the samples were taken from different organs of buffaloes and cattle because some livestock owners did not permit sampling of all organs. This leads to some potential bias, especially in the comparison between the livestock species. Additionally, the animals presented at the abattoir might not be representative of all cattle and buffaloes slaughtered in the region.

AUTHOR CONTRIBUTIONS

Javad Jabbari: Investigation; methodology. **Nasser Hajipour**: Conceptualization; investigation; methodology; supervision; visualization; writing – original draft. **Parviz Hassanzadeh**: Conceptualization; methodology. **Jennifer Ketzis**: Formal analysis; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest relevant to this study.

DATA AVAILABILITY STATEMENT

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

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PEER REVIEW

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ETHICS STATEMENT

This study was approved by the University of Tabriz Ethical Committee (Number Ethical: 2612/43).

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