Prevalence of Toxoplasma gondii in buffaloes (Bubalus bubalis) and cows (Bos taurus) at the Tabriz abattoir, Iran

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Abstract

*Toxoplasma gondii* is a widely prevalent zoonotic protozoan parasite in humans and warm-blooded animals worldwide. Humans usually become infected through consuming water or food contaminated with oocysts or by ingesting tissue cysts in undercooked meat. The aim of this study was to assess the presence of *T. gondii* in the meat of buffaloes and cows slaughtered in Tabriz slaughterhouse, Iran using PCR. Fifty grams of heart, thigh, diaphragm and tongue of 50 buffalo and 100 cows slaughtered at the Tabriz industrial slaughterhouse were randomly sampled and tested using a previously published PCR method. Of the 150 animal samples, *T. gondii* was detected in 10 (6.6%) including one buffalo (2%) and nine cows (9%). The rate of infection was the same for buffalo and cattle (*P* > 0.05). Also, there was no significant relationship between the rate of infection and age, sex or organs assessed (*P* > 0.05). Although the rate of infection of animals slaughtered in Tabriz slaughterhouse is low, the presence of *T. gondii* still poses a risk to human health. 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.

1. Introduction

Foodborne diseases include a wide range of acute and chronic syndromes that vary in severity of infection, clinical symptoms, and prevalence (Gérard et al., 2019). The World Health Organization (WHO) estimates that 31 bacterial, viral, parasitic and chemical global hazards caused a total of 600 million foodborne illnesses in 2010, of which 15% were due to parasites (WHO, 2015). *Toxoplasma gondii* is one of these food born zoonotic parasites with a world-wide distribution, infecting humans, ruminants, and other species of warm-blooded animals (Gérard et al., 2019; Soulsby, 1982). The final host of this protozoan is cats and its intermediate hosts are humans and other warm-blooded animals (Lyons, Lyons, Lyons, Rim, & Roberts, 2002). Humans become infected by ingesting uncooked or under cooked meat containing tissue cysts, by consuming raw milk contaminated with tachyzoites or consumption of soil or water contaminated with oocysts (Jitender P. 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, Kemmeren, & Kortbeek, 2007). The clinical signs of toxoplasmosis in human includes abortion, stillbirth, fetal death in utero, or severe central nervous system involvement in newborns, such as cerebral calcifications and hydrocephalus (Havelaar et al., 2007; Tenter, Heckertho, & Weiss, 2000). In addition to the effects in pregnant women, toxoplasmosis can cause acute disease in immune compromised individuals. In livestock, *T. gondii* is a major cause of abortion, with significant economic losses for ruminants including sheep, goats, cattle, buffalo and camels breeders (Sharif et al., 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, 2014; Cong et al., 2018; Dubey et al., 2005; Ergin et al., 2009; Guo et al., 2015; Ishii et al., 1962; Jackson et al., 1987; Laura et al., 2013; Mahami-Oskouei et al., 2017; Medeiros et al., 2014; Rahdar et
al., 2012; Santos et al., 2013, 2013; Slany et al., 2016; Sroka et al., 2019a, 2019b; Wang et al., 2012). 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 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, 2014; Hamzavi et al., 2007; Mahami-Oskouei et al., 2017; Rahdar et al., 2012). The aim study was to detect *T. gondii* prevalence in the meat of buffalo (*Bubalus bubalis*) and cows (*Bos taurus*) slaughtered at the Tabriz abattoir, Iran.

2. Material And Methods

2.1. Sampling

This study was conducted from April to September 2020. The number of samples was calculated using the following formula, assuming a prevalence of 20-30% of *T. gondii* in animal meat based on previous studies, as well as a 96% confidence interval and an accuracy of 5%.

\[
n = \frac{z_{1-\alpha/2}^2 \times p(1-p)}{d^2}
\]

P: Prevalence, \(\alpha\): Error rate, d: Accuracy

In this study, a 150 animals (50 buffalo and 100 cows) slaughtered at the Tabriz industrial abattoir, were randomly selected for sampling. The abattoir was visited at least eight days per month and a convenience sample from each day selected, based on the number that could be followed pre-slaughter through slaughter with equal numbers of animals examined per seasonal period. Before slaughter, the sex and age (Sex and age (< 2 years old or \(\geq\) 2 years old, based on dentition) were recorded for each animal. Fifty grams of heart, thigh, diaphragm and tongue were sampled from each animal 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 Tissue

DNA was extracted using a commercial kit (MBST, Rapid DNA Isolation Kit from Meat), according to the manufacturer's instructions (Cinna Gen, Iran). 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). Twenty µl proteinase k was added, mixed by vortexing and then incubated at 37°C for 24 hours. Then 580 µl binding buffer was added and the sample incubated for 10 min at 70°C, centrifuged for 1 min by 8000×g and the supernatant transferred into a clean 1.5 ml Eppendorf tube with 440 µl ethanol (100%). Half of the mixture was placed into spin column MBST and centrifuged at 8000 ×g for 1 min. The infiltrate was removed with the remainder 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 20000 xg for 2 min. After discarding the tube containing the infiltrate and placing the spin column MBST in a clean Eppendorf tube, 100 µl elution buffer preheated to 70°C was add, incubated at room temperature for 30 min and then centrifuged at 8000 xg for 1 min. After repeating this step, the infiltrate containing the tube was collected and stored at -20°C for analysis. DNA qualities were 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 used to amplify a fragment of 529 bp (Homan et al., 2000; Tavassoli et al., 2013). The sequences of primers were as follows: TOX4 (CGCTGCAGGGA GGAAGACGAAAGTTG)/ TOX5 (CGCTGCAGACACAGTGCATCTGGATT). PCR buffer, two mM MgCl2, 250 µM of each of the four deoxynucleotide triphosphates, 1.25 U Taq DNA polymerase (Fermentas, Germany), 50 pmol of each primer and 5 µL of the extracted DNA (Homan, Vercammen, De Braekeleer, & Verschueren, 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. Cycling condition was 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 analyzed by 2% agarose gel electrophoresis 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).

2.4. Data Analysis

The results were analyzed with the statistical package SPSS (version 21), using a Chi square test. A P-value of < 0.05 was set as statistically significant.

3. Results

Of the 150 animals studied (50 buffalo and 100 cows), the meat of 10 animals (6.6%) was contaminated with *T. gondii* (Table 1) The rate of contamination with *T. gondii* was not significantly different between buffalo and cows (P > 0.05). Of 40 buffalo over two years old, the meat of one (2.5%) was contaminated with *T. gondii* and no positive cases were observed in buffalo less than two years old, although the tested number in this age group was low. Meat contamination with *T. gondii* also was higher in cows older than 2 (P > 0.05). There was no statistically significant relationship between *T. gondii* infection and animal age or sex (P > 0.05). In both buffalo and cattle, the highest rate of contamination with *T. gondii* was observed in the diaphragm.
Table 1
Number of animals studied, contamination rate of cow and buffalo meat slaughtered in Tabriz slaughterhouse based on age, sex and organ

<table>
<thead>
<tr>
<th>Animal</th>
<th>Variation</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Organ</th>
<th>F</th>
<th>D</th>
<th>To</th>
<th>Th</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≤ 2</td>
<td>&gt; 2</td>
<td>Male</td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>40</td>
<td>38</td>
<td>12</td>
<td>15</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>55</td>
<td>45</td>
<td>65</td>
<td>35</td>
<td>10</td>
<td>45</td>
<td>10</td>
</tr>
<tr>
<td>Buffalo</td>
<td>Examined</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>(n = 50)</td>
<td>No.</td>
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<tr>
<td></td>
<td>Infected NO. (%)</td>
<td>1 (2)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>0 (0)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>0.622</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>Examined</td>
<td>100</td>
<td></td>
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<tr>
<td>(n = 100)</td>
<td>No.</td>
<td></td>
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<tr>
<td></td>
<td>Infected NO. (%)</td>
<td>9 (9)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>3 (5.4)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>0.174</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
| F: Female, D: Diaphragm, To: Tongue, Th: Thigh

4. Discussion

In the study presented herein, contamination rate of buffalo meat (2%) was lower than cattle meat (9%). Lower contamination in buffalo compared to those in cows may be attributed to differences in susceptibility to *T. gondii* and the feeding habits of the animals (Tavassoli, Esmaeilnejad, Malekifard, Soleimanzadeh, & Dilmaghani, 2013). How do the feeding habits differ? Could the difference be due to the different number of diaphragm samples? A higher rate in cattle versus 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. In contrast, in studies conducted in Brazil more buffalo were found to be positive than cattle with *T. gondii* antibodies found in 1.03% and 3.85% of the cattle and buffalo, respectively, in one study and 27.2% of water buffalo and 17.4% of cattle (using an indirect fluorescent antibody test (IFAT) in another study Laura et al., 2013).

In regards to cattle, other studies have found contamination rates of 0%, 4% and 17% in Iran (using latex agglutination (LAT), indirect hemagglutination (IHAT) and IFAT tests in one study and PCR in the latter two studies), 0% in Scotland (using ELISA, modified agglutination assay and PCR), 2.7% (Switzerland using P-30-ELISA), 6.6% (central Ethiopia using an IHAT), and 9% (Indonesia using LAT) (Hashemi-Fesharki, 1996; Sharif et al., 2007; Bekele and Kasali, 1989; Matsuo and Husin, 1996; Plaza et al., 2020; Rahdar et al., 2012; Mahami-Oskouei et al., 2017). Findings of the study presented herein are within the range found in other studies. Also, the contamination rate of meat with *T. gondii* in the examined animals increased with age, which was consistent with the studies conducted by Azizi et al., 2014, and the rate of infection of different tissue samples also 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 (Truppel et al., 2010; Milne et al., 2020; Bigna et al., 2020). Meta-analyses and reviews have shown a wide variation in the rate of meat contamination of 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; Dubey and Jones, 2008). 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 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 analyzed, PCR can result in underestimating prevalence (Liu, Wang, Huang, & Zhu, 2015; Yousefvand et al., 2021). Therefore, while the level of *T. gondii* contamination in the meat of buffalo and cattle slaughtered at the Tabriz abattoir was identified as low, it could be an underestimate and still poses a risk for consumers. While infection was lower in buffalo, they still serve as a potential source of human infection with appropriate and effective preventive measures 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.

**Declarations**

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

**Ethical statement**

The study entailed recording of the normal meat inspection process of animals sent to slaughter for human consumption. No ethical approval was required nor sought as no alteration was required to the normal processing of these animals.

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**Author Contributions**

Nasser Hajipour: Conceptualization, Supervision, Methodology, Project administration, Investigation, Writing- Original Draft preparation, Visualization; Javad Jabbari: Methodology, Investigation, Writing-Original Draft preparation; Parviz Hassanzadeh: Conceptualization, Methodology and Jennifer Ketzis: Formal analysis, Writing - Review & Editing.

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References


Figures
Figure 1

PCR-Amplified Products Using *T. gondii* Specific primers (fragment of 529 bp): Lane 1, 100 bp Ladder (Fermentas, Germany); lane 2: meat of cow, lane 3: meat of buffalo, lane 4: positive control, lane 5: negative control