JOURNAL OF FOOD QUALITY & HAZARDS CONTROL
SHAHID SADOUGHI UNIVERSITY OF MEDICAL SCIENCES & HEALTH SERVICES

Journal of Food Quality and Hazards Control 6 (2019) 153-161

Molecular Identification of *Toxoplasma gondii* in the Native Slaughtered Cattle of Tehran Province, Iran

A. Dalir Ghaffari [1,2], A. Dalimi [1*]

1. Department of Parasitology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2. Student Research Committee, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

HIGHLIGHTS

- Genotype III was the most prevalent genotype of *Toxoplasma gondii* in slaughtered cattle of Tehran, Iran.
- The infection rate in heart muscle samples (16.66%) was significantly higher than the diaphragm samples (4.44%).
- The frequency of *T. gondii* in cattle muscles was high in this area.

Article type
Original article

Keywords
Toxoplasma
Polymerase Chain Reaction
Meat
Cattle
Iran

Article history
Received: 14 Aug 2019
Revised: 10 Oct 2019
Accepted: 27 Oct 2019

Acronyms and abbreviations
PCR=Polymerase Chain Reaction
PCR-RFLP=PCR-Restriction Fragment Length Polymerase

ABSTRACT

Background: Toxoplasmosis, caused by *Toxoplasma gondii*, is a common parasitic disease, affecting almost one-third of the world's population. It is transmitted by ingestion of food or water contaminated with oocysts excreted by cats and the consumption of raw or undercooked meat from ruminants. This study aimed at molecular characterization of *T. gondii* in native cattle from West of Tehran, Iran.

Methods: A total of 180 samples were collected from the cattle diaphragms (n=80) and heart muscles (n=100) from multiple slaughterhouses. The nested Polymerase Chain Reaction (PCR) assay was carried out to amplify the *GRA6* gene of *T. gondii*. The PCR-Restriction Fragment Length Polymerase (PCR-RFLP) assay was also performed on positive samples, using *Tru1*I (*Mse*I) restriction enzyme. Data were statistically analyzed using SPSS (v.15.0).

Results: *T. gondii* was found in 38 out of 180 (21.1%) samples. The infection rate in heart muscle samples (16.66%) was significantly (p<0.05) higher than the diaphragm samples (4.44%). The PCR-RFLP pattern by *Mse*I enzyme showed that 13 (7.22%) samples were genotype II, while 25 (13.88%) were genotype III, having statistically meaningful difference (p<0.05). No genotype I was found in the studied isolates.

Conclusion: Based on our findings, the frequency of *T. gondii* was high in the study area. Therefore, educational programs need to be implemented in order to inform people about the risks of raw or undercooked meat consumption.

Introduction

Toxoplasmosis is a widespread parasitic disease that affects almost one-third human population of the world (Montoya and Liesenfeld, 2004; Robert-Gangneux and Dardé, 2012). Although, the majority of infections are asymptomatic, *Toxoplasma gondii* can lead to severe complications in congenitally infected newborns and immunocompromised individuals (Abdoli et al., 2014; Daryani et al., 2014; Robert-Gangneux and Dardé, 2012). This globally distributed parasite is considered a compulsory intracellular protozoan, infecting all warm-blooded

* Corresponding author (A. Dalimi)
E-mail: dalimi_a@modares.ac.ir
ORCID ID: https://orcid.org/0000-0001-5591-5513

To cite: Dalir Ghaffari A., Dalimi A. (2019). Molecular identification of *Toxoplasma gondii* in the native slaughtered cattle of Tehran Province, Iran. *Journal of Food Quality and Hazards Control*. 6: 153-161.

DOI: 10.18502/jfqhc.6.4.1993 Journal website: http://www.jfqhc.com

mammals including humans (Sarvi et al., 2015; Shariat Bahadori et al., 2019). Wild and domestic felids, as the final hosts, play a key role in the epidemiology of this zoonotic disease. In fact, felids are the only final hosts of *T. gondii*, which excrete oocysts through their feces (Dubey and Jones, 2008; Saadatnia and Golkar, 2012).

Toxoplasmosis is one of the most important problems not only in medicine, but also in veterinary fields (Dubey and Jones, 2008). The toxoplasmosis seroprevalence in the general population of Iran is estimated as 39.3% (95% CI, 33.0-45.7%; Daryani et al., 2014). Some evidences suggest that *T. gondii* imposes a considerable economic burden on livestock industries (Sarvi et al., 2015).

T. gondii may be found in a variety of animals, such as pigs, sheep, rabbits, goats, and domestic hen acting as intermediate hosts (Dubey et al., 2003; Dubey et al., 2008; Mahami-Oskouei et al., 2017). In Iran, *T. gondii* infection has been detected in many domestic animals (e.g., cattle, sheep, and goats), which are used as food sources for people. *T. gondii* is transmitted to humans through exposure to water or food contaminated with cat-excreted oocysts. In addition, eating of raw or undercooked meat can also result in toxoplasmosis in human beings (Dubey and Jones, 2008).

Although, substantial research has been conducted on the transmission of infectious agents through consumption of foods of animal origin (e.g., meat) or ingestion of *T. gondii* oocysts, there are still some ambiguities. It should be noted that epidemiological studies still provide the most helpful strategy for detecting different sources of *T. gondii* infection in humans (Sarvi et al., 2015). Generally, different methods are used to identify *T. gondii*. Molecular methods, like Polymerase Chain Reaction (PCR), have many benefits, including quick identification, high sensitivity, and high specificity (MacPherson and Gajadhar, 1993). These methods can also distinguish between *T. gondii* alleles (Payne and Ellis, 1996). With this background in mind, the main purpose of this study was to genetically characterize *T. gondii* DNA in the native cattle of Tehran, Iran, via PCR-Restriction Fragment Length Polymerase (PCR-RFLP) assay.

Materials and methods

Sample collection

This study was carried out in West of Tehran, Iran (Figure 1). Samples were randomly collected from the diaphragms (n=80) and heart muscles (n=100) of 180 adult cattle from slaughterhouses of Qods, Shahriar, Malard, and Robat Karim counties in West of Tehran Province during March to December of 2017. The study protocol was approved by the Ethics Committee of Tarbiat Modares University, Tehran, Iran (ethics approval number: IR.MODARES.REC.1398.080). All samples were from the Holstein breed, and the majority of slaughtered cattle were local. All cattle were indigenous in the area and intended for human consumption. Sampling was carried out regardless of age and sex.

DNA extraction

First, 250 g of different segments of each muscle was collected and digested under sterile conditions, as described by Dubey (2016) with some modifications. To prepare the digestion solution (pepsin-hydrochloric acid digestion), 1 ml of distilled water was added to 10 ml of Hydrogen Chloride (HCl), 2.5 g of pepsin powder, and 5 g of Sodium Chloride (NaCl). Then, 50 g of each sample was mixed in 100 ml of digestion solution and placed in a warm bath for 30 min at 37 °C. Next, each digested muscles was passed through a two-layer-gauze. The solution was centrifuged for 15 min at 1500 rpm (252 x g), and the sediment was used for DNA extraction. For DNA extraction using a DNA purification kit (YTA Genomic DNA Extraction Kit, Favorgen, Taiwan), 25 g of each sediment solution was transferred to a sterile 1.5 ml microtube, following the manufacturer's instructions. The extracts were stored at -20 °C for molecular analysis.

T. gondii identification by nested-PCR assay

For identification of *T. gondii* DNA, PCR assay was carried out by amplification of *GRA6* gene (dense granular antigens). For amplification of the ~344 bp fragment of *GRA6* gene, two specific primer pairs for *T. gondii* were used including *GRA6*-F1:5'-ATTTGTGTTTCCGAGCAGGT-3' as well as R1:5'-GCACCTTCGCTTGTGGTT-3' for the first round; and GAR6-F2:5'-TTTCCGAGCAGGTGACCT-3' and R2: 5'-TCGCCGAAGAGTTGACATAG-3' for nested-PCR (Khan et al., 2005). In each amplification, the final volume was 20 µl, consisting of 2 µl of each primer (10 pmol of each primer), 5 µl of distilled water, 10 µl of Taq DNA polymerase 2X Master Mix Red (2 mM $MgCl_2$, Amplicon, Denmark), and 3 µl of diluted DNA (100 ng genomic DNA).

In the nested-PCR assay, the primary PCR product (1 µl) was used for amplification of the internal sequences of *GRA6* gene. The positive control contained *T. gondii* RH strain DNA, while the negative control was the nuclease-free water. The first PCR round was set up under the following conditions: one cycle of initial denaturation for five min at 94 °C, followed by 35 cycles of 94 °C for 30 s , 57 °C for 30 s , and 72 °C for 45 s . The final extension was done at 72 °C for 10 min. The nested-PCR condition was similar to the first reaction, whereas the

[DOI: 10.18502/jfqhc.6.4.1993]

annealing temperature in the second round was 56 °C. The amplified PCR products (5 µl) were analyzed on 2% agarose gel.

Genotyping of positive samples by PCR-RFLP assay

The *Tru1*I (*Mse*I) restriction enzyme (Thermo Fisher Scientific, USA) was used in the PCR-RFLP assay on the positive samples (Abdoli et al., 2017). Digestion was performed in the reaction mixture at a final volume of 16 µl, as described by the manufacturer (5 µl of PCR product, 1 µl of 10X Buffer R, 9 µl of nuclease-free water, and 1 unit of *Mse*I endonuclease) and incubated for 4 h at 65 °C. Then, the digested fragments were analyzed with 2% agarose gel. To better characterization, *GRA6* sequences of *T. gondii* from RH type I, ME49 type II, and NED type III were collected from the GenBank (NCBI) and digested virtually by *Mse*I restriction enzyme using NEBcutter (http://nc2.neb.com/NEBcutter2/) as shown in Figure 2.

GRA6 sequencing and phylogenetic analysis

In order to confirm the results of RFLP assay, the secondary PCR product of one positive sample was selected for each genotype and sequenced (Bioneer Company; South Korea). The sequences were edited and aligned using ClustalW program (http://www.ebi.ac.uk/Tools/msa/clustalw2/). The maximum likelihood method was applied to plot the phylogenetic tree using MEGA 7. Also, the sequences were submitted at GenBank, NCBI.

Statistical analysis

Data were analysed by the SPSS.15 version using X^2 test. Results were considered significant at the 95% level (p<0.05).

Results

T. gondii DNA was found in 38 out of 180 (21.1%) cattle samples, which showed the amplicons with the size of ~344 bp (Figure 3). The infection rates in the heart muscle and diaphragm samples were 30 out of 180 (16.66%) and 8 out of 180 (4.44%), respectively, showing significant difference (p<0.05).

Figure 4 demonstrates the results of *GRA6* analysis of *T. gondii* in slaughtered cattle samples. The PCR-RFLP pattern by *Mse*I enzyme showed that 13 (7.22%) samples were genotype II, while 25 (13.88%) were genotype III, having statistically meaningful difference (p<0.05). No genotype I was found in the studied isolates.

The analysis of *GRA6* gene sequences corroborated the results of RFLP assay. The amplified *GRA6* gene of the two isolates were sequenced and submitted to the GenBank under the accession numbers of MK055338 and MK055339. The alignment of our sequences showed 100% homology to other reported sequences in the GenBank. Using a phylogenetic tree, the phylogenetic relation of *T. gondii* isolates was compared with the same target gene in the other strains of *T. gondii* available in the GenBank (Figure 5).

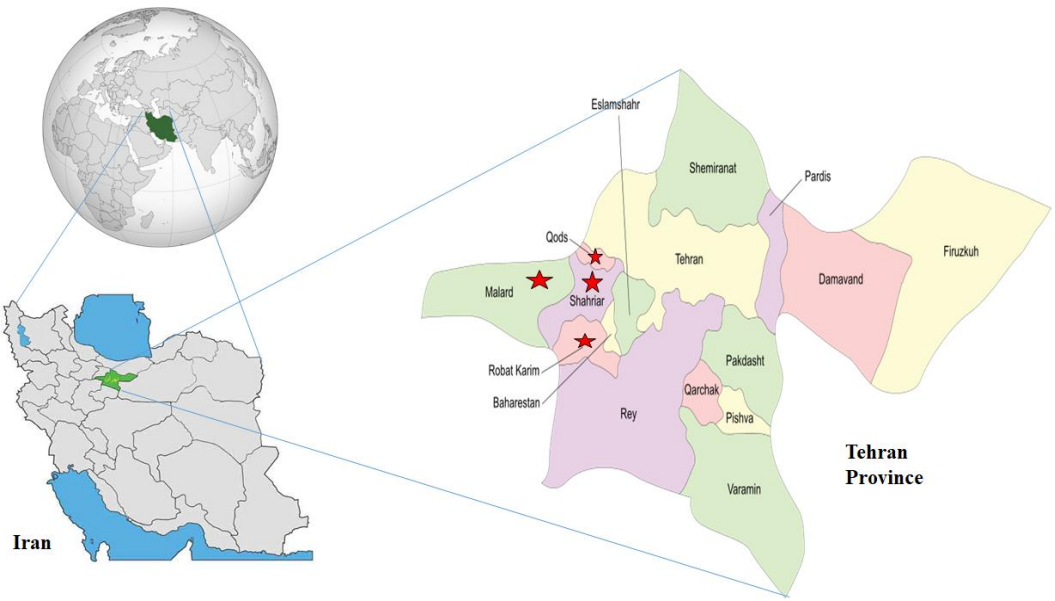

Figure 1: The study region in Tehran Province, Iran is shown with red stars

[DOI: 10.18502/jfqhc.6.4.1993]

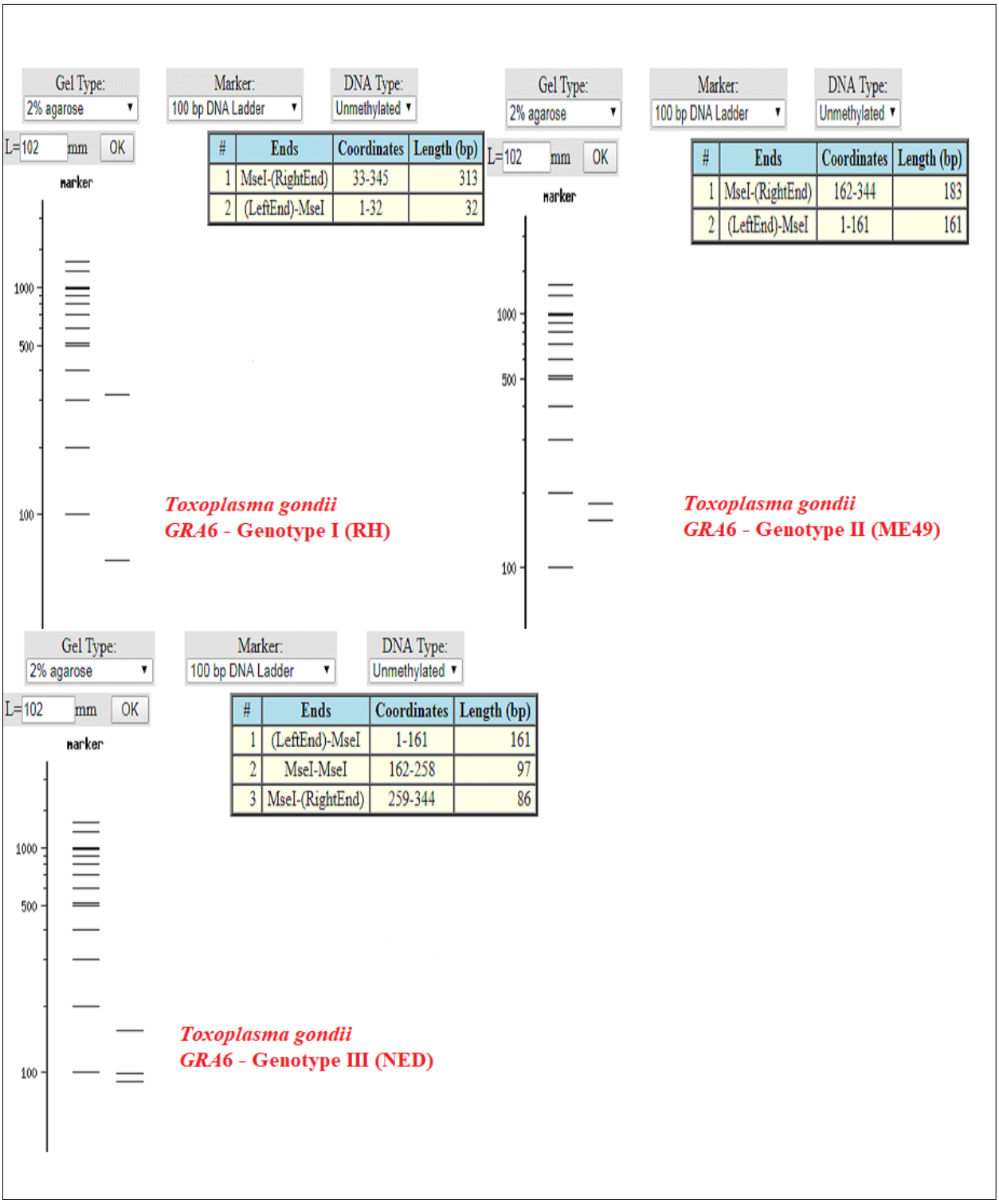

Figure 2: Virtually digestion by *Mse*I restriction enzyme using NEBcutter. The target gene was *GRA6* from the strains of genotype I (RH), genotype II (ME49), and genotype III (NED) obtained from GenBank, NCBI.

[DOI: 10.18502/jfqhc.6.4.1993]

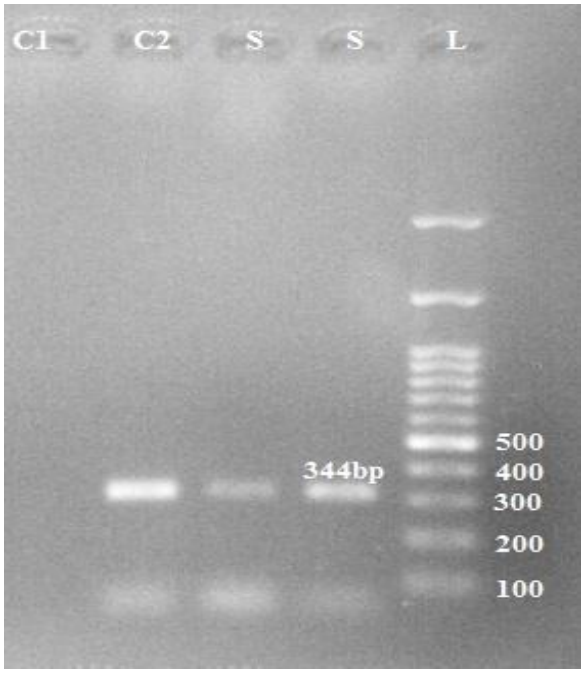

Figure 3: Nested-PCR product of *GRA6* gene of *Toxoplasma gondii*. L: 100 bp DNA ladder; C1: negative control; C2: positive control; S: positive sample. The fragment size with 344 bp was considered as *T. gondii*.

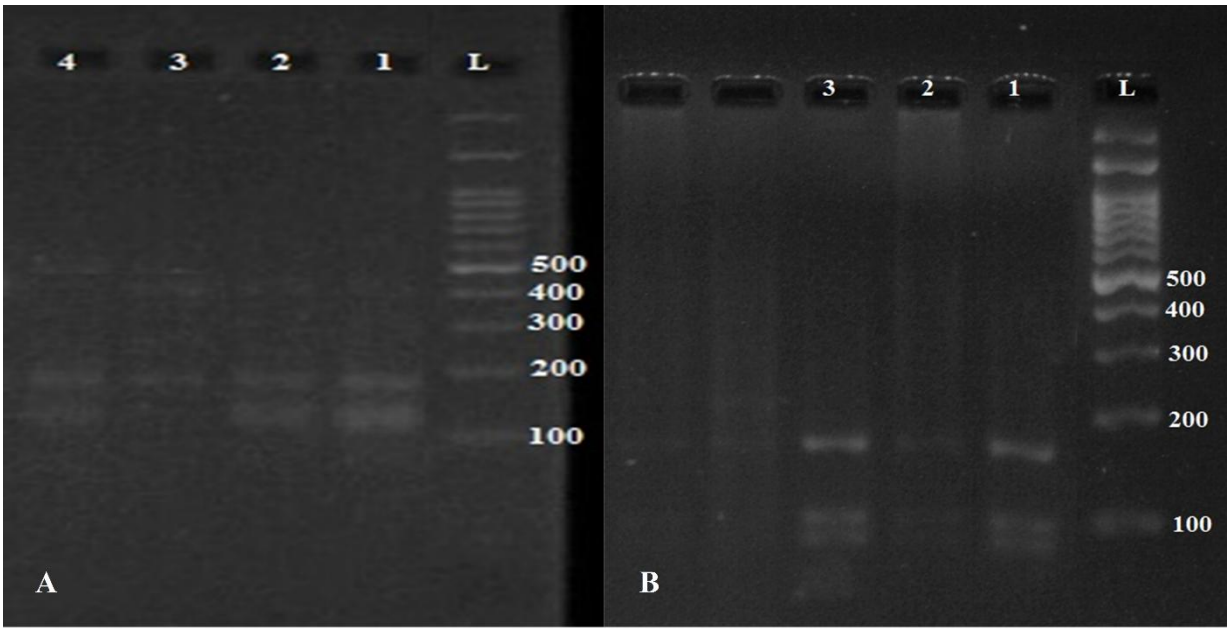

Figure 4: Genotyping of positive sample with the *GRA6* marker of *Toxoplasma gondii* using the restriction enzyme digestion of *Mse*I. A: patterns of genotype II. The pattern with the fragment sizes of 161 and 183 bp in length was considered as genotype II. B: patterns of genotype III. The pattern with the fragment sizes of 161, 97, and 86 bp in length was considered as genotype III; L: ladder marker; lanes 1-4: the studied samples.

[DOI: 10.18502/jfqhc.6.4.1993]

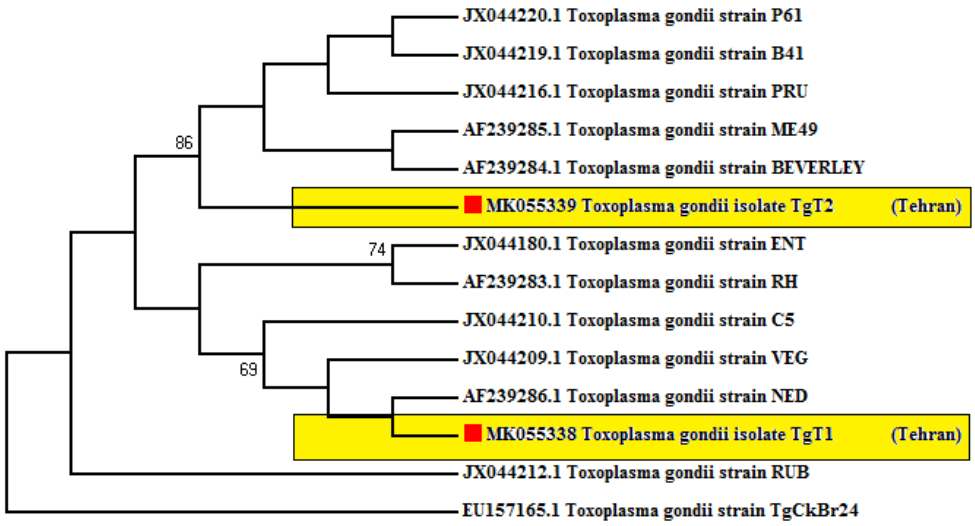

Figure 5: Phylogenetic relationship based on *GRA6* of *Toxoplasma gondii* isolated from cattle muscles in this study (MK055338 and MK055339) compared with other reference in GenBank. Sequences from this study are highlighted with yellow color and bold accession numbers.

Discussion

The present study was carried out to detect and genetically characterize *T. gondii* DNA in the native slaughtered cattle of Tehran Province, Iran. In our study, 38 out of 180 samples (21.1%) were positive based on the *GRA6* gene detection, therefore the prevalence of *T. gondii* in these regions is relatively high. In Iran, various studies have been conducted on different human and animal groups regarding the prevalence of toxoplasmosis. The prevalence rate has been estimated at 39% in the general population (Daryani et al., 2014), 50% in immunodeficiency patients (Ahmadpour et al., 2014), 41% in pregnant women (Foroutan-Rad et al., 2016a), and 33% in blood donors (Foroutan-Rad et al., 2016b). Also, the prevalence rates have been estimated at 34% in felids (Rahimi et al., 2015), 27% in goats, and 31% in sheep (Sharif et al., 2015). The rate of toxoplasmosis in Iranian cattle was 18.1% (9.2-28.5%) during a 30-year period from 1983 to 2012 (Sarvi et al., 2015). The contamination level of *T. gondii* varies in different regions of Iran. In a study by Anvari et al. (2018), 16.0% of different muscles collected from the slaughtered cattle in Zahedan (South-East of Iran) were contaminated with *T. gondii*. In another study from Lorestan Province, Western Iran, the seroprevalence of *T. gondii* in cattle was reported to be 28.73% (Hashemi, 2014). The mentioned findings, in line with the present study, are indicative of the high prevalence of toxoplasmosis in cattle of Iran. The high spread of toxoplasmosis in cattle in some areas may be due to several factors, including weather conditions, lack of routine treatment for feline toxoplasmosis, and contact with contaminated felids and *Toxoplasma* oocysts (Sarvi et al., 2015). However, the prevalence of *T. gondii* infection in the cattle in some provinces of Iran was lower, compared to the current study. For example, in a study in Ahvaz, South-West of Iran, only 4% of cattle were infected to *T. gondii* (Rahdar et al., 2012).

In the present study, the infection rate in heart muscle samples (16.66%) was significantly higher than the diaphragm samples (4.44%). Such findings were previously reported in muscle samples of cattle slaughtered in Switzerland (Berger-Schoch et al., 2011) and Tunisia (Lahmar et al., 2015). Indeed, the heart is known to be one of the most vulnerable to *Toxoplasma* cyst formation.

Also, the prevalence of *T. gondii* varies in different countries. In this way, Amdouni et al. (2017) stated that the infection rate of *T. gondii* was 19.3% in neck muscle samples of slaughtered cattle in North-West Tunisia. Another survey by Lopes et al. (2013) in Portugal showed that prevalence of *T. gondii* infection was 7.5% in cattle. These variations in the prevalence of *T. gondii*

[DOI: 10.18502/jfqhc.6.4.1993]

might be attributed to the type of food sources, methods of detection, geographical location, and sample analysis methods (Azizi et al., 2014). Also, it was shown that there is a meaningful relationship between weather and toxoplasmosis prevalence. In hot and wet climates and lower altitudes, it is typically more prevalent compared to cold and dry districts. This is consistent with longer viability of *T. gondii* oocyst in warm and humid environments (Sarvi et al., 2015).

T. gondii is categorized into three major strain types (type I, type II, and type III) that differ together in their epidemiological patterns, pathogenicity, and virulence (Chaichan et al., 2017; Sharif et al., 2017; Sibley et al., 2009). There was no evidence of genotype I in the present study. Though, in a PCR-RFLP assay conducted in North-West of Iran, 26 out of 150 (17.33%) chicken, beef, and lamb samples were positive for *T. gondii*, which all the samples were identified as genotype I (Mahami-Oskouei et al., 2017). The *T. gondii* genotype II has low pathogenicity and therefore allows the animal to survive until slaughter (Weiss and Kim, 2000). In our study, genotype II was found in 13 (7.2%) samples. In this regard, Lopes et al. (2015) showed that 60% of the cattle were seropositive for *T. gondii*, and three strains of genotype II were found in the heart samples of cattle, with predominant of genotype II (Lopes et al., 2015). In line with the present study, some evidences showed that genotype III was the most dominant genotype of *T. gondii* (Howe and Sibley, 1995).

The results of the current survey confirmed the presence of *T. gondii* in animals. Therefore, the risk of toxoplasmosis transmission due to contaminated meat consumption still needs to be considered as a public health problem (Dubey et al., 2014). To determine risk factors for infection of *T. gondii* in pregnant women, various studies worldwide have shown the significance of undercooked meat consumption as a contributor to human toxicity. It was found that consumption of undercooked meat of cattle and sheep was an important risk factor for infection of French pregnant women (Baril et al., 1999). Generally, there is a significant relationship between the incidence of toxoplasmosis and exposure of cows with cats. Examination of the risk factors for toxoplasmosis in large ruminants indicated that the presence of cats around cattle is a major factor in cattle infection (Ahmad and Qayyum, 2014).

Conclusion

The results showed that the frequency of *T. gondii* is high in cattle muscles in Tehran Province of Iran, and cattle infection plays an important role in the transmission of *T. gondii* to humans. Therefore, educational programs need to be implemented to inform people about the risks of raw/undercooked meat consumption in this area. Also, it is suggested that *T. gondii* infection be studied in felid feces to reach a better understanding of the epidemiological aspects of toxoplasmosis in Iran.

Author contributions

A.D.Gh. and A.D. conceived the study and designed the study protocol; A.D. was supervisor of this research; A.D.Gh. did the experimental work and drafted the manuscript. Both authors read and approved the final version of the manuscript.

Conflicts of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this paper.

Acknowledgements

This research was approved by Ethics Committee of Tarbiat Modares University of Medical Sciences, Tehran, Iran (approval number: IR.MODARES.REC.1398.080). The authors would like to thank all the staff of the Department of Parasitology of Tarbiat Modares University, Tehran, Iran. The present study was carried out with the financial support of Tarbiat Modares University, Tehran, Iran.

References

Abdoli A., Dalimi A.H., Arbabi M., Ghaffarifar F. (2014). Neuropsychiatric manifestations of latent toxoplasmosis on mothers and their offspring. *The Journal of Maternal-Fetal and Neonatal Medicine*. 27: 1368-1374. [DOI: 10.3109/14767058.2013.858685]

Abdoli A., Dalimi A.H., Soltanghoraee H., Ghaffarifar F. (2017). Molecular detection and genotypic characterization of *Toxoplasma gondii* in paraffin-embedded fetoplacental tissues of women with recurrent spontaneous abortion. *International Journal of Fertility and Sterility*. 10: 327-336. [DOI: 10.22074/ijfs.2016.4569]

Ahmad N., Qayyum M. (2014). Seroprevalence and risk factors for toxoplasmosis in large ruminants in northern Punjab, Pakistan. *The Journal of Infection in Developing Countries*. 8: 1022-1028. [DOI: 10.3855/jidc.4405]

Ahmadpour E., Daryani A., Sharif M., Sarvi S., Aarabi M., Mizani A., Rahimi M.T., Shokri A. (2014). Toxoplasmosis in immunocompromised patients in Iran: a systematic review and meta-analysis. *The Journal of Infection in Developing Countries*. 8: 1503-1510. [DOI: 10.3855/jidc.4796]

Amdouni Y., Rjeibi M.R., Rouatbi M., Amairia S., Awadi S., Gharbi M. (2017). Molecular detection of *Toxoplasma gondii* infection in slaughtered ruminants (sheep, goats and cattle) in Northwest Tunisia. *Meat Science*. 133: 180-184. [DOI: 10.1016/j.meatsci.2017.07.004]

Anvari D., Saadati D., Nabavi R., Eskandani M.A. (2018).

[DOI: 10.18502/jfqhc.6.4.1993]

Epidemiology and molecular prevalence of *Toxoplasma gondii* in cattle slaughtered in Zahedan and Zabol districts, South East of Iran. *Iranian Journal of Parasitology*. 13: 114-119.

Azizi H.R., Shiran B., Boroujeni A.B., Jafari M. (2014). Molecular survey of *Toxoplasma gondii* in sheep, cattle and meat products in Chaharmahal va Bakhtiari Province, Southwest of Iran. *Iranian Journal of Parasitology*. 9: 429-439.

Baril L., Ancelle T., Goulet V., Thulliez P., Tirard-Fleury V., Carme B. (1999). Risk factors for *Toxoplasma* infection in pregnancy: a case-control study in France. *Scandinavian Journal of Infectious Diseases*. 31: 305-309. [DOI: 10.1080/00365549950163626]

Berger-Schoch A.E., Herrmann D.C., Schares G., Muller N., Bernet D., Gottstein B., Frey C.F. (2011). Prevalence and genotypes of *Toxoplasma gondii* in feline faeces (oocysts) and meat from sheep, cattle and pigs in Switzerland. *Veterinary Parasitology*. 177: 290-297. [DOI: 10.1016/j.vetpar.2010.11.046]

Chaichan P., Mercier A., Galal L., Mahittikorn A., Ariey F., Morand S., Boumédiène F., Udonsom R., Hamidovic A., Murat J.B., Sukthana Y., Dardé M.L. (2017). Geographical distribution of *Toxoplasma gondii* genotypes in Asia: a link with neighboring continents. *Infection, Genetics and Evolution*. 53:227-238. [DOI: 10.1016/j.meegid.2017.06.002]

Daryani A., Sarvi S., Aarabi M., Mizani A., Ahmadpour E., Shokri A., Rahimi M.T., Sharif M. (2014). Seroprevalence of *Toxoplasma gondii* in the Iranian general population: a systematic review and meta-analysis. *Acta Tropica*. 137: 185-194. [DOI: 10.1016/j.actatropica.2014.05.015]

Dubey J.P. (2016). Toxoplasmosis of animals and humans. CRC press, Boca Raton.

Dubey J.P., Casey S.J., Zajac A.M., Wildeus S.A., Lindsay D.S., Verma S.K., Oliveira S., Kwok O.C.H., Su C. (2014). Isolation and genetic characterization of *Toxoplasma gondii* from alpaca (*Vicugna pacos*) and sheep (*Ovis aries*). *Tropical Animal Health and Production*. 46: 1503-1507. [DOI: 10.1007/s11250-014-0652-z]

Dubey J.P., Graham D.H., Dahl E., Hilali M., El-Ghaysh A., Sreekumar C., Kwok O.C.H., Shen S.K., Lehmann T. (2003). Isolation and molecular characterization of *Toxoplasma gondii* from chickens and ducks from Egypt. *Veterinary Parasitology*. 114: 89-95. [DOI: 10.1016/s0304-4017(03)00133-x]

Dubey J.P., Jones J.L. (2008). *Toxoplasma gondii* infection in humans and animals in the United States. *International Journal for Parasitology*. 38: 1257-1278. [DOI: 10.1016/j.ijpara.2008.03.007]

Dubey J., Velmurugan G.V., Chockalingam A., Pena H.F.J., de Oliveira L.N., Leifer C.A., Gennari S.M., Bahia Oliveira L.M.G., Su C. (2008). Genetic diversity of *Toxoplasma gondii* isolates from chickens from Brazil. *Veterinary Parasitology*. 157: 299-305. [DOI: 10.1016/j.vetpar.2008.07.036]

Foroutan-Rad M., Khademvatan S., Majidiani H.R., Aryamand S., Rahim F., Saki Malehi A. (2016a). Seroprevalence of *Toxoplasma gondii* in the Iranian pregnant women: a systematic review and meta-analysis. *Acta Tropica*. 158: 160-169. [DOI: 10.1016/j.actatropica.2016.03.003]

Foroutan-Rad M., Majidiani H.R., Dalvand S., Daryani A., Kooti W., Saki J., Hedayati-Rad F., Ahmadpour E. (2016b). Toxoplasmosis in blood donors: a systematic review and meta-analysis. *Transfusion Medicine Reviews*. 30: 116-122. [DOI: 10.1016/j.tmrv.2016.03.002]

Hashemi S. (2014). Seroprevalence of toxoplasmosis in cattle, sheep and goat, using ELISA and Indirect Immunoflorescent Antibody (IFA) methods in Lorestan province. *Journal of Large Animal Clinical Science Research*. 7 : 49-55. [Persian with English abstract]

Howe D.K., Sibley L.D. (1995). *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. *Journal of Infectious Diseases*. 172: 1561-1566. [DOI: 10.1093/infdis/172.6.1561]

Khan A., Su C., German M., Storch G.A., Clifford D.B., Sibley L. D. (2005). Genotyping of *Toxoplasma gondii* strains from immunocompromised patients reveals high prevalence of type I strains. *Journal of Clinical Microbiology*. 43: 5881-5887. [DOI: 10.1128/JCM.43.12.5881–5887.2005]

Lahmar I., Lachkhem A., Slama D., Sakly W., Haouas N., Gorcii M., Pfaff A.W., Candolfi E., Babba H. (2015). Prevalence of toxoplasmosis in sheep, goats and cattle in Southern Tunisia. *Journal of Bacteriology and Parasitology*. 6: 1000245. [DOI: 10.4172/2155-9597.1000245]

Lopes A.P., Dubey J.P., Neto F., Rodrigues A., Martins T., Rodrigues M., Cardoso L. (2013). Seroprevalence of *Toxoplasma gondii* infection in cattle, sheep, goats and pigs from the North of Portugal for human consumption. *Veterinary Parasitology*. 193: 266-269. [DOI: 10.1016/j.vetpar.2012.12.001]

Lopes A.P., Vilares A., Neto F., Rodrigues A., Martins T., Ferreira I., Gargate M.J., Rodrigues M., Cardoso L. (2015). Genotyping characterization of *Toxoplasma gondii* in cattle, sheep, goats and swine from the North of Portugal. *Iranian Journal of Parasitology*. 10: 465-472.

MacPherson J.M., Gajadhar A.A. (1993). Sensitive and specific polymerase chain reaction detection of *Toxoplasma gondii* for veterinary and medical diagnosis. *Canadian Journal of Veterinary Research*. 57: 45-48.

Mahami-Oskouei M., Moradi M., Fallah E., Hamidi F., Akbari N.A.R. (2017). Molecular detection and genotyping of *Toxoplasma gondii* in chicken, beef, and lamb meat consumed in Northwestern Iran. *Iranian Journal of Parasitology*. 12: 38-45.

Montoya J.G., Liesenfeld O. (2004). Toxoplasmosis. *The Lancet*. 363: 1965-1976. [DOI: 10.1016/S0140-6736(04)16412-X]

Payne S., Ellis J. (1996). Detection of *Neospora caninum* DNA by the polymerase chain reaction. *International Journal for Parasitology*. 26: 347-351. [DOI: 10.1016/0020-7519(96)00030-6]

Rahdar M., Samarbaf-Zadeh A., Arab L. (2012). Evaluating the prevalence of *Toxoplasma gondii* in meat and meat products in Ahvaz by PCR method. *Jundishapur Journal of Microbiology*. 5: 570-573. [DOI:10.5812/jjm.4280]

Rahimi M.T., Daryani A., Sarvi S., Shokri A., Ahmadpour E., Teshnizi S.H., Mizani A., Sharif M. (2015). Cats and *Toxoplasma gondii*: a systematic review and meta-analysis in Iran. *Onderstepoort Journal of Veterinary Research*. 82: 01-10. [DOI: 10.4102/OJVR.V82I1.823]

Robert-Gangneux F., Dardé M.L. (2012). Epidemiology of and diagnostic strategies for toxoplasmosis. *Clinical Microbiology Reviews*. *25*: 264-296. [DOI: 10.1128/CMR.05013-11]

Saadatnia G., Golkar M. (2012). A review on human toxoplasmosis. *Scandinavian Journal of Infectious Diseases*. 44: 805-814. [DOI: 10.3109/00365548.2012.693197]

Sarvi S., Daryani A., Rahimi M.T., Aarabi M., Shokri A., Ahmadpour E., Mizani A., Sharif M. (2015). Cattle toxoplasmosis in Iran: a systematic review and meta–analysis. *Asian Pacific Journal of Tropical Medicine*. 8: 120-126. [DOI: 10.1016/S1995-7645(14)60301-1]

Shariat Bahadori E., Dalir Ghaffari A., Namroodi S., Mosavipour S.S., Sadraei J. (2019). Electrochemiluminesence epidemiologic detection of *Toxoplasma gondii* infection in pregnant women with direct and indirect diagnostic techniques (ELISA avidity plus biochemical assay), Tehran, Iran. *International Journal of Epidemiologic Research*. 6: 49-54. [DOI: 10.15171/ijer.2019.09]

Sharif M., Amouei A., Sarvi S., Mizani A., Aarabi M., Hosseini S.A., Daryani A. (2017). Genetic diversity of *Toxoplasma gondii* isolates from ruminants: a systematic review. *International Journal of Food Microbiology*. 258: 38-49. [DOI: 10.1016/j.ijfoodmicro.2017.07.007]

Sharif M., Sarvi S., Shokri A., Hosseini Teshnizi S., Rahimi M.T., Mizani A., Ahmadpour E., Daryani A. (2015). *Toxoplasma gondii* infection among sheep and goats in Iran: a systematic review and meta-analysis. *Parasitology Research*. 114: 1-16. [DOI: 10.1007/s00436-014-4176-2]

[DOI: 10.18502/jfqhc.6.4.1993]

Sibley L.D., Khan A., Ajioka J.W., Rosenthal B.M. (2009). Geneticdiversity of *Toxoplasma gondii* in animals and humans. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences.* 364: 2749-2761. [DOI: 10.1098/rstb.2009.0087]

Weiss L.M., Kim K. (2000). The development and biology of bradyzoites of *Toxoplasma gondii*. *Frontiers in Bioscience: a Journal and Virtual Library*. 5: D391-D405.

[DOI: 10.18502/jfqhc.6.4.1993]