



## Molecular genotyping and serological evaluation of *Toxoplasma gondii* in mothers and their spontaneous aborted fetuses in Southwest of Iran

Nasir Arefkhah<sup>a</sup>, Bahman Pourabbas<sup>b</sup>, Qasem Asgari<sup>a</sup>, Abdolali Moshfe<sup>c</sup>, Fataneh Mikaeili<sup>a</sup>, Gordafarin Nikbakht<sup>d</sup>, Bahador Sarkari<sup>a,e,\*</sup>

<sup>a</sup> Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

<sup>b</sup> Professor Alborzi Clinical Microbiology Research Center, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, Iran

<sup>c</sup> Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran

<sup>d</sup> Department of Infectious Diseases, Yasuj University of Medical Sciences, Yasuj, Iran

<sup>e</sup> Basic Sciences in Infectious Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran



### ARTICLE INFO

#### Keywords:

*Toxoplasma gondii*  
Pregnancy  
Aborted fetus  
genotype  
Iran

### ABSTRACT

**Background:** Given the lack of routine screening and the high prevalence of toxoplasmosis in pregnant women in Iran, the current study aimed to find out the rate and features of *Toxoplasma gondii* infection in the spontaneously aborted human fetuses in Kohgiluyeh and Boyer-Ahmad Province, Southwestern Iran.

**Methods:** This cross-sectional study was performed on 100 spontaneously aborted fetuses' tissues and their mother blood samples. The mothers' sera were evaluated for anti-*Toxoplasma* antibodies while their buffy coat and aborted fetuses tissues were evaluated for *Toxoplasma* DNA. PCR product at GRA6 locus was sequenced and phylogenetic analysis was done. Likewise, quantitative Real-Time PCR was performed to find out the parasite burdens in mothers buffy coat and fetuses tissues.

**Results:** Using serological method, anti-*Toxoplasma* IgG and IgM antibodies were detected in 7 (7%) and 3 (3%) out of 100 sera from women with spontaneous abortion. Real-time PCR method detected *T. gondii* DNA in the buffy coat of one seronegative and 2 (out of 3) IgM seropositive cases. None of the samples from aborted fetuses were infected with *T. gondii*. BLAST and phylogenetic analysis showed that the sequenced isolates belonged to type I of *T. gondii* and two identified *T. gondii* isolates were taxonomically grouped into one clade.

**Conclusion:** Our findings revealed type I genotype of *T. gondii* in two mothers with spontaneous abortion, without fetus involvement. It is necessary to examine more aborted fetuses' samples from different geographical areas to determine the association between *Toxoplasma* genotype and abortion.

### 1. Introduction

Abortion is one of the most important challenges in pregnant women around the world. There are ranges of maternal infections which may cause abortion in the human. Toxoplasmosis is a zoonotic disease, caused by the intracellular food-borne pathogen *Toxoplasma gondii*, which may cause abortion in humans as well as farm animals. The disease is usually asymptomatic in most immunocompetent individuals nevertheless; it may cause potentially fatal disease in immunocompromised patients and immature fetuses [1].

Humans can acquire *T. gondii* infection postnatally through the passage of tachyzoites form of the parasite to the placenta and then to the fetus. Seronegative pregnant women who acquire acute primary toxoplasmosis during the first and second trimesters of their pregnancy

are at risk of stillbirth, abortion or delivering fetuses with subclinical infection at birth with a risk of developing ocular or CNS impairment in the next stages of their life [2]. Rate of congenital toxoplasmosis (CT) varies from 1.3 in 1000 to 1 in 30,000 live births in different areas of the world [3–7].

The severity of CT in infants mainly depends on the parasite burden and the time of fetus infection. Results of recent studies have shown that the genotype of *Toxoplasma* may play an important role in the clinical outcome of CT [8]. Human infections are caused by the main three genotypes of *T. gondii* (type I, II, and III). It has also been demonstrated that atypical or non-clonal genotypes of *Toxoplasma* could cause more severe clinical manifestations in comparison with the typical genotypes [9].

In recent years, molecular methods have been used for the diagnosis

\* Corresponding author at: Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.  
E-mail address: [sarkarib@sums.ac.ir](mailto:sarkarib@sums.ac.ir) (B. Sarkari).

of congenital toxoplasmosis and also for the study of parasite genotypes [10–12]. PCR method, using 529-bp repetitive element gene (RE gene), is a valuable specific target with high sensitivity for the diagnosis of *T. gondii* infection. Moreover, the *Toxoplasma* GRA6 gene, with a very high rate of DNA polymorphism, is considered as one of the most suitable markers for evaluation of parasite genotype and polymorphism. Moreover, Real-Time PCR, using the *Toxoplasma* 35-repeat B1 gene with high sensitivity and specificity, has been applied for the diagnosis of congenital toxoplasmosis and quantification of parasite burden in different biological samples [11,13].

The seroprevalence of toxoplasmosis varies from less than 10% to over 90% in the population of different regions of the world, depending on geographical locations, population food habits as well as the environmental factors [10,14,15]. The incidence of congenital toxoplasmosis is an important health concern in pregnant women in areas where the prevalence of *Toxoplasma* infection among pregnant women and newborn is high. In Iran, the results of a systematic review showed that about 47.3% of pregnant women are seropositive for *Toxoplasma* infection [16].

The high prevalence of *Toxoplasma* infection in Iran, as well as, the lack of systematic screening of pregnant women for detection of seroconversion during gestation, have increased the risk of toxoplasmosis in pregnant women.

The paucity of information about the human congenital toxoplasmosis in Iran, especially in the southwest of the country, justified the current study which aimed to find out the serological and molecular status of *T. gondii* infection in human spontaneous aborted fetuses and their mothers in the southwest of the country.

## 2. Materials and methods

### 2.1. Ethical statement

The study was approved by the Ethics Committee of Shiraz University of Medical Sciences (Ethical code: IR.SUMS.REC.1395.S1224). Written informed consent was obtained from each participant.

### 2.2. Study area and sample collection

This cross-sectional survey was carried out in Kohgiluyeh and Boyer-Ahmad province, in the southwest of Iran. Choice of the geographical areas was based on the high prevalence of abortion and early embryonic death in human as reported by the associated organization in the Province. The study was carried out on 100 pregnant women with spontaneous abortion. Blood samples (about 5 mL) from each woman and also tissue samples from their embryos of less than 14 weeks of age were collected. The tissue samples were mainly muscle and brain. In the case of embryos whose tissues were not correctly identified, part of the aborted fetus was collected. The samples were collected from two hospitals in Yasuj and Dehdasht cities, in Kohgiluyeh and Boyer-Ahmad province during 2017 and 2018.

### 2.3. Serological assays

The sera samples were tested for detection of anti-*T. gondii* IgG and IgM antibodies, using a commercial ELISA kit (Acon Biotech, Hangzhou, China) following the manufacturer's instructions. Optical densities were measured by an ELISA reader (BioTek Instruments, Inc., Winooski, VT, USA) at a wavelength of 450 and 630 nm, and values higher than the index value 1.1 were considered positive. Values between 0.9 and 1.1 were considered to be equivocal and retested while index value less than 0.9 were considered as negative.

### 2.4. DNA extraction and polymerase chain reaction

The genomic DNA from all 100 buffy coat samples of the pregnant woman and also the 100 fetuses' tissue samples were extracted, using Tissue Genomic DNA Extraction kit (Favorgene Biotech Corp, Taiwan) following the manufacturer's instructions. The RE gene of *T. gondii* was used for the screening of the samples [14]. The extracted DNA was amplified by PCR, using the forward (ToxoF: 5'-CAG GGA GGA AGA CGA AAG TTG -3') and reverse (ToxoR: 5'-CAG ACA CAG TGC ATC TGG ATT-3') primers. The conventional PCR reactions were conducted in a 25- $\mu$ L reaction volume contained 50 ng of template DNA, 12.5  $\mu$ L of 2 $\times$  Taq PCR mix (Amplicon, Odense, Denmark), 0.5  $\mu$ L of each primer (10 pmol/ $\mu$ L), and 9.5  $\mu$ L of ddH<sub>2</sub>O. The temperature profile of the PCR was one cycle of 95 °C for 5 min, followed by 30 cycles of 95 °C for 45 s, 60 °C for 45 s, and 72 °C for 45 s, and one cycle of 72 °C for 5 min. *T. gondii* DNA, as the positive control, and double-distilled water (DDW) instead of template DNA, as the negative control, were included in each run of the experiment. The PCR products were electrophoretically separated on 1.5% agarose gel and stained with GelRed nucleic acid gel stain (GelRed®, Biotium, CA, USA) for visualization, under a BioDoc gel documentation system (UVP, Upland, CA, USA). The expected length of the amplified DNA fragment was about 529 bp. Afterward, PCR assay was carried out on the positive samples targeting the GRA6 gene using forward (5'-TTTCCGAGCAGGTGACCT-3') and reverse (5'-TCGCCGA AGAGTTGACATAG -3') primers [17]. PCR reaction volumes were the same as described for 529 bp gene. An initial denaturation at 94 °C for 5 min was followed by 35 cycles of denaturation at 94 °C for 35 s, annealing at 56 °C for 45 s, extending at 72 °C for 1 min, and finally a final extension at 72 °C for 10 min. After staining with GelRed nucleic acid gel stain, PCR products were electrophoresed on 1.5% agarose gel. The amplicons were visualized under UV illumination. The expected size of the amplified DNA fragments was 344 bp.

### 2.5. DNA sequencing and phylogenetic analysis

Sequence analysis of GRA6 gene was performed to determine the *T. gondii* genotype. PCR products of GRA6 gene were purified and were sequenced in both directions, using the same primers as used in the PCR. After being edited by the Geneious software, the sequence results were analyzed, using Basic Local Alignment Search Tool (BLAST) system in comparison with GenBank reference sequences. The phylogenetic relationships were constructed among GRA6 gene sequences, obtained in this study, and reference sequences deposited in GenBank and sequence of *Hammondia hammondi* as the out-group, using Maximum-likelihood method, based on the Kimura 2-parameter model in Mega-X software. Bootstrap analyses (using 1000 replicates) were carried out to determine the robustness of the findings.

### 2.6. Real-Time PCR

The Real-Time PCR was used to quantitatively detect *T. gondii* DNA in the mothers' buffy coats and also fetuses' tissues. The forward and reverse sequences of the applied primers which amplified a 130 bp region of *T. gondii*, were 5'-GAAGAGAGGAAACAGGTGGTTCG -3' and 5'-CCGCTCCTTCGTCCGTCGTA -3', respectively [18]. The Real-Time PCR reaction was consisted of a final volume of 20  $\mu$ L including, 50 ng of template DNA, 10  $\mu$ L of 2x SYBR Green PCR Master Mix (Applied Biosystems, US) and 0.5  $\mu$ M of each primer. The reaction was carried out using the StepOnePlus real-time PCR instrument (Applied Biosystems, US) as follow: 10 min hot start at 95 °C, 35 cycles at 95 °C for 10 s, 68 °C for 30 s and 72 °C for 30 s. All assays were performed in duplicates. To evaluate the identity of the amplified product, a melting curve analysis was carried out after completion of any amplification, using 0.5 °C/s as a melt rate with an interval of 70 to 90 °C. For electrophoretic analysis, 10  $\mu$ L of PCR products were run on a 1.5% agarose gel. For the standard curves, serial dilutions of RH-strain of *T. gondii*

tachyzoite, ranging from 10<sup>5</sup> to 10<sup>1</sup> tachyzoites/mL were prepared and the DNA was extracted from the parasite in each dilution. Quantity of parasitemia in each sample was calculated, using the standard curve which plotted by the average CT against the parasite number.

2.7. Statistical analysis

The statistical analysis of the data was performed using SPSS (Ver. 18) software (IBM, Armonk, New York, USA). Chi-square test was employed to determine the association between seropositivity for toxoplasmosis and qualitative variables. A value of less than 0.05 was considered as statistically significant.

3. Results

3.1. Serological results

The age of women with spontaneous abortions ranged from 16 to 46 years old and the mean age was 28 ( ± 6.2) years. The average length of women pregnancy was 12.9 weeks. All of the pregnant women with abortion were in the first trimester of their prenatal period. Generally, 70% of them had no previous history of abortion. The majority of women (92%) were housewives.

Anti-Toxoplasma antibodies were detected in the sera of 10 out of 100 (10%) mothers, including 7 IgG and 3 IgM cases. None of the cases were simultaneously seropositive for both IgG and IgM. Four out of 7 IgG positive cases had a history of repeated abortion.

Results of the univariate analysis showed that *T. gondii* seropositivity was associated with consumption of undercooked meat and with the place of residence (p < 0.05). However, no significant association was found between *T. gondii* seropositivity and other variables such as contact with soil and cat, age and history of abortion (p > 0.05). Table 1 summarizes the seropositivity to *Toxoplasma* infection according to sociodemographic characteristics of the women with spontaneous abortion.

3.2. Molecular detection of *T. gondii* in women with spontaneous abortion and their aborted fetuses

From 100 buffy coats of the pregnant women, *Toxoplasma* DNA was detected in 2 (2%) of cases, targeting either RE or GRA6 genes (Fig. 1). No amplification was observed in 100 fetuses' tissue samples. Considering the serostatus of the 2 PCR-positive cases, both were seropositive for anti-Toxoplasma IgM antibodies.

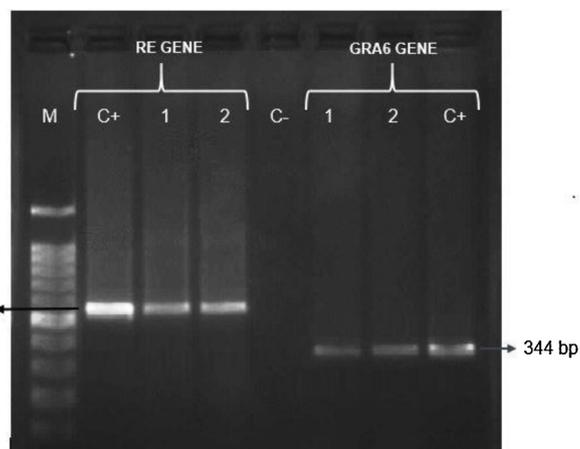


Fig. 1. PCR products of isolated *T. gondii* from the buffy coat of pregnant women with spontaneous abortion. M: 100 bp molecular weight marker; C+, positive control; C-, negative control; lanes 1 and 2: PCR product of GRA6 and RE genes.

Sequence analysis was performed for the two isolates to find out the genotype of *T. gondii*. The *GRA6* sequences obtained in this study were deposited in the GenBank database with accession numbers MK705889 and MK705890. BLAST analysis revealed that both of the isolates had the highest degree of similarity with genotype I of *T. gondii*. Phylogenetic analysis showed that two isolates of *T. gondii* obtained in this study had 100% homologies with each other and also with *T. gondii* isolated from fox in Italy (Accession no. MG587986), in Poland (Accession no. MH429064) and rodents and stray cats from Iran (Accession no. LC414527) (Fig. 2). Intra-species variations of the isolates were 0-2.7%.

The melting curve of reaction products in Real-Time PCR assay showed a single peak with the melting temperature of 81.6 °C. The standard curve was linear over at least five serial dilutions of the parasite DNA with the correlation coefficient (R2) value of 0.99.

Presence of *T. gondii* DNA B1 gene was detected in 3 out of 100 (3%) of buffy coats of pregnant women, using Real-Time PCR. Of these, 2 (2%) were seropositive for IgM and the other one was seronegative for both IgG and IgM. In the IgM seropositive cases (n = 2), the parasite burden varied from 43 to 45 tachyzoite genome-equivalents per milliliter of blood while this ratio was 16 for the seronegative (n = 1) case.

Table 1

Univariate analysis of the variables associated with *T. gondii* seropositivity in the pregnant woman with spontaneous abortion.

Characteristics	Frequency No.	Seropositivity (%)	Odds ratio (95% confidence interval)	p value
<b>Place of residence</b>				
Rural	53	12.8%	7.610 (.881-65.736)	0.033*
Urban	47	1.9%	1	
<b>Eating of semi-cooked meat</b>				
Yes	36	13.9%	5 (0.917-27.248)	0.043*
No	64	3.1%	1	
<b>Contact with soil</b>				
Yes	46	8.7%	1.619(.343-7.641)	0.054
No	54	5.6%	1	
<b>Contact with cat</b>				
Yes	50	8.0%	1.362(.289-6.426)	0.695
No	50	6.0%	1	
<b>History of abortion</b>				
Yes	27	14.8%	4.058(.845-19.492)	0.63
No	73	4.1%	1	
<b>Age group</b>				
< 25	33	3.0	2.286(.133-39.203)	0.510
25-35	52	9.6%	0.671(.072-6.234)	
> 35	15	6.7%	1	

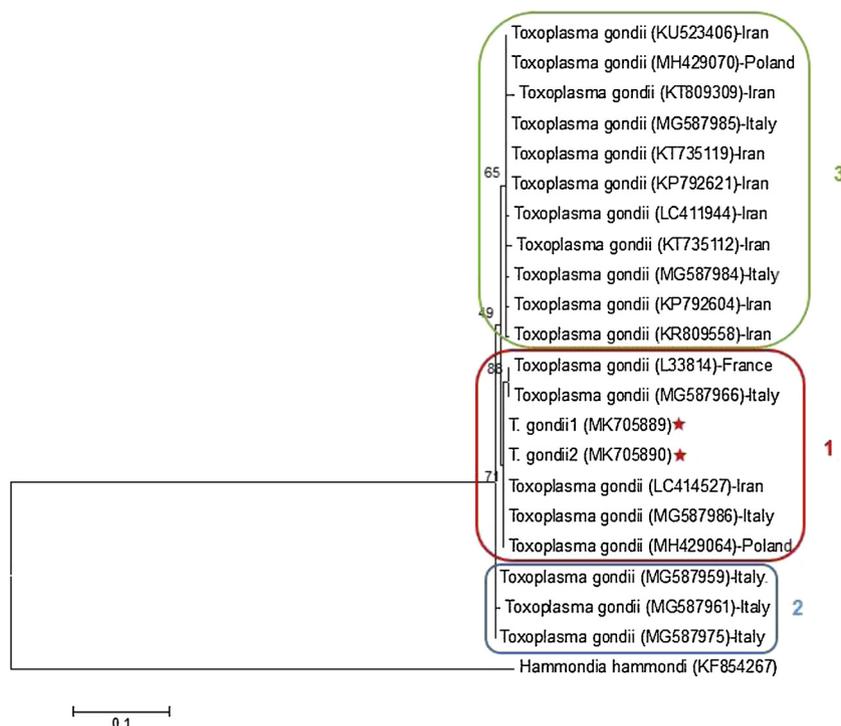


Fig. 2. Phylogenetic relationship of *T. gondii* isolates obtained in this study and reference sequences retrieved from GenBank, using the Maximum Likelihood method based on *GRA6* gene with *Hammondia hammondi* as the out-group. 1: genotype I *T. gondii*, 2: genotype II *T. gondii*, 3: genotype III *T. gondii*.

### 3.3. Agreement between ELISA, PCR and real-time PCR methods

Results of seropositivity, using ELISA, were in good agreement ( $\kappa = 0.656$ ) with Real-Time and conventional PCR ( $\kappa = 0.795$ ). It was also noted a substantial agreement between Real-Time and conventional PCR ( $\kappa = 0.795$ ).

## 4. Discussion

The incidence of congenital human toxoplasmosis is not yet clear in Iran, although the role of *Toxoplasma*-related abortion in the human and sheep has been elucidated in a few studies [19–21]. The current study was conducted on pregnant women with spontaneous abortion in southwest Iran, using serological and molecular approaches. ELISA, Real-Time PCR, and conventional PCR methods revealed *T. gondii* in 10%, 3%, and 2% of the studied pregnant women, respectively. The rate of seroprevalence of *T. gondii* antibodies in this study revealed that 7% of pregnant women who were seropositive for IgG did not have *T. gondii* DNA in their buffy coat when evaluated by either Real-Time or conventional PCR. This mainly indicates previous exposure to *Toxoplasma* in the seropositive cases with no risk of congenital toxoplasmosis in immunocompetent individuals.

Furthermore, anti-*Toxoplasma* IgM antibodies were detected in 3 cases and two of these cases were PCR-positive. Also, the parasite load estimated, using Real-Time PCR, was 43 and 45 parasites/mL in IgM positive cases which indicates a temporary parasitemia in blood and the risk of congenital transmission from mother to the aborted fetus.

In one of the buffy coat samples of the pregnant women for whom the results of serological testing and PCR were negative, *T. gondii* DNA was detected by Real-Time PCR and the parasite load was estimated at 16 parasites/mL. The presence of *T. gondii* DNA by Real-Time PCR in the sample might be due to a more recent infection without having enough antibodies to be detected in the ELISA method. Detection of *Toxoplasma* DNA by Real-Time PCR, but not conventional PCR, in one of the samples could be due to the greater sensitivity of this method in comparison with the conventional PCR.

In the current study, eating undercooked meat and place of residence were associated with seropositivity to toxoplasmosis. This finding is consistent with the previous reports which indicated that *T. gondii* infection is linked with consumption of raw or undercooked meat [14,19,22].

Seroprevalence rate of toxoplasmosis in the studied population was lower than the rate reported among Iranian pregnant women (47.3%), with a history of abortion. However, the prevalence of IgM-positive toxoplasmosis in pregnant women in this study was higher in comparison with some of the studies conducted on pregnant women in Iran and other countries [23,24]. Differences in the lifestyle, nutritional habits, age group, and the studied population, as well as differences in the approaches, applied for the detection of anti-*Toxoplasma* antibodies, may be accounted for the variation in the seroprevalence of toxoplasmosis in this and other similar studies.

*Toxoplasma* DNA was not detected in the tissues of human spontaneous aborted fetuses by either PCR or Real-Time PCR. This finding is in keeping with a previous study on aborted fetuses from Arak, in Iran, where no *T. gondii* DNA was detected in the aborted fetuses.

On the other hand, at least two studies in Iran reported the isolation of *Toxoplasma* DNA in the placenta of women who had experienced abortion. In the first study conducted in Shiraz, southern Iran, *Toxoplasma* DNA has been detected in 14.4% of placenta of women with spontaneous abortion. Also in a case-control study in Tehran, capital of Iran, the rate of *T. gondii* infection in placental tissues of pregnant women with abortion was reported to be 6.4% [25–27]. In other studies on blood samples of pregnant women from Egypt and umbilical cord tissue from human neonates in Libya, *Toxoplasma* prevalence rates of 11.81% and 9.9% have been respectively reported. In all of these studies, the *Toxoplasma* DNA has been detected in the placenta or umbilical cord, but not in the tissues of aborted fetuses. Nevertheless, detection of *Toxoplasma* DNA in the placenta or umbilical cord samples does not necessarily mean the fetus infection [28,29].

Detection of *T. gondii* DNA from buffy coat of women who had experienced abortion, without detection of the parasite DNA in samples from aborted fetuses in our study may be due to a variety of reasons.

Firstly, the risk of transplacental transmission of *T. gondii* to the fetus in the first trimester is as low as 10% to 25% in comparison with the third trimester which ranges from 60% to 90%. Accordingly, the *Toxoplasma* infection might not be transmitted to the fetus, despite the presence of infection in the mother. Secondly, the parasite load in the sample of aborted tissues might be low. Support for this concept comes from Romand study who reported that parasite load greater than 100/mL can cause risk of severe fetal outcome while the parasite load in our cases were much less than this limit [30]. Lastly, as previous studies indicated, *Toxoplasma* type I is less capable of crossing the human placental barrier than the other genotypes and in our study; both of positive cases were infected with type I of *Toxoplasma* [31].

GRA6 marker is a single-copy gene with sufficient polymorphism that well suited for typing as well as the assessment of genetic diversity of *Toxoplasma*. In the current study, sequences of the isolates obtained from the pregnant woman with acute infection had the highest similarity with *T. gondii* type I. This finding is consistent with the findings of previous studies conducted in Iran and other countries. Type I had been recognized in a fatal congenital toxoplasmosis case in Shiraz, Southern Iran, as well as in ovine aborted fetuses in Qazvin and Khorasan Razavi Provinces in Iran [12,20,21].

In contrast to these findings, *Toxoplasma* type II is the most often reported type in the pregnant woman with abortion in Egypt, infants with congenital toxoplasmosis in Poland and France, as well as in Argentina, Mexico, Tunisia, and Brazil. Atypical and mixed genotypes have also been recognized as the causative agent of congenital toxoplasmosis in human [31–35].

Phylogenetic analysis revealed that both of our *Toxoplasma* isolates were closely related to type I of *T. gondii* at the GRA6 locus. Moreover, the findings showed that the GRA6 marker appropriately shows the similarity and differences between our isolates and other isolates from Iran and other countries. Also, intra-divergence was seen in GRA6 gene in this study which is consistent with the previous studies which reported a high degree of genetic variability for GRA6 [13,36,37].

Unlike the present study, previous reports have generally used placenta samples or umbilical cord as an indicator of fetus infection. However, this study was performed on pregnant women and aborted fetuses' tissues, using both molecular and serological methods. This can be considered as the main strength of the study. However, the study has its limitations, including not using the several single-copy genes with high polymorphism and having somewhat relatively small sample size.

## 5. Conclusion

Taken together, our findings revealed *T. gondii* type I in buffy coat samples of two mothers with spontaneous abortion, but the infection was not detected in the fetuses' tissues. Sequence analysis of the isolates revealed homologies with each other and also with isolates from animals and human from other areas of the world. The isolates were taxonomically grouped into one clade where the isolates from Italy, Iran, and Poland were positioned. Findings of the current study also revealed that Real-Time PCR, in comparison with conventional PCR, has greater sensitivity for detection of *T. gondii* DNA in the buffy coat samples.

It is necessary to examine more aborted fetuses' samples from different geographical areas in future studies, with larger sample size, to determine the association between *Toxoplasma* genotype and abortion.

## Funding

The study was financially supported by the vice chancellor for research at Shiraz University of Medical Sciences (Grant No. 95-01-01-12416).

## Declaration of Competing Interest

None to declare.

## Acknowledgment

The study was the subject of the Ph.D. dissertation of Nasir Arefkhan.

## References

- [1] J.P. Dubey, *Toxoplasmosis of Animals and Humans*: CRC Press, 2nd ed., CRC Press, Boca Raton, FL, 2019.
- [2] J.D. Kravetz, D.G. Federman, *Toxoplasmosis in pregnancy*, *Am. J. Med.* 118 (2005) 212–216.
- [3] J.M. Hughes, D.G. Colley, A. Lopez, V.J. Dietz, M. Wilson, T.R. Navin, J.L. Jones, Preventing Congenital Toxoplasmosis. Morbidity and Mortality Weekly Report: Recommendations and Reports, (2000), pp. 57–75.
- [4] D.V. Vasconcelos-Santos, D.O.M. Azevedo, W.R. Campos, F. Oréfice, G.M. Queiroz-Andrade, Carellos ÉVM, R.M.C. Romanelli, J.N. Januário, L.M. Resende, O.A. Martins-Filho, Congenital toxoplasmosis in southeastern Brazil: results of early ophthalmologic examination of a large cohort of neonates, *Ophthalmology* 116 (2009) 2199–2205 e1.
- [5] I. Villena, T. Ancelle, C. Delmas, P. Garcia, A. Brezin, P. Thulliez, M. Wallon, L. King, V. Goulet, Congenital toxoplasmosis in France in 2007: first results from a national surveillance system, *Eurosurveillance* 15 (2010) 19600.
- [6] F. Rosso, J.T. Les, A. Agudelo, C. Villalobos, J.A. Chaves, G.A. Tunubala, A. Messa, J.S. Remington, J.G. Montoya, Prevalence of infection with *Toxoplasma gondii* among pregnant women in Cali, Colombia, South America, *Am. J. Trop. Med. Hyg.* 78 (2008) 504–508.
- [7] M. Paul, E. Petersen, Z.S. Pawlowski, J. Szczapa, Neonatal screening for congenital toxoplasmosis in the Poznań region of Poland by analysis of *Toxoplasma gondii*-specific IgM antibodies eluted from filter paper blood spots, *Pediatr. Infect. Dis. J.* 19 (2000) 30–36.
- [8] A.L. Vallochi, C. Muccioli, M.C. Martins, C. Silveira, R. Belfort Jr, L.V. Rizzo, The genotype of *Toxoplasma gondii* strains causing ocular toxoplasmosis in humans in Brazil, *Am. J. Ophthalmol.* 139 (2005) 350–351.
- [9] S. Hosseini, A. Amouei, M. Sharif, S. Sarvi, L. Galal, J. Javidnia, A. Pagheh, S. Gholami, A. Mizani, A. Daryani, Human toxoplasmosis: a systematic review for genetic diversity of *Toxoplasma gondii* in clinical samples, *Epidemiol. Infect.* (2019) 147.
- [10] A. Rostami, P. Karanis, S. Fallahi, Advances in serological, imaging techniques and molecular diagnosis of *Toxoplasma gondii* infection, *Infection* 46 (2018) 303–315.
- [11] Q. Liu, Z.-D. Wang, S.-Y. Huang, X.-Q. Zhu, Diagnosis of toxoplasmosis and typing of *Toxoplasma gondii*, *Parasit. Vectors* 8 (2015) 292.
- [12] B. Sarkari, S. Abdolahi Khabisi, Severe congenital toxoplasmosis: a case report and strain characterization, *Case Rep. Infect. Dis.* 2015 (2015).
- [13] N. Zia-Ali, A. Fazaeli, M. Khoramizadeh, D. Ajzenberg, M. Dardé, H. Keshavarz-Valian, Isolation and molecular characterization of *Toxoplasma gondii* strains from different hosts in Iran, *Parasitol. Res.* 101 (2007) 111–115.
- [14] A. Moshfe, N. Arefkhan, B. Sarkari, S. Kazemi, A. Mardani, *Toxoplasma gondii* in blood donors: a study in Boyer-Ahmad county, Southwest Iran, *Interdiscip. Perspect. Infect. Dis.* 2018 (2018).
- [15] B. Sarkari, R. Shafiei, M. Zare, S. Sohrabpour, L. Kasraian, Seroprevalence and molecular diagnosis of *Toxoplasma gondii* infection among blood donors in southern Iran, *J. Infect. Dev.* 8 (2014) 543–547.
- [16] M. Foroutan-Rad, S. Khademvatan, H. Majidiani, S. Aryamand, F. Rahim, A.S. Malehi, Seroprevalence of *Toxoplasma gondii* in the Iranian pregnant women: a systematic review and meta-analysis, *Acta Trop.* 158 (2016) 160–169.
- [17] S. Boughattas, R. Ben-Abdallah, E. Siala, O. Souissi, K. Aoun, A. Bouratbine, Direct genotypic characterization of *Toxoplasma gondii* strains associated with congenital toxoplasmosis in Tunisia (North Africa), *Am. J. Trop. Med. Hyg.* 82 (2010) 1041–1046.
- [18] P. Flori, J. Hafid, T. Bourlet, H. Raberin, C. Genin, R.T. Sung, Experimental mode of congenital toxoplasmosis in guinea-pigs: use of quantitative and qualitative PCR for the study of maternofetal transmission, *J. Med. Microbiol.* 51 (2002) 871–878.
- [19] Q. Asgari, M. Fekri, A. Monabati, M. Kalantary, I. Mohammadpour, M.H. Motazedian, B. Sarkari, Molecular genotyping of *Toxoplasma gondii* in human spontaneous aborted fetuses in Shiraz, Southern Iran, *Iran. J. Public Health* 42 (2013) 620–625.
- [20] L. Danehchin, G. Razmi, A. Naghibi, Isolation and genotyping of *toxoplasma gondii* strains in ovine aborted fetuses in Khorasan Razavi Province, Iran, *Korean J. Parasitol.* 54 (2016) 15.
- [21] G. Habibi, A. Imani, M. Gholami, M. Hablolvarid, A. Behroozkhan, M. Lotfi, M. Kamalzade, E. Najjar, K. Esmaeil-Nia, S. Bozorgi, Detection and identification of *Toxoplasma gondii* type one infection in sheep aborted fetuses in Qazvin Province of Iran, *Iran. J. Parasitol.* 7 (2012) 64.
- [22] Q. Asgari, J. Sarnevesht, M. Kalantari, S.J. Sadat, M.H. Motazedian, B. Sarkari, Molecular survey of *Toxoplasma* infection in sheep and goat from Fars province, Southern Iran, *Trop. Anim. Health Prod.* 43 (2011) 389–392.
- [23] J. Saki, N. Mohammadpour, F. Moramezi, S. Khademvatan, Seroprevalence of *Toxoplasma gondii* type one infection in comparison with the women with normal delivery in Ahvaz, southwest of Iran, *Sci. World J.* 2015 (2015).
- [24] L. Nimri, H. Pelloux, L. Elkhatib, Detection of *Toxoplasma gondii* DNA and specific antibodies in high-risk pregnant women, *Am. J. Trop. Med. Hyg.* 71 (2004) 831–835.
- [25] S. Matin, G. Shahbazi, S.T. Namin, R. Moradpour, F. Feizi, H. Piri-dogahe, Comparison of placenta PCR and maternal serology of aborted women for detection

- of *Toxoplasma gondii* in Ardabil, Iran, Korean J. Parasitol. 55 (2017) 607.
- [26] Q. Asgari, M. Fekri, A. Monabati, M. Kalantary, I. Mohammadpour, M.H. Motazedian, B. Sarkari, Molecular genotyping of *Toxoplasma gondii* in human spontaneous aborted fetuses in Shiraz, Southern Iran, Iran. J. Public Health 42 (2013) 620.
- [27] F.S. Ghasemi, S. Rasti, A. Piroozmand, M. Bandehpour, B. Kazemi, S.G.A. Mousavi, A. Abdoli, Toxoplasmosis-associated abortion and stillbirth in Tehran, Iran, J. Matern. Neonatal Med. 29 (2016) 248–251.
- [28] H.M. Ibrahim, A.H. Mohamed, A.A. El-Sharaawy, H.E. El-Shqanqery, Molecular and serological prevalence of *Toxoplasma gondii* in pregnant women and sheep in Egypt, Asian Pac. J. Trop. Med. 10 (2017) 996–1001.
- [29] S.Z. Haq, M.S. Abushahama, O. Gerwash, J.M. Hughes, E.A. Wright, M.S. Elmahaishi, Z.-R. Lun, D. Thomasson, G. Hide, High frequency detection of *Toxoplasma gondii* DNA in human neonatal tissue from Libya, Trans. R. Soc. Trop. Med. Hyg. 110 (2016) 551–557.
- [30] S. Romand, M. Chosson, J. Franck, M. Wallon, F. Kieffer, K. Kaiser, H. Dumon, F. Peyron, P. Thulliez, S. Picot, Usefulness of quantitative polymerase chain reaction in amniotic fluid as early prognostic marker of fetal infection with *Toxoplasma gondii*, Am. J. Obstet. Gynecol. 190 (2004) 797–802.
- [31] D. Ajzenberg, N. Cogné, L. Paris, M.-H. Bessières, P. Thulliez, D. Filisetti, H. Pelloux, P. Marty, M.-L. Dardé, Genotype of 86 *Toxoplasma gondii* isolates associated with human congenital toxoplasmosis, and correlation with clinical findings, J. Infect. Dis. 186 (2002) 684–689.
- [32] D. Abdel-Hameed, O. Hassanein, Genotyping of *Toxoplasma gondii* strains from female patients with toxoplasmosis, J. Egypt. Soc. Parasitol. 38 (2008) 511–520.
- [33] D. Nowakowska, I. Colón, J.S. Remington, M. Grigg, E. Golab, J. Wilczynski, L.D. Sibley, Genotyping of *Toxoplasma gondii* by multiplex PCR and peptide-based serological testing of samples from infants in Poland diagnosed with congenital toxoplasmosis, J. Clin. Microbiol. 44 (2006) 1382–1389.
- [34] C.P. Rico-Torres, L.F. Valenzuela-Moreno, H. Luna-Pastén, R. Figueroa-Damián, V. Gómez-Toscano, L. Hernández-Delgado, M.P. Escobedo-Torres, D. Correa, High heterogeneity, mixed infections and new genotypes in human congenital toxoplasmosis cases in the mega-metropolis of Central Mexico, Acta Trop. 178 (2018) 124–129.
- [35] H. Yera, D. Ajzenberg, F. Lesle, D. Eyrolle-Guignot, M. Besnard, A. Baud, A. Georges, J. Dupouy-Camet, M.L. Dardé, New description of *Toxoplasma gondii* genotypes from French Polynesia, Acta Trop. 134 (2014) 10–12.
- [36] S. Zakimi, H. Kyan, M. Oshiro, C. Sugimoto, X. Xuenan, K. Fujisaki, Genetic characterization of GRA6 genes from *Toxoplasma gondii* from pigs in Okinawa, Japan, J. Vet. Med. Sci. 68 (2006) 1105–1107.
- [37] S. Sousa, D. Ajzenberg, M. Marle, D. Aubert, I. Villena, J.C. da Costa, M.-L. Dardé, Selection of polymorphic peptides from GRA6 and GRA7 sequences of *Toxoplasma gondii* strains to be used in serotyping, Clin. Vaccine Immunol. 16 (2009) 1158–1169.