BMC Research Notes

RESEARCH NOTE Open Access

Serological and molecular survey of *Toxoplasma Gondii* in aborted livestock fetuses from Northeast Iran

Reza Shafiei[1], Nima Firouzeh[1*] and Mohammad Taghi Rahimi[2]

Abstract

Background Toxoplasmosis not only leads to abortion in humans but also in herbivores, which causes significant financial and quality-adjusted life-year losses. The present study aimed to determine the prevalence of toxoplasmosis in aborted fetuses *via* serological and molecular assays. Moreover, the genotypes of the obtained isolates were detected.

Methods Serological and molecular methods were used to study aborted fetuses from Bojnourd City, North Khorasan Province, Iran, which included 52 ovines and 16 bovines. Nested PCR of the B1 gene was used to detect parasite DNA in brain tissues. The PCR-RFLP method for the GRA6 gene was used to determine the genotype of *T. gondii*.

Results Out of 68 aborted fetuses, 16.1% showed the presence of anti-*T. gondii* IgG. Among these, 11.7% were identified in bovine fetuses and 4.4% in ovine fetuses. Additionally, two (2.94%) samples of ovine tested positive for anti-*T. gondii* IgM. Our PCR analysis detected parasite DNA in two cases (2.94%) among 11 IgG-positive samples. All obtained isolates belong to type I of *T. gondii*.

Conclusion Infection with Type I of *T. gondii* during the neonatal period may partly be responsible for abortion and economic losses in livestock farming in our studied region. To understand the molecular epidemiology and genotypes of *T. gondii* associated with abortion, further evaluation of aborted samples from different geographical locations is necessary.

Keywords *Toxoplasma*, Abortion, Molecular, Genotyping, Ruminant fetuses

*Correspondence:
Nima Firouzeh
Nimafirouzeh4@gmail.com
[1]Vector-Borne Disease Research Center, North Khorasan University of Medical Sciences, Bojnurd, Iran
[2]Department of Basic Medical Sciences, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran

Introduction

Abortion in ruminant animals is a multifactorial event with infectious and non-infectious causes. Pathogens like viruses, bacteria, fungi, and protozoa, as well as nutritional deficiencies, physical stressors, toxic substances, and chemical agents, can all lead to abortion in these animals [1–3].This complex interaction of factors increases the risk of abortion, which has detrimental effects on the health and productivity of ruminant animals. The economic consequences for the livestock industry are significant due to the expenses involved in diagnosing, treating, and preventing abortion [4].

The main infectious etiological agents causing sheep and cattle abortion are *Brucella*, *Salmonella*, *Mycoplasma*, *Chlamydia abortus*, *and Coxiella burnetii*, in addition to *Toxoplasma gondii* (*T.gondii*) [5].

T. gondii is a common and significant foodborne zoonotic pathogen with medical and veterinary significance. Felines mainly cats, are definitive hosts in the life cycle of *T.gondii* and excrete millions of resistant oocysts into the environment. Almost all warm-blooded plays a role in transmission cycle as intermediate hosts such as sheep, goat, cattle, pigs and camels or aberrant hosts as humans [6, 7]. The sexual reproduction of this parasite relies on cats, which results in the excretion of oocysts in their feces. These oocysts are consumed by grazing animals like sheep or chickens and small wild animals like rats. Once the parasite infects these animals, it typically forms cysts in their brain and muscles. The Toxoplasma life cycle is completed when a cat consumes an infected rat. This cat-rat interaction plays a vital role in transmitting Toxoplasma in the ecosystem and has implications for the health of domestic animals and humans [8].

T. gondii can be transmitted through three main routes. The first is the ingestion of oocysts that are shed by the definitive host. The second mechanism involves consuming raw or undercooked meat that contains tissue cysts of the parasite. The third mechanism is the congenital transmission [9–11].

T. gondii infection in ruminants is typical without clinical signs. However, it could be dangerous for the developing fetus during pregnancy, potentially resulting in mummification, macerated, aborted, or stillborn [12]. Toxoplasma-related abortion is a persistent problem globally, affecting human populations and sheep and cattle breeding industries. Additionally, *T. gondii* infection in ruminants can pose a risk to human health through the consumption of contaminated meat or milk products, making it a public health concern [13].

The estimated monetary burden due to congenital toxoplasmosis in the UK ranges from $1.2 million to $12 million, and Brazil's annual economic loss caused by ovine toxoplasmosis is estimated at around $1.491 million [14, 15]. Toxoplasmosis contributes to a substantial disease burden globally, with an estimated 1.87 million Disability-Adjusted Life Years (DALYs) attributed to food- and vector-borne parasitic zoonoses, as outlined in the WHO roadmap for 2030 [16].

The diagnosis of toxoplasmosis in ruminants (or aborted fetuses) typically involves a combination of serological, molecular, bioassay and histopathology examinations. Most epidemiological studies on *T. gondii* have primarily relied on extensive serological testing conducted worldwide, including in Iran [17, 18].

The global prevalence varies with seroprevalence rates ranging from 75% in dogs, 11–36% in pigs, 11–61% in goats, less than 10% in cows, and 35–73% in cats and humans [19, 20].In comparison, this value in Iran has been documented in various regions of Iran, with sheep ranging from 13.8 to 35%, goats from 13.1 to 30% and cattle from 0 to 16% [21].

Against serological methods, molecular surveys are effective in confirming the causative role of *T. gondii* in abortion cases and providing information on the genetic diversity of the parasite [18].

The molecular surveys about genetic diversity of *T. gondii* have demonstrated that this parasite can be classified into three distinct groups: genotype I, characterized by its virulent nature, and genotypes II and III, consisting of non-virulent strains [22, 23].

Considering the significant impact of *T. gondii* infection on abortion in livestock and indefinite prevalence of infection in aborted fetuses in our studied area, present research was designed to determine the infection rate by both serological molecular and methods. Additionally, the genetic diversity of isolated strains was investigated.

Methods

Study area

This cross-sectional study was performed from March to April 2021 in Bojnurd City in North Khorasan Province in northeastern Iran (Fig. 1). Bojnurd City is the province's capital (37° 28′ N and 57° 20′ E) and has an area of about 17.68 km^2 and over 230,000 people. The city is located in a semi-arid area, with an elevation of 1070 m above sea level, with hot summers and cool winters. Bojnourd weather station data shows rainfall and average annual temperature are 243–292 mm and 7–15 °C, respectively [24, 25].

Sampling

The present survey was conducted on 68 spontaneously aborted fetuses (52 ovine and 16 bovines) in Bojnurd City, Iran, from March 2020 to February 2021. All fetuses were referred to the North Khorasan Veterinary Organization. Next, blood and brain tissues of aborted fetuses (gestational age of ≥120) were collected and preserved

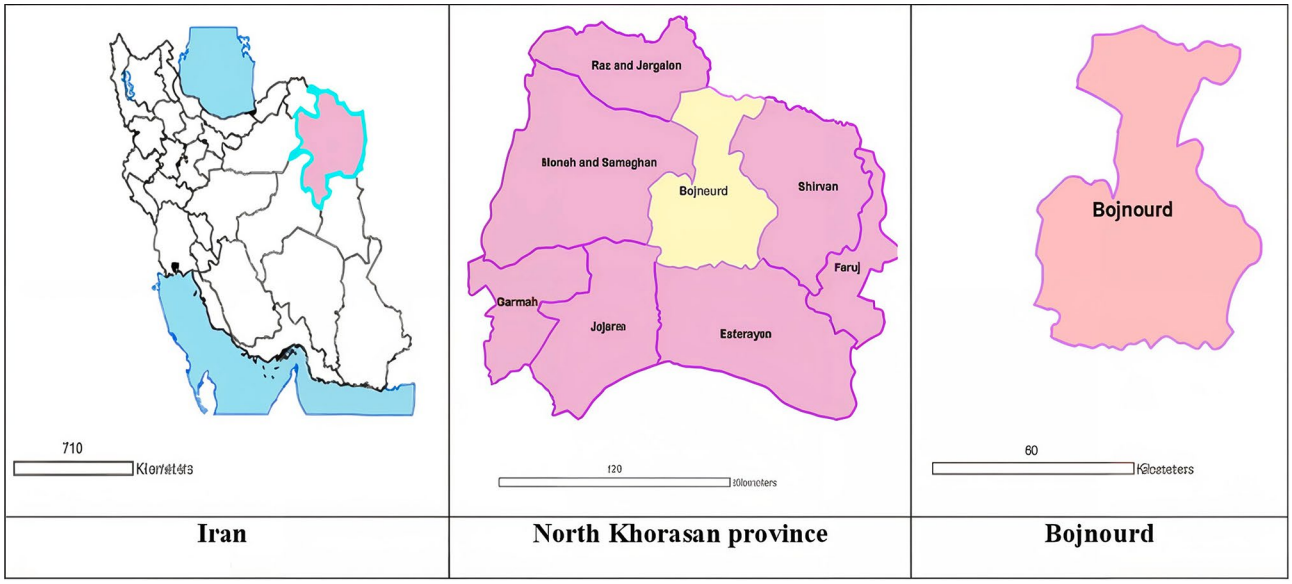

Fig. 1 Map of North Khorasan province as the study area

at −20 °C until serological and molecular examination, respectively.

Serological survey

The presence of IgG and IgM antibodies of *T. gondii* was determined *via* conventional ELISA technique based on the manufacturer's instructions (ID VET Innovative Diagnostic, Montpellier, France).

DNA extraction

All Toxo IgG-positive samples were investigated by PCR assay on the brain tissue of the same fetuses. The DNA extraction was done using (Gene All, Exgene, Cell SV mini, Korea) kit and according to the manufacturer`s instruction.

Nested-PCR for B1 gene

The Nested-PCR assay was performed *via* two repeated genomic targets, B1, to detect T. gondii DNA in fetuses' brain tissues [26].

Two PCR primer pairs of the B1 gene, S1 (5′-CGACA GAAAGGGAGCAAGAG-3′) and AS1 (5′-ACGCTGTG TCTCCTCTAGGC-3′), S2 (5′-TCTTCCCAGACGTGG ATTTC-3′) and AS2 (5′-CTCGACAATACGCTGCTTG A-3′), were employed to amplifying 531 bp fragment.

The first amplification was carried out in 20 µl of reaction mixture containing one µl of each primer (*S1* and *AS1*), ten µl Master mix (Ampliqon Company, Denmark), two µl extracted DNA from heart or diaphragm samples and 6 µl sterilized distilled water. The first PCR was performed in a thermocycler (Flex Cycler) for initial denaturation at 94 °C for 3 min; this step was followed by 35 cycles of denaturation at 94 degrees for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 2 min and a final extension step at 30 °C for 1 min. The second amplification was performed in 20 µl reaction mixture. The first PCR product was diluted with a ratio of 1:40 to distilled water and then used as a template. The twenty-µl reaction mixture contained 1 µl of each primer (*S2* and *AS2*), 8 µl Master mix (Ampliqon Company, Denmark), one µl of our new template and nine µl distilled water sterilized. The second PCR was performed in 30 cycles.

Nested PCR for GRA6 gene

The positive samples of Nested-PCR of the B1 gene were selected for Nested-PCR of GRA6 gene. GRA6, a highly polymorphic gene, is repeated in the genome of *T. gondii*. It is particularly suitable for distinguishing between three distinct types: type I, II and III, from each other, primarily type III, which is close to type I. Two PCR primer pairs of the GRA6 gene, GRA6FO (5′GGCAAACAAAACGA AGTG-3′) and GRA6RO (5′-CGACTACAAGACATAG AGTG-3′) used in first amplification, and GRA6R (5′-G TAGCGTGCTTGTTGGCGAC-3′) and GRA6 (5′TACA AGACATAGAGTGCCCC-3′) used in second amplification. All procedures were performed based on previously published works [21, 27].

The first amplification was carried out in 25 µl of reaction mixture containing 1 µl of each primer (GRA6FO and GRA6RO), 8 µl Master mix (Ampliqon Company, Denmark), 5 µl extracted DNA of heart or diaphragm samples and 10 µl sterilized distilled water. The first PCR was performed in a thermocycler (Flex Cycler) for initial denaturation at 94 °C for 5 min; this step was followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 60 s, extension at 72 °C for 90 s and a final

extension step at 72 °C for 7 min. The second amplification was performed in 25 µl reaction mixture. The first PCR product was used as a template while diluted with a ratio of 1:10 to distilled water. Twenty-five microliters reaction mixture contained 1 µl of each primer (GRA6R and GRA6), 8 µl Master mix, 1 µl of our new template and 14 µl Distilled water sterilized.

The second PCR was performed at the annealing temperature of 60 °C for the 60s [15]. The PCR products were electrophoresed in a 1.5% agarose gel in tris-borate-EDTA 0.5X (TBE 0.5X) buffer and stained with ethidium bromide. All positive samples of Nested PCR for the GRA6 gene were used to perform the PCR-RFLP technique to differentiate the three types (I, II, III) of *T. gondii.*

PCR-RFLP assay

The GRA6 gene amplified product was digested with *MseI* with *MseI* restriction endonuclease (10 U/μl, 300 units), (Fermentas, Thermo Scientific, USA). A total volume of 30 µl, including 10 µl of GRA6 PCR product, was added with 1 µl of either *Msp*I, 2 µl of 10x Tango buffer (Fermentas, Lithuania) and 17 µl of DD-H2O. According to the manufacturer's instructions, the tubes were incubated at 37 °C for 2–3 h to ensure full cutting of fragments. Five µl of each product and 1 µl of loading buffer were run in 2% gel electrophoresis to analyze the digestion products. This restriction enzyme can differentiate between various genotypes of *T.gondii* by cutting products to 168 and 544 bp, 75 and 623 bp and 97 and 544 bp fragments in type I, II and III, respectively.

Results

Serological data

Table 1 depicts the results of serological tests. From 68 aborted fetuses, 16.17% (11 cases) showed anti-*T. gondii* IgG that 11.7% (8 cases) and 4.4% (3 cases) were in bovine and ovine fetuses, respectively. anti-*T. gondii* IgM was found in 2 aborted ovine fetuses (2.94%).

Nested PCR B1 data

Two positive IgG (11 cases) samples were positive in Nested PCR B1. It's worth mentioning that both of these samples also had positive results for the IgM test (Fig. 2a). We observed the 791 bp fragment of GRA6 gene after Nested PCR on positive samples for B1 gene.

Nested-PCR of GRA6 & PCR-RFLP data

Figure 2b demonstrates the related results of Nested-PCR for the GRA6 gene that was performed on two positive samples of Nested-PCR of B1 gene. Figure 2c exhibits the PCR-RFLP technique data (to determine the genotypes of *T.gondi*) that carry out on positive Nested-PCR for GRA6 gene products. Our data approved that both obtained isolates belong to type I of *T. gondii*.

Discussion

Toxoplasmosis poses a significant public health challenge globally, especially in societies such as Iran [28, 29], where cattle and sheep products are primary food sources [26]. Few studies have confirmed the role of T. gondii in inducing fetal abortion in sheep [30].

Our study revealed that seropositivity in aborted fetuses was 16.17% and molecular findings detected parasite DNA in nearly 3% of examined fetuses. The prevalence of toxoplasmosis-induced abortions in sheep is between 10.6 and 23.1% in the US and Europe [31, 32]. Documents suggest that less than 2% of sheep experience congenital toxoplasmosis and <4% of pregnant sheep transmit the infection to the next generation [17, 33]. Adult Sheep have seropositivity from 4.4% in China to 99.2% in France. In Iran, it was 13–35% for sheep and 18.1% for cattle. [26, 34, 35].

The newest systematic review and meta-analysis report in 2021 showed that based on molecular and serological tests, the prevalence rates of *T. gondii* infection in sheep aborted fetuses and stillbirths globally were 42% (95% CI: 17–67%) and 16% (95% CI: 11–22%), respectively. In this published work, the similar value for cattle was not estimated due to the small number of studies and animals studied [36].The same report with high *T.gondi* antibodies (97.4%) has been documented in sheep herds that experienced abortion outbreaks in Iran [37].

Our findings differ from previous studies in other parts of Iran. In aborted ruminant fetuses, Khorasan Razavi, Hamedan, and Ardebil report 5.2%, 5.9%, and 28% seropositivity against *T. gondii*, respectively. [38–40].

The prevalence of *T. gondii* in aborted livestock fetuses in Iran varies from 2 to 66% across different regions [30, 39, 41–44]. This variation could be attributed to differences in diagnostic methods, sample sizes, parasite strains, ecological circumstances, and hygiene standards [44].

Table 1 Results of serological assays in the examined samples based on animal type

Aborted fetus	Number	ELISA IgG		ELISA IgM	
		Positive	Negative	Positive	Negative
Ovine	52	3 (5.76%)	47 (90.38%)	2 (3.84%)	50 (96.16%)
Bovine	16	8 (50%)	8 (50%)	0	16 (100%)
Total	68	11 (16.17%)		2 (2.94%)	

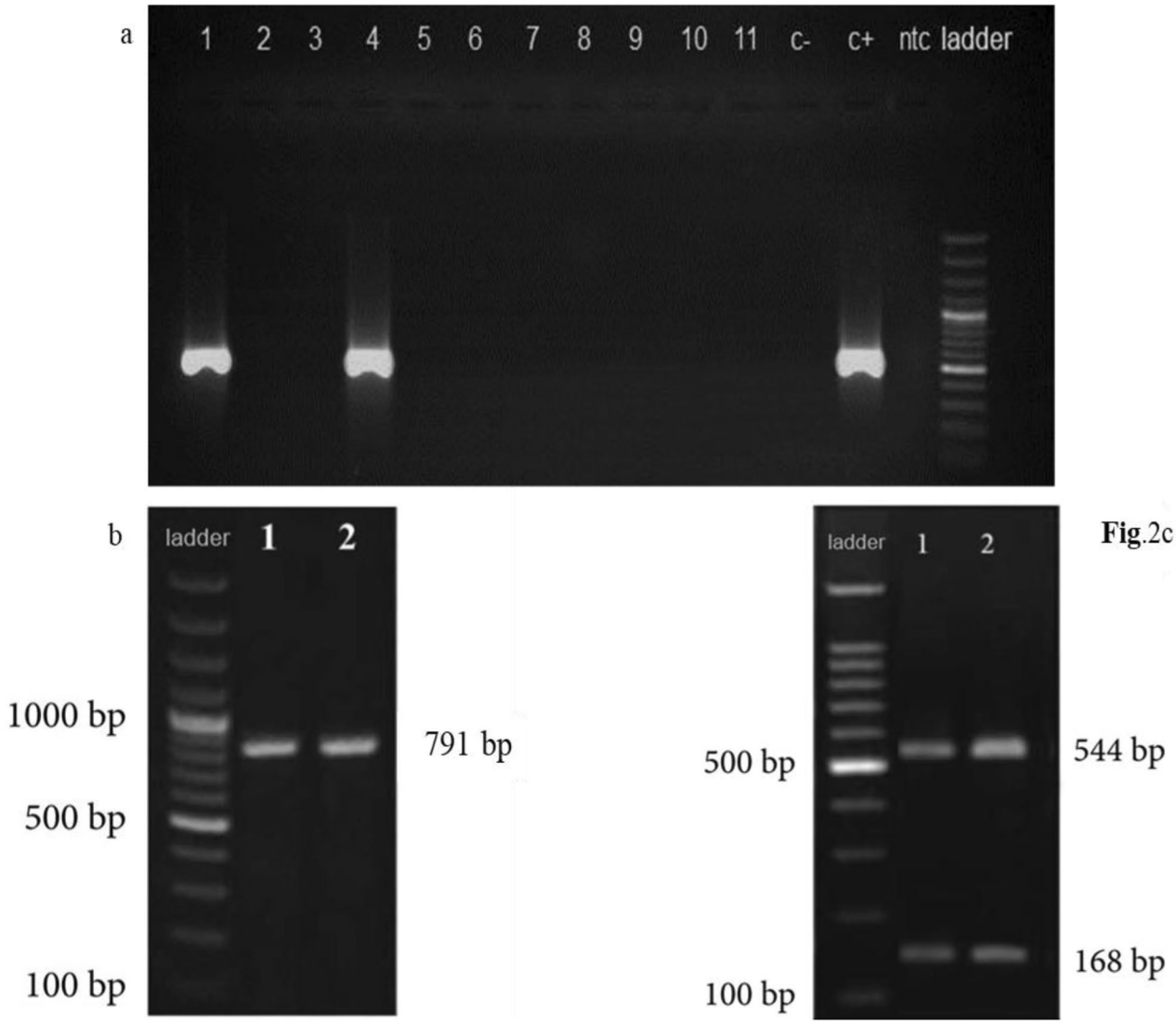

Fig. 2 **(a)** Agarose gel separation of representative nested PCR products of the B1 gene (531 bp). Lane 1and 4, positive isolates; c^-: negative control, c^+: positive control, ntc: Negative sample, DNA ladder 100 bp. **(b)** Electrophoretic pattern of the Nested PCR products of GRA6 gene from tissue samples. Lanes 1,2: samples from B1 positive samples Ladder: DNA marker. **(c)** PCR-RFLP analysis of GRA6 gene coding region with *Msel* endonuclease., Lanes 1–2 are *Toxoplasma gondii*, type I pattern

In Iran, research on aborted bovine fetuses has been limited to two studies. However, they reported nearly similar findings (5.9%, 13.4%), but neither of these studies used serological and molecular approaches concurrently [30, 44]. In this regard, the novelty of our research is the simultaneous usage of both approaches to examine studied fetuses.

In the present study, seropositivity in bovine cases was more than ovine (11.4% versus 4.4%), whereas we did not find parasite DNA in bovine samples. Similar data was found in previous surveys [45, 46]. In contrast, the positive PCR data from various regions of the globe necessitate additional research into the potential role of *T.gondii* as a causative agent of abortion among cattle offspring [44, 47]. It seems cattle are resistant to toxoplasmosis, making their significance in infection epidemiology negligible and challenging to assess [12].

Diagnosing *T.gondii* infection in pasture-raised livestock is challenging due to several factors like limited submission of aborted fetuses for diagnosis and frequent fetal autolysis [43]. Therefore we used the combination of diagnostic techniques for accurate diagnosis, including the serological technique as a screening step and followed up with the molecular approach as a confirmatory method to detect *T.gondii* presence.

Different genotypes of *T.gondii* may have varying levels of virulence, with genotype I being the most virulent [26]. While genotypes II and III can result in latent

toxoplasmosis, it is unclear if the same is valid for sheep infection [48]. Mice inoculated with type I separated from sheep did not exhibit clinical symptoms of toxoplasmosis [43].GRA6, the single-copy gene is more polymorphic than other markers and can distinguish three genotypes (I, II, and III) using one PCR reaction, followed by single endonuclease (MseI) digestion [49].

Based on our knowledge, only three studies have been carried out in various regions of Iran regarding the genotyping of ruminant aborted fetuses. These reports are limited to ovine samples and consistent with our genotyping analysis and their obtained *T.gondii* isolates also belong to type I [30, 42, 43]. Iranian adult sheep mostly have genotype II, while the most common genotype in Europe is type III [26]. It indicates that various genotypes of *T. gondii* are prevalent in distinct areas, possibly responsible for inducing sheep abortion.

Two surveys on toxoplasmosis in aborted fetuses in North Khorasan Province had limitations, as they only used serological or molecular approaches and focused on ovine fetuses without genotyping the isolates [50, 51].

Understanding the nutritional pattern, rapid detection of *T. gondii*, keeping feed and water sources clean for small ruminants, controlling rodent and domestic cat populations, practicing hygiene and identifying the circulating genotypes in nature are crucial for infection control/prevention. Vaccination of pregnant animals can also effectively prevent reproductive losses due to *T. gondii* infection [52].

Collectively, it is recommended to establishing efficient prevention and control health programs against toxoplasmosis in adult sheep and cattle and their fetuses, which humans commonly consume. This study lends weight to the argument that toxoplasmosis cases within sheep and cattle populations might be caused by congenital transmission of *T. gondii*. This transmission mode may also maintain infection levels within certain family lines.

Conclusion

Type I genotype of *T. gondii* may be a possible cause of abortion in domestic livestock. Additionally, the presence of *T.gondii* DNA in the tissues of studied aborted fetuses from northeastern Iran implies that meat consumption might pose the risk of human infection. Further research is necessary to obtain more precise details about the molecular epidemiology and genotypes of *T. gondii*.

Limitations

The present study has several limitations, as follows:

We detected only *T. gondii* and its genotype without exploring other factors that could affect animal abortion. The sample size was relatively small, and the fetuses were stored for two days, which could lead to contamination. More polymorphic markers (such as GRA6) are recommended for genotyping, and different tissues could also be used for diagnosis5. Here, we focused on the brains of fetuses as the preferred tissue for diagnosing *T. gondii*, but skeletal muscle, cardiac muscle, liver, spleen and lung could also serve as further choices for diagnostic purposes.

Acknowledgements

We would like to acknowledge all staff from North Khorasan Veterinary Organization and also Vector-Borne Disease Research Center, North Khorasan University of Medical Sciences, Bojnurd, Iran.

Author contributions

Conceptualization, N.F.; methodology, S.R.; software, M.R; writing _original draft preparation. All authors have read and agreed to the published present manuscript version.

Funding

The research leading to these results was financially supported by a grant from the project No. 990205 from North Khorasan University of Medical Sciences, Bojnord, Iran.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

All methods were carried out in accordance with relevant guidelines and regulations.

Ethics approval and consent to participate

The present work is approved by the ethical committee of North Khorasan, University of Medical Sciences. The ethical approval Code is IR.NKUMS.REC.1400.117.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 22 August 2023 / Accepted: 27 August 2024
Published online: 03 October 2024

References

1. Pretzer SD. Bacterial and protozoal causes of pregnancy loss in the bitch and queen. Theriogenology. 2008;70:320–6.
2. Giakoumelou S, Wheelhouse N, Cuschieri K, Entrican G, Howie SEM, Horne AW. The role of infection in miscarriage. Hum Reprod Update. 2016;22:116–33.
3. Dereje T, Benti D, Feyisa B, Abiy G. Review of common causes of abortion in dairy cattle in Ethiopia. J Vet Med Anim Heal. 2018;10:1–13.
4. Sebastiani C, Curcio L, Ciullo M, Cruciani D, Crotti S, Pesca C, et al. A multiscreening fast qPCR approach to the identification of abortive agents in ruminants. J Microbiol Methods. 2018;148:12–7.
5. Zhang H, Deng X, Cui B, Shao Z, Zhao X, Yang Q, et al. Abortion and various associated risk factors in dairy cow and sheep in Ili, China. PLoS ONE. 2020;15(10 October):e0232568.
6. Firouzeh N, Ziaali N, Sheibani V, Doustimotlagh AH, Afgar A, Zamanpour M, et al. Chronic toxoplasma gondii infection potentiates parkinson's disease course in mice model. Iran J Parasitol. 2021;16:527–37.
7. Hill D, Dubey JP. Toxoplasma Gondii: transmission, diagnosis, and prevention. Clin Microbiol Infect. 2002;8:634–40.
8. Shafiei R, Riazi Z, Sarvghad M, Sharifdini MG, Mahmoodzadeh A, Hajia M. Prevalence of IgG and IgM anti-toxoplasma gondii antibodies in HIV positive patients in northeast of Iran. Iran J Pathol. 2011;6:68–72.

9. Sheikhshoaee S, Taheri F, Esmaeilpour K, Firouzeh N, Fard SRN. Aggravation of cognitive impairments in the valproic acid-induced animal model of autism in BALB/c mice infected with Toxoplasma gondii. Int J Dev Neurosci. 2024;84:64–74.
10. Shojaee S, Firouzeh N, Keshavarz H, Jafar-Pour Azami S, Salimi M, Mohebali M. Nanosilver Colloid inhibits Toxoplasma Gondii tachyzoites and bradyzoites in vitro. Iran J Parasitol. 2019;14:362–7.
11. Zarean M, Shafiei R, Gholami M, Fata A, Balaghaleh MR, Karimi A, et al. Seroprevalence of anti–toxoplasma gondii antibodies in healthy voluntary blood donors from Mashhad city, Iran. Arch Iran Med. 2017;20:441–5.
12. Dubey JP, Jones JL. Toxoplasma Gondii infection in humans and animals in the United States. Int J Parasitol. 2008;38:1257–78.
13. Iacobucci E, Taus NS, Ueti MW, Sukhbaatar L, Bastsukh Z, Papageorgiou S, et al. Detection and genotypic characterization of Toxoplasma gondii DNA within the milk of Mongolian livestock. Parasitol Res. 2019;118:2005–8.
14. Bennett R, IJpelaar J. Updated estimates of the costs associated with thirty four endemic livestock diseases in Great Britain: a note. J Agric Econ. 2005;56:135–44.
15. de Oliveira FC, de Oliveira PA, da Cunha Filho NA, Aguiar CLG, Pappen FG, Ruas JL, et al. Incidência E significância econômica Da toxoplasmose ovina no Sul do Brasil. Cienc Rural. 2016;46:1618–21.
16. Anisuzzaman, Hossain MS, Hatta T, Labony SS, Kwofie KD, Kawada H et al. Food- and vector-borne parasitic zoonoses: Global burden and impacts. In: Advances in Parasitology. 2023. pp. 87–136.
17. Dubey JP. Toxoplasmosis in pigs-the last 20 years. Vet Parasitol. 2009;164:89–103.
18. Liu Q, Wang ZD, Huang SY, Zhu XQ. Diagnosis of toxoplasmosis and typing of Toxoplasma Gondii. Parasites Vectors. 2015;8:292.
19. Tegegne D, kelifa A, Abdurahaman M, Yohannes M. Seroepidemiology and associated risk factors of Toxoplasma Gondii in sheep and goats in Southwestern Ethiopia. BMC Vet Res. 2016;12:280.
20. Tonouhewa ABN, Akpo Y, Sessou P, Adoligbe C, Yessinou E, Hounmanou YG, et al. Toxoplasma Gondii infection in meat animals from Africa: systematic review and meta-analysis of sero-epidemiological studies. Vet World. 2017;10:194–208.
21. Habibi GR, Imani AR, Gholami MR, Hablolvarid MH, Behroozikhah AM, Lotfi M, et al. Detection and identification of Toxoplasma gondii type one infection in sheep aborted fetuses in Qazvin province of Iran. Iran J Parasitol. 2012;7:64–72.
22. Taylor S, Barragan A, Su C, Fux B, Fentress SJ, Tang K, et al. A secreted serine-threonine kinase determines virulence in the eukaryotic pathogen Toxoplasma Gondii. Sci (80-). 2006;314:1776–80.
23. Bastos da Silva I, Batista TP, de Martines A, Kanamura RB, Ferreira CT, Vidal IMR. Genotyping of Toxoplasma Gondii: DNA extraction from formalin-fixed paraffin-embedded autopsy tissues from AIDS patients who died by severe disseminated toxoplasmosis. Exp Parasitol. 2016;165:16–21.
24. Piri J, Kisi O. Modelling solar radiation reached to the Earth using ANFIS, NN-ARX, and empirical models (case studies: Zahedan and Bojnurd stations). J Atmos Solar Terr Phys. 2015;123:39–47.
25. Parsipour H, Popović SG, Behzadfar M, Skataric G, Spalevic V. Cities expansion and land use changes of agricultural and garden lands in peri-urban villages (Case study: Bojnurd). Agric for. 2019;65:173–87.
26. Nima F, Foroughi Borj H, Ziaali N, Tavakoli Kareshk A, Ahmadinejad M, Shafiei R. Genetic Diversity of Toxoplasma Gondii by Serological and Molecular analyzes in different Sheep and Goat tissues in northeastern Iran. Iran J Parasitol. 2023;18:217–28.
27. Fazaeli A, Carter PE, Darde ML, Pennington TH. Molecular typing of Toxoplasma gondii strains by GRA6 gene sequence analysis. Int J Parasitol. 2000;30:637–42.
28. El Bissati K, Levigne P, Lykins J, Adlaoui EB, Barkat A, Berraho A, et al. Global initiative for congenital toxoplasmosis: an observational and international comparative clinical analysis. Emerg Microbes Infect. 2018;7:1–14.
29. Edwards JF, Dubey JP. Toxoplasma Gondii abortion storm in sheep on a Texas farm and isolation of mouse virulent atypical genotype T. Gondii from an aborted lamb from a chronically infected ewe. Vet Parasitol. 2013;192:129–36.
30. Arefkhah N, Sarkari B, Asgari Q, Moshfe A, Khalafi MH, Mohammadpour I. Molecular genotyping of toxoplasma gondi in sheep aborted fetuses reveals predominance of type i infection in southwest of Iran. Iran J Parasitol. 2020;15:374–82.
31. Steuber S, Niu A, Bauer C, Reetz J, Roth A, Janitschke K. The detection of Toxoplasma Gondii in abortion tissues of sheep using the polymerase chain reaction. Dtsch Tierarztl Wochenschr. 1995;102:91–3.
32. Pereira-Bueno J, Quintanilla-Gozalo A, Pérez-Pérez V, Álvarez-García G, Collantes-Fernández E, Ortega-Mora LM, Erratum. Evaluation of ovine abortion associated with Toxoplasma gondii in Spain by different diagnostic techniques (Veterinary Parasitology (2004) 121 (33–43) DOI: 10.1016/j.vetpar.2004.02.004). Vet Parasitol. 2004;121:353.
33. Buxton D, Maley SW, Wright SE, Rodger S, Bartley P, Innes EA. Toxoplasma Gondii and ovine toxoplasmosis: New aspects of an old story. Vet Parasitol. 2007;149:25–8.
34. Guo M, Dubey JP, Hill D, Buchanan RL, Ray Gamble H, Jones JL, et al. Prevalence and risk factors for toxoplasma gondii infection in meat animals and meat products destined for human consumption. J Food Prot. 2015;78:457–76.
35. Khademvatan S, Foroutan M, Hazrati-Tappeh K, Dalvand S, Khalkhali H, Masoumifard S, et al. Toxoplasmosis in rodents: a systematic review and meta-analysis in Iran. J Infect Public Health. 2017;10:487–93.
36. Nayeri T, Sarvi S, Moosazadeh M, Daryani A. The global prevalence of Neospora caninum infection in Sheep and Goats that had an abortion and aborted fetuses: a systematic review and Meta-analysis. Front Vet Sci. 2022;9:109370.
37. Movassaghi AR, Rassouli M, Fazaeli A, Salimi-Bejestani MR. Outbreak of ovine congenital toxoplasmosis in Iran, confirmed by different diagnostic methods. J Parasit Dis. 2016;40:152–6.
38. Gharekhani J. Seroprevalence of Neospora Caninum and Toxoplasma gondii infections in aborted cattle in Hamedan, Iran. J Adv Vet Anim Res. 2014;1:32–5.
39. Shahbazi G, Hoghooghi Rad N, Madani R, Matin S, Mortazavi P, Jangjou AH. Toxoplasma Gondii in aborted fetuses of sheep in Ardebil Area, North-West of Iran. Iran J Parasitol. 2019;14:430–5.
40. Razmi GR, Ghezi K, Mahooti A, Naseri Z. A serological study and subsequent isolation of toxoplasma gondii from aborted ovine fetuses in Mashhad area, Iran. J Parasitol. 2010;96:812–4.
41. Rassouli M, Razmi GR, Bassami MR, Movassaghi AR, Azizzadeh M. Study on ovine abortion associated with Toxoplasma Gondii in affected herds of Khorasan Razavi Province, Iran based on PCR detection of fetal brains and maternal serology. Parasitology. 2011;138:691–7.
42. Danehchin L, Razmi G, Naghibi A. Isolation and genotyping of toxoplasma gondii strains in ovine aborted fetuses in Khorasan Razavi Province, Iran. Korean J Parasitol. 2016;54:15–20.
43. Sharifiyazdi H, Haghkhah M, Behroozikhah AM, Nematgorgani E. Bacteriological and molecular investigation of B. melitensis in dairy cows in Iran. Comp Clin Path. 2012;21:269–73.
44. Azimi K, Amouei A, Sharif M, Sarvi S, Shams N, Mizani A, et al. Diagnosis of toxoplasmosis in ruminants aborted fetuses in northern Iran using molecular and bioassay techniques. Iran J Parasitol. 2021;16:229–35.
45. Moore DP, Regidor-Cerrillo J, Morrell E, Poso MA, Cano DB, Leunda MR, et al. The role of Neospora Caninum and Toxoplasma Gondii in spontaneous bovine abortion in Argentina. Vet Parasitol. 2008;156:163–7.
46. Reitt K, Hilbe M, Voegtlin A, Corboz L, Haessig M, Pospischil A. Aetiology of bovine abortion in Switzerland from 1986 to 1995 - a retrospective study with emphasis on detection of Neospora Caninum and Toxoplasma Gondii by PCR. J Vet Med Ser Physiol Pathol Clin Med. 2007;54:15–22.
47. Sager H, Fischer I, Furrer K, Strasser M, Waldvogel A, Boerlin P, et al. A Swiss case-control study to assess Neospora caninum-associated bovine abortions by PCR, histopathology and serology. Vet Parasitol. 2001;102:1–15.
48. Genot S, Franck J, Forel JM, Rebaudet S, Ajzenberg D, De Paula AM, et al. Severe Toxoplasma gondii I/III recombinant-genotype encephalitis in a human immunodeficiency virus patient. J Clin Microbiol. 2007;45:3138–40.
49. Dardé ML, Mercier A, Su C, Khan A, Grigg ME. Molecular epidemiology and population structure of Toxoplasma Gondii. Toxoplasma Gondii: the Model Apicomplexan - perspectives and methods. Elsevier; 2020. pp. 63–116.
50. Salehi M, Nezami H. A survey of Toxoplasma Gondii infection in aborted fetuses of sheep using ELISA method in different cities of North Khorasan Province. J Vet Res. 2019;74:304–10.
51. Salehi M, Nezami H, Niazkar HR. Investigation of Toxoplasma gondii Infection in Aborted Fetuses of Sheep Using PCR: A Study in North Khorasan Province, Iran. Vet Med Int. 2020;2020.
52. Aguirre AA, Longcore T, Barbieri M, Dabritz H, Hill D, Klein PN et al. Correction to: The One Health Approach to Toxoplasmosis: Epidemiology, Control, and Prevention Strategies (EcoHealth, (2019), 16, 2, (378–390), https://doi.org/10.1007/s10393-019-01405-7). Ecohealth. 2019;16:391.

Publisher's note