



# Molecular detection and phylogenetic characterization of *Neospora caninum* in naturally infected sheep in Alborz and Qazvin provinces, the north of the central region of Iran

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## Abstract

*Neospora caninum* is a protozoan coccidian parasite that can act as a cause of abortion in sheep. The aim of this study was to investigate the presence of this parasitic agent and its role in causing abortion in sheep of Iran. Between June 2019 and February 2022, 100 samples [brain ( $n = 39$ ), placenta ( $n = 8$ ), embryonic membrane ( $n = 7$ ), cotyledon ( $n = 7$ ), umbilical cord ( $n = 2$ ), homogenate mixture of tissues (heart, liver, spleen and digestive track) ( $n = 37$ )] that were collected following the necropsies of 39 aborted ovine fetuses from different parts of the Alborz and Qazvin provinces, the north of the central region of Iran were employed for DNA extraction. Nc-5 was selected as the target gene sequence for amplification of DNA by using four pairs of primers in two semi-nested PCR. Samples considered positive for the presence of the NC-5 gene were examined to further confirm the presence of the ITS1 gene. Sequence of NC-5 gene was detected from the 27 tissue samples of 23 aborted ovine fetuses. The ITS1 gene sequence was detected in all of the 27 tissue samples that were positive for the NC-5 gene analysis. Brain tissue was the most studied tissue, and the highest number of positive cases was observed in this tissue. The present study updated the situation of ovine neosporosis in the central region of Iran and confirmed the presence of the *N. caninum* among sheep flocks' abortion.

**Keywords** *Neospora caninum* · Abortion · Sheep · Iran

## Introduction

*Neospora caninum* is the causative agent of polysystemic parasitic disorder and a coccidial protozoan parasite with worldwide distribution (Dubey 2003). This disease was first identified in puppies in Norway (Bjerkås et al. 1984). Other canine hosts, including domestic dogs (*Canis familiaris*), coyotes (*C. latrans*), dingoes (*C. domesticus*), and gray wolves (*C. lupus*) are definitive hosts for *N. caninum*

(Dubey et al. 2005). Oocysts, tachyzoites, and tissue cysts were all thought to be infectious stages in the life cycle of the parasite (Dubey 2003). *Neospora caninum* can be transmitted to intermediate hosts following ingestion of oocysts through contaminated food or water or can be transferred vertically from mothers to offspring via the placenta (McAllister et al. 1998). Primary intermediate hosts, such as goats, sheep, and cattle may experience stillbirths, abortions, and neonatal mortalities (Nie et al. 2018).

*Neospora caninum* was first detected in sheep in England in a congenitally infected lamb (Dubey et al. 1990). In Japan, South America, and Switzerland, ovine neosporosis has been documented as naturally occurring (Kobayashi et al. 2001; Koyama et al. 2001; Hässig et al. 2003a; Moore 2005), and seroprevalence reports suggest that the parasite is present worldwide (Liu et al. 2015; Villagra-Blanco et al. 2017; Dahourou et al. 2019).

It has been demonstrated that experimentally infecting pregnant ewes with tachyzoites can cause congenital infection or abortion (McAllister et al. 1996; Howe et al. 2012), and the parasite could cause mortality in newborn lambs

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and induce congenital infection in naturally exposed sheep (Dubey et al. 1990; Buxton et al. 1998; Hurtado et al. 2001; Helmick et al. 2002). Sheep can also be infected by ingesting oocysts shed in the feces of the definitive carnivore hosts, demonstrating the possibility of horizontal infection (O’Handley et al. 2002).

Sheep breeding industry has an important economic role in Iran. Sheep farming has become increasingly favored because of its higher production levels. Unfortunately, these kinds of farms are regularly not supported by proper feeding and management, and they are not able to avoid stressful situations, and animals have lower resistance to opportunistic pathogens like *N. caninum* (Selim et al. 2021).

In Iran, *N. caninum* was documented for the first time in dairy cattle in the Mashhad region, the northeast of the country (Sadrebazzaz et al. 2004), and from that time, many different researches have been carried out on some species of mammals and birds (Gharekhani et al. 2020).

Certain scientists have suggested that neosporosis could be responsible for inducing abortion in sheep as *N. caninum* DNA was detected in 9.9% and 8.5% of brain samples obtained from aborted fetuses in Mashhad and Tabriz regions, respectively (Asadpour et al. 2013; Sasani et al. 2013; Razmi and Naseri 2017). During the scrutiny, genetic material of *N. caninum* was identified in 6.7% and 0.7% of cardiac and cerebral specimens, correspondingly, in asymptomatic animals (Arbabi et al. 2016).

In the present study, we attempted to provide information on the role that this parasite is playing in ovine reproductive failure by establishing the prevalence of *N. caninum* DNA in ovine fetal material present in sheep farms with ongoing unexplained abortion problems.

## Material and methods

### Study duration and sample preparation

From June 2019 to February 2022, tissue samples were collected following the necropsies of aborted ovine fetuses

from private farms situated in Alborz and Qazvin provinces (Fig. 1). These two provinces are located in the north of the central region of Iran at the skirts of the Alborz Mountain ranges. The Alborz Mountains have an impact on these provinces’ weather, which has cold winters and mild summers. The south of these provinces has hot and dry weather because of being adjacent to the desert, and on one side, it reaches Alborz Mountain, and on the other side, it ends in one of the driest deserts of Iran. Sheep and goat breeding in Iran, also in these two provinces, is mostly by traditional methods, and flocks under these conditions often have direct contact with stray dogs, but, despite all the above points, the clinical and economic reality of neosporosis in sheep and goats is unclear (Gharekhani et al. 2020). Tissue samples (brain, placenta, embryonic membrane, cotyledon, umbilical cord, heart, liver, spleen, and digestive track) were sent to the Protozoology Laboratory, Parasitology Department, Razi Vaccine and Serum Research Institute. In the laboratory, tissue samples were stored under freezing conditions ( $-20^{\circ}\text{C}$ ) until they were used for molecular analysis.

### Questionnaire data collection of risk factors

A questionnaire asking about the history of abortion in livestock has been completed by participating farm owners. The data obtained from the owners included fetal age (the fetus died prior to or during labor), size of flock, type of farming (traditional or industrial), type of flocks (containing sheep, goat, cow, or a combination of them), source of feeding (in the stable, possibility to graze or both), source of water (stagnant water source or municipal water), type of habitat (mountainous or plain), and presence of the dog and cat at the farm.

### DNA extraction

A portion of the tissues from the aborted fetus including brain, placenta, embryonic membrane, cotyledon, umbilical cord, and a mixture of various tissues (heart, liver, spleen, and digestive track) were removed, homogenized,

**Fig. 1** A map showing the sampling site’s geographical location. Alborz and Qazvin provinces are marked with a red circle



and utilized for DNA extraction by the phenol-chloroform extraction procedure, as described previously (Biase et al. 2002; Jameie et al. 2022; Nasiri and Jameie 2023). The resulting DNA was kept at  $-20\text{ }^{\circ}\text{C}$  for following PCR assessments.

### PCR analyses

The Nc-5 gene that has been shown to be highly specific to *N. caninum* was chosen for detection of this parasite as the target sequence by using combinations of four pairs of primer in two nested PCR (Hughes et al. 2006).

#### Procedure I

Briefly, as described previously (Hughes et al. 2006; Nasiri and Jameie 2023), first round of PCR (337b) was done using a pair of *N. caninum* Nc-5 gene specific primers: Np21 PLUS and Np6 PLUS (Liddell et al. 1999), and then the second round of PCR(225b) were performed with a pair of primers: Np7 and Np6 (Yamage et al. 1996; Baszler et al. 1999). Amplification of the first round reaction was performed with initial denaturation for 5 min at  $95\text{ }^{\circ}\text{C}$ , followed by 40 cycles of  $94\text{ }^{\circ}\text{C}$  for 40 s,  $63\text{ }^{\circ}\text{C}$  for 40 s, and  $72\text{ }^{\circ}\text{C}$  for 70 s, and final extension at  $72\text{ }^{\circ}\text{C}$  for 10 min. The conditions of the reaction were identical to the first round, with the exception of annealing temperature of  $56\text{ }^{\circ}\text{C}$ .

#### Procedure II

As described previously (Nasiri and Jameie 2023), in the preliminary PCR (275bp), detection was executed using Np4 and Np7 primers. The amplification involved an initial denaturation at  $95\text{ }^{\circ}\text{C}$  for 5 min, followed by 35 cycles at  $95\text{ }^{\circ}\text{C}$  for 30 s,  $57\text{ }^{\circ}\text{C}$  for 30 s,  $72\text{ }^{\circ}\text{C}$  for 60 s, and a final extension at  $72\text{ }^{\circ}\text{C}$  for 10 min. The second set of primers, Np6 and Np7, were used for conducting semi-nested PCR, which targeted a fragment of 225 base pairs. The conditions of the second reaction was identical to the first PCR, except that the annealing temperature was decreased to  $56\text{ }^{\circ}\text{C}$  (Baszler et al. 1999).

#### A single-tube nested PCR for ITS1 gene (249 bp)

As described previously (Nasiri and Jameie 2023), samples that were NC-5 gene positive, were analyzed to validate the existence of the ITS1 gene. Single-tube nested-ITS1 PCR using the external primers (ITS-1-N-F1 and ITS-1-N-R1) and the internal primers (ITS-1-N-F2 and ITS-1-N-R2) was used to find parasite ITS1 gene. The amplification conditions involved 1 cycle of  $94\text{ }^{\circ}\text{C}$  for 3 min, 15 cycles of  $94\text{ }^{\circ}\text{C}$  for 30 s,  $65\text{ }^{\circ}\text{C}$  for 45 s, and  $72\text{ }^{\circ}\text{C}$  for 1 min, 35 cycles of  $94\text{ }^{\circ}\text{C}$  for 30 s,  $56\text{ }^{\circ}\text{C}$  for 30 s,  $72\text{ }^{\circ}\text{C}$  for 30 s, and a

final cycle lasting 5 min at  $72\text{ }^{\circ}\text{C}$ . For all PCR reactions, a positive control (DNA extracted from the brain of the experimentally infected inbred BALB/c mouse and DNA extracted from cultures of NC1 strain of *N. caninum*) and a negative control (DNA extracted from the brain of inbred BALB/c mice (confirmed to be serologically and molecularly negative for neosporosis) and double distilled water) were included (Nasiri and Jameie 2023; Buxton et al. 1998; Hurtado et al. 2001; Regidor-Cerrillo et al. 2014; García-Sánchez et al. 2020).

### ITS1 gene phylogenetic analysis

The positive PCR products of the four amplified ITS1 gene were sequenced in both the reverse and forward directions. The results were aligned with the ITS1 gene partial sequences of *N. caninum* from previous researches that were presented in GenBank using the BioEdit Sequence Alignment Editor program (Hall 1999). The maximum-likelihood method was used to perform the phylogenetic tree and evolutionary analyses by using MEGA11 software. The bootstrap results were computed for 1000 replicates (Tamura et al. 2013).

### Statistical analysis

The data about the relationship between the different risk factors and molecular results were analyzed using the non-parametric Chi-square ( $\chi^2$ ) test (SPSS 26.0, Chicago, IL, USA) software, and the results were considered significant when the probability score was  $< 0.05$ .

### Ethics statement

The Animal Care Committee of Razi Vaccine and Serum Research Institute, Alborz, Iran, approved all animal experimentation procedures. All animals used in our experiment were treated humanely and in accordance with National Research Council's Guide for the Care and Use of Laboratory Animals.

### Results

In collaboration with active veterinarians in the field and veterinary organizations, 100 samples [brain ( $n = 39$ ), placenta ( $n = 8$ ), embryonic membrane ( $n = 7$ ), cotyledon ( $n = 7$ ), umbilical cord ( $n = 2$ ), and homogenate mixture of all tissues (heart, liver, spleen, and digestive track) ( $n = 37$ )] were collected. Tissue homogenates contained portions of each of the tissues submitted from the cases. These samples were obtained following the necropsies of 39 individual aborted ovine fetuses.

## Analysis of risk factors associated with *N. caninum* infection

In total, 27 questionnaires containing epidemiological information related to the collected aborted fetus were received from investigating farms. There was no relationship between some factors like the type of farming, type of flocks, source of feeding, type of habitat, and presence of dogs or cats with the positive results of the molecular detection of *N. caninum* ( $p > 0.05$ ). This analysis indicated that there was significant variation only between the source of water and the positive molecular results ( $p = 0.005$ ) (Table 1).

## Molecular analyses

By the molecular assessment of the NC-5 gene in different tissue samples, this gene was amplified from the 27 tissue

samples belonging to the 23 aborted ovine fetuses that all 27 positives tissue samples for the NC-5 gene were also positive for the ITS1 gene.

Brain tissue was the most frequently submitted tissue, and the highest number of positive cases were observed in this tissue.

The results of molecular analysis of the presence of parasite in the different tissues are shown in Table 2.

The results of molecular analysis of the presence of the parasite by number and code of the aborted fetus sample and the type of tissue examined in each case are presented in Table 3.

## Phylogenetic analysis

After sequencing, four positive sequences were submitted to GenBank (accession numbers OL825739, OL825740,

**Table 1** Statistical analysis of data obtained from questionnaires compared to molecular results

Risk factors		Molecular findings +	–	<i>p</i> value
Type of farming	Traditional	14	10	0.411
	Industrial	1	2	
Dog presence	Have dog	14	9	0.501
	No dog	1	3	
Type of flocks	Sheep	10	5	0.183
	Sheep and goat	3	3	
	Sheep and cow	1	1	
	Sheep, goat, and cow	1	3	
Cat presence	Have cat	12	9	0.756
	No cat	3	3	
Source of feeding	In stable	2	1	0.207
	Access to graze	1	4	
	Both feeding	12	7	
Source of water	Stagnant water	6	12	<b>0.005</b>
	Municipal water	5	0	
	Stagnant and municipal	4	0	
How to dispose an aborted fetus	Consumed by carnivores	8	6	0.103
	Abandoned	2	4	
	Buried	5	0	
	Abandoned and consumed by carnivores	0	1	
	Buried and consumed by carnivores	0	1	
Approximate age of pregnant sheep at the time of abortion	Under 1 year	2	1	0.880
	1–5 year	10	9	
	Different ages	3	2	
The approximate month of pregnancy when the abortion occurred	Mostly in the first month of pregnancy	4	1	0.533
	2 months	3	3	
	4 months	3	4	
	Up to 5 months	0	1	
	Late pregnancy	5	3	
Habitat type	Mountainous	6	4	0.722
	Plain	9	8	

Texts in bold font style signifies variation between the source of water and the positive molecular results ( $p = 0.05$ )

**Table 2** The number of tissues examined with the number and percentage of molecular results for each tissue

Tissue type	No. of examined tissue	No. of infected tissue	Infected cases (%)
Brain	39	17	43.58
Placenta	8	1	12.5
Embryonic membrane	7	2	28.57
Cotyledon	7	1	14.28
umbilical cord	2	1	50
mixture of all tissues	37	5	13.51
Total	100	27	27

OM484267, and OM484268). To characterize the most resembling sites, existing sequences were subjected to the basic local alignment search tool (BLAST), and after alignment with BioEdit, the findings indicated that our sequences exhibited likeness to one another and to other affirmative instances of *N. caninum* NC-5 gene recorded in Gen Bank (Supplementary Table S1).

The phylogeny of the *N. caninum* was investigated by computerized analysis of the NC-5 gene sequences of 44 *N. caninum* isolates that four samples obtained in the present work, plus those of 40 other studies about *N. caninum* and other apicomplexan parasites (Fig. 2). The phylogenetic results indicated that the grouping of the *N. caninum* lineages of this study could be divided into the same clades. Maximum likelihood was employed to construct phylogenetic trees (Fig. 2), which revealed resemblance between *N. caninum* isolates investigated in this research and other *N. caninum* found in animals from various regions across the globe, which were archived in the GenBank.

Phylogenetic tree showed more similarity among *N. caninum* isolates in this study with *N. caninum* isolated from cattle in Iran, human umbilical cord blood from Brazil, and from coyote and sheep hosts (Fig. 3).

As shown in Table 3, evolutionary differences of *N. caninum* that were collected from the current study (1–4) with other recently detected samples that were expressed as pairwise distance (*p* distance) varied from 0.00 to 2.571.

### Discussion

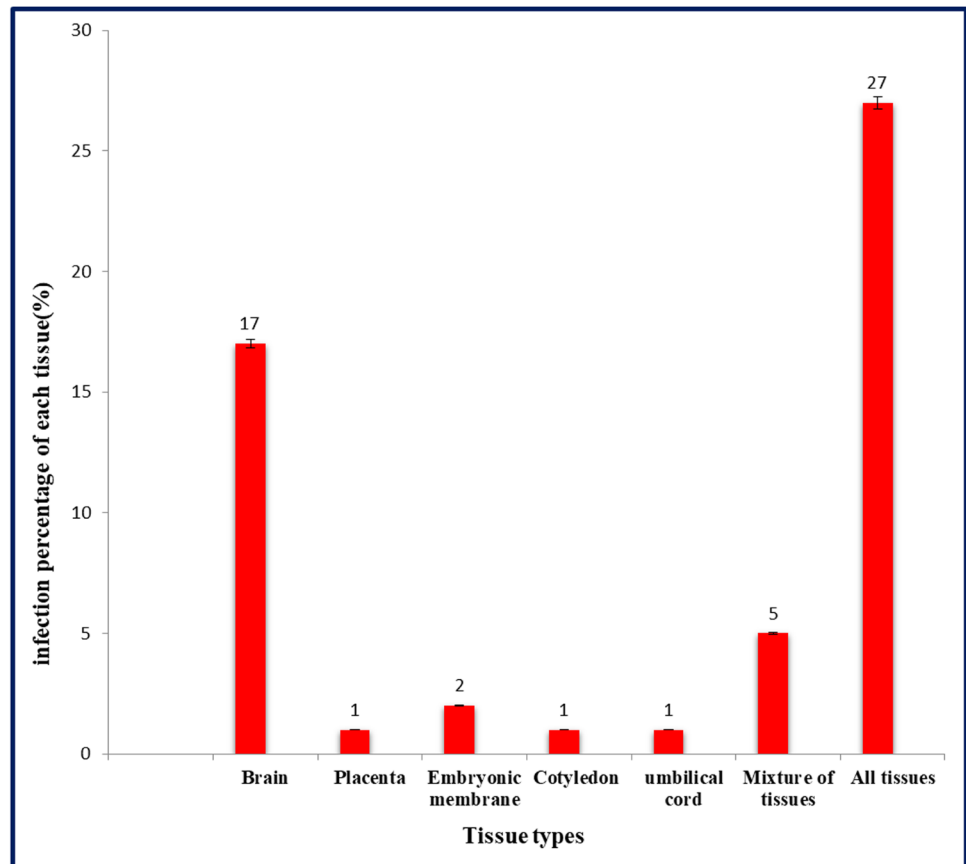
The efficiency of sheep herds relies on their ability to reproduce, but unfortunately, sheep abortion is a significant contributor to financial losses (Hecker et al. 2019). Many infectious agents may lead to ruminants’ abortions, either due to non-specific path (such as cause maternal stress or due to febrile disease), or due to fatal infection of the fetus. Some of the infectious organisms are mostly opportunistic, while others are specific ovine fetus pathogens leading

**Table 3** Evolutionary differences of detected *Neospora caninum* in the aborted fetus (*p* distance)

Accession number	Animal host	Organ	Country	1	2	3	4	5	6	7	8	9	10	11	12	13
1	OL825739	Sheep	Iran													
2	OL825740	Sheep	Iran	0.007												
3	OM484267	Sheep	Iran	<b>0.000</b>	0.007											
4	OM484268	Sheep	Iran	0.007	<b>0.000</b>	0.007										
5	MK944312.1	Human	Brazil	1.297	1.268	1.297	1.268									
6	AY459289.1	Cow	New Zealand	0.0182	0.033	0.018	0.033	1.492								
7	KR106184.1	Sheep	Iran	1.297	1.268	1.297	1.268	0.013	1.430							
8	MN972458.1	Sheep	Mongolia	0.055	0.055	0.055	0.055	1.242	0.035	1.242						
9	KF649848.1	Gray wolf	USA	1.998	1.958	1.998	1.958	0.000	2.053	0.013	1.654					
10	MF802335.1	Bat	China	0.036	0.036	0.036	0.036	1.483	0.024	1.345	0.092	1.418				
11	KU253799.1	Dog	Australia	1.996	1.953	1.996	1.953	0.008	2.043	0.008	1.698	0.018	1.425			
12	MH795879.1	Ruminants	Iran	0.040	0.040	0.040	0.040	1.484	0.034	1.342	0.077	1.799	0.032	1.806		
13	MZ272470.1_	Donkey	Iran	1.152	1.178	1.152	1.178	2.736	1.141	2.370	1.413	2.557	1.053	2.571	1.132	
14	KP702735.1	Sparrow	Iran	1.308	1.277	1.308	1.277	0.004	1.445	0.017	1.248	0.004	1.363	0.008	1.354	2.487

Texts in bold font style signifies variation between the source of water and the positive molecular results (*p* = 0.05)

**Fig. 2** The infection percentage of each examined tissue



to characteristic syndromes of abortion (Tirosh-Levy et al. 2022). Specific infectious agents that cause ovine abortions include some bacterial species, such as *Chlamydophila abortus* (enzootic abortion of ewes), *Campylobacter fetus fetus*, *Campylobacter jejuni*, *Listeria monocytogenes*, and *Brucella medinensis*; viruses including *Simbu serogroup* and *Blue tongue*; Border disease; and protozoa including *Sarcocystis* spp., *N. caninum*, and *T. gondii* (Buxton and Henderson 1999; Menzies 2011; Lindsay and Dubey 2020).

From different infectious agents that cause abortion in sheep, we can point to the *N. caninum* (Buxton and Henderson 1999). This parasite causes abortions, and clinical manifestation of ovine neosporosis is like ovine toxoplasmosis (Hecker et al. 2019). Due to the significant economic impact of neosporosis in ruminants and the absence of any cure or immunization, effective prevention and control measures represent the optimal means of minimizing *N. caninum* transmission (Hemphill et al. 2016; Machado 2019). Accordingly, the evaluation of related risk factors affecting its prevalence is crucial to reduce the dissemination of the disease (Wang et al. 2018; Mendonça et al. 2019; Selim and Ali 2020; Selim and Radwan 2020).

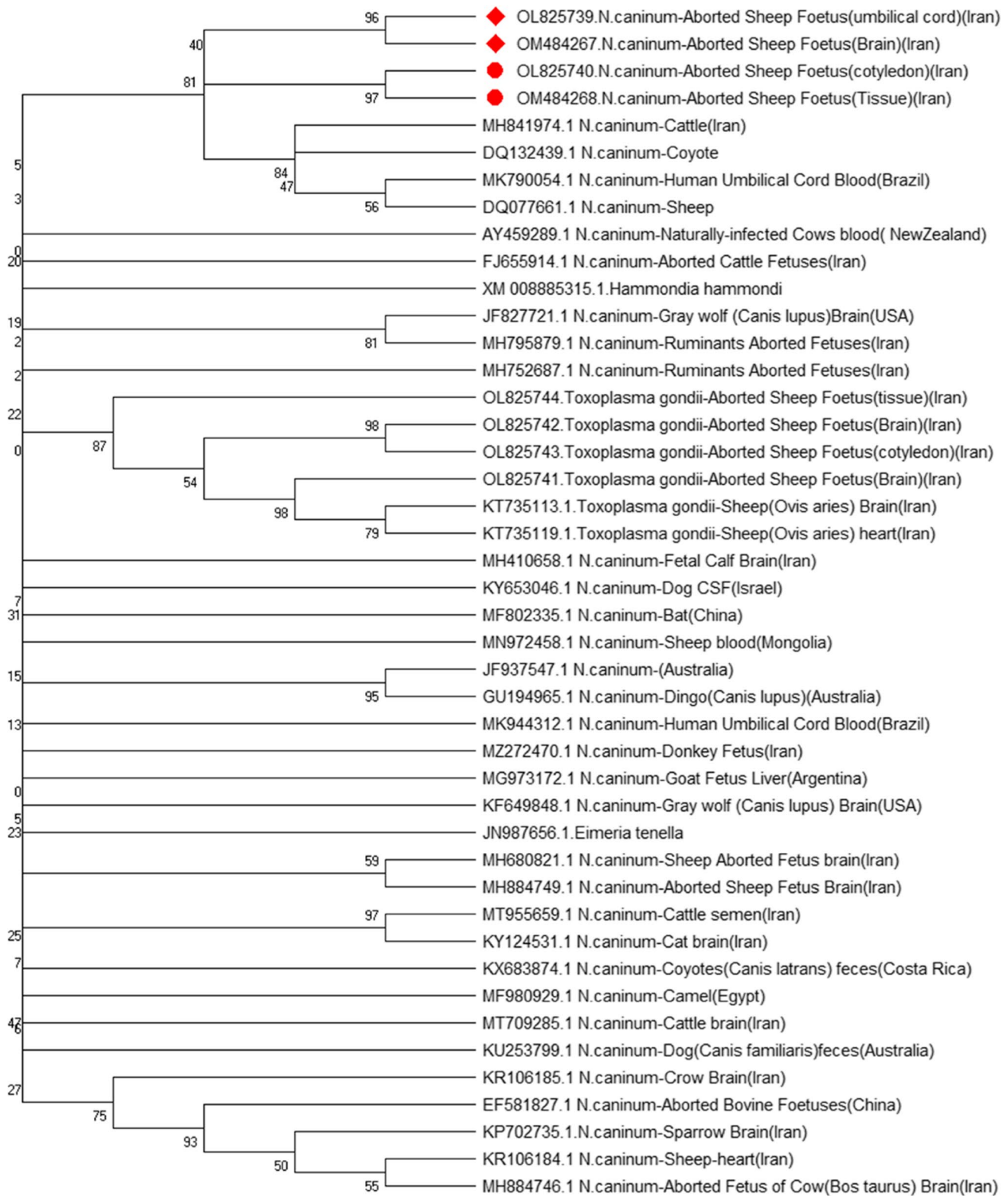
Different studies indicate that the diagnosis of neosporosis as the cause of an abortion cluster should be done with caution, due to its high seroprevalence in the general sheep

population irrelevant to the abortion history. Comprehensive epidemiological investigation of the entire flock is necessary for an accurate diagnosis of reproductive failure in sheep (Tirosh-Levy et al. 2022).

*Neospora caninum* infection has been sporadically reported as a cause of reproductive failures such as abortion and neonatal mortality in sheep in Iran, similar to toxoplasmosis (Dubey and Schares 2011; Razmi and Naseri 2017). In the present study, no statistically significant correlation between contact with dogs and abortion was present.

In this research, *N. caninum* was detected in examined aborted fetuses which was higher than the results of other studies in Iran (1 to 8.5%) (Asadpour et al. 2013; Sasani et al. 2013). In other countries, *N. caninum* was found in Switzerland 25% (Hässig et al. 2003b), in Spain 4.7% (Moreno et al. 2012), and in Italy 2% (Masala et al. 2007).

Among the limitations of the present research, it can be pointed out that the number of aborted fetuses examined was not large enough to be statistically valid for comparisons, and sometimes it was not possible to clearly express the statistical significance of the difference in the results. Another limitation of this study is that only one cause of infectious abortion pursued which is a major limitation, and not an exhaustive evaluation of potential causes of reproductive losses in these flocks was done.



**Fig. 3** Phylogenetic relationships obtained for four *Neospora caninum* sequences (OL825739, OL825740, OM484267, and OM484268) (red rhombus and circle) using the maximum likelihood method

based on their NC-5 gene sequences supported by 1000 bootstrap replicates. They represented the sequences obtained in this study

## Conclusions

The above results are important regardless of the percentage and tissues involved in the presence of this parasite and show that *N. caninum* can be one of the main causes of abortion in sheep. But what is important is to provide a suitable solution to farmers to prevent future abortions. Sheep and goats infected with *N. caninum* may abort or give birth to defective lambs. In the control of the disease, it is recommended to control dogs by not having access to infected tissues, in particular embryonic membranes. Studies have shown an association between abortion epidemics and the presence of dogs in livestock, and therefore, dogs should be prevented from approaching sheep and accessing infected aborted tissues, and aborted tissues should be removed from the livestock so that dogs and other intermediate hosts cannot access them. Different control methods should be used to prevent an increase in the number of stray dogs, and every effort should be made to prevent the contamination of water and food of livestock with dog feces.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00436-023-07980-7>.

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**Author contribution** VN, BSh, and FJ were involved in the conception of the research idea and methodology design, supervision and performed data analysis and interpretation and in the methodology and data analysis; VN, ShK, and FJ prepared and critically revised the manuscript for publication and revision. All authors read and approved the final manuscript.

**Data availability** All data generated or analyzed during this study are included in this article, and its supplementary files.

## Declarations

**Ethics approval** All animals used in our experiment were treated humanely and in accordance with National Research Council's Guide for the Care and Use of Laboratory Animals. The Animal Care Committee of Razi Vaccine and Serum Research Institute, Alborz, Iran, approved all animal experimentation procedures.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.

## References

Arbabi M, Abdoli A, Dalimi A, Pirestani M (2016) Identification of latent neosporosis in sheep in Tehran, Iran by polymerase chain reaction using primers specific for the nc-5 gene. *J Vet Res* 83:1–7. <https://doi.org/10.4102/ojvr.v83i1.1058>

- Asadpour R, Jafari-Joozani R, Salehi N (2013) Detection of *Neospora caninum* in ovine abortion in Iran. *J Parasit Dis* 37:105–109. <https://doi.org/10.1007/s12639-012-0141-0>
- Baszler TV, Gay LJC, Long MT, Mathison BA (1999) Detection by PCR of *Neospora caninum* in fetal tissues from spontaneous bovine abortions. *J Clin Microbiol* 37:4059–4064. <https://doi.org/10.1128/jcm.37.12.4059-4064.1999>
- Biase FH, Franco MM, Goulart LR, Antunes RC (2002) Protocol for extraction of genomic DNA from swine solid tissues. *Genet Mol Biol* 25:313–315
- Bjerkås I, Mohn SF, Presthus J (1984) Unidentified cyst-forming Sporozoon causing encephalomyelitis and myositis in dogs. *Zeitschrift für Parasitenkd Parasitol Res* 70:271–274. <https://doi.org/10.1007/BF00942230>
- Buxton D, Henderson D (1999) Infectious abortion in sheep. *In Pract* 21:360–368. <https://doi.org/10.1136/inpract.21.7.360>
- Buxton D, Maley SW, Wright S et al (1998) The pathogenesis of experimental neosporosis in pregnant sheep. *J Comp Pathol* 118:267–279. [https://doi.org/10.1016/S0021-9975\(07\)80003-X](https://doi.org/10.1016/S0021-9975(07)80003-X)
- Dahourou LD, Gbati OB, Savadogo M et al (2019) Prevalence of *Toxoplasma gondii* and *Neospora caninum* infections in households sheep “Elevage en case” in Dakar, Senegal. *Vet world* 12:1028
- Dubey JP (2003) Review of *Neospora caninum* and neosporosis in animals. *Korean J Parasitol* 41:1
- Dubey JP, Hartley WJ, Lindsay DS, Topper MJ (1990) Fatal congenital *Neospora caninum* infection in a lamb. *J Parasitol*:127–130
- Dubey JP, Knickman E, Greene CE (2005) Neonatal *Neospora caninum* infections in dogs. *Acta Parasitol* 50:176–179
- Dubey JP, Schares G (2011) Neosporosis in animals—the last five years. *Vet Parasitol* 180:90–108. <https://doi.org/10.1016/j.vetpar.2011.05.031>
- García-Sánchez M, Moreno-Gonzalo J, González-Warleta M et al (2020) Isolation and genetic characterization of *Neospora caninum* from naturally infected sheep. *Vet Parasitol* 280:109091. <https://doi.org/10.1016/j.vetpar.2020.109091>
- Gharekhani J, Yakhchali M, Berahmat R (2020) *Neospora caninum* infection in Iran (2004–2020): a review. *J Parasit Dis* 44:671–686. <https://doi.org/10.1007/s12639-020-01266-w>
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic acids symposium series*, pp 95–98
- Hässig M, Sager H, Reitt K et al (2003a) *Neospora caninum* in sheep: a herd case report. *Vet Parasitol* 117:213–220
- Hecker YP, Morrell EL, Fiorentino MA et al (2019) Ovine Abortion by *Neospora caninum*: first case reported in Argentina. *Acta Parasitol* 64:950–955. <https://doi.org/10.2478/s11686-019-00106-z>
- Helmick B, Otter A, McGarry J, Buxton D (2002) Serological investigation of aborted sheep and pigs for infection by *Neospora caninum*. *Res Vet Sci* 73:187–189
- Hemphill A, Aguado-Martinez A, Mueller J (2016) Approaches for the vaccination and treatment of *Neospora caninum* infections in mice and ruminant models. *Parasitology* 143:245–259
- Howe L, Collett MG, Pattison RS et al (2012) Potential involvement of *Neospora caninum* in naturally occurring ovine abortions in New Zealand. *Vet Parasitol* 185:64–71. <https://doi.org/10.1016/j.vetpar.2011.10.033>
- Hughes JM, Williams RH, Morley EK et al (2006) The prevalence of *Neospora caninum* and co-infection with *Toxoplasma gondii* by PCR analysis in naturally occurring mammal populations. *Parasitology* 132:29–36. <https://doi.org/10.1017/S0031182005008784>
- Hurtado A, Aduriz G, Moreno B et al (2001) Single tube nested PCR for the detection of *Toxoplasma gondii* in fetal tissues from naturally aborted ewes. *Vet Parasitol* 102:17–27
- Jameie F, Nasiri V, Paykari H (2022) Morphological detection and molecular characterization of Hepatozoon spp. from venomous



- terrestrial snakes in Iran. *Exp Parasitol* 239:108309. <https://doi.org/10.1016/j.exppara.2022.108309>
- Kobayashi Y, Yamada M, Omata Y et al (2001) Naturally-occurring *Neospora caninum* infection in an adult sheep and her twin fetuses. *J Parasitol* 87:434–437
- Koyama T, Kobayashi Y, Omata Y et al (2001) Isolation of *Neospora caninum* from the brain of a pregnant sheep. *J Parasitol* 87:1486–1488. [https://doi.org/10.1645/0022-3395\(2001\)087\[1486:ION-CFT\]2.0.CO;2](https://doi.org/10.1645/0022-3395(2001)087[1486:ION-CFT]2.0.CO;2)
- Liddell S, Jenkins MC, Dubey JP (1999) A competitive PCR assay for quantitative detection of *Neospora caninum*. *Int J Parasitol* 29:1583–1587. [https://doi.org/10.1016/S0020-7519\(99\)00101-0](https://doi.org/10.1016/S0020-7519(99)00101-0)
- Lindsay DS, Dubey JP (2020) Neosporosis, toxoplasmosis, and sarcocystosis in ruminants: an update. *Vet Clin Food Anim Pract* 36:205–222
- Liu Z-K, Li J-Y, Pan H (2015) Seroprevalence and risk factors of *Toxoplasma gondii* and *Neospora caninum* infections in small ruminants in China. *Prev Vet Med* 118:488–492
- Machado GP (2019) Neosporosis in small ruminants. *Silva* 4:211–214
- Masala G, Porcu R, Daga C et al (2007) Detection of pathogens in ovine and caprine abortion samples from Sardinia, Italy, by PCR. *J Vet Diagnostic Investig* 19:96–98. <https://doi.org/10.1177/104063870701900116>
- McAllister MM, Dubey JP, Lindsay DS et al (1998) Dogs are definitive hosts of *Neospora caninum*. *Int J Parasitol* 28:1473–1479. [https://doi.org/10.1016/S0020-7519\(98\)00138-6](https://doi.org/10.1016/S0020-7519(98)00138-6)
- McAllister MM, McGuire AM, Jolley WR et al (1996) Experimental neosporosis in pregnant ewes and their offspring. *Vet Pathol* 33:647–655
- Mendonça CED, Munhoz AD, de Santana RD et al (2019) Factors associated with the seroprevalence of *Neospora caninum* (Apicomplexa: Toxoplasmatinae) in sheep from the State of Sergipe, Brazil. *Brazilian J Vet Med* 41:e002819
- Menzies PI (2011) Control of important causes of infectious abortion in sheep and goats. *Vet Clin Food Anim Pract* 27:81–93
- Moore DP (2005) Neosporosis in South America. *Vet Parasitol* 127:87–97
- Moreno B, Collantes-Fernández E, Villa A et al (2012) Occurrence of *Neospora caninum* and *Toxoplasma gondii* infections in ovine and caprine abortions. *Vet Parasitol* 187:312–318. <https://doi.org/10.1016/j.vetpar.2011.12.034>
- Nasiri V, Jameie F (2023) Snake neosporosis : molecular detection and phylogenetic characterization of *Neospora caninum* DNA from Iranian venomous snakes. *Eur J Wildl Res* 1–7. <https://doi.org/10.1007/s10344-023-01699-7>
- Nie LB, Cong W, Zou Y et al (2018) First report of seroprevalence and risk factors of *Neospora caninum* infection in Tibetan sheep in China. *Biomed Res Int* 2018. <https://doi.org/10.1155/2018/2098908>
- O’Handley R, Liddell S, Parker C et al (2002) Experimental infection of sheep with *Neospora caninum* oocysts. *J Parasitol* 1120–1123. [https://doi.org/10.1645/0022-3395\(2002\)088\[1120:EIO-SWN\]2.0.CO;2](https://doi.org/10.1645/0022-3395(2002)088[1120:EIO-SWN]2.0.CO;2)
- Razmi G, Naseri Z (2017) Molecular detection of *Neospora caninum* infection in ovine aborted foetuses in the Mashhad area Iran. *Ann Parasitol* 63:45–47
- Regidor-Cerrillo J, Arranz-Solís D, Benavides J et al (2014) *Neospora caninum* infection during early pregnancy in cattle: how the isolate influences infection dynamics, clinical outcome and peripheral and local immune responses. *Vet Res* 45:1–15
- Sadrebazzaz A, Haddadzadeh H, Esmailnia K et al (2004) Serological prevalence of *Neospora caninum* in healthy and aborted dairy cattle in Mashhad Iran. *Vet Parasitol* 124:201–204
- Sasani F, Javanbakht J, Seifori P et al (2013) *Neospora caninum* as causative agent of ovine encephalitis in Iran. *Pathol Discov* 1(5)
- Selim A, Ali A-F (2020) Seroprevalence and risk factors for *C. burnetii* infection in camels in Egypt. *Comp Immunol Microbiol Infect Dis* 68:101402
- Selim A, Khater H, Almohammed HI (2021) A recent update about seroprevalence of ovine neosporosis in Northern Egypt and its associated risk factors. *Sci Rep* 11:14043. <https://doi.org/10.1038/s41598-021-93596-9>
- Selim A, Radwan A (2020) Seroprevalence and molecular characterization of West Nile virus in Egypt. *Comp Immunol Microbiol Infect Dis* 71:101473
- Tamura K, Stecher G, Peterson D et al (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725–2729
- Tirosh-Levy S, Savitsky I, Blinder E, Mazuz ML (2022) The involvement of protozoan parasites in sheep abortions—a ten-year review of diagnostic results. *Vet Parasitol* 303:109664. <https://doi.org/10.1016/j.vetpar.2022.109664>
- Villagra-Blanco R, Wagner H, Dolz G et al (2017) First report on the seroprevalence of *Neospora caninum* in goats from the federal state of Hesse, Germany. *Berl Munch Tierarztl Wschr* 130:517–522
- Wang S, Li L, Lu Y et al (2018) Seroprevalence and risk factors of *Neospora caninum* infection among domestic sheep in Henan province, central China. *Parasite* 25:15. <https://doi.org/10.1051/parasite/2018019>
- Yamaga M, Flechtner O, Gottstein B (1996) *Neospora caninum*: Specific oligonucleotide primers for the detection of brain “cyst” DNA of experimentally infected nude mice by the polymerase chain reaction (PCR). *J Parasitol* 82(2):272–279. <https://doi.org/10.2307/3284160>

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