Simultaneous infection of bovine aborted fetuses by Infectious Bovine Rhinotracheitis and *Neospora caninum* in a dairy farm around Tehran, Iran

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Abstract

Infectious bovine rhinotracheitis (IBR) is a predominant viral abortifacient agent and *Neospora caninum*, has been documented as a primary etiological agent of reproductive losses in dairy herds. Cattle co-infected with IBR and *N. caninum* exhibit elevated abortion rates compared to herds infected with a single pathogen. The present study aimed to investigate the concurrent infection of bovine fetuses with IBR and *N. caninum*, emphasizing the critical need for implementing control measures. A total of 23 blood samples, 23 placentas, 23 vaginal secretions and 7 fetuses from aborted cattle, were collected for the identification of *N. caninum* and IBRV. Real-time PCR developed for the detection of *N. caninum* and bovine herpesvirus 1 (BoHV-1). The presence of *N. caninum* and IBRV specific antibodies was assessed using commercially available ELISA kits. Serological analysis revealed a higher prevalence of IBRV infection compared to *N. caninum*, whereas aborted fetuses demonstrated greater frequency of *N. caninum* infection. ELISA results indicated that 60.9% and 43.5% of cattle sera tested positive for antibodies against IBRV and *N. caninum*, respectively. IBRV was detected in 34.8% of the fetuses, while *N. caninum* infection was identified in 43.5%. Concurrent infection with both pathogens was observed in 26.1% of the fetuses, and 4.3% of placental and vaginal discharge samples tested positive for both agents. All positive fetuses and the infected placental sample originated from seropositive cattle. Effective control of neosporosis and IBR infection in cattle requires vaccination and biosecurity measures. Implementation of comprehensive hygienic measures is critical which should include rigorous environmental disinfection, isolation of suspected cases, control of stray dogs (the definitive host of *Neospora* spp.), and proper disposal of reproductive tissues to effectively limit disease transmission.

Keywords: Abortion, Cattle, Dog, Infectious bovine rhinotracheitis, Neospora caninum

1. Introduction

Abortion is a major contributor to economic losses in the dairy cattle industry, adversely impacting both production efficiency and reproductive performance. Uncontrolled abortion in dairy herds can result in fetal mortality, reproductive inefficiency, diminished milk yield, premature culling of affected animals, and decreased body condition (1). Globally, the estimated economic cost of abortions in dairy cattle ranges from $90 to $1,900 (2). Among abortion cases in dairy cattle, infectious agents represent the predominant etiology, attributed to their high abortifacient potential, diagnostic accessibility, and zoonotic implications (3).

Infectious bovine rhinotracheitis (IBR) is a bovine Alphaherpesvirus type 1 (BoAHV-1), which belongs to the *Varicellovirus* genus, a member of the family *Herpesviridae*, subfamily *Alphaherpesvirinae* (4). IBR is recognized as a reportable disease by the World Organization for Animal Health (WOAH) (5). Nevertheless, despite its economic significance and impact on bovine reproduction, many nations have yet to establish standardized control programs for the prevention and mitigation of the disease. IBR virus is a predominant viral abortifacient agent that induces placentitis, impairing placental function and disrupting nutrient and oxygen exchange between the dam and fetus (6, 7). In addition, respiratory and ocular disorders are the other complication of the virus infection (8). The abortion rate associated with IBR virus varies from 5% to 60%, resulting in significant economic losses every year (9). Tadeg et al. (10) in 2021 reported a seroprevalence rate of 25.6% for IBR virus in the Dessie and Kombolcha districts of Ethiopia, with all seropositive cases resulting in abortion (100% abortion incidence).

Beyond IBR virus, multiple other abortifacient pathogens substantially compromise reproductive efficiency in dairy production systems, requiring integrated control measures (11,12). *Neospora caninum* (*Sarcocystidae* family), has been extensively documented as a primary etiological agent of reproductive losses in dairy herds, contributing to significant economic burdens (13). Canids serve as critical epidemiological reservoirs for *N. caninum*, facilitating its maintenance and transmission within dairy cattle populations (14). Studies in Iran have reported seropositivity ranging from 3.8% to 76.2% in cattle infected with *Neospora*, highlighting widespread exposure to the parasite (15). A review study by Nayeri et al. (16) in 2022, reported consistent *N. caninum* identification in abortion cases through serological testing, with 47% prevalence via ELISA and 45% via immunofluorescence assay (IFA). The study by Kaveh et al. (17) in 2017, demonstrated that *Neospora* infection accounted for 30.47%

of bovine abortions (39/128 cases) in Qazvin province, Iran, representing the most frequently identified cause of reproductive loss. This substantial epidemiological variation demonstrates significant consequences for bovine reproductive health, necessitating immediate implementation of targeted prevention and control strategies.

Cattle co-infected with different pathogens exhibit elevated abortion rates compared to herds infected with a single agent, despite their distinct pathogenic mechanisms (1). Epidemiological investigations, including studies conducted in *Brucella*-free Algerian dairy herds, demonstrate that concurrent seropositivity for IBR virus and other abortifacient agents including *N. caninum* is significantly associated with increased abortion incidence (18). The present study aimed to investigate the concurrent infection of bovine fetuses with IBR and *N. caninum*, emphasizing the critical need for implementing control measures to address their high prevalence and associated reproductive and economic impacts.

2. Materials and methods

2.1. Study design and sample collection

Samples comprising 23 placentas and vaginal secretions, as well as 7 aborted fetuses, were collected for the identification of *N. caninum* and IBRV using real-time PCR. The samples were transported to the laboratory under cold chain conditions. Additionally, 23 blood samples were collected via jugular venipuncture from the aborted cattle using sterile syringes and transferred into EDTA-free tubes to preserve serum integrity.

2.2. Real-time PCR

DNA and RNA were extracted from homogenized tissue samples and swabs using SinaClon extraction kit according to the manufacturer's protocol. A Real-time PCR developed to amplify region of the Nc5 gene (GenBank accession: X84238.10) for the detection of *N. caninum*. Custom-designed primers and a probe were employed for amplification: forward primer (5´-CTGTGCTCGCTGGGACTTC-3´), reverse primer (5´-CGATTTACGACATACGGTGTTCA-3´), and a FAM-BHQ1-labeled probe (5´-[FAM]CATCGGAGGACATCGCTCACTGACTG[BHQ1]-3´).

Reactions were performed in a total volume of 25 µl, containing 400 nM of each primer, 120 nM of probe, and 5 µL of extracted nucleic acid template. Amplifications were conducted with the following thermal profile: initial denaturation at 95°C for 10 min, followed by 40 cycles of

95°C for 15 s and 60°C for 45 s. All reactions were performed in triplicate to ensure reproducibility.

A real-time RT-PCR assay was then performed to detect bovine herpesvirus 1 (BoHV-1) by targeting a highly conserved region of the glycoprotein B gene. The oligonucleotide sequences used were as follows: Forward primer (gB-F): 5′-TGT GGA CCT AAA CCT CAC GGT-3′ , Reverse primer (gB-R): 5′-GTA GTC GAG CAG ACC CGT GTC-3′ , TaqMan probe: 5′-AGGACCGCGAGTTCTTGCCGC-3′. The real-time RT-PCR reaction was set up in a final volume of 25 µl containing 12.5 µl of 2× Platinum Quantitative PCR SuperMix-UDG (Invitrogen), 1 µl each of forward and reverse primers (final concentration 80 nM), 1 µl of a TaqMan probe (final concentration 120 nM) labeled with FAM at the 5′ end and TAMRA at the 3′ end, 4 µl of nuclease-free water, and 5 µl of the extracted DNA.

Negative controls using nuclease-free water were included at all stages, while positive controls were provided by standardized BoHV-1 DNA. Amplification was carried out on a Rotor-Gene Q Series (QIAGEN) under the following cycling conditions: an initial UDG activation at 50°C for 2 minutes, a denaturation step at 95°C for 10 minutes, followed by 45 cycles of 15 seconds at 95°C and 45 seconds at 60°C for annealing and extension.

2.3. Enzyme-Linked Immunosorbent Assay (ELISA)

The presence of *N. caninum* and IBRV specific antibodies was assessed using commercially available ELISA kits (IDVET, Grabels, France) in strict accordance with the manufacturer's protocol. Optical density values were measured at 450 nm using an automated ELISA reader, with reference filters set at 620 nm.

3. Results:

The results of *N. caninum* and IBRV detection in different samples are shown in Table 1. Serological analysis revealed a higher prevalence of IBRV infection compared to *N. caninum*, whereas aborted fetuses demonstrated greater frequency of *N. caninum* infection. All the positive fetuses and the placenta sample were belonged to the seropositive cattle.

Table 1. The frequency of IBRV and *N. caninum* detection in the aborted materials and serum samples

Sample type	Sample (N)	IBRV (N)	IBRV (%)	N. caninum (N)	N. caninum (%)	Co-Infection (N)	Co-Infection (%)
Fetus	23	8	34.8%	10	43.5%	6	26.1%
Placenta	23	1	4.3%	1	4.3%	1	100%
Vaginal Discharge	23	1	4.3%	1	4.3%	1	100%
Serum	23	14	60.9%	10	43.5%	7	30.4%

4. Discussion:

It is evident that *N. caninum* and IBRV are endemic in Iranian cattle populations and play a significant role in bovine abortion (19,20). Notably, the current results demonstrated a high prevalence of these pathogens, with molecular analysis revealing IBRV and *N. caninum* infection rates of 34.8% and 43.5% among aborted fetuses, respectively (Table 1). These findings are consistent with those reported by Ansari-Lari (21) in 2021, who identified *N. caninum* in 34.8% of bovine fetuses collected across nine Iranian provinces. A recent study in Khorasan Razavi, Iran detected *N. caninum* in 24.76% of bovine fetuses, with 100% seropositivity among aborted cattle, suggesting endemic parasite transmission in the region (15). The serological results from this study demonstrated a high seroprevalence of both pathogens. All the samples that tested positive via molecular methods were derived from seropositive cattle.

Most studies investigating *N. caninum* in Iranian cattle populations have relied on sero-epidemiological approaches, primarily using ELISA to detect antibodies against the parasite (16). Reported seroprevalence rates vary widely, ranging from 3.8% to 76.2%, highlighting significant variability in infection levels across studies (15). This variation may be due to factors beyond those examined, such as climatic and weather changes, breed, size of herd and test type (22). The molecular investigation conducted in the present study provides a more

reliable approach for determining the parasite involvement in fetal abortion compared to serological methods, which merely indicates prior exposure to the pathogen.

IBRV have been identified as a leading cause of bovine abortion in numerous regions of Iran, particularly in dairy farms lacking a structured vaccination program (9, 23, 24). High number of seropositive aborted cattle (60.9%) in the current study was in accordance with the study of Khaneabad et al.(23) in 2023, which showed 96.7% of pregnant cattle of Shahrekord, Iran were seropositive for the virus. Another research conducted in Khorasan Razavi, Iran detected IBRV in 84 out of 618 aborted fetuses using conventional PCR (9). Previous studies have determined BHV-1 in Iran to be 58.74% at the individual level and 82.93% at the herd level. Furthermore, intra-herd prevalence exhibited considerable variation, ranging from 16.70% to 100% (24).

In contrast to the high prevalence of the studied pathogens in aborted fetuses and serum samples, only one out of 23 placental and vaginal secretion samples (4.3%) tested positive for each pathogen. This discrepancy may be attributed to both environmental contaminations of placental and vaginal samples and limited pathogen shedding in vaginal discharges, potentially resulting in lower detection rates compared to other sample types.

The current study identified co-infection rates involving Bovine Herpesvirus-1 (BoHV-1) and *Neospora caninum* ranging from 4.3% in placental and vaginal swab samples to 26.1% in aborted fetuses and 30.4% in sera from the affected cattle. Recurrent abortions, reduced herd productivity, and economic losses are significant consequences of co-infections (25, 26). Multiple studies have reported co-infections involving various abortifacient pathogens, including bovine viral diarrhea virus (BVDV) and IBRV, as well as *N. caninum* and BVDV, along with other viral and bacterial agents (27). However, the present study represents the first documented detection of concurrent IBRV and *N. caninum* infections in bovine aborted fetuses, demonstrating a notably high frequency of co-infection (Table 1). While Bovine Herpesvirus-1 (BoHV-1) induces necrotizing placentitis, *N. caninum* triggers inflammatory responses and tissue damage (28, 29). The independent immunosuppressive effects of *N. caninum* and IBRV create a permissive environment for both latent infection recrudescence and new pathogen establishment (28, 29). Further investigation is required to establish causal relationships and characterize the precise pathophysiological mechanisms underlying the possible synergistic effect of IBRV and *Neospora*.

Effective control of neosporosis and IBR infection in cattle requires a multipath approach among which vaccination and biosecurity measures have a significant role (4). Available

vaccines against IBRV provide clinical protection to cattle upon infection and significantly reduce subsequent shedding of the field virus (4). Although vaccination may not prevent field virus infection in individual animals, it effectively limits the transmission of wild-type virus within infected herds (5). While vaccination and eradication programs remain important components of disease management, implementation of comprehensive hygienic measures is equally critical. These should include rigorous environmental disinfection, isolation of suspected cases, control of stray dogs (the definitive host of *Neospora* spp.), and proper disposal of reproductive tissues to effectively limit disease transmission.

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6. Authors contribution:

Study concept and design: M. H.

Acquisition of data: M. H., S. V.

Analysis and interpretation of data: M. H.

Drafting of the manuscript: S. V., A. L. G.

Critical revision of the manuscript for important intellectual content: A. M., P. B.

Administrative, technical, and material support: M. H.

Study supervision: A. M., P. B.

7. Ethics

Mona Hamedi, Saber Vahedi, Amirabbas Lak Ghasemabadi, Alireza Mortazavinia and Parimah BourBour confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

8. Conflict of interest and Data availability

Mona Hamedi, Saber Vahedi, Amirabbas Lak Ghasemabadi, Alireza Mortazavinia and Parimah BourBour confirm that there are no known conflicts of interest associated with this publication.

All data and supplementary information would be available upon request from the corresponding author.

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10. Reference

1. Vindas-van der Wielen E, Romero-Zúñiga JJ, Monti G. Productive and reproductive losses associated with abortion in specialized dairy cattle from Costa Rica. J Dairy Sci. 2025;108(5):5287-95.
2. Neupane M, Hutchison JL, Cole JB, Van Tassell CP, VanRaden PM. Genomic evaluation of late-term abortion in cows recorded through Dairy Herd Improvement test plans. JDS Commun. 2023;4(5):354-7.
3. Hajibemani A, Sheikhalislami H. Zoonotic pathogens cause of animal abortion and fetal loss. Journal of Zoonotic Diseases. 2020;4(3):1-19.
4. Rimayanti R, Khairullah AR, Lestari TD, Moses IB, Utama S, Damayanti R, et al. Infectious bovine rhinotracheitis: Unveiling the hidden threat to livestock productivity and global trade. Open Vet J. 2024;14(10):2525-38.
5. World Organisation for Animal H. Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV). 2023.
6. Givens MD, Marley M. Infectious causes of embryonic and fetal mortality. Theriogenology. 2008;70(3):270-85.
7. Pirrone A, Antonelli C, Mariella J, Castagnetti C. Gross placental morphology and foal serum biochemistry as predictors of foal health. Theriogenology. 2014;81(9):1293-9.
8. Mottaghian, P. , Raoofi, A. , Madadgar, O. , Badiei, A. and Ashrafi Tamai, I. (2023). A Study on Mycoplasmal and Viral Infections in Bovine Keratoconjunctivitis. Iranian Journal of Veterinary Medicine, 17(4), 345-352.
9. Mohammadian M, Mirshokraei P, editors. Evaluation of the Infectious Bovine Rhinotracheitis Frequency in Aborted Bovine Fetuses over a Seven-Year Period In North-East of Iran. The 2nd Congress of Infection and Immunity; 2024.
10. Tadeg WM, Lemma A, Yilma T, Asgedom H, Reda AA. Seroprevalence of infectious bovine rhinotracheitis and brucellosis and their effect on reproductive performance of dairy cattle. Journal of Veterinary Medicine and Animal Health. 2021;13(2):106-13.
11. Okumu TA, John NM, Wabacha JK, Tsuma V, VanLeeuwen J. Seroprevalence of antibodies for bovine viral diarrhoea virus, Brucella abortus and Neospora caninum, and their roles in the incidence of abortion/foetal loss in dairy cattle herds in Nakuru District, Kenya. BMC veterinary research. 2019;15(1):95.

12. Bahreinipour, A. , Bahonar, A. , Boluki, Z. , Rahimi Foroshani, A. , Lotfollah Zadeh, S. and Amiri, K. (2023). Bovine Brucellosis Infection in Iranian Dairy Farms: A Herd-level Case-control Study. Iranian Journal of Veterinary Medicine, 17(4), 383-392
13. Liu Y, Reichel MP, Lo W-C. Combined control evaluation for Neospora caninum infection in dairy: Economic point of view coupled with population dynamics. Veterinary Parasitology. 2020;277:108967.
14. Villa L, Allievi C, Di Cerbo AR, Zanzani SA, Sommariva F, Zanini L, et al. Neospora caninum antibodies in bulk tank milk from dairy cattle herds in Italy in relation to reproductive and productive parameters and spatial analysis. Acta Tropica. 2024;254:107194.
15. Shahrestanaki AK, Nourani H, Razmi G. Pathological and Molecular Studies of Neospora caninum Infection in Aborted Bovine Foetuses in Khorasan Razavi Province, Iran. Veterinary Medicine and Science. 2025;11(3):e70329.
16. Nayeri T, Moosazadeh M, Sarvi S, Daryani A. Neospora caninum infection in aborting bovines and lost fetuses: A systematic review and meta-analysis. PLoS One. 2022;17(5):e0268903.
17. Kaveh A, Merat E, Samani S, Danandeh S, Soltan Nezhad S. Infectious Causes of Bovine Abortion in Qazvin Province, Iran. Arch Razi Inst. 2017;72(4):225-30.
18. Yahiaoui F, Kardjadj M, Mohammedi S, Ben-Mahdi MH. Seroprevalence of Abortion-Related infectious diseases and associated risk factors among Brucellosis-free herds in Northern central Algeria. BMC Vet Res. 2025;21(1):185.
19. Hassani M, Menzies P, Alamian S, Esmaeili H. Prevalent Infectious Causes of Abortion in the Ruminant Population in Iran-A. Archives of Razi Institute Journal. 2025;80(3).
20. Hadad zadeh, H. R. , Shayan, P. , Vojgani, M. and Bolorchi, M. (2010). Serological study of Neospora caninum in pregnant dairy cattle in Tehran, Iran. Iranian Journal of Veterinary Medicine, 4(2)
21. Ansari-Lari M. Neospora caninum in aborted bovine fetuses in Iran: a systematic review and meta-analysis. Annals of Parasitology. 2021;67(3):357-66.

22. Haji Hajikolaei, M. R. , Hamidinejat, H. , Ghorbanpoor, M. and Goraninejad, S. (2008). Serological study of Neospora caninum infection in cattle from Ahvaz area, Iran. Iranian Journal of Veterinary Medicine, 2(2), 63-66.
23. Khaneabad A, Taktaz T, Goodarzi S, Momtaz H. BoHV-1 affects abortion and progesterone in dairy cows Bovine alphaherpesvirus 1 (BoHV-1) seropositivity, progesterone levels and embryo loss of 30-day-old pregnant dairy cows in Zagros Industrial Dairy Farm in Shahrekord: Examination and analysis. Veterinary Medicine and Science. 2023;9(4):1934-9.
24. Bahari A, Gharekhani J, Zandieh M, Sadeghi-Nasab A, Akbarein H, Karimi-Makhsous A, et al., editors. Serological study of bovine herpes virus type 1 in dairy herds of Hamedan province, Iran. Veterinary Research Forum; 2013.
25. Gädicke P, Monti G. Factors related to the level of occurrence of bovine abortion in Chilean dairy herds. Prev Vet Med. 2013;110(2):183-9.
26. Robi DT, Bogale A, Urge B, Aleme M, Temteme S. Neglected zoonotic bacteria causes and associated risk factors of cattle abortion in different agro-ecological zones of southwest Ethiopia. Veterinary Immunology and Immunopathology. 2023;259:110592.

27. Hou Z, Wang J, Tan B, Zhang S. A Systematic Study of Bovine Viral Diarrhoea Virus Co-Infection with Other Pathogens. Viruses. 2025;17(5):700.
28. Graham DA. Bovine herpes virus-1 (BoHV-1) in cattle–a review with emphasis on reproductive impacts and the emergence of infection in Ireland and the United Kingdom. Irish veterinary journal. 2013;66(1):15.
29. Miqueo E, Cruz MS, Moore DP, Campero LM. Oxidative and inflammatory status, daily weight gain, intake and feed efficiency on natural congenitally Neospora caninum infected dairy calves. Veterinary Parasitology. 2025:110530.