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Prevalence of bovine respiratory disease viruses in calves from the central desert of Iran

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Abstract

Bovine respiratory disease (BRD) is a significant disease in the cattle industry worldwide. The interaction between environmental factors, hosts, livestock management, and viral and bacterial pathogens causes this disease. Viruses are crucial in the initiation and progression of BRD. This study was the first to investigate the prevalence of BRD viruses using the reverse transcription polymerase chain reaction method in nasal and eye conjunctival swabs and blood samples of 115 BRD calves in the central desert of Iran. At least one investigated virus was detected in 44 animals (38.26%). The detection rates of bovine viral diarrhea virus, bovine coronavirus, bovine adenovirus, bovine respiratory syncytial virus, bovine herpes virus-1, and bovine para influenza virus-3 were 20.00, 14.78, 5.21, 0.86, 0.00, and 0.00%, respectively. Three animals (2.60%) had a simultaneous infection with two viruses. Detection of bovine viral diarrhea virus, bovine coronavirus, and bovine adenovirus was correlated. The virus infection rates were 31.81 and 44.66% in five sampled cities. The virus detection rate in infected animals was related to the nose (26 animals; 50.09%), nose and eyes (seven animals; 15.90%), eyes (seven animals; 15.90%), nose, eyes, and blood (three animals; 6.81%), and blood (one animal; 2.27%) samples. The virus detection rate in different samples was in separate clusters. Monitoring and controlling the circulation of bovine viral diarrhea virus and bovine coronavirus in the central desert of Iran is vital due to the high detection rate. Our results highlight the necessity of investigating other viruses and bacterial agents related to the BRD in the study area.

Introduction

Bovine respiratory disease (BRD) is one of the most common diseases in beef and dairy cattle herds. The BRD causes a lot of economic losses by reducing performance (reducing milk and meat production), animal mortality, and treatment and labor costs. The widespread use of antibiotics in the treatment and prevention of BRD has also raised concerns about the rise of antibiotic resistance and threat to human and animal health.[1-6] The BRD is a general term referring to a range of respiratory diseases affecting the lower respiratory tract of cattle.[2] Anorexia, depression, fever, nasal and eye discharges, excessive salivation, rapid breathing, shortness of breath, and coughing are manifestations of the disease. The disease usually affects young animals under the age of one.[2,7,8]

The BRD is multi-factorial, resulting from the interaction between environmental factors (humidity, temperature, dust, ventilation, and livestock congestion), host (age, sex, and immune status), management (colostrum management, maternity pen, pathogen population composition, and vaccination), and pathogen factors (bacteria and viruses).[1,2,7,9-16] At the beginning of the last century, researchers believed that the disease was caused only by a bacterial infection (bovine pasteurellosis).[2] Today, viruses play an essential role in the induction of BRD.[2,17] A mixture of bacteria and viruses is isolated from the respiratory secretions of BRD animals. Viruses that are usually associated with BRD include bovine alpha herpes virus-1 (BoHV-1), bovine viral diarrhea virus (BVDV), bovine para influenza virus-3 (BPIV3), bovine respiratory syncytial virus (BRSV), bovine

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adenovirus (BAdV), and bovine coronavirus (BCoV). Bacterial infections mainly include *Mannheimia haemolytica, Histophilus somni, Pastuerella multocida,* and *Mycoplasma bovis*.[4,5,18-23]

The BRD pathogenesis includes a complex interaction between pathogenic bacteria and viruses under the influence of stressful factors (weaning, diet changes, and transportation), environmental factors, and the immune status of the animals.[2,3,12] A primary viral infection may increase bacterial adherence and colonization in the respiratory tract. Viruses and stressful factors suppress the immune defense and lead to the growth of bacteria in the upper respiratory tract and invasion of pathogens in the lower respiratory tract. Viruses play a role in increasing the activity of inflammatory factors, causing damage to the infected epithelial cells and reducing mucosal transmission.[1,11,21-25]

The leading causes of death in dairy cattle calves in Iran are gastrointestinal disorders and respiratory diseases.[26] Determining the prevalence and associations of viruses linked with BRD can help develop effective therapeutic interventions to prevent and control the disease, thus reducing the damage it causes.[3,27,28] New molecular methods are increasingly replacing tests for isolating respiratory tract pathogens. These methods are of interest due to their cost-effectiveness, high specificity and sensitivity, the volume of test samples, the labor force, and test speed.[13,29,30] So far, no study has been conducted for the molecular diagnosis of viruses related to BRD in the central desert of Iran. The purpose of this study was to molecularly determine the frequency of selected respiratory viruses in calves suffering from BRD and pinpoint the relationship between one or more viral factors in the central desert of Iran.

Materials and Methods

Study area and animal sampling. The study was carried out in Yazd, a province situated in central Iran (31° 30′ 0″ N, 54° 40′ 0″ E), covering an area of approximately 76,469 km^2. Yazd is part of the global dry belt and experiences significant seasonal variation. The summers are extremely hot and arid, with temperatures reaching up to 46.00 ˚C, while the winters are mild with occasional humidity. The average annual precipitation is approximately 110 mm. Data collection occurred between December 2022 and December 2023, spanning all four seasons. No vaccination programs for BRDs had been implemented in the region at the time of the study. Based on reports from the Iranian Veterinary Organization and local veterinarians regarding the prevalence of respiratory disease symptoms, several cities within the province were visited. A total of 115 Holstein calves, aged between 6 days and 10 months (83 females and 32 males), were selected for sampling. Calves were included in the study if their clinical symptoms had been present for no more than three days. The biological samples, including nasal and conjunctival eye swabs and blood samples, were collected. These samples were preserved in viral transport medium (BM-VTM™; Bio-Med, Ghaziabad, India) and anti-coagulant tubes, respectively. They were promptly transported on ice to the Virology Department of the Razi Vaccine and Serum Research Institute, Karaj, Iran, for further analysis. The presence of six specific viruses, including BVDV, BCoV, BAdV, BRSV, BoHV-1, and BPIV3, was tested using reverse transcription polymerase chain reaction (RT-PCR). This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the Razi Vaccine and Serum Research Institute, Karaj, Iran (Date: October 25, 2021; Ethics Committee Code: 3-18-1857-028-000370).

Viral RNA extraction. To extract viral RNA, 500 µL of blood or 100 µL of swab solution was pipetted into a 2.00 mL micro-centrifuge tube that was free of RNase and DNase. One milliliter of cold RNX solution (SinaClon, Tehran, Iran) was added to the tube. The mixture was vortexed for 5 - 10 sec and then, incubated at room temperature for 5 min. Next, 200 µL of chloroform was added, and the solution was mixed gently by hand for 15 sec (Merck, Darmstadt, Germany). The tube was incubated on ice or at 4.00 ˚C for 5 min, followed by centrifugation at 12,000 rpm at 4.00 ˚C for 15 min. The upper aqueous phase was carefully transferred to a new tube, and an equal volume of isopropanol (Merck, Darmstadt, Germany) was added. After mixing, the solution was incubated on ice for 15 min, followed by a second round of centrifugation at 12,000 revolutions *per* min at 4.00 ˚C for 15 min. The supernatant was discarded, and the RNA pellet was washed with 1.00 mL of 75.00% ethanol before being centrifuged at 7,500 rpm at 4.00 ˚C for 8 min. The supernatant was discarded again, and the pellet was allowed to air-dry at room temperature. The dried pellet was re-suspended in 50.00 µL of diethyl pyrocarbonate-treated water (Merck, Darmstadt, Germany) and stored at – 70.00 ˚C if not used immediately. For RT-PCR analysis, typically 6.00 µL of the RNA solution was utilized.

Reverse transcriptase reaction. First-strand cDNA synthesis was performed using the Revert Aid™ First Strand cDNA Synthesis Kit (Waltham, Massachusetts, USA). To initiate the reaction, 6.00 µL of RNA, 1.00 µL of Random Hexamer primer, and 5.00 µL of diethyl-pyrocarbonate-treated water were mixed to a final volume of 12.00 µL. The mixture was incubated at 65.00 ˚C for 5 min and cooled to 4.00 ˚C. To this, 4.00 µL of 5.00 x buffer, 1.00 µL of RNase inhibitor (200U μL^{-1}), 2.00 µL of dNTP mix (10.00 mM), and 1.00 µL of Revert Aid™ M-MuLV Reverse Transcriptase (200 U μL^{-1}) were added. The final volume was adjusted to 20.00 µL. The reaction was incubated in a thermocycler (42.00 ˚C for 60 min and 70.00 ˚C for 5 min).

Polymerase chain reaction. Two microliters of the synthesized cDNA was used as a template for PCR amplification.

Virus DNA extraction. The DNA was extracted from viral genomes present in blood and swab samples using the DNP™ Kit (SinaClon), a high-efficiency DNA purification kit. The extraction was carried out according to the manufacturer's instructions. For PCR amplification, 2.00 µL of the extracted DNA was used. The primers were designed using the Oligo7 Software (Molecular Biology Insights, Inc., Cascade, Colorado, USA). Details regarding the primers and PCR conditions for virus detection are presented in Table 1.

Statistical analysis. Statistical analyses were performed using R Software (version 4.3.1; R Foundation for Statistical Computing, Vienna, Austria). Pearson correlation coefficients were visualized using the Corrplot Package, and principal component analysis (PCA) was conducted using the Factoextra R Package.

Results

Forty-four animals (38.26%) from the sampled calves were infected with the virus. The detection rates of BVDV, BCoV, BAdV, and BRSV were 20.00, 14.78, 5.21, and 0.86%, respectively. The BoHV-1 and BPIV3 were not detected in any of the samples. Simultaneous infection of two viruses was reported in three animals (2.60%). Infection with the investigated viruses was observed in 12 males (37.50%) and 32 females (38.55%). The infection rates with different viruses in the sampled cities were 31.81 and 46.66% (Table 2).

Figure 1 shows the number of sampled animals, number of infected animals with the viruses, and detection rate of the examined viruses in infected animals. The virus was detected in the nose samples of 31 animals (26.95%), nose and eyes of five animals (4.34%), eyes of four animals (3.47%), nose, eyes, and blood of three animals (2.60%), and blood from one animal (0.86%).

Table 1. The primers and programs for virus detection.

Viruses	Primer	Product size (bp)	Program
Bovine viral diarrhea virus	F: ATG CCC (T/A)TA GTA GGA CTA GCA R: TCA ACT CCA TGT GCC ATG TAC	288	95.00 ˚C, 3 min; 95.00 ˚C, 40 sec; 56.00 ˚C, 30 sec; 72.00 ˚C, 20 sec; Repeat = 35 cycles, 72.00 ˚C, 30 sec
Bovine viral diarrhea virus nested	F: TCGTGGTGAGATCCCTGAG R: GCAGAGATTTTTTATACTAGCCTATRC	225	95.00 ˚C, 3 min; 95.00 ˚C, 40 sec; 55.00 ˚C, 35 sec; 72.00 ˚C, 14 sec; Repeat = 33 cycles, 72.00 ˚C, 30 sec
Bovine herpes virus-1	F : CACGGACCTGGTGGACAAGAAG R : CTACCGTCACGTGAGTGGTACG	468	95.00 ˚C, 4 min; 95.00 ˚C, 45 sec; 60.00 ˚C, 30 sec; 72.00 ˚C, 20 sec; Repeat = 33 cycles, 72.00 ˚C, 2 min
Bovine herpes virus-1 nested	F : AGCCGAGTACCTGCGCAG R : AGCCCTCGATCTGCTGGA	383	95.00 ˚C, 5 min; 95.00 ˚C, 50 sec; 55.00 ˚C, 40 sec; 72.00 ˚C, 20 sec; Repeat = 30 cycles, 72.00 ˚C, 1 min
Bovine respiratory syncytial virus	F: GGGAGAGGTGGCTCCAGAATACAGGC R: AGCATCACTTGCCCTGAACCATAGGC	350	95.00 ˚C, 3 min; 95.00 ˚C, 50 sec; 60.00 ˚C, 40 sec; 72.00 ˚C, 30 sec; Repeat = 35 cycles, 72.00 ˚C, 30 sec
Bovine para influenza virus-3	F: ACCAGGAAACTATGCTGCAGAACGGC R: GATCCACTGTGTCACCGCTCAATACC	230	95.00 ˚C, 3 min; 95.00 ˚C, 50 sec; 60.00 ˚C, 30 sec; 72.00 ˚C, 15 sec; Repeat = 35 cycles, 72.00 ˚C, 30 sec
Bovine adenovirus	F: GAGATGGATGTGAACAGCGA R: ACATTCTGATGCTGGTACTG	644	95.00 ˚C, 3 min; 95.00 ˚C, 40 sec; 55.00 ˚C, 30 sec; 72.00 ˚C, 30 sec; Repeat = 35 cycles, 72.00 ˚C, 30 sec
Bovine coronavirus	F: GCA ATC CAG TAG TAG AGC GT R: CTT AGT GGC ATC CTT GCC AA	566	95.00 ˚C, 3 min; 95.00 ˚C, 40 sec; 52.00 ˚C, 30 sec; 72.00 ˚C, 30 sec; Repeat = 32 cycles, 72.00 ˚C, 10 sec

Table 2. The number of sampled animals, infected animals, and the type of viruses detected (number of infected animals) in the sampled cities.

Cities	No.	Infected animals	Detected viruses (number of infected animals)
Yazd	25	11 (44.00%)	BVDVD (5), BCoV (4), and BAdV (2)
Zarch	15	7 (44.66%)	BVDV (4), BCoV (2), and BCoV + BVDV (1)
Ashkezar	22	7 (31.81%)	BVDVD (3), BCoV (3), and BAdV + BVDVD (1)
Taft	26	10 (38.46%)	BVDVD (5), BCoV (4), and BRSV (1)
Mehriz	27	9 (33.33%)	BVDVD (4), BCoV + BVDV (1), BAdV (2), and BCoV(2)
Total	115	44 (38.26%)	BVDV (21), BCoV (15), BAdV (4), BRSV (1), BCoV + BVDV (2), BVDV + BAdV (1), BoHV-1 (0), and BPIV3 (0)

BoHV-1: Bovine alpha herpes virus-1; BVDV: Bovine viral diarrhea virus; BPIV3: Bovine para influenza virus-3; BRSV: Bovine respiratory syncytial virus; BAdV: Bovine adenovirus; BCoV: Bovine coronavirus.

The highest percentage of virus detection was related to nose (39 animals; 33.91%), eye (12 animals; 43.10%), and blood (four animals; 47.30%) samples. Detection rate of investigated viruses by sample type in infected animals is specified in Figure 2.

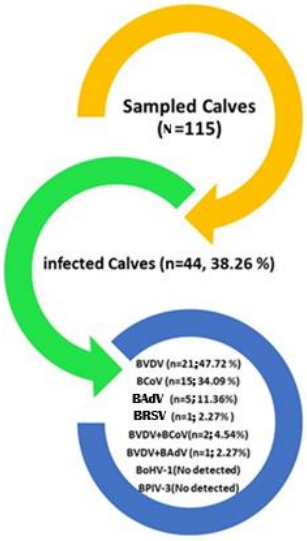

Fig. 1. The number of sampled animals, number of infected animals with the viruses, and detection rate of the examined viruses in infected animals. BVDV: Bovine viral diarrhea virus; BCoV: Bovine coronavirus; BAdV: Bovine adenovirus; BRSV: Bovine respiratory syncytial virus; BoHV-1: Bovine alpha herpes virus-1; BPIV-3: Bovine para influenza virus-3.

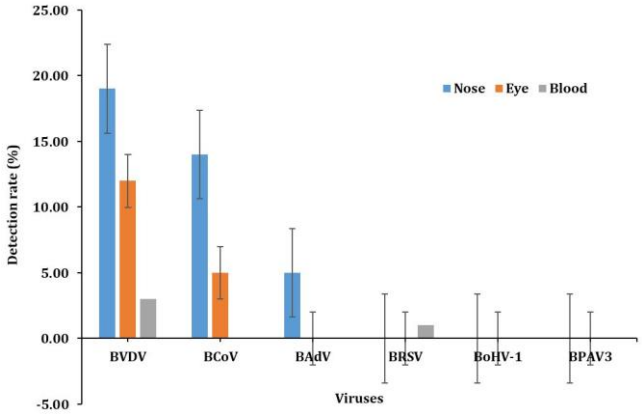

Fig. 2. Detection rate of investigated viruses by sample type in infected animals. BVDV: Bovine viral diarrhea virus; BCoV: Bovine coronavirus. BAdV: Bovine adenovirus; BRSV: Bovine respiratory syncytial virus; BoHV-1: Bovine alpha herpes virus-1; BPAV3: Bovine para influenza virus-3.

Among the detected viruses, a positive correlation was observed between BVDV, BCoV, and BAdV. On the other hand, the detection of these three viruses had a negative correlation with BRSV ($p < 0.05$; Fig. 3).

The PCA of 44 infected calf samples (nose, eyes, and blood) showed that the first and second components accounted for 53.07 and 26.34% of the variance in the data, respectively. Viruses found in the eye had a high correlation only with the first component of the data. However, when detected in the blood and nose, there was a high correlation between both components. The diagnostic ratio of the nose to blood was also low. The results of the PCA showed three different factors (nose, blood, and eye; Fig. 4).

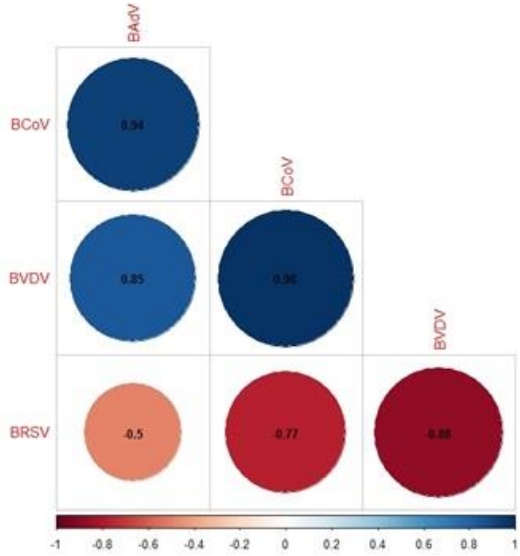

Fig. 3. The correlation coefficients of detected viruses. BVDV: Bovine viral diarrhea virus; BRSV: Bovine respiratory syncytial virus; BAdV: Bovine adenovirus; BCoV: Bovine coronavirus.

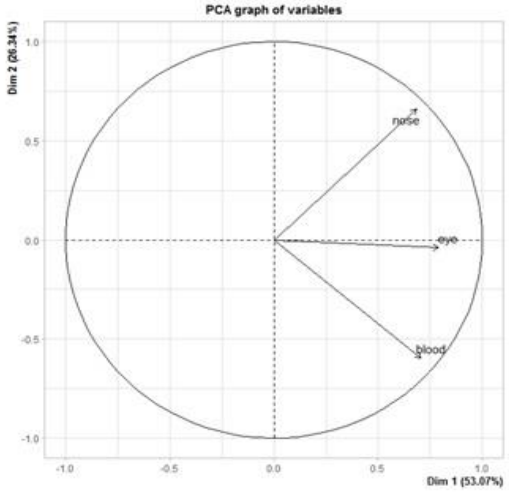

Fig. 4. Principal component analysis (PCA) of nose, eye, and blood samples of 44 infected calves.

Discussion

Bovine respiratory disease is one of the most critical diseases in the cattle industry. The prevalence of this disease in cattle farms, in addition to the economic losses caused by the decrease in performance, losses, and costs of treatment and prevention, raises the risk of increasing antibiotic resistance. Viruses play a vital role in the

initiation and progression of disease.[5,21] This study was conducted to identify common respiratory viruses in diseased calves in the central desert of Iran.

In this study, 38.26% of the sampled cattle were infected with investigated viruses. Ambrose *et al*.,[1] found that 43.30% (63 animals) of BRD cattle had attained at least one of the ten viruses investigated. Guo *et al*.,[3] with a molecular analysis of nasal swap samples of 302 beef cattle suffering from BRD, announced the detection rate of at least one of the four viruses (BRSV, BVDV, BPIV3, and BoHV-1) in six different Chinese cities from 54.00 to 80.00%. Molecular analysis of cumulative broncho-alveolar lavage samples of 28 dairy herds involved in BRD in Belgium showed a detection rate of respiratory viruses (BCoV, BRSV, and BPIV3) as 58.60%.[12] Toker and Yeşilbağ reported the detection rate of the investigated viruses (BRSV, BoH-V, BVDV, and BPIV3) in nasal swab (133 samples) and lung tissue (60 samples) samples of BRD cattle as 74.60%.[31]

According to our study, BVDV had the highest virus detection rate (20.00%) among BRD animals. The BVDV is a single-stranded RNA virus belonging to the Flaviviridae family and Pestivirus genus. The virus has two types, including Pestivirus A (BVDV1) and Pestivirus B (BVDV2), and two biotypes, including cytopathic and non-cytopathic. Fetal infection with a non-cytopathic strain between days 40 and 120 of pregnancy causes permanent calf infection. Permanent calf infection animals do not produce anti-virus antibodies, play a vital role in transmitting the virus to the herd, and shed a disproportionate amount throughout their lives. Infection with BVDV often manifests as respiratory and gastrointestinal illness. This virus plays an essential role in developing respiratory disease by suppressing the immune system and paving the way for secondary bacterial and viral super-infections.[2,31,32] Karimi *et al*.,[33] reported that the seroprevalence of BVDV in cattle in the studied area (central desert of Iran) was high (66.83% at the individual level and 91.60% at the herd level). The high prevalence of BVDV infection in BRD animals in this study matches the reports of Guo *et al.* (44.70%),[3] Klima *et al*. (69.00%),[34] Oliveria *et al*. (28.60%),[13] and Fulton *et al*. (12.30%).[30] In contrast, İnce *et al.*,[23] Deepak *et al.*,[35] Headley *et al.*,[36] Frucchi *et al.*,[37] and Ng *et al.*,[28] did not detect BVDV infection in BRD cattle.

In this study, the highest virus detection rate was related to BCoV (14.78%), after BVDV. Two of the cattle infected with BCoV had a double infection with BVDV simultaneously. The BCoV is a single-stranded positive-sense RNA virus with a lipid coating, belonging to the Nidovirales order, Coronaviridae family, Orthocoronavirinae subfamily, and Betacoronavirus genus. The clinical manifestations of BCoV in cattle are enteric and respiratory.[2,38-40] Molecular detections of BCoV in BRD animals by Ambrose *et al*. (1.40%),[1] Deepak *et al*. (9.70%),[35] Guo *et al*. (34.80%),[3] Lachowicz-Wolak *et al*. (32.43%),[27] Oliveria *et al*. (33.33%),[13] Orozco-Cabrera *et al*. (16.66%),[40] and Saegerman *et al*. (25.20%),[19] were reported. Nevertheless, Ng *et al*., did not detect BCoV in the samples of cattle with respiratory disease.[28] The role of coronaviruses in BRD is controversial, because they have been isolated from asymptomatic and BRD animals.[38,41] During an experimental study, Ridpath *et al*., have suggested that co-infection with BCoV and BVDV may play an essential role in the pathogenesis of the disease, and the timing of infection with these viruses plays a significant role in the severity of lesions.[41]

The detection rate of BAdV in our study was 5.21%. One of the cattle infected with BAdV had a double infection with BVDV simultaneously. The Adenoviride family includes this virus, and it is classified into ten stereotypes and two genera (Mastadenovirus and Atadenovirus). The BAdV causes respiratory and digestive problems in calves. Infection with the virus can lead to ocular or systemic symptoms and contribute to respiratory disease.[42-44] In the report by Ng *et al.*,[28] the virus detection rate in nasal swab samples of 50 cattle with BRD was related to BAdV3 (48.00%). This virus had a significant relationship with the disease. Pratelli *et al*.,[44] Mitra *et al*.,[45] and Paller *et al*.,[15] have reported the detection rate of BAdV3 in cattle with BRD as 13.80, 11.10, and 75.00%, respectively.

In our study, none of the BRD animals were infected with BoHV-1. This virus is one of the most critical pathogens in cattle and has a global prevalence. The Herpesviridae family includes BoHV-1, a DNA virus belonging to the Alphaherpesvirinae subfamily and Herpesvirales order. The BoHV-1.1 and BoHV-1.2 are virus subgroups, both of which are linked to BRD. The BoHV harms the immune system and leads to increased susceptibility to other pathogens. This virus can cause a latent infection, and with its reactivation by stressful factors, a new cycle of virus replication and spread in the herd begins.[2,42] Our results that BRD animals were not infected with BoHV-1 are in agreement with the results of Headley *et al*.,[36] İnce *et al*.,[23] Deepak *et al*.,[35] Oliveria *et al*.,[13] Klima *et al*.,[34] Ng *et al*.,[28] Zhang *et al*.,[24] Frucchi *et al*.,[37] and Kamdi *et al*.[21] In Mexico, only one cattle with respiratory disease (3.70%) was found to be infected with BoHV-1. The virus has not been detected in any BRD animals in the United States.[45] On the other hand, other researchers have reported the detection of BoHV in BRD cattle.[1,3,4,15,16,18-20,46-48] Karimi *et al*., reported that the seroprevalence of BoHV-1 in cattle in the studied area (central desert of Iran) was high (50.00% at the individual level and 65.00% at the herd level).[46] The animals being sampled in our research were mostly calves that were under nine months old. It is possible that maternal antibodies could have protected the calves against BoHV-1. On the other hand, BoHV-1-associated BRD usually occurs in mature animals. There is also the possibility of latent infection if the calves were infected with the virus before sampling.[13,34]

In our study, two respiratory paramyxoviruses (BRSV and BPIV3) were investigated in the nose, eye conjunctiva, and blood samples of BRD calves. These viruses are classified as Mononegavirales. The viruses of this line are covered with non-definitive single-stranded RNA with a negative sense. The BRSV belongs to the Pneumoviridae family and Orthopneumovirus genus. Although about 40.00% of the BRSV nucleotide identity is similar to that of the human respiratory syncytial virus, it is only diagnosed in cattle and small ruminants and not as a zoonotic pathogen. The BPIV3 is classified in the family of Paramyxoviridae and genus of Respirovirus. The virus is also known as the official bovine respirovirus 3.[2,3,11,20,] In this study, only one animal's blood sample had a BRSV infection. The BPIV3 was not detected in any of the samples. Ng *et al*.,[28] and Frucchi *et al*.,[37] did not detect BRSV and BPIV3 in the samples of BRD cattle. Mitra *et al*.,[45] examined 47 breeding cattle with BRD and detected BRSV in only one animal. The BPIV3 infection was not detected in any of the animals. Küçük and Yildrim,[48] tested nasal and eye conjunctival swabs and blood samples of 190 BRD cattle by molecular method to detect BPIV3 infection. The virus was detected only in two nasal swab samples (1.05%). Toker and Yeşilbağ,[31] Ambrose *et al*.,[1] Guo *et al*.,[3] Pratelli *et al*.,[44] Saegerman *et al*.,[19] and Timurkan *et al.,*[49] reported the detection rate of BRSV and BPIV3 2.59 and 0.52%, 0.70 and 2.10%, 32.60 and 12.40, 6.95 and 5.63, 14.50 and 4.30, 13.90 and 5.30, 1.29 and 1.93%, respectively.

The investigated viruses were not present in 61.73% of the sampled animals in our study. Furthermore, infections with BoHV-1 and BPIV3 were not identified. Different reports have indicated that the prevalence of BRD infectious agents can vary greatly, even within the same country.[27] This can be due to a viral load lower than the detectable level, the presence of the virus in the deeper parts of the respiratory tract, the infection alone with pathogenic bacterial agents, the infection with unexamined viral agents,[23,28] the limitations of the molecular method used in terms of sensitivity and specificity, and the place of sampling.[29,48] In addition, the pathogens of the upper and lower respiratory tracts of cattle are diverse and varied, and samples from the upper respiratory tract may not be a good representative of the lower respiratory tract.[24] The prevalence of respiratory viruses is greatly influenced by geographic regions, seasons, temperatures, age of cattle, livestock immunity status, and herd management.[3]

Very little information is available regarding the correlation between viral agents and the course of the BRD. In our study, a positive correlation was observed between BVDV, BCoV, and BAdV. On the other hand, the detection of these three viruses had a negative correlation with BRSV. Lachowicz-Wolak *et al*., have reported an average correlation level between the detection of BRSV and BPIV3.[27] In our study, three animals were infected with two viruses at the same time. Two animals were infected with BVDV and BCoV simultaneously, and one animal was infected with BVDV and BAdV. Co-infection with BRSV and BPIV3, as well as BCoV and BPIV3 has been reported previously.[27]

The PCA results suggest three distinct factors influencing virus detection, including nose, blood, and eye. This suggests that the virus may be present in different locations due to the different underlying factors. Our findings suggest that nasal samples are more suitable than eye and blood samples for detecting BRD viruses.

The results show that the sampling site (nose, eye conjunctiva, or blood) plays a role in detecting viral infection. Nasal samples had the highest rate of virus detection. The high detection rate of BVDV and BCoV indicates the importance of monitoring and controlling the circulation of these viruses in the central desert of Iran. Our results highlight the necessity of investigating other viruses and bacterial agents related to the respiratory disease of cattle in the study area.

Use of next-generation sequencing methods for unbiased screening of viral and bacterial agents of BRD in symptomatic and asymptomatic animals and performing serological tests and molecular detection of BRD viruses to confirm infection are highly suggested for upcoming research.

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Conflict of interest

The authors declare no competing interests.

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