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Diarrheal pathogens in calves: Rotavirus and co-infection with Coronavirus and Norovirus in selected provinces of Iran

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Abstract

Calf diarrhoea is a leading cause of mortality and morbidity among neo-natal calves in the cattle industry, with viral pathogens such as bovine rotavirus (BRoV), bovine coronavirus (BCoV), and bovine norovirus (BNoV) playing prominent roles. Present study investigated the occurrence and co-infection pattern of BRoV, BCoV, and BNoV in diarrheic calves in seven main livestock-producing provinces of Iran. A total of 320 fecal samples from diarrheic calves were examined with ELISA and RT-PCR. The results revealed that BRoV was the most prevalent pathogen (68.8%), followed by BCoV (56.5%), and BNoV (25.9%). The most common co-infections were BRoV+BCoV (22.5%) and BRoV+BNoV (12.5%), whereas 6.9% cases revealed triple infection. Notably, BNoV mono-infection was rare (1.6%), suggesting its limited pathogenic role single-handedly but with a potential synergistic effects in co-infections. BRoV detection rate was significantly higher during colder months (77.9%), whereas no clear seasonal patterns were observed for BCoV and BNoV. The study revealed intensive management systems as a significant risk factor for BRoV infection. These findings expound the complexity of viral enteric diseases in calves and emphasize the need for specific management and control strategies. Further investigations are recommended to examine the interactions of viruses, genetic variations, and potential zoonotic risk of these viruses.

1. Introduction

Calf diarrhea is a common and multifaceted health problem that poses formidable health and economic issues in the animal husbandry sector, mostly within the initial weeks of a calf's life (Carter et al. 2021). Research results indicate that acute diarrhea causes over 50% of neonatal mortality among dairy calves and substantially contributes adverse to early growth and weight gain, and induces prolonged reproductive losses and poor productivity (Jessop et al. 2024; Potter 2011). Although infectious agents carry the greatest blame, nutrition, management practices, and environmen can aggravate the calf diarrhea (Gichile 2022). Among the viral pathogens, Bovine Rotavirus (BRoV) is known to be the predominating etiological agent of calf diarrhea, particularly in dairy farms (Geletu et al. 2021). BRoV is a double-stranded RNA virus within the Reoviridae family with a three-layered capsid and an eleven-segment genome. The virus is primarily transmitted through the fecal-oral route and preferentially infects the epithelial cells lining the intestinal villi, causing malabsorption, dehydration, and watery diarrhea (Lockhart et al. 2022). The zoonotic potential of BRoV has been increasingly recognized due to antigenic and genetic similarities between viral strains isolated from animals and humans (Geletu et al. 2021). The symptoms can vary between subclinical infection to acute enteritis and mortality, mostly impacting calves below eight weeks of age, with increased detection during colder months (Dhama et al. 2009; Patel et al. 2013). So far, specific antiviral therapy is not known to exist, and care is mostly supportive (Maclachlan and Dubovi 2010; Murphy 1999).

Bovine Coronavirus (BCoV), another significant cause of enteritis among calves, belongs to the *Betacoronavirus* genus within the *Coronaviridae* family (Vlasova and Saif 2021). BCoV can infect calves between the period of their birth and three months of life, with highest infection rate in calves between the age of 1 to 2 weeks (Seid et al. 2020). The virus damages absorptive cells of the small and large intestine, causing outcomes identical to the BRoV infection, including malabsorption, electrolytic disturbance, and dehydration. Environmental contamination and vertical transmission through infected dams add to its infective capability (Seid et al. 2020; Vlasova and Saif 2021). Furthermore, bovine Noroviruses (BNoV) are new emerging pathogens of the intestine that have been found across various nations and are associated with prolonged diarrhea, with its occurrence being typically seen between 2-5 days post-infection (Guo et al. 2018). The viruses disseminate through the fecal-oral route and co-circulate with multiple other viruses of the intestine. Despite their widespread presence and established pathogenic capability, BNoV are significantly less studied than BRoV and BCoV, particularly concerning their role in co-infections. Lack of epidemiological and molecular data, specifically within areas such as Iran, is an important knowledge gap relating to dynamics of disease that affect neonatal calves (Castells et al.

2020; Guo et al. 2018). A myriad of studies showed that co-infections comprising BRoV, BCoV, and BNoV are frequent and complicate diagnostic and treatment interventions with profound negative health outcomes (Cho and Yoon 2014; Mohteshamuddin et al. 2020). Much of the available research, however, has been individual-pathogen oriented, and therefore very limited data are available relating to molecular epidemiology of co-infections, specifically in animal systems within Iran (Delling and Daugschies 2022; Mohteshamuddin et al. 2020).

Considering the economic significance of the livestock sector in Iran and higher prevalence of enteric disease, studying occurrence of BRoV and its co-infections with BCoV and BNoV at molecular level represents a key step to developing prevention and control strategies. Tehran, Qazvin, Hamadan, Qom, Golestan, and Alborz provinces were chosen here because of their productive livestock activities (Pourasgari et al. 2016). Despite numerous global studies, limited data exist on the molecular epidemiology of these pathogens in Iran's livestock systems, particularly regarding co-infections. Therefore, the aim of this study was to determine the molecular detection rate and co-infection patterns of BRoV, BCoV, and BNoV in neonatal calves across major livestock-producing provinces in Iran. Understanding these patterns is vital for developing targeted prevention and control strategies to reduce calf mortality and improve herd health.

2. Materials and Methods

2.1 Description of the study area

This study was conducted between October 2023 to May 2024 across seven major livestock-producing provinces of Iran: Tehran, Alborz, Qom, Qazvin, Golestan, Hamadan, and Kermanshah. Fourteen industrial and semi-industrial dairy farms, selected to represent a range of production scales, were included. These provinces span diverse climatic zones, ranging from semi-arid (Tehran, Qazvin, Kermanshah) and desert (Qom) to humid subtropical (Golestan) and mountainous cold (Hamadan) regions, providing ecological variability relevant to pathogen distribution and transmission dynamics.

2.2 Study design and study population

A cross-sectional design was applied. In the intensive and semi-intensive managed target farms Holstein friesian calves of either sex up to 90 days of age were clinically examined for signs of diarrhea, dehydration, and lethargy and fecal samples were collected for diagnostic testing. Relevant information for each calf was recorded using the appropriate registration form.

2.3 Sampling technique and sample size determination

A total of 320 diarrheic samples were collected from calves, the distribution of samples across the provinces was as follows: 120 samples from Tehran, 32 from Qom, 52 from Qazvin, 76 from Alborz, 8 from Golestan, 8 from Hamadan, and 24 from Kermanshah. In large-scale farms (>200 head), at least 10% of the calves were randomly selected for sampling. In small-scale (<50 head) and medium-scale (50–200 head) farms, a cluster sampling method was employed. The sampling framework was designed and implemented based on records maintained by dairy cooperatives in each province. The sampling distribution was proportionate to the size of the farm population and previous regional reports of diarrheal incidence to enhance the accuracy of the study and allow for comparisons of disease occurrence across farms of different sizes. All the collected samples were included in the final analysis. This approach ensured a more precise and scientifically robust assessment of disease occurrence.

2.4 Collection and preparation of fecal samples

Diarrheal fecal samples were collected after cleaning the anus and performing rectal stimulation. Approximately 50 grams fecal material was directly obtained from the rectum of calves. The collected samples were placed in containers with ice packs and transported to the virology laboratory at the Faculty of Veterinary Medicine, University of Tehran. The samples were stored at −80°C until further processing. For sample preparation, 10% suspension of fecal samples were prepared in phosphate-buffered saline (PBS) and homogenized using a mortar. Then, 100 μL of the fully homogenized suspensions were transferred into 1.5 mL microtubes. The microtubes were centrifuged at 3000 rpm for 15 minutes at 4°C and the supernatants were used for the assays.

2.5 Viral isolation

A representative subset of 15 fecal samples that tested positive for BRoV only by RT-PCR were subjected to viral isolation, given the known ability of BRoV to be cultured *in vitro*. Samples were clarified by centrifugation and filtration (0.45 µ) before inoculation onto Vero cell monolayers. Cultures were maintained at 37°C with 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) supplemented with 2% heat-inactivated fetal bovine serum (FBS), 1% penicillin–streptomycin, and 1% L-glutamine. For viral adsorption, inoculates were incubated with Vero cells for 1 h at 37 °C, after which the cultures were maintained in serum-free DMEM containing 10 µg/mL trypsin to facilitate viral replication. Control flasks were included in which no inoculum was added. Cultures were monitored daily for the development of cytopathic effects (CPE) for up to 48 h, including cell rounding, detachment, and monolayer disruption. suspected positive cultures were subjected to two blind passages to confirm viral replication. Due to the limited or no *in vitro* growth capacity of BCoV and BNoV in standard cell culture systems, viral isolation was not attempted for these pathogens.

2.6 Immunoassay (ELISA)

Bovine rotavirus antigen was screened using a validated commercial ELISA kit (BIO-X Diagnostics, Belgium), following the manufacturer's instructions. This method was selected due to its diagnostic relevance and proven field utility. This ELISA kit has been validated for bovine fecal samples and provides reliable sensitivity and specificity under field conditions (Hamedian-Asl et al. 2022; Mayameei et al. 2010). ELISA was not performed for bovine coronavirus or norovirus owing to the lack of commercially available and validated antigen detection kits for these viruses in bovine fecal samples.

2.7 Molecular analysis

Total viral RNA was extracted from fecal samples using RNAplus reagent (SinaGen Inc., Iran) according to the manufacturer's protocol. cDNA synthesis was conducted using the PrimeScript RT Reagent Kit (SinaClone, Iran). The extracted RNA was screened for BRoV using a specific primer pair targeting the VP6 gene. To detect potential co-infections with other major diarrheagenic viruses, previously established and validated RT-PCR assays in our laboratory were employed for BNoV and BCoV. The primer pairs for these viruses target conserved regions of the RNA-dependent RNA polymerase (RdRp) gene for BNoV and the nucleocapsid (N) gene for BCoV (Table 1). RT-PCR assays used had been previously validated in our laboratory using known positive control samples and sequencing-confirmed

Table 1. RT-PCR conditions for the detection of BRoV, BCoV, and BNoV

Virus	Primer	Primer sequence (5′ → 3′)	Position (nt) & Target gene	Amplicon size	Annealing Temperature (°C)	Reference
Bovine Rotavirus	VP6-F3	GACGGVGCRACTACATGGT	737–755 (VP6 gene)	~379 bp	50 °C	(Di Bartolo et al. 2011; Gouvea et al. 1990)
	VP6-R3	GTCCAATTCATNCCTGGTG	1116–1098 (VP6 gene)			
Bovine Coronavirus	BCoV-F	ACTCAATGGTGATGTTGGTG	508–527 (N gene)	~407 bp	55 °C	(Socha et al. 2022; Takiuchi et al. 2006)
	BCoV-R	CAGGAGAGGTGACACATAGC	914–895 (N gene)			
Bovine Norovirus	BNoV-F	GGGAGGGCGATCGCAATCT	4570–4588 (RdRp)	~327 bp	52 °C	(Di Bartolo et al. 2011; Otto et al. 2011)
	BNoV-R	CCTTAGACGCCATCATCATCATT	4896–4874 (RdRp)			

BRoV: Bovine Rotavirus; BCoV: Bovine Coronavirus; BNoV: Bovine Norovirus; nt: Nucleotides

reference strains (virology lab., university of Tehran). Negative controls (nuclease-free water) were included in each run to monitor contamination.

PCR products were visualized on 1% agarose gel prepared in TBE buffer (containing Tris base, boric acid, and EDTA) and stained with ethidium bromide. The buffer pH was adjusted to 8. Electrophoresis was conducted at 90 V for 55 minutes. A 100 bp Plus DNA Ladder (GeneRuler, Thermo Fisher Scientific, Germany) was used as a molecular size marker. Samples exhibiting bands corresponding to the expected amplicon sizes (~379 bp for BRoV, ~327 bp for BNoV, and ~407 bp for BCoV) were considered positive.

2.8 Epidemiological data collection & statistical analysis

For each calf, individual metadata – including sex, age, season of sampling, and province of origin were recorded. Additional farm-level factors such as housing system (intensive or semi-intensive) and colostrum intake quality were also recorded through structured interviews with farm personnel and direct field observation. Descriptive statistics summarized detection rates, with categorical variables expressed as frequencies and percentages. Associations between risk factors and viral occurrence were assessed using chi-square or Fisher's exact tests ($p < 0.05$). All analyses were performed using SPSS (IBM.SPSS.27).

3. Results

To validate the specificity of the RT-PCR results, the amplicons were visualized on agarose gels. Clear bands of the expected sizes for BRoV (379 bp), BCoV (407 bp), and BNoV (327 bp) were observed, indicating successful amplification of the target genes and validating the reliability of the molecular detection (Fig. 1).

Cytopathic effects indicative of rotavirus infection in Vero cell cultures were observed after 48 hours, including cell rounding, detachment, and monolayer disruption. However, uninfected control cultures maintained normal morphology (Fig. 2).

Among 320 fecal samples tested with ELISA, 220 (68.8%) were positive for BRoV antigen. The mean optical density of the positive samples (1.20 ± 0.29) was significantly higher than that of the negative samples (0.10 ± 0.05). A boxplot clearly illustrates the distribution of OD values, with a cut-off of 0.3 separating most positive samples from the negatives (Fig. 3).

Laboratory analysis revealed a high burden of viral enteric pathogens, with many samples exhibiting co-infection by more than one virus. The overall detection rates for each pathogen, regardless of co-infection, were as follows; BNoV 83/320 (25.9%), BCoV 181/320

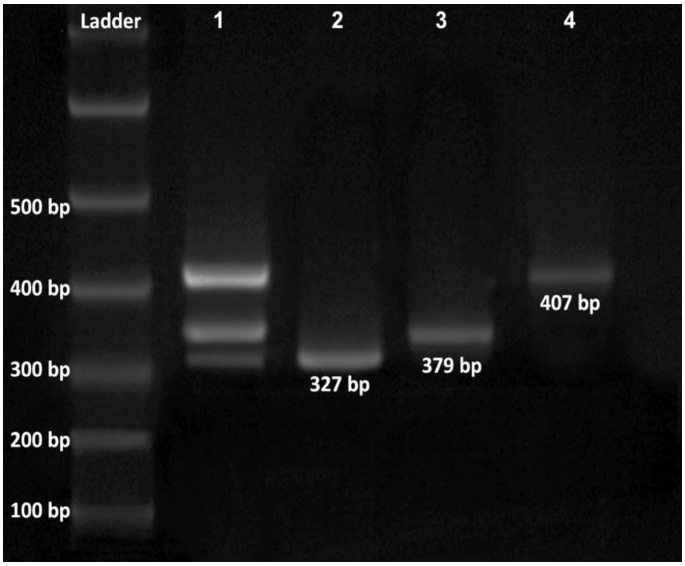

Fig. 1. Agarose gel electrophoresis of RT-PCR products.
Lane 1: Positive control (BRoV, BCoV, BNoV); Lane 2: BNoV (~327 bp); Lane 3: BRoV (~379 bp); Lane 4: BCoV (~407 bp); 100 bp DNA ladder

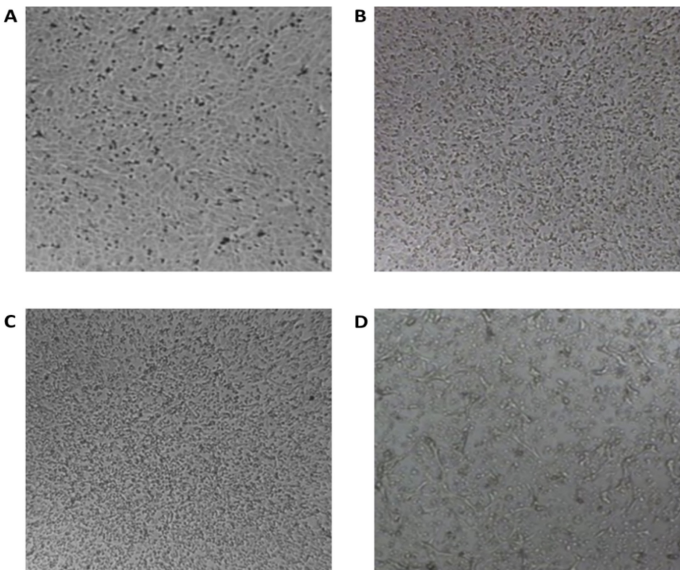

Fig. 2. Cytopathic effects of Rotavirus infection in Vero cell culture
Panel A displays uninfected, healthy cells with normal morphology, while panel B shows cells infected with rotavirus, although no significant cytopathic effects (CPE) are yet visible at this early stage. Panel C depicts uninfected cells at 48 hours, where increased confluency and minor cell death are observed due to overgrowth. In contrast, panel D shows infected cells at 48 hours post-infection, where clear cytopathic effects are evident, including cell rounding, detachment, and loss of monolayer integrity

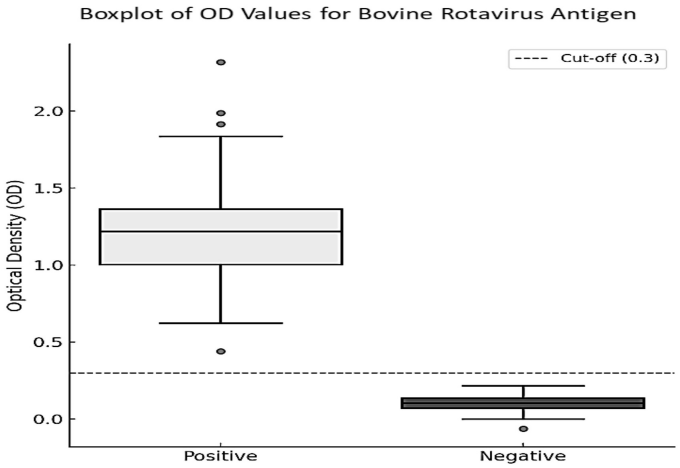

Fig. 3. Boxplot of optical density (OD) values in fecal samples tested for bovine rotavirus antigen (n = 320)
A total of 220 samples (68.8%) tested positive, showing significantly higher OD values (mean ± SD: 1.20 ± 0.29) compared to negative

(56.5%), and BRoV 220/320 (68.7%) (Fig. 4).

The overall detection rate of single infections was 26.9% for BRoV, 22.2% for BCoV, and 1.6% for BNoV across all provinces. However, co-infections were frequent with the combination of BCoV and BRoV exhibiting the highest occurrence at 22.5%, followed by BNoV and BRoV co-infection (12.5%). Triple infections involving all three viruses were detected in only 6.9% of the total samples. These findings highlight the complex etiology of viral enteric infections and the common occurrence of multi-pathogen involvement. Notably, the detection rate of each virus varied across provinces. Among them,

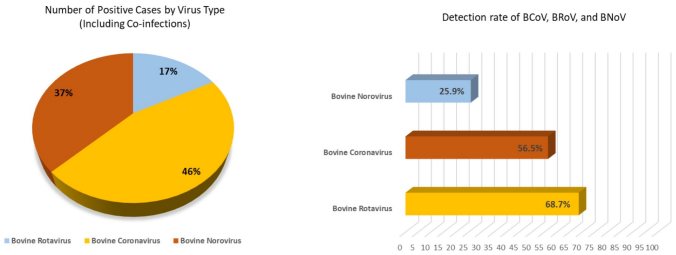

Fig. 4. Occurrence of bovine Rotavirus, bovine Coronavirus, and bovine Norovirus in diarrheic calves across seven provinces in Iran.
The pie chart illustrates the total percentage of positive cases for each virus, including co-infections. The bar graph represents the overall percentage of calves positive for each pathogen, regardless of whether the infection was single or mixed

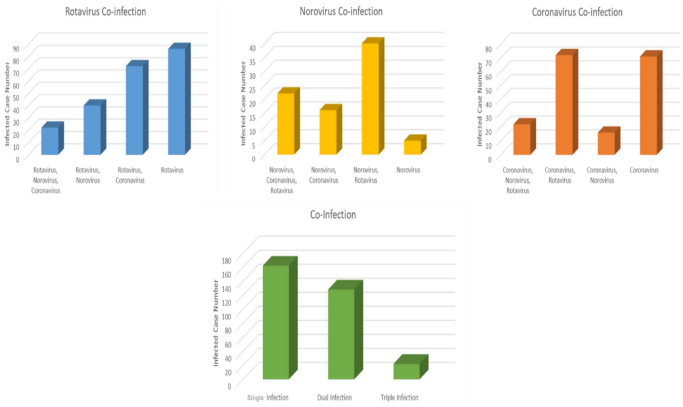

Fig. 5. Distribution of co-infections among BRoV, BCoV, and BNoV in diarrheic calves across studied provinces

Golestan, Hamedan, and Qazvin exhibited the highest overall levels of contamination (Table 2, Fig. 5).

While the assessment of epidemiological risk factors, no significant association was observed between the sex of the calves and the detection rates of BRoV, BCoV, or BNoV ($p > 0.05$). However, a significant seasonal effect was observed on BRoV, with a higher occurrence during the colder months (77.9%) compared to the warmer season (41.3%) ($p < 0.05$). In contrast, seasonal variation did not significantly influence the detection rates of BCoV or BNoV, although the number of positive samples was higher in the colder seasons. Colostrum intake did not show any significant effect on infection status. Although calves with poor colostrum intake exhibited slightly elevated infection rates for all three viruses, the differences were not statistically significant ($p > 0.05$). Notably, the type of management system showed a strong association with BRoV occurrence. Calves raised in intensive farming systems had a significantly higher rate of infection (83.8%) compared to those in semi-intensive systems (40.0%) ($p < 0.05$). However, no such association was found between the management system and the occurrence of BCoV or BNoV ($p > 0.05$) (Table 3).

4. Discussion

The present study provides the first comprehensive molecular investigation of BRoV, BCoV, and BNoV co-infections in diarrheic calves across multiple livestock-producing provinces of Iran. The dominance of BRoV as an etiological agent, with a detection rate exceeding two-thirds (68.7%) of all samples coupled with its high frequency in mixed infections (>40%), indicates a complicated viral

Table 2. BRoV, BCoV, and BNoV single and mixed occurrence in diarrheic calves from different provinces of Iran

Virus/ Province	Tehran (n = 120)	Qom (n = 32)	Qazvin (n = 52	Alborz (n = 76)	Kermanshah (n = 24)	Golestan (n = 8)	Hamedan (n = 8)	Total (n = 320)
BRoV+	28 (23.3%)	7 (21.9%)	15 (28.8%)	22 (28.9%)	7 (29.2%)	4 (50.0%)	3 (37.5%)	86 (26.9%)
BCoV+	24 (20.0%)	5 (15.6%)	13 (25.0%)	18 (23.7%)	6 (25.0%)	3 (37.5%)	2 (25.0%)	71 (22.2%)
BNoV+	2 (1.7%)	0 (0.0%)	1 (1.9%)	1 (1.3%)	0 (0.0%)	1 (12.5%)	0 (0.0%)	5 (1.6%)
BRoV+ BCoV+	25 (20.8%)	5 (15.6%)	12 (23.1%)	20 (26.3%)	5 (20.8%)	3 (37.5%)	2 (25.0%)	72 (22.5%)
BCoV+ BNoV+	6 (5.0%)	1 (3.1%)	3 (5.8%)	3 (3.9%)	1 (4.2%)	1 (12.5%)	1 (12.5%)	16 (5.0%)
BNoV+ BRoV+	12 (10.0%)	3 (9.4%)	8 (15.4%)	10 (13.2%)	2 (8.3%)	3 (37.5%)	2 (25.0%)	40 (12.5%)
BRoV+ BCoV+ BNoV+	6 (5.0%)	1 (3.1%)	4 (7.7%)	6 (7.9%)	1 (4.2%)	2 (25.0%)	2 (25.0%)	22 (6.9%)

BRoV: Bovine Rotavirus; BCoV: Bovine Coronavirus; BNoV: Bovine Norovirus

Table 3. Association between selected risk factors and the occurrence of BRoV, BCoV, and BNoV infections in diarrheic calves

Risk factor	Bovine Rotavirus			Bovine Coronavirus			Bovine Norovirus		
	Positive	Negative	P-value	Positive	Negative	P-value	Positive	Negative	P-value
Gender									
Male (n=160)	110 (68.8%)	50 (31.2%)	> 0.05	90 (56.3%)	70 (43.8%)	> 0.05	42 (26.3%)	118 (73.8)	> 0.05
Female (n=160)	110 (68.8%)	50 (31.2%)		90 (56.3%)	70 (43.8%)		42 (26.3%)	118 (73.8)	
Season									
Cold (n=240)	187 (77.9%)	53 (22.1%)	< 0.05*	109 (45.4%)	131 (54.6%)	> 0.05	50 (20.8%)	190 (79.2%)	> 0.05
Warm (n=80)	33 (41.3%)	47 (58.8%)		72 (90.0%)	8 (10.0%)		33 (41.3%)	47 (58.8%)	
Colostrum intake									
Well (n=12)	4 (33.3%)	8 (66.7%)	> 0.05	9 (75.0%)	3 (25.0%)	> 0.05	2 (16.7%)	10 (83.3%)	> 0.05
Moderate (n=60)	40 (66.7%)	20 (33.3%)		27 (45.0%)	33 (55.0%)		17 (28.3%)	43 (71.7%)	
Poor (n=248)	176 (71.0%)	72 (29.0%)		145 (58.5%)	103 (41.5%)		65 (26.2%)	183 (73.8%)	
Management system									
Intensive (n=210)	176 (83.8%)	34 (16.2%)	< 0.05*	100 (47.6%)	110 (52.4%)	> 0.05	50 (23.8%)	160 (76.2%)	> 0.05
Semi-intensive (n=110)	44 (40.0%)	66 (60.0%)		81 (73.6%)	29 (26.4%)		33 (30.0%)	77 (70.0%)	

BRoV: Bovine Rotavirus; BCoV: Bovine Coronavirus; BNoV: Bovine Norovirus

environment underlying calf diarrhea with potential implications for disease severity, diagnostic strategies, and control measures. The findings of present study establish BRoV as the most predominant enteric pathogen, which corroborates observations from other national and international studies (Madadgar et al. 2015; Nazaktabar and Madadgar 2020; Ranjbar et al. 2021). The fact that a high detection rate of BCoV (56.5%) and an increasing detection rate of BNoV (25.9%) indicates that calf diarrhea in Iran tends to be caused by multiple factors, thus confirming earlier research that single-pathogen diagnostics may not reflect the true disease etiology (Cho et al. 2013; Cho and Yoon 2014; Kim et al. 2021).

A key strength of this study is the detailed description of co-infection patterns. The most frequent double infection, BRoV + BCoV (22.5%), suggests potential synergistic effects that may aggravate clinical outcomes. Triple infections (6.9%), although less common, are a serious clinical concern owing to their potential in exacerbation of symptoms, diagnosis, and treatment. Similar observations have been made by earlier researchers, who highlighted the diagnostic and therapeutic challenge posed by viral co-infections in neonatal calves (Gomez and Weese 2017; Hou et al. 2025; Tulu Robi et al. 2024). In contrast, the extremely low detection rate of BNoV mono-infection (1.6%) indicates that its pathogenicity may be increased when co-infecting together with other viruses, which is in agreement with other studies from China and Uruguay supporting the hypothesis of BNoV as a passenger virus. Additional pathogenesis studies are needed to clarify its role (Castells et al. 2020; Chen et al. 2022; Qin et al. 2022; Shi et al. 2019; Turan et al. 2018).

The geographical distribution of infections revealed provincial variation. While overall BRoV detection rate did not vary between provinces, some provinces such as Golestan and Hamadan featured disproportionately higher rate of mixed infections and might be accounted for on ecological or management-related grounds. Seasonality played a significant role in the prevalence of BRoV, with higher rates in colder months (77.9%), a pattern also observed globally and ascribed to greater viral stability and susceptibility of calves under cold stress conditions (Kong et al. 2025; Nonnecke et al. 2009). However, BCoV and BNoV lacked significant seasonal correlation, contrary to some other studies and warranting additional ecological modeling (Alotaibi et al. 2022; Mohebbi et al. 2017; Pourasgari et al. 2016; Pourasgari et al. 2018).

Among the risk factors quantified, the management system is the most significant predictor of BRoV prevalence (Uddin Ahmed et al. 2022). In present study, calves reared under intensive systems had very significant infection rates (83.8%) when compared to animals reared under semi-intensive systems (40.0%). Results highlight the potential role of overcrowding, environmental contamination, and absence of biosecurity measures in promoting viral transmission (Fritzen et al. 2019). Surprisingly, neither sex nor colostrum intake was a statistically significant predictor of viral detection, although trends suggested that compromised colostrum quality may increase susceptibility, a theory worthy of exploration using immunoglobulin titration studies. The BRoV detection rate reported here (68.7%) is significantly greater than earlier Iranian estimates (~26–34%) derived from ELISA or limited RT-PCR protocols. These discrepancies may reflect enhanced detection sensitivity, greater sample size, broader geographic representation, or genuine epidemiologic variation over time. Nevertheless, it must be stated that this research targeted diarrheic samples alone and wasn't an entire screening of the overall cattle population, which can also account for the high detection rate. Likewise, the detection rate of BCoV documented herein is consistent with findings from research studies in Argentina and Turkey but greater than local reports previously, which points toward either an increase in prevalence or underreporting in past assessments (Gomez and Weese 2017; Yasir et al. 2023). In contrast, BNoV is less characterized, and while detection rate is quite high in this study, the low mono-infection rate indicates its co-infective nature is more significant than its mono pathogenicity (Castells et al. 2020).

However, there are also limitations to this study. The absence of genotyping or whole genome sequencing constrains the investigation of genetic diversity and zoonotic potential, which is especially relevant to BRoV due to its close genetic similarity with human strains. Also, the lack of quantitative viral load testing precludes the correlation of viral burden with disease severity.

5. Conclusion

The present study demonstrates that calf diarrhea among Iranian dairy operations is commonly multivariate and that BRoV is the dominant pathogen, but BCoV and BNoV have key roles through co-infections. The important connection between concentrated management systems

and infection with BRoV emphasizes the key role of biosecurity, accommodation quality, and farm-level sanitation on disease burden. The seasonal patterns of BRoV also suggest that seasonally specific preventive measures during the cold season might improve calf survival. Longitudinal studies should be prioritized to determine the clinical significance of co-infections, genetic characterization of strains that circulate through dairies, and their zoonotic potential. Integration of molecular epidemiology with management practice and vaccine programs will be important to reducing calf morbidity and mortality, improving productivity, and ensuring public health.

Declarations

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Conflict of interest: Authors declare that they have no conflicts of interest

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

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