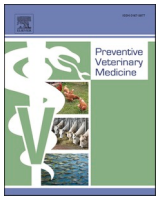
Preventive Veterinary Medicine 254 (2026) 106917



Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed





Molecular characterization of genetically related co-circulating bovine viral diarrhea virus in cattle and water buffalo within mixed-herd production systems

Eaftekhar Ahmed Rana [a,b], Jully Gogoi-Tiwari [a,c], Subir Sarker [d], Jasim M. Uddin [a,c,e,*]

[a] School of Veterinary Medicine, Murdoch University, WA 6150, Australia
[b] Department of Microbiology and Veterinary Public Health, Chattogram Veterinary and Animal Sciences University, Khulshi, Chattogram 4225, Bangladesh
[c] Centre for Biosecurity and One Health, Harry Butler Institute, Murdoch University, WA 6150, Australia
[d] Biomedical Sciences & Molecular Biology, College of Medicine and Dentistry, James Cook University, Townsville, QLD 4811, Australia
[e] Centre for Animal Production and Health, Food Futures Institute, Murdoch University, WA 6150, Australia

ARTICLE INFO

Keywords:
BVDV
Cattle
Water-buffalo
Genetic diversity
Biotypes
Risk factors
Mixed farming

ABSTRACT

Interspecies transmission and co-circulation of BVDV among bovines occur frequently, contributing to complex epidemiological dynamics in mixed-herd environments. A cross-sectional study was conducted in mixed bovine herds in the southern region of Bangladesh. A total of 219 blood samples were collected from cattle and water-buffalo across 12 mixed herds. Serum samples were tested for BVDV-specific antibodies using Ab-ELISA, while RT-qPCR on blood to detect seropositivity and active infection, respectively. PCR-positive samples were then subjected to 5′-UTR-based sequencing to identify circulating species and subgenotypes. Biotyping was conducted using the MDBK cell line. At the herd level, the prevalence of BVDV seropositivity and active infection were 75.0% and 66.7%, respectively. At the animal level, seropositivity was 15.9% in cattle and 14.0% in buffalo, while active infection was detected at 9.5% and 7.5%, respectively. Among infected animals,18 (94.7%) were transiently infected (TI), and one calf was persistently infected (PI). Of the 19 isolates, 12 (63.2%) were classified as BVDV-1, representing eight subgenotypes (BVDV-1a, 1b, 1c, 1d, 1j, 1k, 1o, and 1p) detected in both species, indicating co-circulation and high nucleotide identity (93–100%). All BVDV-2 and HoBi-like isolates belonged to subgenotypes BVDV-2a and HoBiPeV-a, respectively. Ten isolates (10/15; 66.7%) were non-cytopathic, and five (5/15; 33.3%) were cytopathic. Stunted growth ($p = 0.036$) and a history of clinical diarrhoea ($p = 0.032$) were associated with BVDV seropositivity. At the farm level, close proximity between farms ($p = 0.001$), larger farm size ($p = 0.034$), and introduction of recently purchased animal ($p = 0.034$) were significantly associated with seropositivity. The co-circulation of diverse BVDV genotypes at the cattle-water buffalo interface presents challenges for control and eradication in mixed-farming systems.

1. Introduction

Bovine viral diarrhea virus (BVDV) is a highly contagious pathogen capable of infecting a wide range of livestock and wild animal species (Rana et al., 2025a; Ridpath, 2010). BVDV is designated as a statutory notifiable animal disease and classified as a Class III animal disease for livestock species (WOAH., 2023). The virus can lead to significant economic losses by inducing multisystemic damage in susceptible hosts and adversely impacting overall production performance (Riley et al., 2019). BVDV primarily affects bovine species and leads to various reproductive disorders, including decreased fertility, and transplacental infection often causes early embryonic death and resorption, abortion, foetal mummification, stillbirths, and teratogenesis in foetus (Ridpath, 2010). Additionally, BVDV infection can cause gastrointestinal and respiratory disorders, leukopenia, and insidious immunosuppression. Based on the duration of viremia within the susceptible host, BVDV infection is classified into transient infection (TI) and persistent infection (PI) (Houe, 1995). Following TI, animals mount an effective humoral immune response and develop BVDV-specific antibodies (Chase, 2013). In contrast, PI animals remain immunotolerant, without developing

* Corresponding author at: School of Veterinary Medicine, Murdoch University, WA 6150, Australia.
E-mail addresses: eaftekhar.rana@murdoch.edu.au (E.A. Rana), Jully.GogoiTiwari@murdoch.edu.au (J. Gogoi-Tiwari), subir.sarker@jcu.edu.au (S. Sarker), jasim.uddin@murdoch.edu.au (J.M. Uddin).

https://doi.org/10.1016/j.prevetmed.2026.106917
Received 25 February 2026; Received in revised form 9 May 2026; Accepted 15 May 2026
Available online 17 May 2026
0167-5877/© 2026 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

BVDV-specific antibodies, maintain constant viremia, and act as continuous sources of viral shedding (Ridpath, 2010). The coexistence of TI and PI infections, together with viral immunotolerance, immunosuppression, and reproductive system involvement, poses a significant challenge to the profitability of the livestock industry.

BVDV is an enveloped, positive-sense, single-stranded RNA virus with an approximately 12.3 kb genome, classified within the genus *Pestivirus* of the family *Flaviviridae*. Its genome contains a single open reading frame (ORF) flanked by 5′ and 3′ untranslated regions (UTRs), that is non polyadenylated, without 5′ cap (Tautz et al., 2015). The envelope of the virus consists of four structural proteins, including nucleocapsid protein C, Erns, E1, and E2 (Tautz et al., 2015). Among them, E2 is the predominant glycoprotein and the major inducer of neutralizing antibodies. Additionally, the single ORF encodes eight non-structural proteins (Npro, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B), which are processed by viral and host proteases during replication (Tautz et al., 2015). Recently, the genus *Pestivirus* have been taxonomically reclassified into 11 distinct virus species based on the analyses of 5′ UTR together with partial E2 and Npro, and complete genomic sequences (Postler et al., 2023; Walker et al., 2022). Under this revised nomenclature, BVDV-1, BVDV-2, and HoBi-like pestiviruses are designated as *Pestivirus bovis*, *Pestivirus tauri*, and *Pestivirus brazilense*, respectively (Postler et al., 2023). However, BVDVs are genetically highly diverse, even within the individual species. The 5′ UTR is a highly conserved region and has mainly been utilized to detect different sub-genotypes of BVDV (Deng et al., 2020). Based on phylogenetic analysis of the 5′ untranslated region (5′ UTR), 24 distinct subgenotypes (1a-1v) have been identified for BVDV-1, while five subgenotypes (2a-2e) of BVDV-2 and four subgenotypes of HoBi-like pestiviruses (HoBiPeV-a to HoBiPeV-d) have been documented worldwide (Rana et al., 2025b; Rivas et al., 2022). Based on *in-vitro* lytic activity in epithelial cell culture, BVDV is classified into cytopathic and non-cytopathic biotypes (Ridpath et al., 2006). However, the non-cytopathic biotype predominates in nature and is commonly associated with diverse clinical manifestations in animals, as well as the development of persistent infection following fetal exposure between 40 and 120 days of gestation (Darweesh et al., 2015). Notably, superinfection with a cytopathic biotype in persistently infected animals carrying an antigenically related non-cytopathic strain can lead to the development of life-threatening mucosal disease (MD) (Darweesh et al., 2015).

Due to the virus' broad tissue tropism and the presence of functionally similar cellular receptors for BVDV across domestic and wild species (Rana et al., 2025a), it can co-circulate among a wide range of host species, thereby increasing epidemiological complexity. Moreover, the circulating wild strain and cross infection of BVDV among different animals may facilitate the mixing of viral genetic material, potentially promoting the evolution and emergence of novel genotypes or strains. Although cattle are the primary hosts and reservoirs, mixed-species farming increases the susceptibility and vulnerability of animals to BVDV infection (Rana et al., 2025b). In Bangladesh, mixed-species farming particularly the rearing of water buffalo alongside dairy or beef cattle is common across most regions. Consequently, interspecies transmission of BVDV between bovine species is biologically plausible and epidemiologically significant (Rana et al., 2025b; Deng et al., 2015). Active BVDV infection and seroprevalence have been reported in cattle herds in Bangladesh (Rana et al., 2026; Sajeeb et al., 2025); however, epidemiology and molecular evidence of BVDV on other animal species, including cattle–buffalo mixed production systems, remain unreported. Therefore, the primary aim of the present study was to investigate the co-circulation and genetic relatedness of BVDV in cattle and water buffalo within mixed production systems. In addition, the study aimed to determine the serostatus of the host (bovine) populations, characterize the circulating species, subgenotypes, and biotypes of BVDV, and identify associated risk factors in mixed farming systems.

2. Materials and methods

2.1. Ethical approval and owner consent

To conduct the current study, a complete study design and protocol were reviewed and approved by the Animal Ethics Committees (ACE) of Murdoch University (MU), Australia, and Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh [Permit No. OS3581/24 & Approval No. CVASU/Dir (R&E) EC/2024/763/1, respectively]. Additionally, the study goals and procedures were explained to all individual farm owners, and informed verbal and written consent was obtained during sample and data collection following the AEC guidelines.

2.2. Study design and farm selection

This study was conducted and reported in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines, and the completed checklist is provided as a supplementary file 1. A cross-sectional study was conducted in the southern region of Bangladesh between November 2024 and July 2025. The selection process of farms and animals, including exclusion criteria and resampling of animals, is summarized in the study flow diagram presented in supplementary Fig. 1. The southern region was purposively selected due to its high livestock density and the predominance of integrated mixed-species farming systems, facilitated by extensive low-lying marshy landscapes and diverse livestock management practices. Only mixed-species farms rearing both cattle and buffalo under the same management and production system were eligible for inclusion (Fig. 1). Initially, lists of livestock farms and their locations were obtained from the respective district livestock services (DLS) office, The Government of People's Republic of Bangladesh. In consultation with local veterinarians, farms practising mixed-species farming that reared a minimum of 10 dairy cattle and 5 buffaloes were identified and selected for inclusion in the study. These herd-size thresholds were chosen to ensure a sufficiently large population for reliable sampling and to allow meaningful detection of BVDV exposure within mixed-species systems. Farms with fewer than 10 cattle or buffalo, or those rearing only beef cattle, were excluded from sampling.

2.3. Sample size calculation and sampling procedure

The sample size was calculated to estimate prevalence in a cross-sectional study using the standard statistical formula described by Thrusfield, (2018). Since, there was no published prevalence data for BVDV in mixed cattle–buffalo farming systems in Bangladesh, an expected prevalence of 50% was considered based on a previous seroprevalence study (Uddin et al., 2017) to enumerate the sample size. The calculation was performed considering a 95% confidence level and a desired precision of 5%. Based on the standard formula for cross-sectional studies, a minimum of 384 animals was required to obtain a reliable estimate of BVDV prevalence. Although the minimum calculated sample size was 384 animals, the final sample size of 219 was constrained by eligibility criteria and farm-level availability. This may have slightly reduced the precision of the prevalence estimates. A stratified random sampling strategy was followed to ensure representation across production stages, age groups, and sex as these factors may influence BVDV exposure. Within each stratum, animals were selected randomly from the eligible population in the selected farms (Tables 1 and 2). Sampling was conducted during the study period across the selected farms. Pregnant dams were excluded to avoid stress or potential injury, in accordance with AEC guidelines.

2.4. Blood sample collection and processing

After proper restraint, approximately 5 mL of blood was collected



Fig. 1. Schematic of the mixed farming system showing cattle and buffalo raised together on the same premises.

Table 1
Distribution of Pestivirus species, subgenotypes, and biotypes identified from mixed cattle–water buffalo farming systems, with corresponding GenBank accession numbers of 5′-UTR sequences.

Farm id	Animal	BVDV antigen status in RT-qPCR	Animal category	Identified Pestivirus species	Identified sub-genotype	BVDV Biotype	Infection status	Accession number
MF–01	Cattle	Positive	Heifer–1	BVDV-1	1b	Ncp	TI	PV453976
			Cow–42	BVDV-1	1p	Fail to propagate	TI	PV874948
	Buffalo	Negative	–	–	–	–	–	–
MF–02	Cattle	Positive	Bull calf–15	BVDV-1	1b	Ncp	TI	PV569115
			Bull calf–02	BVDV-2	2a	Cp	TI	PV580258
	Buffalo		Bull calf –barishal	BVDV-1	1j	Ncp	TI	PV492127
MF–03	Cattle	Positive	Bull calf–7	BVDV-1	1k	Ncp	PI	PV569118
	Buffalo		Bull calf–jayful	BVDV-1	1c	Ncp	TI	PX448386
MF–04	Cattle	Positive	Cow 22.2	HoBiPeV	HoBiPeV– a	Fail to propagate	TI	PV874964
			Bull calf–13	BVDV-2	2a	Cp	TI	PV817803
	Buffalo		Bufalo cow–1280	HoBiPeV	HoBiPeV–a	Fail to propagate	TI	PV535837
MF–05	Cattle	Positive	Cow–41	BVDV-2	2a	Ncp	TI	PV874960
			Cow–44	BVDV-1	1b	Cp	TI	PV874955
	Buffalo	Negative	–	–	–	–	–	–
MF–08	Cattle	Positive	Female calf–15	BVDV-1	1a	Ncp	TI	PV345782
	Buffalo		Adult bull–1271	BVDV-1	1j	Ncp	TI	PV492126
MF–09	Cattle	Positive	Bull calf–24	BVDV-2	2a	Cp	TI	PV437272
	Buffalo		Bull calf–bali	BVDV-1	1c	Cp	TI	PV535838
MF–10	Cattle	Positive	Cow–60	BVDV-1	1o	Ncp	TI	PV874954
			Bull calf–3	BVDV-1	1d	Ncp	TI	PX447476
	Buffalo		Adult bull-11	HoBiPeV	HoBiPeV–a	Fail to propagate	TI	PX448387

MF: Mixed farming; BVDV-1: bovine viral diarrhea virus 1 (*Pestivirus bovis*); BVDV-2: bovine viral diarrhea virus 2 (*Pestivirus tauri*); HoBiPeV: HoBi-like pestivirus (*Pestivirus brazilense*). Ncp: Noncytopathic; Cp: Cytopathic. TI: Transiently infected and PI: Persistently infected.

from the jugular vein of each selected animal using sterile 10 mL syringes (JMI Group, Bangladesh). Immediately after collection, the blood was divided into two portions: half portion transferred into an anticoagulant-containing vacutainer tube and the remaining blood into a clot-activated tube (BD Vacutainer®, Bangladesh). Subsequently, both tubes were centrifuged at 3000 rpm for 5 min to separate the serum and buffy coat from the respective samples. The serum samples were stored at −20 °C for serological testing, while the buffy coat samples were preserved at −80 °C for subsequent virus detection and characterisation.

2.5. Animal demographic, farm management, and biosecurity practices data collection

A structured questionnaire was developed based on previously reported epidemiological risk factors for BVDV and was adapted to reflect regional mixed-farming practices in Bangladesh. The questionnaire was reviewed and approved by the AEC (Supplementary file 2). Face-to-face interviews were conducted during farm visits using a standardized and pre-tested questionnaire. The farm owner was selected as the principal respondent because they were directly responsible for herd management, biosecurity practices, and overall farm operational decisions. Demographic details for individual cattle and buffalo were collected,

Table 2
Serological and virological status of BVDV at farm-level and individual animal-level in mixed cattle and water buffalo production systems.

Total number mixed farms sampled	Mixed farm positive for Ab-BVDV (%; 95% CI)	Mixed farm positive for active BVDV infection (%; 95% CI)	Total number of animal (Cattle and buffalo)	BVDV antibody positive animals (%; 95% CI)	PCR confirmed BVDV (%; 95% CI)	Different pestivirus species	Prevalence of pestivirus species (%; 95% CI)
12	9 (75.0; 42.81–94.51)	8 (66.67; 34.89 –90.08)	Cattle (126)	20 (15.87; 9.97 –23.44)	12 (9.52; 5.02 –16.05)	*BVDV-1 *BVDV-2 *HoBiPeV	7 (5.56; 2.26 –11.11) 4 (3.17; 0.87–7.93) 1 (0.80; 0.02–4.34)
			Buffalo (93)	13 (13.98; 7.66 –22.72)	7 (7.52; 3.08 –14.90)	BVDV-1 BVDV-2 HoBiPeV	5 (5.38; 1.77–12.10) 0 2 (2.15; 0.26 –7.55)
Total			219	33 (15.07; 10.61 –20.51)	19 (8.68; 5.30 –13.22)		

BVDV-1: bovine viral diarrhea virus 1 (*Pestivirus bovis*); BVDV-2: bovine viral diarrhea virus 2 (*Pestivirus tauri*); HoBiPeV: HoBi-like pestivirus (*Pestivirus brazilense*); CI: confidence interval.

covering breed, age, sex, physiological status, and previous clinical and reproductive history. Moreover, farm management information including the herd size, farming duration, distance between herds, composition of mixed species, production status, housing system, grazing practice, source of animal, and animal movement. Additionally, biosecurity practices were assessed at the farm level through direct observation and farmer information at the time of sample collection. Data were collected on the presence and implementation of quarantine practices for newly introduced animals, isolation of diseased animals, use of footbaths, cleaning and disinfection of farm equipment, sharing of equipment between farms, breeding practices, use of protective clothing and footwear by workers, and contact with neighboring farms, etc. These variables were documented to evaluate their potential relevance as risk factors for BVDV transmission and infection in mixed farming settings.

2.6. Detection of BVDV-Ab in cattle and buffalo

Serum samples collected from cattle and buffalo were tested for antibodies (Abs) against BVDV using a commercial Ab-ELISA kit (Cat. No. abx055874, Abbexa Ltd., Cambridge, UK). Briefly, prior to testing, serum samples were thawed at room temperature and gently mixed. A total of 10 μL of serum was mixed with 40 μL of sample diluent buffer according to the manufacturer's instructions. Subsequently, 50 μL of the prepared samples were dispensed into antigen-coated microplate wells and incubated to allow binding of BVDV-specific antibodies. Positive and negative controls supplied with the kit were included in predefined wells of each plate to ensure assay validity. Subsequently, the optical density (OD) of each well was measured spectrophotometrically at 450 nm using a microplate reader (BioTek® 800™ Microplate Reader, USA). Results were interpreted according to the manufacturer's guidelines, with the cut-off value calculated as the mean OD of the negative control plus 0.15. The test run was considered valid when the mean OD of the positive control was ≥ 1.0 and that of the negative control was ≤ 0.2. Based on these criteria, samples were classified as seropositive, seronegative, or inconclusive.

2.7. Viral RNA extraction and complementary DNA (cDNA) synthesis

All buffy coat samples from both cattle and buffalo were subjected to viral RNA extraction using a commercial kit (ELK-EP017, ELK Biotechnology, Denver, USA) following the manufacturer's instructions. A total of 200 μL of each sample was processed, and total RNA was eluted in 30 μL of RNase-free water. The concentration and purity of the extracted RNA were determined using a NanoDrop spectrophotometer (Thermo Scientific NanoDrop™ 1000, USA). All extracted RNA samples were immediately stored at −80 °C until further processing. cDNA was then synthesized from the extracted RNA using a commercially available ABScript II cDNA First Strand Synthesis Kit (ABclonal, San Francisco, USA). Briefly, 20 μL of extracted RNA was mixed with random hexamer primers and the reverse transcription master mix according to the manufacturer's instructions. Finally, the reaction was incubated under the following thermal conditions: 25 °C for 5 min, 42 °C for 60 min, and 80 °C for 5 min. The synthesized cDNA was stored at −20 °C for subsequent PCR amplification and sequencing.

2.8. Real-time reverse transcription quantitative PCR (RT-qPCR)

The presence of BVDV in cattle and buffalo samples was detected using a one-step RT-qPCR assay targeting the conserved 5′ untranslated region (5′-UTR) of the BVDV genome. Amplification was performed using the forward primer (BVD-SF: 5′-AGGCTAGCCATGCCCTTAGT-3′) and reverse primer (BVD-SR: 5′-CTCCATGTGCCATGTACAGC-3′), as described by Peddireddi et al. (2018). To amplify the target region, the PCR reaction was prepared in a total volume of 25 μL. The reaction mixture contained 12.5 μL of SYBR Green PCR SuperMix (ELK Biotechnology, Denver, USA), 1.0 μL (10 μM) of each primer, 1.0 μL of ROX reference dye, 2.0 μL of cDNA template, and 7.5 μL of nuclease-free water. After that, the PCR cycling was performed on a real-time thermal cycler system (Applied Biosystems® 7500 Fast, Thermo Fisher Scientific, Inc., USA) under the following running conditions: initial denaturation at 95 °C for 15 min, followed by 40 cycles of denaturation at 95 °C for 20 s, annealing at 58 °C for 30 s, and extension at 72 °C for 45 s, with fluorescence acquisition during amplification. At the end of the PCR cycle, samples with a cycle threshold (Ct) value ≤ 35 were considered BVDV-positive, whereas samples showing no detectable amplification curve or Ct values > 35 were considered negative. The amplified PCR products were further confirmed by 1% agarose gel electrophoresis to verify the expected amplicon size of 293 bp.

2.9. Detection of PI and TI animals

Animals that tested positive for BVDV in the initial RT-qPCR assay were further subjected to repeat blood sample collection at a three-week interval to differentiate PI from TI status. Subsequently, the buffy coat was separated from whole blood, and viral RNA extraction, cDNA synthesis, and RT-qPCR were performed using the same protocol as described above. Animals that remained RT-qPCR-positive in the second test and were seronegative for BVDV antibodies by Ab-ELISA were classified as PI. In contrast, animals that tested positive for BVDV during the first sampling but were negative in the follow-up RT-qPCR test were classified as TI (Lanyon et al., 2014).

2.10. Sequencing of amplified 5′-UTR and identification of BVDV species

To identify the circulating BVDV species, RT-qPCR–positive samples with the expected 5′-UTR amplicon were selected for nucleotide sequencing. The amplified PCR products were subjected to bidirectional sanger sequencing using the same forward and reverse primers. PCR product purification and sequencing tasks were performed

commercially through third-party (BD Genome, Bangladesh). Then, the obtained forward and reverse sequence chromatograms were inspected, edited, and assembled to generate reliable consensus sequences using BioEdit® v7.2 software. All consensus sequences were subsequently compared in the GenBank database using the BLASTn algorithm to identify and confirm the corresponding BVDV species. Finally, all sequences were submitted to the GenBank database using the BankIt submission tool, and individual accession numbers were assigned to each sequence (Table 1).

2.11. Phylogenetic analysis and detection of BVDV sub-genotypes

To explore the genetic relatedness, sequence data (5′-UTR) obtained from both cattle and buffalo species in mixed production system were subjected to phylogenetic analysis. Additionally, to reveal the sub-genotypes of circulating field isolate, representative reference sequences of known BVDV sub-genotypes retrieved from the GenBank database and previously reported by Deng et al. (2020) and Xiao et al. (2025) **(**Supplementary Table 1**)**. Initially, all consensus sequence data were compiled, and multiple sequence alignment was performed using the ClustalW program. Subsequently, the neighbor-joining method was used to construct the phylogenetic tree using the MEGA version 11 software (DNAStar Inc., USA). The evolutionary distances among different sequences in the phylogenetic tree were estimated using the Kimura 2-parameter model with default settings, as described by Bhambri and Gupta, (2012). The robustness of each phylogenetic branch was assessed by bootstrap analysis using 1000 replicates. The matrix algorithm numbers (bootstrap values) displayed over the branches represent the percentage of genomic similarity supporting each phylogenetic branch. Nucleotide (nt) identity percentages were estimated using pairwise distance analysis based on aligned sequences. Nucleotide identity was calculated as the percentage similarity between cattle- and buffalo-derived BVDV sequences and across different subgenotypes relative to reference sequences, as determined from the pairwise distance matrix.

2.12. Virus propagation and biotype characterisation

RT-qPCR–positive buffy coat samples were subjected to virus isolation and biotyping using Madin–Darby bovine kidney (MDBK) cells. Firstly, MDBK cells (Passage no 29) were propagated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% BVDV-free fetal bovine serum (FBS), 1% antibiotic solution (10,000 units/mL penicillin and 10 mg/mL streptomycin), and 0.25% antifungal agent (amphotericin B, 250 μg/mL). When the MDBK cell monolayers reached approximately 90% confluency, the prepared samples were inoculated. Briefly, immediately after thawing, 300 μL of buffy coat sample was mixed with 200 μL of DMEM and added over the cell culture monolayers. Subsequently, the inoculum was evenly distributed over the cell monolayers, and the flasks were incubated at 37 °C for 90 min to allow viral adsorption to susceptible cell receptors, with gentle agitation performed at 15-minute intervals. The infected cultures were incubated at 37 °C and examined at 24-hour intervals to check the development of cytopathic effects (CPE) under an inverted microscope for up to 9 days post-infection (pi). MDBK cultures that did not exhibit visible CPE were subjected to blind passaging for up to three passages. Briefly, 1 mL of infected culture supernatant was collected and re-inoculated onto fresh MDBK cell monolayers at approximately 90% confluency, following the same protocol as described above. MDBK cell cultures showing characteristic CPE, as well as cultures without visible CPE, were harvested, and viral replication was confirmed by measuring cycle threshold (Ct) values using RT-qPCR on cell culture supernatants (Supplementary Table 2), following the same protocol described above. BVDV-positive isolates that produced visible cytopathic changes were identified as cytopathic biotypes, whereas isolates that replicated without inducing visible CPE were classified as non-cytopathic biotypes, as described previously (Birk et al., 2008).

2.13. Statistical analysis

All epidemiological data and laboratory findings were stored in Microsoft Excel-10 spreadsheets and subsequently all analyzed using STATA software version 18 (StataCorp LLC, College Station, TX, USA). Descriptive statistics were used to calculate the seroprevalence and molecular prevalence of BVDV as the number of positive farms and animals divided by the total number of farms and animals tested, respectively. Subsequently, animal-level demographic factors and herd-level epidemiological variables were analyzed separately to identify risk factors associated with two outcomes, such as the presence of BVDV antibodies and active BVDV infection in cattle and buffalo. Initially, the associations between different categorical explanatory variables and each targeted outcome were tested using the chi-square test. Thereafter, univariable logistic regression analysis was performed for each variable to identify the individual association with the two outcomes mentioned. Any variables with a p-value ≤ 0.20 in univariable analysis were further analyzed using a multivariable logistic regression model to identify independent predictors of BVDV seropositivity and active infection. A backward stepwise elimination approach was followed to retain significantly associated variables with the related outcomes. Finally, the results of the multivariable analysis and degree of association between independent variables were presented as odds ratios (OR) with 95% confidence intervals (CI), and statistical significance was set at $p < 0.05$. However, some categories of independent variables were omitted or appeared empty in the logistic regression analysis due to very low or zero cell counts (no events/sparse data), which precluded reliable estimation of model parameters. Notably, multicollinearity among the risk factor variables was assessed prior to model construction using Goodman and Kruskal's Gamma statistic, as described by Barbiero and Hitaj (2020). Moreover, potential confounding effects were assessed by including relevant variables in the multivariable model. A variable was considered a confounder if its inclusion resulted in a change of $\geq 25\%$ in the adjusted odds ratio (aOR), as described by Rothman et al. (2008). Additionally, a heat map was generated to visualize the overall status and distribution of BVDV in mixed farming systems using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Samples and distribution of animals in mixed production system

In total, 219 animals, including 126 (57.53%) cattle and 93 (42.47%) buffaloes from 12 mixed farms, were sampled, with representation of different age categories and sexes (Tables 2 and 3).

3.2. Seroprevalence of BVDV

Among the 12 mixed farms, 9 farms were seropositive for BVDV, resulting in a farm-level seroprevalence of 75.0% (95% CI: 42.81–94.51) (Table 2**;** Fig. 2). At the animal level, 33 of 219 animals were seropositive, corresponding to an overall seroprevalence of 15.07% (95% CI: 10.61–20.51) **(**Table 2**)**. Of these, 20 cattle (15.87%; 95% CI: 9.97–23.44) and 13 buffaloes (13.98%; 95% CI: 7.66–22.72) were positive for BVDV antibodies. Within animal categories, the highest seroprevalence was observed in cattle calves (11/58; 18.97%; 95% CI: 9.87–31.41), whereas among buffaloes, buffalo bulls showed the highest seropositivity (6/26; 23.08%; 95% CI: 8.97–43.65) (Table 3).

3.3. Molecular prevalence of BVDV

Among the 12 mixed farms investigated, 8 farms (66.67%; 95% CI: 34.89–90.08) were positive for active BVDV infection (Table 2**;** Fig. 2). Within the BVDV-positive mixed-species farms, 19 of 219 animals

Table 3
Seroprevalence and active BVDV infection (viremic) status among different animal categories in mixed farming production systems.

Animal Species	Animal category	No of animal	BVDV antibody positive (%; 95% CI)	BVDV active infection (%; 95% CI)	Pestivirus species	Prevalence of Pestivirus species (%; 95% CI)
					BVDV-1	3 (5.17; 1.08–14.38)
	Calves	58	11 (18.97; 9.87–31.41)	5 (8.62; 2.86–18.98)	BVDV-2	2 (3.45; 0.42–11.91)
					HoBiPeV	0
					BVDV-1	1 (33.34; 0.84–90.57)
Cattle	Heifers	3	0	1 (33.34; 0.84–90.57)	BVDV-2	0
					HoBiPeV	0
					BVDV-1	3 (4.62; 0.96–12.90)
	Cows	65	9 (13.85; 6.53–24.67)	6 (9.23; 3.46–19.02)	BVDV-2	2 (3.08; 0.37–10.68)
					HoBiPeV	1 (1.54; 0.04–8.28)
					BVDV-1	4 (7.41; 2.06–17.89)
	Calves	54	4 (7.41; 2.06–17.89)	4 (7.41; 2.06–17.89)	BVDV-2	0
					HoBiPeV	0
					BVDV-1	1 (3.85; 0.10–19.64)
Buffalo	Bull	26	6 (23.08; 8.97–43.65)	2 (7.69; 0.95–25.13)	BVDV-2	0
					HoBiPeV	1 (3.85; 0.10–19.64)
					BVDV-1	0
	Cows	13	3 (23.07; 5.04–53.81)	1 (7.69; 0.19–36.03)	BVDV-2	0
					HoBiPeV	1 (7.69; 0.19–36.03)
Total		219	33 (15.07; 10.61–20.51)	19 (8.68; 5.30–13.22)		

BVDV-1: bovine viral diarrhea virus 1 (*Pestivirus bovis*); BVDV-2: bovine viral diarrhea virus 2 (*Pestivirus tauri*); HoBiPeV: HoBi-like pestivirus (*Pestivirus brazilense*); CI: confidence interval.

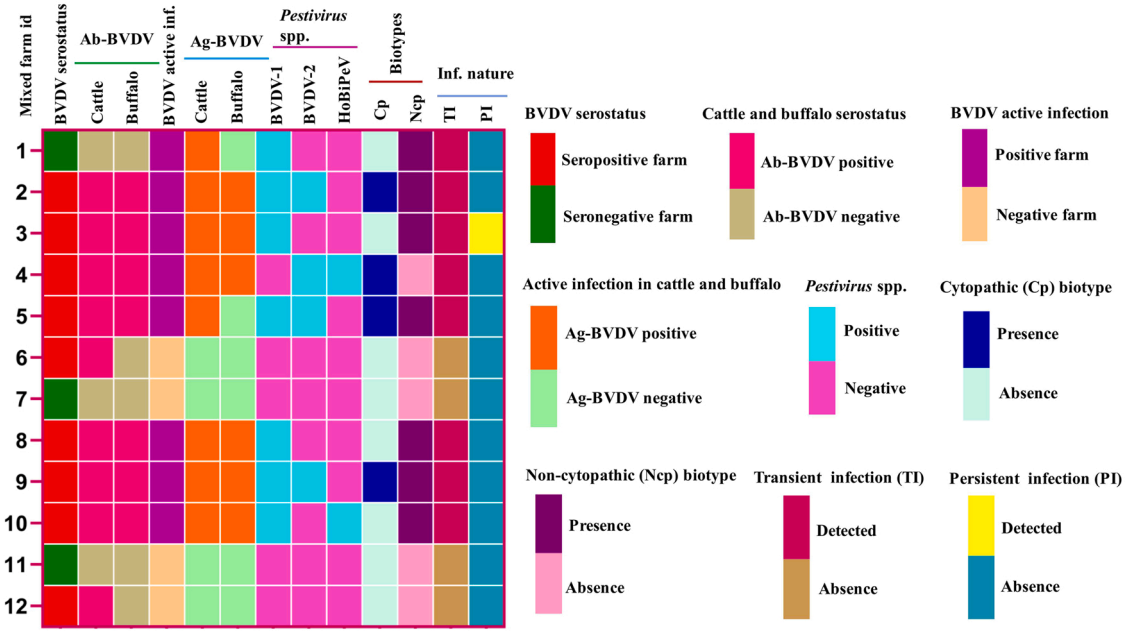


Fig. 2. Heat map illustrating BVDV serostatus and active infection in mixed-herd production systems. The figure also shows the distribution of circulating Pestivirus species, BVDV biotypes, and transient (TI) and persistent (PI) infections in cattle and buffalo, indicating the overall farm-level epidemiological scenario.

(8.68%; 95% CI: 5.30–13.22) were identified as viremic, indicating active infection across all eight farms. At the animal level, 12/126 cattle (9.52%; 95% CI: 5.02–16.05) and 7/93 buffaloes (7.52%; 95% CI: 3.08–14.90) were found to be viremic (Tables 2 and 3). Within the cattle population, the highest molecular prevalence was observed in dairy cows 6/65 (9.23%; 95% CI: 3.46–19.02) and calves 5/58 (8.62%; 2.86–18.98). In contrast, buffalo calves showed a molecular prevalence of 7.41% (4/54; 95% CI: 2.06–17.89) (Table 3).

3.4. Detection rate of TI and PI animals

Among the 19 viremic animals, 18 (94.74%; 95% CI: 73.97–99.87) cattle and buffaloes were classified as TI based on the follow-up RT-qPCR test. In contrast, only one cattle calf was identified as PI (Table 1; Fig. 2).

3.5. Prevalence of Pestivirus species

Among the 19 RT-qPCR–positive samples, 12 isolates (63.16%; 95% CI: 38.36–83.71) were identified as BVDV-1 from both cattle and buffalo, representing the predominant virus species detected in the study population (Tables 1 and 2). At the bovine species level, the highest proportion of BVDV-1 positive animals was observed in calves of both cattle and buffalo, with detection rates of 5.17% (3/58; 95% CI: 1.08–14.38) and 7.41% (4/74; 95% CI: 2.06–17.89), respectively (Table 3). Moreover, 4 of 19 isolates (21.05%; 95% CI: 6.05–45.57) were identified as BVDV-2 in cattle, while no buffalo samples tested positive for BVDV-2. In addition, 3/19 isolates (15.79%; 95% CI: 3.38–39.58) were confirmed as HoBi-like pestivirus, with infections detected in two buffaloes and one cattle (Tables 2 and 3).

3.6. Phylogenetic analysis

Phylogenetic analysis revealed that all BVDV-1 isolates obtained from cattle and buffalo in mixed-farm environments clustered together with high genetic relatedness, indicating potential viral transmission between species (Fig. 3). Within the BVDV-1 cluster, sequences derived from cattle and buffalo formed closely related sub-clusters, representing significant genetic similarity across viral sequence. Comparative analysis of the 5′-UTR revealed that BVDV-1 isolates from cattle and water buffalo shared 92–98% nucleotide (nt) identity, reflecting high genetic conservation across bovine species. In contrast, all BVDV-2 sequences derived from cattle grouped within a single clade with high bootstrap support, and showed 96–97% nucleotide similarity and suggesting strong genetic homogeneity. No BVDV-2 isolates were obtained from buffalo in the mixed production system. Additionally, three HoBi-like pestivirus sequences formed a distinct clade in the phylogenetic tree. The cattle-origin HoBi-like pestivirus isolate similarly exhibited 94–98% identity with buffalo-origin HoBiPeV isolates. This nucleotide identity indicates a high degree of genetic conservation (Fig. 3).

3.7. Detection of circulating subgenotypes and genetic relatedness

The circulating BVDV subgenotypes identified through phylogenetic analysis in isolates obtained from mixed-farm settings are presented in Fig. 4 **and** Table 1. Phylogenetic analysis showed that all 12 field-derived BVDV-1 isolates clustered within the BVDV-1 lineage. Within this group, eight distinct subgenotypes were identified, namely BVDV-1a, 1b, 1c, 1d, 1j, 1k, 1o, and 1p (Fig. 4 **and** Table 1). Among these, three isolates belonged to subgenotype BVDV-1b, two isolates were detected as BVDV-1j, and the remaining subgenotypes were each represented by a single isolate. All four BVDV-2 isolates clustered within a single lineage and were identified as BVDV-2a subgenotypes (Fig. 4 **and** Table 1). Similarly, the three HoBi-like pestivirus isolates grouped together in the phylogenetic tree and were all identified as HoBiPeV-a subgenotypes (Fig. 4 **and** Table 1). Moreover, in pairwise nucleotide sequence analysis of the 5′-UTR demonstrated high genetic relatedness between the study isolates and their respective reference strains across all BVDV-1 subgenotypes (Table 1 **and** Supplementary Table 1). The BVDV-1a isolate shared ~99–100% nt identity. BVDV-1b isolates exhibited 96–99% nt identity, while BVDV-1c buffalo isolates showed 95–99% identity, reflecting high conservation with minimum regional variations. The BVDV-1d and BVDV-1k isolates showed ~99% and 97–100% nt identity, respectively, whereas BVDV-1j buffalo isolates demonstrated ~93–97% identity. Notably, BVDV-1p and BVDV-1o isolates exhibited 94–96% and 98–99% nt identity, respectively, indicating a plausible co-circulation of high genetically related virus within mixed bovine herds.

3.8. In-vitro propagation and biotyping of BVDV

All 19 PCR-confirmed isolates were cultured onto MDBK cell lines to differentiate cytopathic and non-cytopathic biotypes, and the distribution of biotypes at both herd and animal levels is presented in Table 1 **and** Fig. 5. Of these, 15 isolates (78.95%; 95% CI: 54.43–93.94) were successfully propagated in cell culture, whereas four isolates from three different farms (Farm-01, Farm-04, Farm-10) failed to propagate, possibly due to low viral load or the presence of non-infective viral particles (Table 1). Among the 15 isolates, 10 (66.67%; 95% CI: 38.38–88.18) were identified as non-cytopathic, while the remaining 5 (33.33%; 95% CI: 11.82–61.62) belonged to the cytopathic biotype (Table 1 **and** Fig. 5). Cytopathic biotypes exhibited characteristic cytopathic effects in MDBK cell culture, including distinct cytoplasmic vacuolation with small vacuoles coalescing into larger vacuoles, disruption and irregularity of the cell monolayer, increased cytoplasmic density and granularity due to cellular degeneration, cell rounding and detachment, and eventual cell death with floating cells in the culture medium. These effects were typically evident within 7–9 days post-infection (Fig. 5). Notably, among the 12 BVDV-1 isolates, 9 (75%; 95% CI: 42.81–94.51) were non-cytopathic, whereas of the 4 BVDV-2 isolates, 3 (75%; 95% CI: 19.41–99.37) were cytopathic (Table 1). Notably, all three HoBi-like pestivirus isolates detected from two mixed farms (Farm-04 and Farm-10) failed to propagate in MDBK cell culture

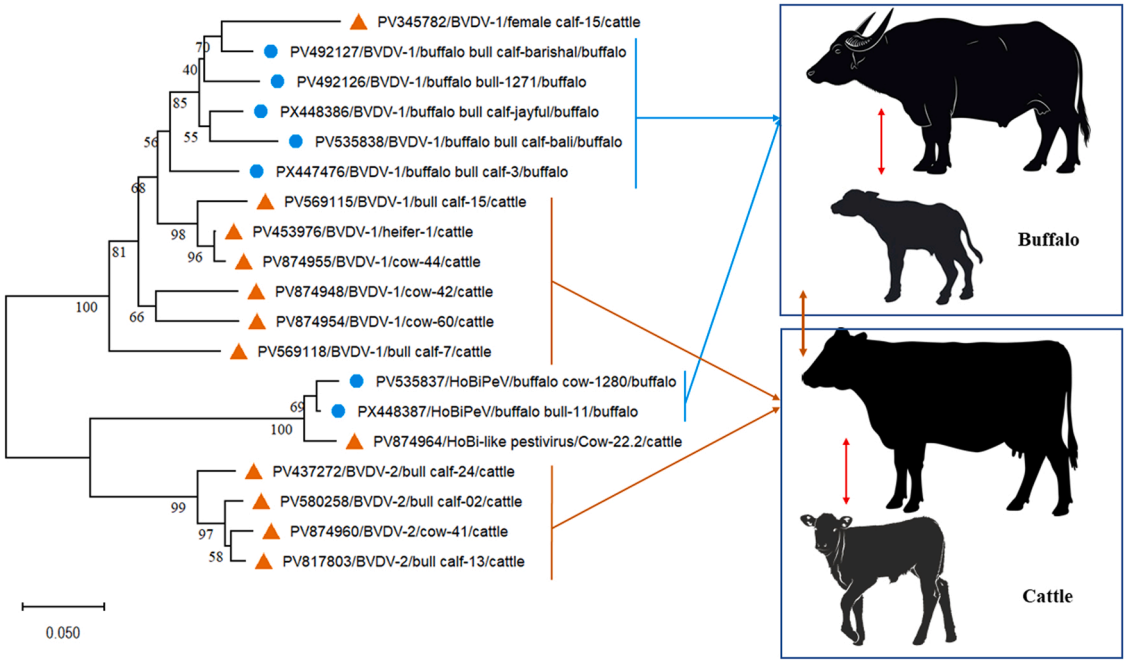
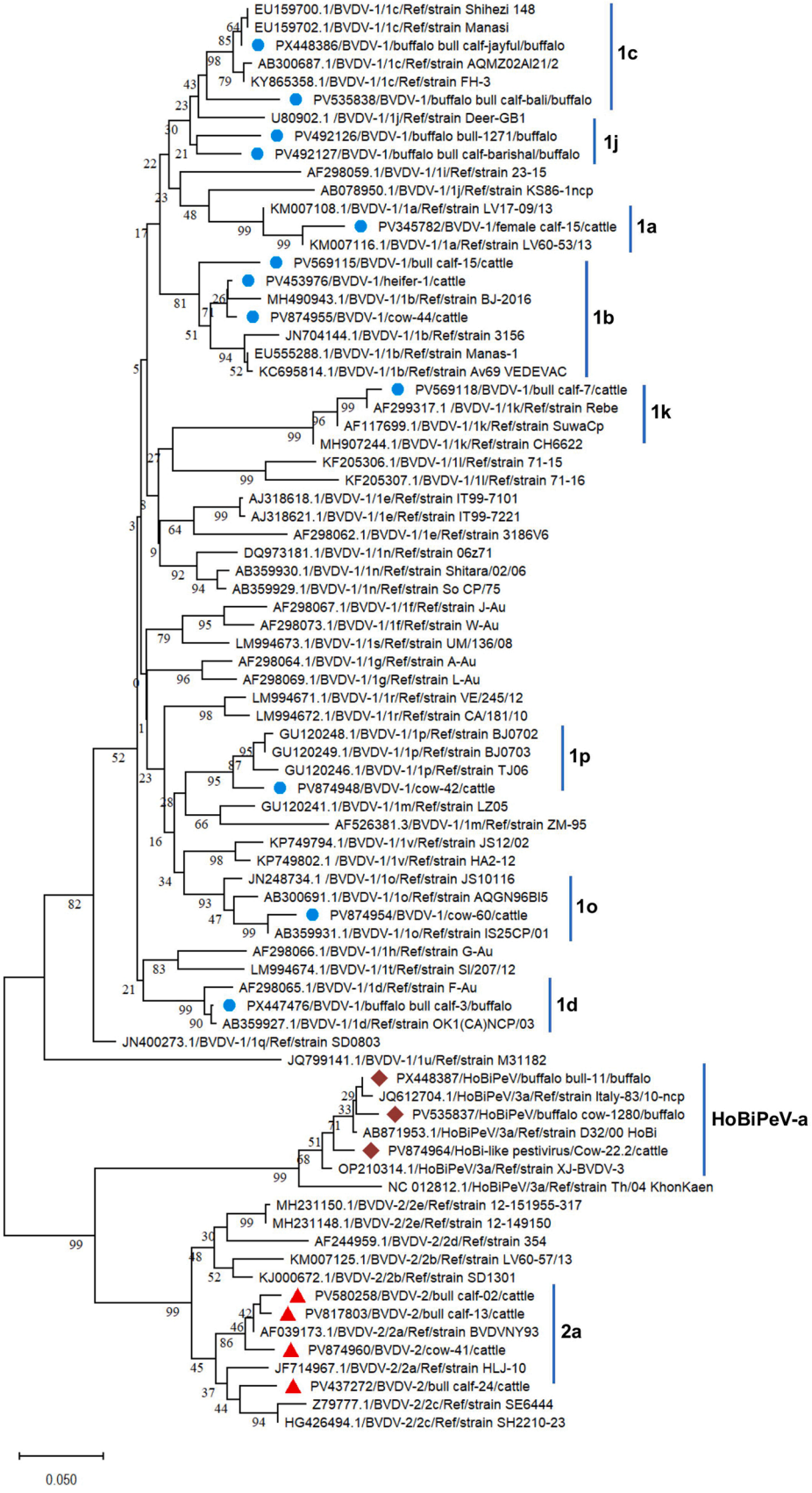


Fig. 3. Phylogenetic analysis based on 5′-UTR sequences obtained from cattle and buffalo in mixed herds (Table 1). The tree was constructed using the neighbor-joining method in MEGA 11 software with 1000 bootstrap replicates. Cyan solid circles represent sequences from buffalo, whereas orange triangles indicate sequences from cattle. Distinct clustering of BVDV-1, BVDV-2, and HoBi-like pestivirus obtained from both cattle and buffalo, along with high genetic similarity, indicates potential intra- and inter-species transmission.



(caption on next page)

Fig. 4. BVDV subgenotypes were identified through phylogenetic analysis based on 5′-UTR nucleotide sequences. A total of 19 Bangladeshi sequences obtained from cattle and buffalo in mixed herds (Table 1) were compared with 60 reference sequences representing different subgenotypes (Supplementary Table 1). The phylogenetic tree was constructed using the neighbor-joining method in MEGA 11 software. Node values indicate the percentage of 1000 bootstrap replicates supporting each cluster. Cyan solid circles represent different BVDV-1 subgenotypes, red triangles indicate BVDV-2a subgenotypes, and maroon diamond symbols highlight HoBiPeV–a subgenotypes.

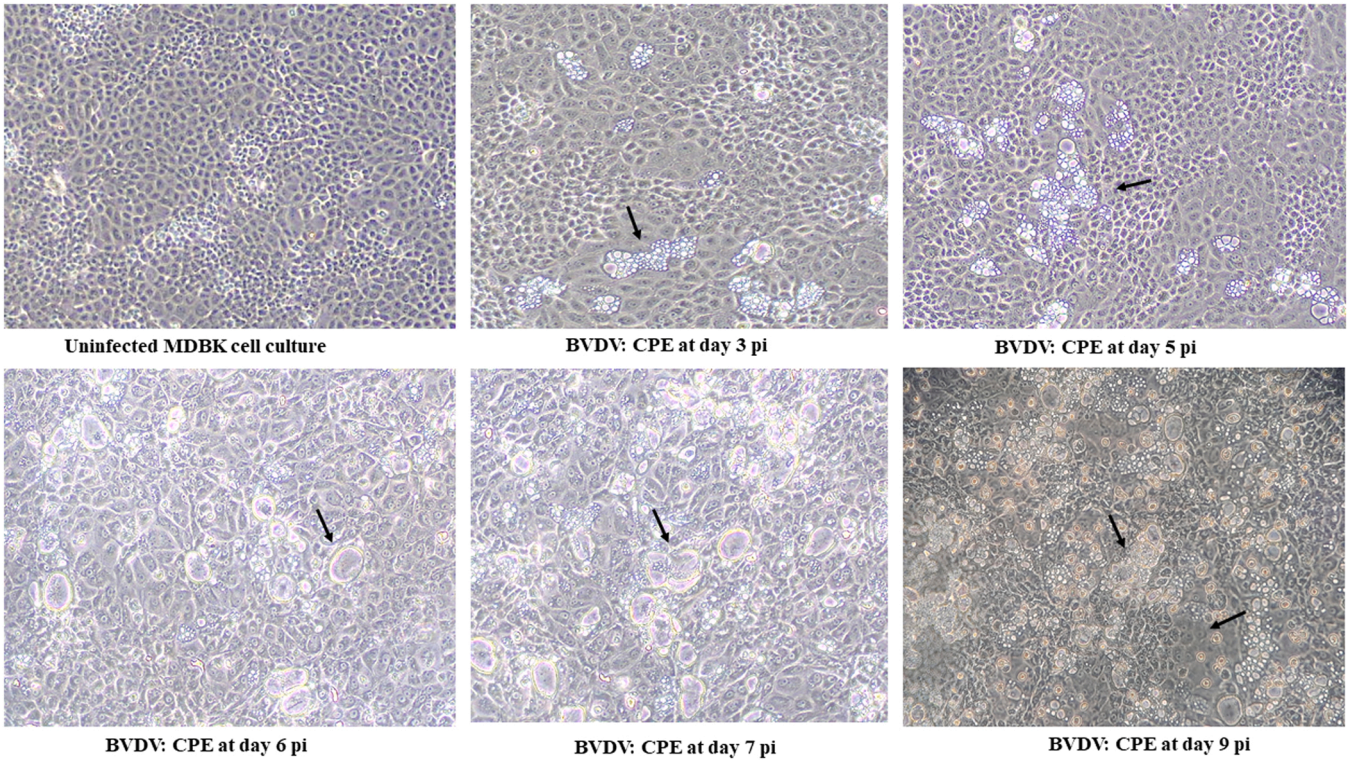


Fig. 5. Progressive cytopathic effects (CPE) of BVDV in MDBK cell culture following inoculation with positive samples. At 3 days post-infection (dpi), observable cytoplasmic vacuolation appeared in the MDBK cell monolayer. By day 5 dpi, vacuolation became more widespread and distinct, with occasional cell fusion. At days 6–7 dpi, extensive cytoplasmic vacuolation, cell fusion, cellular morphological changes, and widespread viral replication were evident in the monolayer. By day 9 dpi, the monolayer was severely disrupted, with infected cells showing rounding, shrinkage, detachment, and floating dead cells. Black arrows indicate characteristic CPE, confirming the cytopathic biotype of BVDV.

and, consequently, could not be biotyped (Table 1).

4. Risk factors associated with the seropositivity and active infection of BVDV

4.1. Analysis of animal demographic and previous clinical conditions

In total, 11 variables related to animal demographic characteristics and clinical history were evaluated using chi-square tests and univariable logistic regression to assess associations between potential risk factors and BVDV seropositivity in cattle and buffalo from mixed-farming systems **(**Supplementary Table 3**)**. Of these, four variables met the eligibility criterion ($p \leq 0.20$) and were included in the subsequent multivariable logistic regression analysis. In the final multivariable model, cattle and buffalo with 'poor or stunted growth' rates were 3.22 times more likely to be seropositive for BVDV compared with other animals (95% CI: 1.75–13.80; $p = 0.036$) (Table 4). Additionally, the 'presence of clinical diarrhoea within the last three months' in cattle and buffalo was significantly associated with BVDV seropositivity (OR = 2.33; 95% CI: 1.08–5.06; $p = 0.032$) (Table 4).

Moreover, for active (viremic) BVDV infection, two variables initially met the inclusion criterion ($p \leq 0.20$) for multivariable analysis; however, none remained significant in the multivariable logistic regression model (Supplementary Table 4). In univariable analysis, young-aged animals were the only variable significantly associated with active BVDV infection (OR: 5.88; 95% CI: 1.93–37.15; $p = 0.050$) (Supplementary Table 4).

4.2. Analysis of management and biosecurity factors

A total of 46 farm management and biosecurity–related factors were analysed using chi-square tests and univariable logistic regression to identify factors associated with previous BVDV exposure in mixed cattle–buffalo herds (Supplementary Table 5). Among these, 26 variables

Table 4
Animal-level multivariable logistic regression analysis of demographic factors independently associated with BVDV seropositivity in commercial mixed farming systems.

Outcome variable	Explanatory variable	Description	OR (95% CI)	*p*-value
Presence of BVDV antibody	Growth rate	Good	1	Reference
		Moderate	2.37 (1.06–5.31)	0.116
		Poor/ Stunted	3.22 (1.75–13.80)	0.036*
	History of clinical diarrhoea within last 3 months	Yes	2.33 (1.08–5.06)	0.032*
		No	1	Reference

Abbreviation: OR: Odds ratio; CI: Confidence interval; statistically significant *$p \leq 0.05$

($p \leq 0.20$) met the initial screening criterion and were included in the multivariable logistic regression analysis. In the final model, three farm factors remained significantly associated with BVDV seropositivity (Table 5). Among the significant factors, 'close distance (proximity)' (OR: 68.76; 95% CI: 5.69–830.82; $p = 0.001$) and 'moderately distance' between neighboring farm (OR: 14.03; 95% CI: 1.36–144.18; $p = 0.026$) were significantly associated with BVDV seropositivity. Additionally, 'large farm size' was 22 times more likely to be BVDV seropositive (95% CI: 1.27–379.02; $p = 0.034$), and 'recent introduction of purchased animals' (OR: 3.27; 95% CI: 1.18–9.09; $p = 0.023$) was also significantly associated with previous exposure of BVDV (Table 5).

For active BVDV infection, nine variables met the inclusion criterion ($p \leq 0.20$) and were initially selected for multivariable logistic regression analysis (Supplementary Table 6). However, due to multicollinearity among the variables, none were retained in the final multivariable model. In bivariate analysis, chi-square tests indicated significant associations between active BVDV infection and quarantine practices ($p = 0.039$) as well as between active BVDV infection and disinfection practices before farm entry ($p = 0.039$) (Supplementary Table 6).

5. Discussion

Bovine viral diarrhea virus (BVDV) affects a wide range of food-producing animals and poses threat to profitable and sustainable livestock farming. In response to increasing demand for milk, meat, and dairy products over the past two decades, cattle–buffalo mixed dairy farming has expanded rapidly in Bangladesh, potentially influencing the transmission epidemiology of BVDV. The widespread distribution and broad host tropism of BVDV make it a persistent and significant challenge for mixed-livestock production systems (Martínez-Burnes et al., 2024). Multiple livestock species particularly cattle and buffalo are reared in the same premises or in close proximity in mixed farming systems, where BVDV poses an increased risk of interspecies transmission (Deng et al., 2015; Rana et al., 2025b). Despite extensive reporting on the molecular epidemiology and genetic diversity of BVDV in dairy herds (Rana et al., 2026), evidence of co-circulation of BVDV from mixed-livestock production systems remains limited in Bangladesh. Given the widespread practice of mixed-livestock farming in Bangladesh, this study investigates the molecular epidemiology and viral characteristics in cattle and buffalo mixed-herds.

Table 5
Farm-level multivariable logistic regression analysis of different risk factors independently associated with BVDV seropositivity in commercial mixed farming systems.

Outcome variable	Explanatory variable	Description	OR (95% CI)	*p*-value
Presence of BVDV antibody	Distance between neighboring farm	Very close distance	1.56 (0.41–5.86)	0.513
		Close distance (proximity)	68.76 (5.69–830.82)	0.001*
		Moderately distance	14.03 (1.36–144.18)	0.026*
		Far distance	1	Reference
	Farm size	No nearby farms	5.08 (0.54–47.07)	0.153
		Large (≥100 animals)	21.94 (1.27–379.02)	0.034*
		Medium (≥50 – <99 animals)	8.41 (0.87–80.89)	0.065
		Small (10 – < 50 animals)	1	Reference
	Introduction of recently purchased animal	Yes	3.27 (1.18–9.09)	0.023*
		No	1	Reference

Abbreviation: OR: Odds ratio; CI: Confidence interval; statistically significant *$p \leq 0.05$

In this study, high herd-level seropositivity (75%) and active infection rates (66.67%) were observed in mixed-farm settings. These findings indicate widespread exposure to BVDV in Bangladeshi dairy herds and are consistent with previous reports of herd-level seroprevalence of 72.5–83.3% (Rana et al., 2026; Sajeeb et al., 2025). At the individual animal level, BVDV seropositivity in mixed farms was 15.87% in cattle and 13.98% in buffalo. As BVDV vaccination is not practiced in cattle or buffalo populations in Bangladesh, the observed serostatus likely reflects natural exposure to wild-type virus circulating within herd environments. However, BVDV seroprevalence in cattle shows considerable variation across Asia, ranging from 11.7% to 66.8% in countries (Rana et al., 2025b). Additionally, high BVDV seroprevalence (73.3–87.1%) has been reported in several European countries, as well as in African countries (53.6–66.4%) (Rana et al., 2025b; Scharnböck et al., 2018). In contrast, BVDV seropositivity in buffalo has been reported as 19.4% in China, 20.4% in Iran, 27% in India and Colombia, 30.6% in Pakistan, and 30.33% in Brazil (Lotfi et al., 2016; Assunção et al., 2022; Selvaraj et al., 2007; Rana et al., 2025b). Based on previous epidemiological evidence, BVDV seropositivity is generally lower in buffalo than in cattle population; however, mixed-farming systems showed almost close seropositivity rate in both species, suggesting that there might be interspecies transmission contribute to high infection rates within herds. Moreover, the active infection rates of BVDV in cattle (9.52%) and buffalo (7.52%) observed in the present study were broadly comparable, where cattle and buffalo are co-housed or managed under common grazing, watering, husbandry and biosecurity practices. Such mixed interspecies contact may encourage virus transmission among bovine species (Deng et al., 2015).

In the present study, 94.74% of viremic cattle and buffaloes were identified as TI, whereas only a single calf was confirmed as PI based on 21-day follow-up antigen testing. The high rate of TI animals is consistent with findings from cross-sectional and surveillance studies conducted in BVDV-endemic regions (Scharnböck et al., 2018). However, the development of PI animals requires time-specific exposure of BVDV to the pregnant dam, particularly through in utero infection during early gestation, typically between 40 and 120 days (Darweesh et al., 2015). However, limited follow-up or small sample sizes may hinder accurate estimation of the true prevalence of PI (Scharnböck et al., 2018). Notably, PI animals remain epidemiologically critical, as their infections are often subclinical or asymptomatic, allowing continuous viral replication and serving as a major source of virus shedding and transmission within cattle and buffalo populations in mixed herd (Houe, 1995). Moreover, following confirmation of a PI calf in the mixed herd, the farm owner was notified of the significant epidemiological risk associated with PI animals. Consequently, immediate implementation of control measures, including strict segregation and prompt culling of the PI calf, was strongly recommended to mitigate further viral transmission and endemicity.

In the present study, BVDV-1 (*Pestivirus bovis*) was the predominant pestivirus, representing 63.16% of infections in both cattle and buffaloes. This finding is consistent with the global molecular epidemiological pattern in which BVDV-1 remains the most widely circulating and genetically diverse Pestivirus in bovine herds (Rana et al., 2025b, Rivas et al., 2022). Several studies have also reported the presence of BVDV-1 in water buffalo, including both in subclinical and clinical cases (Martínez-Burnes et al., 2024; Martucciello et al., 2009). The dominance of BVDV-1 within bovine populations could be associated with its evolutionary adaptation to bovine hosts and its ability to efficiently maintain viral circulation among cattle and buffalo herds through the establishment of PI animals. Notably, all BVDV-2 isolates were detected in cattle only, despite mixed-farming conditions, whereas HoBi-like pestivirus was identified in both bovine species. This host-restricted detection of BVDV-2 may reflect differences in host susceptibility, limited exposure, and lower virus burden in farm environments, or may

be because of small sample size. Both BVDV-2 and HoBi-like pestivirus are capable of producing pathogenic effects similar to those observed in cattle and have been reported in buffalo populations in different geographical regions, such as Argentina and Italy (Pecora et al., 2017; Rana et al., 2025b). BVDV-2 appears to have a lower propensity to establish PI calves (Letellier et al., 2010), which may limit its long-term circulation within herd environments compared with BVDV-1.

Phylogenetic analysis and nucleotide identity assessment of 5′-UTR sequences from cattle and buffalo showed that BVDV-1 and HoBi-like pestivirus isolates clustered with high bootstrap support, indicating close genetic relatedness and bidirectional transmission. Shared housing, animal movement, and common husbandry practices create ecological overlap that may facilitates co-circulation within mixed-farm environments and potential interspecies virus transmission (Rana et al., 2025a). Additionally, bovine species share cellular receptors, such as CD46 and glycosaminoglycans, involved in viral attachment and entry, which may contribute to similar susceptibility to BVDV infection and suggests the potential for co-circulation and likelihood of cross-transmission of BVDV among bovine hosts (Rana et al., 2025a; Maurer et al., 2004). Notably, failure to detect BVDV-2 in buffalo may be influenced by sample size and temporal sampling. Nevertheless, broad-scale molecular surveillance programs are strongly recommended to better understand the epidemiology of BVDV-2 in mixed farming systems. Identifying the primary reservoir host, whether cattle or buffalo remains critical, as both species are susceptible to BVDV infection.

Subsequent genetic analysis of the three Pestivirus species, using reference subgenotype sequences, revealed that BVDV-1 comprised eight distinct subgenotypes (1a, 1b, 1c, 1d, 1j, 1k, 1o, and 1p) co-circulating in mixed-herds. The result indicates extensive genetic diversity of BVDV-1 within mixed herd environments. High genetic variability has also been reported in dairy herds from different countries, where 24 distinct BVDV-1 subgenotypes (1a to 1 v) have been identified (Rana et al., 2025b; Rivas et al., 2022; Yeşilbağ et al., 2017). The co-circulation of multiple subgenotypes suggests ongoing viral mutation and evolution in bovine herds (Rana et al., 2025b). Notably, the extensive genetic diversity of BVDV may compromise vaccine efficacy due to antigenic variation among circulating strains and the resulting limitation of cross-protective immune responses. In the absence of antigenic compatibility assessments, virus neutralization data, or direct evidence of vaccine efficacy against the circulating strains, the implications of our findings for immunization should be interpreted with caution. Although BVDV-2 comprises five subgenotypes (2a–2e) and HoBi-like pestivirus includes four subgenotypes (HoBiPeV-a to d), only BVDV-2a and HoBiPeV-a were detected in the mixed herds in this study. This nucleotide identity indicates a high degree of genetic conservation and suggests limited mutation events (Deng et al., 2015). Moreover, the high nucleotide identity observed between field strains detected in cattle and buffalo suggests that both species may contribute to potential bidirectional transmission and shared co-circulation of genetically related BVDV. Currently available commercial vaccines, mainly based on BVDV-1a, 1b, and 2a subgenotypes (Tesfaye et al., 2025), may provide limited protection against the diverse circulating subgenotypes identified in mixed herds; however, firm conclusion cannot be drawn without virus neutralisation data. The high level of viral genetic diversity observed within the region likely reflects the complexity of mixed-farming systems, which may facilitate interspecies BVDV transmission; therefore, reducing mixed bovine farming and adopting separate production systems is recommended to help limit viral spread and evolution. Notably, no BVDV vaccine is currently available in Bangladesh, and vaccination is not practiced under field conditions in either cattle or buffalo population. Therefore, further studies integrating antigenic and immunological evaluations are required before proposing vaccination recommendations, while current findings primarily support strengthening general preventive and biosecurity measures in mixed-bovine production systems. Nonetheless, broader longitudinal molecular surveillance and deep sequencing of virus whole genome is essential to accurately define the epidemiological dynamics and mutation events of BVDV in bovine populations.

Based on cytopathic effects observed in MDBK cells, the non-cytopathic biotype was predominant (66.7%) in mixed herds, which is supported by a previous study reported by Zhang et al., (2022). Both biotypes are pathogenic, able to induce clinical disease and play critical role in the development of fatal MD (Ridpath et al., 2000; Chai et al., 2023). The characteristic CPE, including cytoplasmic vacuolation with small vacuoles coalescing into larger vacuoles, disruption and irregularity of the cell monolayer, increased cellular degeneration, cell rounding, death and detachment are supported by the observations described by Birk et al. (2008). However, the co-circulation of both biotypes within the same herds has important virological implications and disease pathogenesis. Specifically, non-cytopathic BVDV–infected animals can develop acute MD when superinfected with a cytopathic virus (Darweesh et al., 2015). Notably, the non-cytopathic biotype is generally endemic in nature, whereas the emergence of the cytopathic biotype is associated with specific genomic recombination events, such as cleavage of the viral NS2/3 protein into NS2 and NS3 (Ridpath et al., 2006; Chai et al., 2023). Therefore, the occurrence of the cytopathic biotype is often limited in herd environments due to its complex evolutionary process. In contrast, some non-cytopathic viruses that do not produce CPE in MDBK cells may still be virulent and cause cell death in cultured lymphoid cells, a phenotype often classified as lymphocytopathogenic biotype (Ridpath et al., 2006). Notably, based on the effects of BVDV on cultured lymphoid cells, a third biotype, termed lymphocytopathogenic, has been recognized, which does not produce cytopathic effects in epithelial cells (Ridpath et al., 2006). However, this third biotype was not evaluated in the present study, should be addressed in future investigations. Moreover, the failure of all three HoBi-like pestivirus isolates to propagate in MDBK cell culture may reflect differences in host cell tropism and replication efficiency, as well as potential technical factors such as low viral load or loss of infectivity during sample handling. This limitation indicates a bias inherent to culture-dependent methods, which may underestimate the presence and diversity of poorly adapted viral strains, and underscores the need for complementary molecular and alternative culture approaches; therefore, the characterization of HoBi-like pestiviruses warrants further investigation.

The potential risk factors were identified in the mixed farming condition. Among the animal demographic factors, poor or stunted growth in cattle and buffalo calves, as well as the history of clinical diarrhoea, were significantly associated with BVDV seropositivity. The higher seropositivity observed in calves may reflect increased susceptibility to infection due to their immature immune system, as well as early-life exposure to BVDV through vertical transmission or close contact with infected dams, leading to early seroconversion (Darweesh et al., 2015). Additionally, management practices in mixed farming systems, including shared housing and limited biosecurity, may facilitate early exposure to BVDV in young animals. Moreover, these associations also represent the pathogenic consequences of BVDV infection and the host's immunological response to viral exposure (Brodersen, 2014). However, BVDV can induce viremia, systemic inflammatory responses, and damage to the intestinal epithelium during replication, which cause profuse diarrhoea in infected hosts (Brodersen, 2014; Zhang et al., 2022). Such pathological alterations can hinder nutrient absorption and metabolic efficiency, ultimately leading to poor growth performance and growth retardation in affected animals (Al-Kubati et al., 2021; Brodersen, 2014). However, it is not possible to determine whether BVDV infection directly caused the observed clinical conditions or whether prior exposure, as indicated by seropositivity, was the definitive cause of historical diarrhoea in the animals. Further longitudinal studies are required to clarify temporal relationships and better understand the role of BVDV in clinical disease expression under field conditions.

During the analysis of risk factors associated with active BVDV infection in mixed herds, none of the variables remained significant

($p \leq 0.05$) in the multivariable model after statistical adjustment. This suggests that the associations observed in the univariable regression analysis may have been influenced by the lower sample size, low outcome frequency, as well as confounding and interrelationships among explanatory variables, thereby reducing their independent effects in the final multivariable model. Such attenuation after statistical adjustment is often evidenced in epidemiological modelling, where host factors, management practices, and biosecurity parameters are analysed combinedly rather than independently (Mainar-Jaime et al., 2001). However, in the univariable analysis, younger animals were identified as being at higher risk and more vulnerable to active BVDV infection (OR: 5.88) compared to other age groups. Younger animals are immunologically naïve, possess lower levels of protective immunity, and have greater opportunities for viral exposure in mixed-herd environments (Rana et al., 2026; Brodersen, 2014). Additionally, the lack of immediate testing for PI calves after birth, overlapping age-group management, and shared housing of young animals may facilitate viral transmission (Rana et al., 2025b). Although younger age appeared to be an important epidemiological risk factor for active BVDV infection, further longitudinal studies are required to better disentangle host, management, and environmental determinants of viremia in mixed production systems.

Among the identified farm-level risk factors, close distance (proximity) between neighboring farms was significantly associated with increased BVDV seropositivity in mixed herds. This relationship indicates ongoing viral circulation within farm environments, where repeated exposure over time likely promotes seroconversion (Al-Kubati et al., 2021). Additionally, close proximity between farms increases the likelihood of frequent human movement, shared grazing land and water sources, environmental exposures such as farm waste discharge, and the transmission of active infections between herds. The association between larger farm size and seropositivity is likely due to higher animal density and more frequent contact rates, which increase opportunities for viral transmission within the herd. Moreover, mixed-species production systems may facilitate a higher burden of contagious pathogens, especially viruses with broad host ranges, thereby increasing the risk of overlapping or co-infections (Rana et al., 2025b; Hou et al., 2025). Moreover, the introduction of recently purchased animal into the farm was identified as a significant risk factor for BVDV seropositivity, emphasizing the role of animal trade and marketing in disease epidemiology. Unorganized livestock markets in the study region, where different animal species from diverse epidemiological backgrounds are frequently mixed, create favourable conditions for viral transmission (Rana et al., 2025b; Galindo-González, 2022). The presence of TI or PI animals increases the likelihood of virus spread in live market environments (Galindo-González, 2022). Consequently, the reintroduction of recently purchased or return of unsold market animals without prior testing or an adequate quarantine, may create a favourable condition for potential interspecies transmission (Houe, 1995).

Although the present study explored the role of mixed bovine farming in the epidemiology of BVDV infection, long-term and longitudinal surveillance programs are strongly recommended to identify the exact reservoir hosts and to better understand interspecies transmission patterns among bovine populations and its impact on viral ecology. The cross-sectional design limits causal inference and provides a snapshot of BVDV circulation within the study population. This study was conducted on 12 mixed bovine herds comprising 219 animals, selected purposively due to the availability of mixed cattle–buffalo production systems. Although appropriate for the study objectives, this sampling strategy may have introduced selection bias, limiting the strength of prevalence estimates and potentially influencing risk factor associations. Moreover, the reported odds ratios may have been influenced by potential herd-level dependence and clustering effects, which could affect the precision and stability of the estimated associations. Given the limited number of clusters (12 herds), the regression analysis should be considered exploratory, particularly for farm-level predictors. Additionally, more complex cluster-based statistical approaches, such as mixed-effects models, generalized estimating equations (GEE), or cluster-robust standard errors, were not applied to account for herd-level dependence. This may have introduced some uncertainty, particularly in farm-level estimates, due to unequal sample sizes across herds. Therefore, future studies should incorporate larger sample sizes and broader geographic coverage to enhance robustness of mixed herds and provide a better understanding of the epidemiological dynamics of circulating virus in mixed bovine populations. The single follow-up at three weeks may have limited the detection of PI animals; regular screening is recommended to accurately identify PI individuals. Additionally, this study sequenced the 5′-UTR region; therefore, future studies should incorporate higher-resolution genomic approaches, including whole-genome sequencing of diverse BVDV wild strains, to provide a deeper understanding of viral evolution, enable better reconstruction of phylogenetic relationships and transmission chains, identify potential reservoir hosts, and strengthen the development of effective prevention and control strategies.

6. Conclusion

The serological and virological findings from this study indicates that BVDV infection is widespread in mixed cattle–water buffalo production systems. All three Pestivirus species BVDV-1, BVDV-2, and HoBi-like pestivirus were identified, with BVDV-1 predominating in both bovine species. Notably, the detection of ten distinct subgenotypes indicates high genetic diversity within the mixed animal population. Moreover, the high nucleotide identity (93–100%) observed among subgenotypes suggests a plausible co-circulation of BVDV between cattle and buffalo within mixed production systems. Therefore, the development of vaccines targeting the predominant circulating Pestivirus is strongly recommended for effective immunization and control programme. Although TI is highly prevalent, the presence of PI animals in mixed-herds acts as a continuous source of infection and maintains the epidemic trends of BVDV. The isolation of both cytopathic and non-cytopathic biotypes in mixed herd indicates a potential risk for the development of fatal MD. Additionally, the detection of multiple risk factors in mixed production systems may help development of specific biosecurity control measures and national guidelines for BVD prevention.

CRediT authorship contribution statement

Jasim M Uddin: Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization. **Eaftekhar Ahmed Rana:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Subir Sarker:** Writing – review & editing, Supervision. **Jully Gogoi-Tiwari:** Writing – review & editing, Validation, Supervision.

Funding

This study received financial support from the Murdoch University Higher Degree by Research (HDR) Fund (Project Code: 002217).

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgements

The authors gratefully acknowledge the staff of the Department of Microbiology and Veterinary Public Health and the Poultry Research and Training Center (PRTC), Chattogram Veterinary and Animal Sciences University, Bangladesh, for their excellent laboratory assistance.

We also thank the farm owners for their cooperation in sample collection and epidemiological data acquisition. The HDR candidate (EAR) was supported by an Australian Government Research Training Program (RTP) Scholarship (doi.org/10.82133/C42F-K220).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2026.106917.

References

Al-Kubati, A.A., Hussen, J., Kandeel, M., Al-Mubarak, A.I., Hemida, M.G., 2021. Recent advances on the bovine viral diarrhea virus molecular pathogenesis, immune response, and vaccines development. Front. Vet. Sci. 8, 665128. https://doi.org/10.3389/fvets.2021.665128.

Assunção, S.F., Antos, A., Barbosa, J.D., Reis, J.K., Larska, M., Oliveira, C.H., 2022. Diagnosis and phylogenetic analysis of bovine viral diarrhea virus in cattle (Bos taurus) and buffaloes (Bubalus bubalis) from the Amazon region and Southeast Brazil. Pesq. Vet. Bras. 42, e06955. https://doi.org/10.1590/1678-5150-PVB-6955.

Barbiero, A., Hitaj, A., 2020. Goodman and Kruskal's gamma coefficient for ordinalized bivariate normal distributions. Psychometrika 85 (4), 905–925. https://doi.org/10.1007/s11336-020-09730-5.

Bhambri, P., Gupta, O.P., 2012. Development of phylogenetic tree based on Kimura's Method. 2012 2nd IEEE International Conference on Parallel, Distributed and Grid Computing. IEEE, pp. 721–723. https://doi.org/10.1109/PDGC.2012.6449910.

Birk, A.V., Dubovi, E.J., Cohen-Gould, L., Donis, R., Szeto, H.H., 2008. Cytoplasmic vacuolization responses to cytopathic bovine viral diarrhoea virus. Virus Res. 132 (1-2), 76–85. https://doi.org/10.1016/j.virusres.2007.10.017.

Brodersen, B.W., 2014. Bovine viral diarrhea virus infections: manifestations of infection and recent advances in understanding pathogenesis and control. Vet. Pathol. 51 (2), 453–464.

Chai, C., Zhang, F., Diao, Y., Zhou, Y., Li, S., Yin, H., Zhang, J., Niu, R., Wang, W., 2023. Characterization of a highly virulent noncytopathic bovine viral diarrhea virus 2b isolate detected in cattle in Inner Mongolia, China. Transbound. Emerg. Dis. 2023 (1), 3821435. https://doi.org/10.1155/2023/3821435.

Chase, C.C., 2013. The impact of BVDV infection on adaptive immunity. Biologicals 41 (1), 52–60. https://doi.org/10.1016/j.biologicals.2012.09.009.

Darweesh, M.F., Rajput, M.K., Braun, L.J., Ridpath, J.F., Neill, J.D., Chase, C.C., 2015. Characterization of the cytopathic BVDV strains isolated from 13 mucosal disease cases arising in a cattle herd. Virus Res 195, 141–147. https://doi.org/10.1016/j.virusres.2014.09.015.

Deng, M., Ji, S., Fei, W., Raza, S., He, C., Chen, Y., Chen, H., Guo, A., 2015. Prevalence study and genetic typing of bovine viral diarrhea virus (BVDV) in four bovine species in China. PloS One 10 (4), e0121718. https://doi.org/10.1371/journal.pone.0134777.

Deng, M., Chen, N., Guidarini, C., Xu, Z., Zhang, J., Cai, L., Yuan, S., Sun, Y., Metcalfe, L., 2020. Prevalence and genetic diversity of bovine viral diarrhea virus in dairy herds of China. Vet. Microbiol. 242, 108565. https://doi.org/10.1016/j.vetmic.2019.108565.

Galindo-González, J., 2022. Live animal markets: Identifying the origins of emerging infectious diseases. Curr. Opin. Environ. Sci. Health 25, 100310. https://doi.org/10.1016/j.coesh.2021.100310.

Hou, Z., Wang, J., Tan, B., Zhang, S., 2025. A systematic study of bovine viral diarrhoea virus co-infection with other pathogens. Viruses 17 (5), 700. https://doi.org/10.3390/v17050700.

Houe, H., 1995. Epidemiology of bovine viral diarrhea virus. Vet. Clin. N. Am. Food Anim. Pr. 11, 521–547. https://doi.org/10.1016/S0749-0720(15)30465-5.

Lanyon, S.R., Hill, F.I., Reichel, M.P., Brownlie, J., 2014. Bovine viral diarrhoea: pathogenesis and diagnosis. Vet. J. 199 (2), 201–209. https://doi.org/10.1016/j.tvjl.2013.07.024.

Letellier, C., Pardon, B., Van Der Heyden, S., Deprez, P., 2010. Circulation in Belgium of a bovine viral diarrhoea virus type 2 closely related to North American hypervirulent viruses. Vet. Rec. 166 (20), 625. https://doi.org/10.1136/vr.b4792.

Lotfi, M.A., Morteza, K.B., Navidpour, S.C., Javadi, S.D., 2016. Seroepidemiological assay of water buffalo enzootic pneumonia agents (BVDV, BHV-1, bPI3V) in Khuzestan province of Iran. J. Adv. Agric. Technol. 3, 213–216. https://doi.org/10.18178/joaat.3.3.213-216.

Mainar-Jaime, R.C., Berzal-Herranz, B., Arias, P., Rojo-Vázquez, F.A., 2001. Epidemiological pattern and risk factors associated with bovine viral-diarrhoea virus (BVDV) infection in a non-vaccinated dairy-cattle population from the Asturias region of Spain. Prev. Vet. Med. 52 (1), 63–73. https://doi.org/10.1016/S0167-5877(01)00239-2.

Martínez-Burnes, J., Barrios-García, H., Carvajal-de la Fuente, V., Corona-González, B., Obregón Alvarez, D., Romero-Salas, D., 2024. Viral diseases in water buffalo (Bubalus bubalis): new insights and perspectives. Animals 14 (6), 845. https://doi.org/10.3390/ani14060845.

Martucciello, A., De Mia, G.M., Giammarioli, M., De Donato, I., Iovane, G., Galiero, G., 2009. Detection of bovine viral diarrhea virus from three water buffalo fetuses (Bubalus bubalis) in southern Italy. J. Vet. Diagn. Invest 21, 137–140. https://doi.org/10.1177/1040638709021001.

Maurer, K., Krey, T., Moennig, V., Thiel, H.J., Rümenapf, T., 2004. CD46 is a cellular receptor for bovine viral diarrhea virus. J. Virol. 78 (4), 1792–1799. https://doi.org/10.1128/jvi.78.4.1792-1799.2004.

Pecora, A., Pérez Aguirreburualde, M.S., Malacari, D.A., Zabal, O., Sala, J.M., Konrad, J. L., Caspe, S.G., Bauermann, F., Ridpath, J., Dus Santos, M.J., 2017. Serologic evidence of HoBi-like virus circulation in Argentinean water buffalo. J. Vet. Diagn. Investig. 29 (6), 926–929. https://doi.org/10.1177/1040638717720246.

Peddireddi, L., Foster, K.A., Poulsen, E.G., An, B., Hoang, Q.H., O'Connell, C., Anderson, J.W., Thomson, D.U., Hanzlicek, G.A., Bai, J., Hesse, R.A., 2018. Molecular detection and characterization of transient bovine viral diarrhea virus (BVDV) infections in cattle commingled with ten BVDV persistently infected cattle. J. Vet. Diagn. Investig. 30 (3), 413–422. https://doi.org/10.1177/1040638717753962.

Postler, T.S., Beer, M., Blitvich, B.J., Bukh, J., De Lamballerie, X., Drexler, J.F., Imrie, A., Kapoor, A., Karganova, G.G., Lemey, P., Lohmann, V., 2023. Renaming of the genus Flavivirus to Orthoflavivirus and extension of binomial species names within the family Flaviviridae. Arch. Virol. 168 (9), 224. https://doi.org/10.1007/s00705-023-05835-1.

Rana, E.A., Prodhan, M.A., Aleri, J.W., Akter, S.H., Annandale, H., Abraham, S., Sarker, S., Gogoi-Tiwari, J., Uddin, J.M., 2025a. A critical review of bovine viral diarrhea virus: spotlights on host plasticity and potential spillover events. Viruses 17 (9), 1221. https://doi.org/10.3390/v17091221.

Rana, E.A., Gogoi-Tiwari, J., Aleri, J., Prodhan, M.A., Akter, S.H., Annandale, H., Sarker, S., Abraham, S., Uddin, J.M., 2025b. Molecular epidemiology and control strategies for BVDV: a global systematic review from 2000 to 2025. Vet. Med. Int. 2025 (1), 6732453. https://doi.org/10.1155/vmi/6732453.

Rana, E.A., Islam, M.S., Hossain, B., Ahad, A., Hampson, D.J., Abraham, S., Sarker, S., Gogoi-Tiwari, J., Uddin, J.M., 2026. Seroprevalence, molecular characterization, biotyping, and associated risk factors of bovine viral diarrhea in dairy cattle in Bangladesh. Res. Vet. Sci., 106065 https://doi.org/10.1016/j.rvsc.2026.106065.

Ridpath, J.F., 2010. Bovine viral diarrhea virus: global status. Vet. Clin. N. Am. Food Anim. Pr. 26, 105–121. https://doi.org/10.1016/j.cvfa.2009.10.007.

Ridpath, J.F., Neill, J.D., Frey, M., Landgraf, J.G., 2000. Phylogenetic, antigenic and clinical characterization of type 2 BVDV from North America. Vet. Microbiol. 77 (1-2), 145–155. https://doi.org/10.1016/S0378-1135(00)00271-6.

Ridpath, J.F., Bendfeldt, S., Neill, J.D., Liebler-Tenorio, E., 2006. Lymphocytopathogenic activity in vitro correlates with high virulence in vivo for BVDV type 2 strains: criteria for a third biotype of BVDV. Virus Res 118, 62–69. https://doi.org/10.1016/j.virusres.2005.11.014.

Riley, J.M., Peel, D.S., Raper, K.C., Hurt, C., 2019. Invited review: economic consequences of beef cow-calf disease mismanagement: bovine viral diarrhea virus. Appl. Anim. Sci. 35 (6), 606–614. https://doi.org/10.15232/aas.2019-01861.

Rivas, J., Hasanaj, A., Deblon, C., Gisbert, P., Garigliany, M.M., 2022. Genetic diversity of bovine viral diarrhea virus in cattle in France between 2018 and 2020. Front. Vet. Sci. 9, 1028866. https://doi.org/10.3389/fvets.2022.1028866.

Rothman, K.J., Greenland, S., Lash, T.L., 2008. Modern epidemiology (Vol. 3). Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins. https://doi.org/10.1016/j.annemergmed.2008.06.461.

Sajeeb, M.S.M., Alam, M.S., Islam, M.N., Islam, M.M., Adhikari, B.J., Islam, S., Rahman, M.S., Rahman, A.A., 2025. Prevalence and Risk Factors of Bovine Viral Diarrhea Virus Antibodies in Dairy Herds of Bangladesh. Vet. Sci. 12 (8), 739. https://doi.org/10.3390/vetsci12080739.

Scharnböck, B., Roch, F.F., Richter, V., Funke, C., Firth, C.L., Obritzhauser, W., Baumgartner, W., Käsbohrer, A., Pinior, B., 2018. A meta-analysis of bovine viral diarrhoea virus (BVDV) prevalences in the global cattle population. Sci. Rep. 8 (1), 14420. https://doi.org/10.1038/s41598-018-32831-2.

Selvaraj, J., Manohar, B.M., Balachandran, C., Mishra, N., Pradhan, H.K., 2007. Seroprevalence of bovine viral diarrhoea in buffaloes at Chennai. Indian J. Vet. Pathol. 2007 31, 180.

Tautz, N., Tews, B.A., Meyers, G., 2015. 2015. The molecular biology of pestiviruses. Adv. Virus Res 93, 47–160. https://doi.org/10.1016/bs.aivir.2015.03.002.

Tesfaye, M.A., Sisay Tessema, T., Paeshuyse, J., 2025. Host immune response to bovine viral diarrhea virus (BVDV): insights and strategies for effective vaccine design. Vaccines 13 (5), 456. https://doi.org/10.3390/vaccines13050456.

Thrusfield, M., 2018. Veterinary Epidemiology, fourth ed. John Wiley & Sons, Hoboken, NJ. https://doi.org/10.1002/9781118280249.

Uddin, M.A., Ahasan, A.L., Islam, K., Islam, M.Z., Mahmood, A., Islam, A., Islam, K.M.F., Ahad, A., 2017. Seroprevalence of bovine viral diarrhea virus in crossbred dairy cattle in Bangladesh. Vet. World 10 (8), 906. https://doi.org/10.14202/vetworld.2017.906-913.

Walker, P.J., Siddell, S.G., Lefkowitz, E.J., Mushegian, A.R., Adriaenssens, E.M., Alfenas-Zerbini, P., Dempsey, D.M., Dutilh, B.E., García, M.L., Curtis Hendrickson, R., Junglen, S., 2022. Recent changes to virus taxonomy ratified by the International Committee on Taxonomy of Viruses (2022). Arch. Virol. 167 (11), 2429–2440. https://doi.org/10.1007/s00705-022-05516-5.

World Organisation for Animal Health (WOAH). Bovine viral diarrhoea. Terrestrial Animal Health Code, Diseases, infections and infestations listed by WOAH. 31st edition 2023. ⟨https://www.woah.org/en/disease/bovine-viral-diarrhoea/⟩.

Xiao, Y., Liu, Y., Chi, T., Jiang, W., He, T., Xu, L., Dong, Q., Chen, R.Q., An, Z., Sun, X., Sheng, J., 2025. Prevalence and genetic characterization of bovine viral diarrhea

virus in dairy cattle in northern China. BMC Vet. Res. 21 (1), 250. https://doi.org/10.1186/s12917-025-04491-8.
Yeşilbağ, K., Alpay, G., Becher, P., 2017. Variability and global distribution of subgenotypes of bovine viral diarrhea virus. Viruses 9 (6), 128. https://doi.org/10.3390/v9060128.
Zhang, K., Zhang, J., Qiu, Z., Zhang, K., Liang, F., Zhou, Q., Wang, L., Li, J., 2022. Prevalence characteristic of BVDV in some large scale dairy farms in Western China. Front. Vet. Sci. 9, 961337. https://doi.org/10.3389/fvets.2022.961337.