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Seroprevalence, molecular characterization, biotyping, and associated risk factors of bovine viral diarrhea in dairy cattle in Bangladesh

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ABSTRACT

Bovine viral diarrhea virus (BVDV) is globally endemic, with the ability to establish persistent infection (PI) being central to its complex epidemiology. Currently the genetic variability of BVDV in Bangladesh remains poorly understood. This study involved a survey in commercial dairy herds in the south-eastern part of Bangladesh in 2024/2025. A total of 373 blood samples were collected from cattle in 24 dairy herds. Serum and buffy coat samples were analyzed using antibody-ELISA and RT-qPCR targeting the 5′-UTR region, followed by sequencing. The MDBK cell line was used for virus isolation and biotyping. Herd and animal-level seroprevalences were 83.3% and 15.3%, respectively, while the corresponding viremic rates were 79.2% and 11.0%. Analysis of 41 sequences identified nine distinct BVDV-1 subgenotypes (1a, 1b, 1c, 1d, 1e, 1 k, 1p, 1o, and 1v), with BVDV-1b (41.5%) and BVDV-2a (14.6%) predominating. Additionally, five HoBiPeV-a pestiviruses were detected. Among antigen-positive cattle, 38 (92.68%) were identified as transiently infected and 3 (7.3%) were confirmed as PI. Six (14.6%) and 27 (65.9%) were identified as cytopathic and non-cytopathic biotypes, respectively. Risk factors for BVDV seropositivity included: female sex (OR: 3.0), clinical disease in the past three months (OR: 2.4), crowding (OR: 2.9), and lack of dedicated clothing for farm workers (OR: 5.7). Active infection was associated with calves (OR: 6.2), heifers (OR: 2.3), stunted growth (OR: 3.0), technician-performed artificial insemination (OR: 10.4), and frequent neighboring farm visits (OR: 3.1). This study has provided data crucial for formulating prevention and control strategies against BVDV to safeguard the Bangladeshi dairy industry.

1. Introduction

Bovine viral diarrhea virus (BVDV) is a positive-sense single-stranded RNA (+ssRNA) virus belonging to the family *Flaviviridae* and genus *Pestivirus* (ICTV, 2020). Following its first report in 1946, BVDV has become globally endemic, causing widespread infections and economic losses in bovine herds (Reichel et al., 2018). Persistent infections (PI) may occur, with the infection rate estimated to be 60% to 80% in cattle populations in endemic regions (Ridpath, 2010). BVDV exhibits extensive genetic diversity, broad host tropism, and the ability to establish both acute and persistent infections (Deng et al., 2020; Rana et al., 2025). Infected cattle may present with diarrhea, mucosal erosion and necrosis, respiratory distress, fetal deformities, abortion, and significant leukopenia with immunosuppression, leading to increased morbidity and mortality (Peddireddi et al., 2018; Ridpath, 2010).

Based on genomic and antigenic variation, particularly in the 5′ UTR conserved regions, envelope glycoprotein ($E^{rns \text{ and } E2}$), and N-terminal autoprotease (N^{pro}), BVDV is classified into three major species, including BVDV-1 (*Pestivirus bovis*), BVDV-2 (*Pestivirus tauri*), and HoBi-like pestivirus (*Pestivirus brazilense*, previously BVDV-3) (ICTV, 2020). BVDV-1 is the most widely distributed, comprising 25 sub-genotypes (BVDV-1a to BVDV-1× and the Chinese strain ZM-95) (Rivas et al.,

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2022; Deng et al., 2020). BVDV-2 includes five sub-genotypes (BVDV-2a to 2e), while HoBi-like pestivirus (previously referred as BVDV-3) consists of four sub-genotypes (BVDV-3a to 3d) (Rivas et al., 2022; Giammarioli et al., 2015). Moreover, BVDV is classified into cytopathic and non-cytopathic biotypes based on their cyto-pathogenic effects on susceptible cell cultures (Tautz et al., 2015). A third biotype, referred to as lymphocytopathic, has also been detected, which induces cytopathic changes and cell death in cultured lymphocytes (Ridpath et al., 2006). Although the biotype of BVDV does not directly determine its virulence, the cytopathic biotype is more frequently associated with acute clinical disease, whereas the non-cytopathic form is primarily linked to silent or asymptomatic infections (Ridpath et al., 2006). Infection of pregnant cows with non-cytopathic BVDV between 40 and 120 days of gestation may give rise to the birth of PI calves, often exhibiting stunted growth (Darweesh et al., 2015). PI animals discharge abundant active virus throughout their lives without developing antibodies against BVDV, making them the key source of virus transmission (Darweesh et al., 2015). Notably, when a PI animal becomes superinfected with a cytopathic BVDV, it may develop mucosal disease (MD), which is often a fatal infection (Darweesh et al., 2015). On the other hand, animals with sporadic or transient infections (TI) show mild clinical signs, shedding virus for a short period, and mostly leading to lifelong immunity (Houe, 1995). Despite both PI and TI animals generally being viremic (VI), differentiating PI from TI animals requires two sensitive antigen detection tests conducting at least three weeks apart (Scharnböck et al., 2018). Two complementary diagnostic test methods are recommended, one to identify the virus during active infection (antigen test) and another to detect antibodies against BVDV, indicating seroconversion resulting from previous exposure. Nevertheless, BVDV has diverse genetic variants leading to differ in their antigenicity, virulence properties, pathogenicity, and host tropism (Rana et al., 2025). Moreover, the continuous evolution of emerging genotypes, subgenotypes or strains influences transmission dynamics, increases host susceptibility, and expands host range and infection burden. Emerging strains may compromise vaccine efficacy and host immunity. Therefore, identification of the circulating strains is essential for strain-specific vaccine development, mitigating infection burden and developing eradication policy.

Dairy farming in Bangladesh is a critical sector that supports the livelihoods of millions of farmers and directly contributes to the national economy and food security (Datta et al., 2019). Despite the global significance of BVDV, it remains a neglected and poorly studied disease in Bangladesh, with only two studies focusing solely on its seroprevalence (Uddin et al., 2017; Sajeeb et al., 2025). To date, there are no detail reports describing circulating BVDV species, biotypes, genetic diversity, epidemiology, and associated risk factors in dairy herds in Bangladesh. To address this knowledge gap, an extensive seroprevalence and molecular study of BVDV infection in dairy herds in Bangladesh was conducted. This study is the first to provide data on active infection, predominant circulating biotypes, sub-genotype, and associated risk factors in dairy herds in Bangladesh.

2. Materials and methods

2.1. Statement of ethics approval and farm owner consent

Animal and human ethics approvals were obtained from Murdoch University (MU, Perth, Australia) and Chattogram Veterinary and Animal Sciences University (CVASU, Chattogram, Bangladesh) [Permit No. OS3581/24; 2025/048; CVASU/Dir (R&E) EC/2024/763/1]. All procedures strictly adhered to animal welfare guidelines and ethical standards.

2.2. Study areas and farm selection

The study was conducted in the southern-eastern (SE) part of Bangladesh, covering the three districts of Chattogram, Feni, and Cumilla (Fig. 1). These regions have a dense population of livestock, particularly dairy cattle, and supply a significant portion of the country's milk and meat demand (DLS, 2025). At the outset of the study, a list of dairy farms was obtained from the Divisional Livestock Services (DLS) office. Farms were selected based on herd size, including those with at least 10 lactating cows and a minimum of five calves, to ensure representative coverage of the target population. Almost all dairy farms are privately owned, and the majority of cattle are Zebu or crossbreeds of Holstein-Friesian (HF), Sahiwal, Jersey, and Red Sindhi. Mixed farming practices are common, as traditionally farmers often rear buffaloes, sheep, and goats together with cattle on the same premises.

2.3. Study design and sample size estimation

A cross-sectional study was conducted from the period of November 2024 to August 2025. The sample size was calculated based on the previous BVDV seroprevalence of 51.1% (Uddin et al., 2017), following the guidelines of Thrusfield (2018), with a 95% confidence level and 5% desired precision. Based on this calculation, a total of 384 animals were targeted for sampling. Within each farm, individual animals were selected randomly and proportionally to herd size, ensuring adequate coverage of each farm for blood sample collection. According to the Animal Ethics guideline, pregnant cows were excluded to avoid potential stress or risk to the dam and fetus, while non-pregnant lactating cows, heifers, and calves were included to ensure representative sampling across age and production groups. Although 384 animals were calculated for initial sampling, 11 were excluded due to restraining difficulties and insufficient blood collection, ensuring adherence to animal welfare guidelines, with the result that the final sample number was 373.

2.4. Sample collection and processing

In total, 5 mL of blood was aseptically collected from the jugular vein of each restrained animal using a sterile 10 mL syringe with 18-gauge needle (JMI Group, Bangladesh). Each sample was immediately divided into two separate vacutainers tubes (BD Vacutainer®, Bangladesh), one containing a clot activator for serum separation and the other containing an anticoagulant for subsequent analysis. Samples were transported to the laboratory in ice boxes, with the temperature maintained at 4 °C to ensure sample stability. Both vacutainers were centrifuged at 3500 rpm for 5 min (4 °C) to separate the serum (clot activator tube) and the buffy coat (anticoagulant tube). All serum samples were preserved at −20 °C, while the buffy coat samples were stored at −80 °C for further serological and virological analysis.

2.5. Data collection for risk factor analysis

A predesigned validated and structured questionnaire (**Supplementary File-1**) was administered to collect individual animal data, including age, breed, sex, body condition score, previous disease history, production and reproductive records, breeding methods, pregnancy and lactation status, abortion history, and conception failure. Additionally, farm-related information, including herd size, housing system, calf management, animal movement history, routine laboratory tests, vaccination records, farm hygiene, and other biosecurity practices, was gathered to assess various risk factors associated with BVDV infection. An animal was classified as having poor or stunted growth if its body weight or body condition score (BCS) was below the expected standard for its age, breed, and sex, or if visibly poor growth was reported by the farm owner or attending veterinarian. Information on animals, farm management, and biosecurity practices was obtained directly from the respective dairy farm owners during the same visit as sample collection.

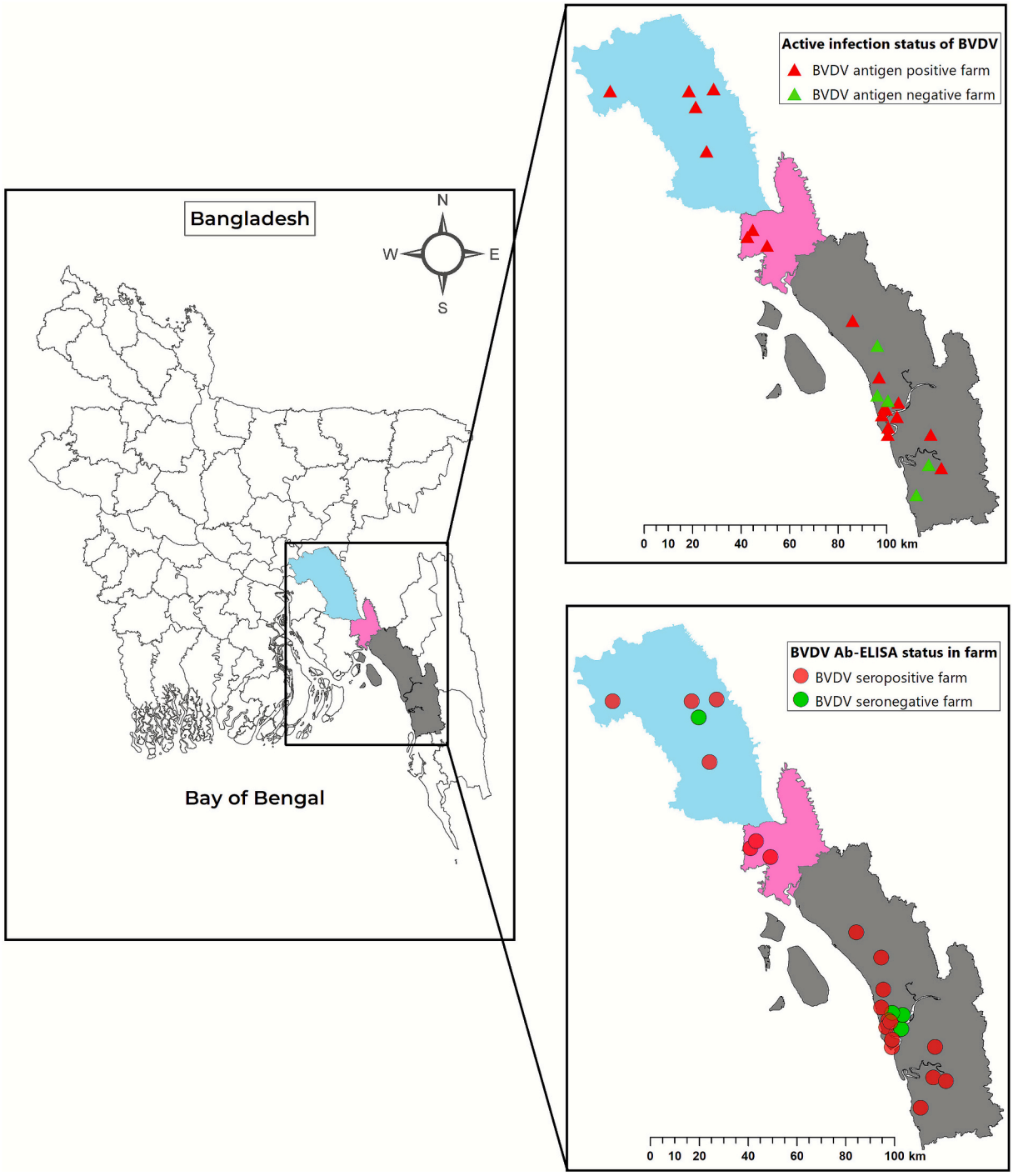

Fig. 1. The Choropleth map showing the geographical study area and the corresponding farm location. This map was generated using ArcGIS version 10.8 software (ESRI, Redlands, CA, USA).

2.6. Serological assay (Ab-ELISA) for detecting previous exposure to BVDV

All serum samples were examined with a competitive commercial enzyme-linked immunosorbent assay (ELISA) kit for the detection of BVDV-specific antibody (cat. no: abx055874, Abbexa Ltd., Cambridge, UK). The assay was performed according to the manufacturer's instructions. In total, 10 µL of serum was diluted to 40 µL of sample diluent buffer to make 50 µL total. After completing the sequential assay protocol, the optical density (OD) of the test plate was measured spectrophotometrically at 450 nm using a microplate reader (BioTek® 800™ microplate Reader, USA). Each run included manufacturer-supplied positive and negative controls, and samples were tested in duplicate. The assay was considered valid when the mean OD of the positive control was $\geq$1.0 and that of the negative control was $\leq$0.2. The cut-off value was calculated as the mean OD of the negative control plus 0.15. Samples with OD values below the cut-off were classified as negative, whereas those equal to or above the cut-off were classified as positive.

2.7. Viral RNA extraction and cDNA synthesis

All seronegative blood samples (buffy coat) were thawed at room

temperature and subjected to total RNA extraction. A total of 200 μL of buffy coat was extracted using a commercial Viral RNAclean Kit (ELK-EP017, ELK Biotechnology, Denver, USA) by following the manufacturer's instructions. A total of 30 μL of RNA was eluted from the purification column, and it's concentration was estimated using a NanoDrop spectrophotometer (Thermo Scientific NanoDrop™ 1000 Spectrophotometer, USA). The extracted RNA was converted into cDNA using the commercial ABScript II cDNA First Strand Synthesis Kit (ABclonal, San Francisco, USA). Immediately after cDNA synthesis, all reaction products were preserved at −20 °C for subsequent molecular analysis.

2.8. RT-qPCR for the detection of BVDV

The RT-qPCR assay was performed by targeting a 293-bp fragment of the BVDV 5′-UTR region, as described by Peddireddi et al., 2018. To conduct the RT-qPCR, 25 μL of PCR reaction mixture was prepared, comprising 12.5 μL of SYBR Green PCR SuperMix (ELK Biotechnology, Denver, USA), 1.0 μL (10 μM) of both forward primer ((BVD-SF: 5′-AGGCTAGCCATGCCCTTAGT-3′) and reverse primer (BVD-SR: 5′-CTCCATGTGCCATGTACAGC-3′), 1.0 μL of passive reference ROX dye, 2 μL of cDNA as a template, and the remaining 7.5 μL of RNase-free water to prepare the final reaction volume. The RT-PCR was performed in an Applied Biosystems® 7500 Fast Thermal Cycler (Thermo Fisher Scientific, Inc., Waltham, MA, U.S.A.) with initial denaturation at 95 °C for 15 min, followed by 40 cycles of 95 °C for 20 s, 58 °C for 30 s, and 72 °C for 45 s. Sequenced-confirmed field isolates and nuclease-free water were used as positive and negative controls, respectively, and all samples were tested in duplicate. The amplified PCR products were assayed and visualized by 1% agarose gel electrophoresis stained with ethidium bromide to confirm that only a single amplicon (293 bp) was generated. A 100 bp DNA ladder was used as a molecular size marker (Supplementary Fig. 1).

2.9. Screening of persistently infected (PI) and transiently infected (TI) cattle

To differentiate between PI and TI calves, animals that initially tested positive for BVDV antigen through RT-qPCR were re-sampled after an interval of 21 days. Fresh blood samples were collected, and RNA was extracted, followed by cDNA synthesis and RT-qPCR, using the same protocol described above. Calves that remained BVDV-positive upon retesting were classified as PI, while those found negative in the second sampling were considered TI (Lanyon et al., 2014).

2.10. Virus isolation, propagation, and bio-typing of BVDV in MDBK cells

All RT-qPCR positive samples were inoculated into Madin-Darby bovine kidney (MDBK) cell culture for propagation and bio-typing of BVDV. BVDV-free MDBK cells at passage 128 were sourced from the collection held at the Veterinary Virology Laboratory at Murdoch University. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Sigma-Aldrich®, Germany) supplemented with BVDV-free 10% fetal bovine serum (Sigma-Aldrich®, Germany) and antibacterials (100 IU/mL penicillin and 100 μg/mL streptomycin) and antifungals (250 μg/mL amphotericin B) at 37 °C in a humidified incubator with a 5% CO_2 atmosphere. The cells were maintained for 5 to 7 days when cell growth reached approximately 90 to 95% confluence.

2.11. BVDV positive sample inoculation on MDBK cell line (first passage)

BVDV-positive whole blood buffy coats (frozen at −80 °C) were thawed, vortexed, and centrifuged at 12000 rpm for 10 min at 4 °C. Each blood supernatant was diluted 1:1 with DMEM, and 500 μL was inoculated into 90% to 95% confluent MDBK cell culture flasks (Peddireddi et al., 2018). Duplicate flasks were prepared for each sample. Prior to infection, the MDBK cells were gently washed with phosphate-buffered saline (PBS) to remove dead cells and residual medium. MDBK cell cultures were incubated with samples in 1 mL of DMEM containing 10% FBS and antimicrobials at 37 °C for 1.5 h to facilitate viral adsorption. The cell culture flasks were gently swirled every 15 min to ensure even distribution of the inoculum across the monolayer. The final volume was adjusted to 10 mL with prepared DMEM. Infected cultures were examined under an inverted microscope at 24-h intervals for evidence of cytopathic effects (CPE) for up to 9 days post-infection (dpi). Uninfected MDBK cells were maintained as negative controls alongside inoculated cultures.

2.12. Serial passages of BVDV in the MDBK cell line

Following the initial inoculation, infected cultures were subjected to three consecutive passages to enhance viral propagation and detection of CPE. In each passage, 1 mL supernatant from the infected cells were transferred to fresh 90% confluent MDBK cells and subsequently monitored for the evidence of cytopathic effects (CPE) at 24-h intervals for 9 dpi. Subsequently, duplicate RT-qPCR was performed using the same primers to confirm the presence of active BVDV replication in every inoculated cell culture flask irrespective of evidence of CPE. A progressive decrease in cycle threshold (Ct) values indicate an increase in viral copy number, reflecting active viral replication (**Supplementary Table 1**). If an infected cell culture displayed characteristic CPE, the isolate was classified as the cytopathic biotype. In contrast, BVDV isolates that replicated in culture without producing visible CPE were identified as the non-cytopathic biotype (Birk et al., 2008).

2.13. Partial sequencing of 5′-UTR and species identification

For the accurate identification of BVDV species and virus subgenotypes the amplified 5′-UTR gene was targeted for sequencing. Following gel electrophoresis in 1% agarose gels the amplified PCR products were submitted for automated bidirectional sequencing (Sanger sequencing) through a third party (BD Genome Bangladesh). Subsequently, all nucleotide sequences were individually checked using the NCBI Nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) program to detect and differentiate the different BVDV species. Finally, the consensus nucleotide sequences of the BVDV isolates were individually submitted to the GenBank database and accession numbers obtained (Table 1).

2.14. Phylogenetic analysis and the detection of circulating sub-genotype

The nucleotide sequences of selected pestivirus species available in GenBank were retrieved and subjected to phylogenetic analysis as described by Deng et al. (2020). The multiple nucleotide sequences of the 5′-UTR (293 bp) fragments were aligned using the ClustalW algorithm in MEGA 11.0 software, with the corresponding regions of BVDV-1, BVDV-2, HoBi-like pestivirus (Table 1), and the reference sequences in GenBank (**S.** Table 2). A total of 41 5′-UTR gene sequences were gained from the field isolates (including 26 BVDV-1, 10 BVDV-1, and 5 HoBi-like pestiviruses). The phylogenetic tree was constructed with the neighbor-joining method using the MEGA 11.0 program (DNAStar Inc., USA). The evolutionary distances and the robustness of the phylogenetic analysis were estimated using the Kimura 2-parameter and bootstrapping methods, respectively. The confidence of the tree branch order was calculated using 1000 replicates.

2.15. Statistical analysis

Collected data were divided into individual animal level and dairy herd-level information. Data were cleaned, organized, and stored in Microsoft Excel spreadsheets (Microsoft Excel, 2010) for further analysis. All laboratory results and associated metadata were exported to STATA software version 18 (StataCorp LLC, College Station, TX, USA)

Table 1
Distribution of Pestivirus species, subgenotypes, and biotypes isolated from different dairy farms, with corresponding GenBank accession numbers for 5′-UTR sequences.

Farm id	Farm name	BVDV antigen status in RT-qPCR	Animal category	Identified species	Identified sub-genotype	BVDV Biotype	Infection status	Accession number
F-01	MB dairy	Positive	Heifer-1	BVDV-1	1b	Ncp	TI	PV453976
			Cow-42	BVDV-1	1p	Fail to propagate	TI	PV874948
F-02	Mohiuddin dairy	Positive	Bull calf-3	BVDV-1	1c	Ncp	TI	PV453977
			Female calf-126	BVDV-1	1b	Ncp	PI	PV817801
F-03	SM dairy	Positive	Cow-47	BVDV-2	2a	Ncp	TI	PV874959
F-04	Molla dairy	Positive	Female calf-464	BVDV-1	1d	Ncp	TI	PV569116
F-05	Anjuman dairy	Positive	Bull calf-5	BVDV-1	1b	Cp	TI	PV569114
			Cow-602	HoBiPeV	HoBiPeV a	Fail to propagate	TI	PV569117
F-06	Red meat and dairy	Positive	Female calf-8	BVDV-1	1b	Ncp	TI	PV580257
			Cow-10	BVDV-1	1b	Ncp	TI	PV874949
F-07	Ahmed Agro	Positive	Female calf-2	BVDV-1	1e	Ncp	TI	PV874950
			Cow-Ghora	HoBiPeV	HoBiPeV a	Fail to propagate	TI	PV874963
F-08	Ayesha dairy	Positive	Bull calf-15	BVDV-1	1b	Ncp	TI	PV569115
			Bull calf-02	BVDV-2	2a	Cp	TI	PV580258
			Female calf-15	BVDV-1	1a	Ncp	TI	PV345782
			Cow-10	BVDV-2	2c	Fail to propagate	TI	PV580259
			Female calf-9	BVDV-1	1b	Ncp	TI	PV874951
F-09	Shah Amanat Dairy	Positive	Bull calf-7	BVDV-1	1 k	Ncp	PI	PV569118
F-10	Umme Aman agro	Negative	–	–				–
F-11	Abdullah dairy	Positive	Female calf-7	BVDV-1	1b	Ncp	TI	PV874953
			Heifer-1	BVDV-1	1v	Fail to propagate	TI	PV874952
F-12	J.H agro	Positive	Bull calf-24	BVDV-2	Unclassified	Cp	TI	PV437272
			Female calf-196	BVDV-2	Unclassified	Ncp	TI	PV453975
			Cow 22.2	HoBiPeV	HoBiPeV a	Fail to propagate	TI	PV874964
F-13	Nur nobi dairy	Positive	Bull calf-41	BVDV-1	1c	Ncp	TI	PV368849
F-14	Joynal Abedin dairy	Positive	Female calf-3	HoBiPeV	HoBiPeV a	Fail to propagate	TI	PV874965
			Female calf-12	BVDV-1	1b	Ncp	TI	PV297898
			Bull calf-7	HoBiPeV	HoBiPeV a	Ncp	TI	PV476731
F-15	Hasan Agro	Positive	Cow-41	BVDV-2	2a	Ncp	TI	PV874960
			Cow-44	BVDV-1	1b	Cp	TI	PV874955
			Cow-60	BVDV-1	1o	Ncp	TI	PV874954
F-16	Mukta dairy	Positive	Female calf-49	BVDV-1	1b	Ncp	TI	PV476732
F-17	Monoroma Dairy	Positive	Female calf-1	BVDV-1	1b	Ncp	TI	PV476733
			Cow-Nagin	BVDV-1	1b	Ncp	TI	PV874956
F-18	Akhondo dairy	Negative	–	–	–	–	–	–
F-19	Homeland dairy	Positive	Female calf-17	BVDV-1	1b	Ncp	PI	PV817805
			Female calf-8	BVDV-2	Unclassified	Fail to propagate	TI	PV874961
			Bull calf-13	BVDV-2	2a	Cp	TI	PV817803
			Female calf-9	BVDV-2	2a	Cp	TI	PV817804
			Bull calf-29	BVDV-1	1b	Ncp	TI	PV874957
			Heifer-213	BVDV-2	2a	Ncp	TI	PV874962
F-20	Johura Dairy farm	Negative	–	–	–	–	–	–
F-21	Sahanaj Dairy	Positive	Female calf-2	BVDV-1	1b	Ncp	TI	PV817802
F-22	Joynab Dairy farm	Negative	–	–	–	–	–	–
F-23	Ashfaq dairy farm	Positive	Cow-609	BVDV-1	1b	Ncp	TI	PV874958
F-24	CVASU dairy farm	Negative	–	–	–	–	–	–

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.

for analysis. Any dairy farm with at least one seropositive or actively BVDV-infected animal was categorized as a positive herd. Descriptive statistics were used to estimate the seroprevalence of BVDV antibodies and the prevalence of active BVDV infection at both the herd and animal levels. Herd-level prevalence was calculated as the proportion of herds with at least one positive animal relative to the total number of herds sampled, whereas animal-level prevalence was determined as the proportion of cattle testing positive for BVDV antibodies or antigen relative to the total number of animals examined.

Data on both animal demographic factors and herd-related risk

Table 2
Seroprevalence and active infection rates of BVDV at the farm and individual animal levels across different dairy farms.

Total number of dairy farms sampled	Farm positive for Ab-BVDV (%, 95% CI)	Farm positive for active BVDV infection (%, 95% CI)	Total number of cattle	BVDV antibody positive cattle (%, 95% CI)	PCR confirmed BVDV (%, 95% CI)	Different pestivirus species	Prevalence of pestivirus species (%, 95% CI)
24	20 (83.3; 62.62–95.26)	19 (79.2; 57.85–92.87)	373	57 (15.3; 11.78–19.34)	41 (11.0; 8.00–14.62)	BVDV-1 BVDV-2 HoBiPeV	26 (7.0; 4.60–10.05) 10 (2.7; 1.29–4.88) 5 (1.3; 0.44–3.10)

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.

factors were analyzed separately, targeting two outcomes: the presence of BVDV antibodies and active BVDV infection. Initially, chi-square tests were performed for all variables to identify potential associations with the targeted outcomes. Variables with a *p*-value ≤0.05 were considered significantly associated with the presence of BVDV antibodies (indicating previous infection) and active infection (viremic status). Subsequently, univariable logistic regression analysis was performed for each predictor variable to assess individual association with the two outcomes mentioned. After that, a multivariable logistic regression model was applied, including variables that showed a p-value ≤0.20 in the univariable analysis. A stepwise forward selection approach was followed to construct the final model, and the degree of association between independent variables and BVDV seropositivity and active infection was computed by means of an odds ratio (OR). Finally, variables with a p-value ≤0.05 were retained in the final model and considered significantly associated with the outcomes. Potential confounding effects were assessed by monitoring changes in the odds ratio (OR); a variable was considered a confounder if the adjusted OR changed by 25% (> 0.25) or more. Additionally, all predictor variables were assessed for multicollinearity using Goodman and Kruskal's Gamma statistic, as described by Barbiero and Hitaj (2020). In all statistical analyses, the 95% confidence interval (CI) was computed following the modified Wald method. A choropleth dot map representing the study locations, along with the corresponding seropositive and active BVDV infection positive and negative farms, was constructed using ArcGIS version 10.8 (ESRI, Redlands, CA, USA). Additionally, a heat map was generated using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Sample and animal category

A total of 373 blood samples were collected from different categories of animals, including dairy cows ($n = 195$), heifers ($n = 13$), and calves ($n = 165$), across 24 commercial dairy herds.

3.2. Sero-prevalence of BVDV at the herd and animal level

Among the 24 dairy herds, 20 (83.3%; 95% CI: 62.62–95.26) were found seropositive for BVDV (Table 2, Figs. 1 and 2). At the animal level, out of 373 cattle, 57 (15.3%; 95% CI: 11.78–19.34) tested positive for BVDV antibodies. Of these, dairy cows showed the highest seropositivity (18.5%; 95% CI: 13.28–24.63), compared to heifers (15.4%; 95% CI: 1.92–45.44) and calves (11.5%; 95% CI: 7.07–17.40) (Table 3).

3.3. Prevalence of active BVDV infection at the herd and animal level

In terms of active infection or viremia, BVDV was detected in 19 herds (79.8%; 95% CI: 57.85–92.87), indicating ongoing viral circulation (Table 2, Figs. 1 and 2). A total of 41 cattle (11.0%; 95% CI: 8.00–14.62) were found to be viremic. Among them, 25 calves (15.2%;

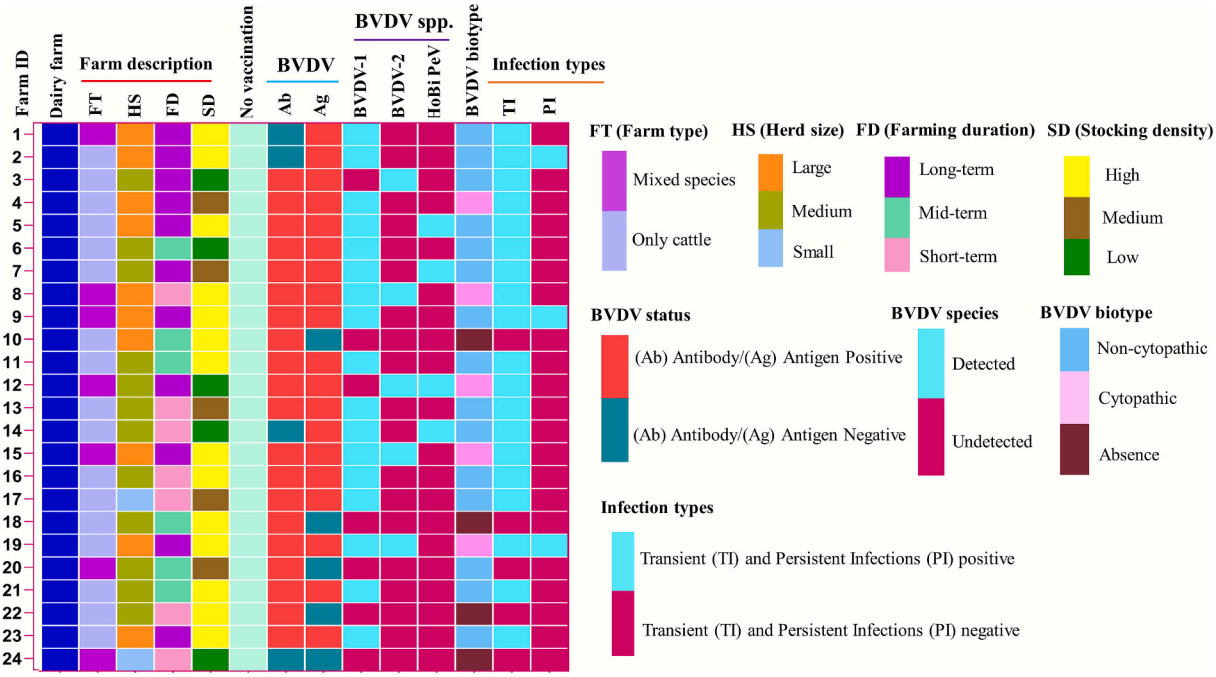

Fig. 2. The heat map illustrates the demographic profiles of different dairy farms, indicating BVDV seropositivity and active infections status. It also depicts the circulating pestivirus species, BVDV biotypes, and the distribution of persistent (PI) and transient (TI) infections among different farms.

Table 3
Seroprevalence and active infection status of BVDV among calves, heifers, and cows.

Animal category	No of animal	BVDV antibody positive (%; 95% CI)	BVDV active infection (%; 95% CI)	BVDV species	Prevalence of BVDV species (%; 95% CI)
Calves	165	19 (11.5; 7.07–17.40)	25 (15.2; 10.05–21.54)	BVDV-1	17 (10.3; 6.12–15.98)
				BVDV-2	6 (3.7; 1.35–7.75)
				HoBiPeV	2 (1.2; 0.15–4.31)
Heifers	13	2 (15.4; 1.92–45.44)	4 (30.8; 9.09–61.43)	BVDV-1	3 (23.1; 9.09–61.42)
				BVDV-2	1 (7.7; 0.19–36.03)
				HoBiPeV	–
Cows	195	36 (18.5; 13.28–24.63)	12 (6.2; 3.21–10.50)	BVDV-1	6 (3.1; 1.14–6.58)
				BVDV-2	3 (1.5; 0.32–4.43)
				HoBiPeV	3 (1.5; 0.32–4.43)
Total	373	57 (15.3; 11.78–19.34)	41 (11.0; 8.00–14.62)	–	–

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.

95% CI: 10.05–21.54) and 12 dairy cows (6.2%; 95% CI: 3.21–10.50) tested positive for active BVDV infection (Table 3).

3.4. Herd and animal level prevalence of different pestivirus species

A total of 41 amplicons derived from RT-qPCR were successfully sequenced, enabling the detection of different species of pestiviruses. Within the 24 dairy herds, 17 (70.8%; 95% CI: 48.91–87.38) were positive for BVDV-1, five (20.8%; 95% CI: 7.13–42.15) for BVDV-2, and four (16.7%; 95% CI: 4.74–37.38) for HoBi-like pestivirus (HoBiPeV) (Table 1**,** Fig. 2). Notably, multiple viral infections were detected across seven (29.2%; 95% CI: 12.62–51.09) distinct farms. Specifically, BVDV-1 and BVDV-2 co-circulated in three farms, BVDV-1 and HoBiPeV in another three farms, and one farm showed concurrent infection with BVDV-2 and HoBiPeV (Fig. 2).

Moreover, among the 41 PCR-confirmed viremic cattle (out of 373), 26/373 (7.0%; 95% CI: 4.60–10.05) were identified as BVDV-1, while 10/373 (2.7%; 95% CI: 1.29–4.88) and 5/373 (1.3%; 95% CI: 0.44–3.10) were confirmed as BVDV-2 and HoBiPeV, respectively **(**Table 2**,** Fig. 2). Among the animal groups, BVDV-1 was detected in 17 out of 165 calves (10.3%; 95% CI: 6.12–15.98), 3 out of 13 heifers (23.1%; 95% CI: 9.09–61.42), and 6 out of 195 dairy cows (3.1%; 95% CI: 1.14–6.58). Additionally, 6/165 (3.6%; 95% CI: 1.35–7.75) calves and 3/195 (1.5%; 95% CI: 0.32–4.43) dairy cows were found to carry BVDV-2. Notably, a lower prevalence was observed for HoBiPeV, with 3/195 (1.5%; 95% CI: 0.32–4.43) dairy cows and 2/165 (1.2%; 95% CI: 0.15–4.31) calves being infected **(**Table 3**,** Fig. 2**)**.

3.5. Detection rates of TI and PI animals

Among the 41 viremic cattle, only three calves (7.3%; 95% CI: 1.54–19.92) were identified as PI, while the remaining 38 cattle (92.7%; 95% CI: 80.08–98.46) were classified as TI (Table 1**;** Fig. 2). Notably, out of the 19 BVDV-positive herds, only 3 herds (15.8%; 95% CI: 3.38–39.58) were found to contain PI animals (Table 1**;** Fig. 2).

3.6. Biotyping of BVDV based on cytopathic effects in MDBK cells

Among the 41 confirmed BVDV isolates, six (14.6%; 95% CI: 5.57–29.17) were identified as having a cytopathic biotype (Figs. 2 and 3), while 27 (65.9%; 95% CI: 49.41–79.92) were classified as non-cytopathic (Table 1; Fig. 2). Notably, eight isolates failed to propagate in the MDBK cell line. Among the CP biotypes, two were identified as BVDV-1 and the remaining four as BVDV-2 (Table 1). BVDV-infected MDBK cells exhibited characteristic cytopathic effects, including cytoplasmic vacuolation, with large, encapsulated vacuoles fusing to form even larger vacuoles, granulation of the cytoplasm, rounding up, detachment, and eventual cell death (Fig. 3). In contrast, all the Ncp biotypes infected MDBK cells without inducing any visible morphological alterations.

3.7. Phylogenetic analysis and detection of BVDV sub-genotypes

To explore the subgenotypes or genetic diversity within the circulating BVDV species, all submitted sequences (**S.** Table 2) were included in the phylogenetic analyses with the sequence of the reference strain (**S.** Table 2). The phylogenetic tree grouped the BVDV-1 field isolates into nine distinct subgenotypes: 1a, 1b, 1c, 1d, 1e, 1 k, 1p, 1o, and 1v (Fig. 4**,** Table 1). Among these, BVDV-1b was predominant, with 17 isolates (41.5%) forming the largest cluster, while two isolates were classified as 1c, representing 4.9% of the total. The remaining subgenotypes 1a, 1d, 1e, 1 k, 1p, 1o, and 1v were each identified only once, accounting for 2.4% of the total (Fig. 4**,** Table 1).

Further analysis of the BVDV-2 sequences revealed three distinct clusters of wild isolates (Fig. 5). Among these, six sequences clustered within the BVDV-2a sub-genotype, accounting for 14.6% of the total isolates, while a single sequence was classified as BVDV-2c. Notably, three (7.3%) BVDV-2 sequences grouped into unclassified subgenotypes. All five (12.2%) HoBi-like pestivirus sequences were classified within the same sub-genotype, HoBiPeV-a (previously designated BVDV-3a) (Fig. 6).

3.8. Risk factors associated with antibody presence and active BVDV infection at the animal level in cattle herds

Eight different variables relating to animal demographic factors were analyzed using chi-square and univariable logistic regression models to assess the seropositivity of animals **(S.** Table 3**).** Of these, six met the eligibility criterion ($p < 0.20$) for inclusion in the subsequent multivariable logistic regression analysis to assess the presence of previous BVDV exposure (antibody presence). In the multivariable model, animals with a history of clinical disease in the past three months were 2.4 times more likely to be BVDV seropositive (95% CI: 1.35–4.28, $p < 0.00$) compared to animals without such a history. Furthermore, the odds of a BVDV seropositive result were 3.0 times higher in female animals (95% CI: 1.04–8.73, $p < 0.04$) than in males (Table 4).

Moreover, in the case of active infection (viremic status), four of the eight variables related to animal demography were initially identified as potential risk factors ($p \leq 0.20$) associated with active BVDV infection in cattle herds **(S.** Table 4**).** In the multivariable model, calves and heifers were found to have 6.2 (95% CI: 1.61–23.45, $p = 0.00$) times and 2.3 (95% CI: 1.04–4.90, $p = 0.03$) times higher risk, respectively. Furthermore, "poor or stunted growth rate" (OR = 3.0, 95% CI: 0.99–3.95, $p = 0.05$) demonstrated a notable association with active BVDV infection (Table 4).

3.9. Risk factors associated with antibody presence and active infection in relation to cattle herd factors and management practices

A total of 55 variables related to herd factors and management practices were initially assessed for BVDV seropositivity in cattle herds, of which five were omitted due to completely negative responses from their co-variables. The remaining variables were analyzed using chi-square and univariable logistic regression **(S.** Table 5**).** Among these, 22 potential risk factors ($p \leq 0.20$) were identified as being associated

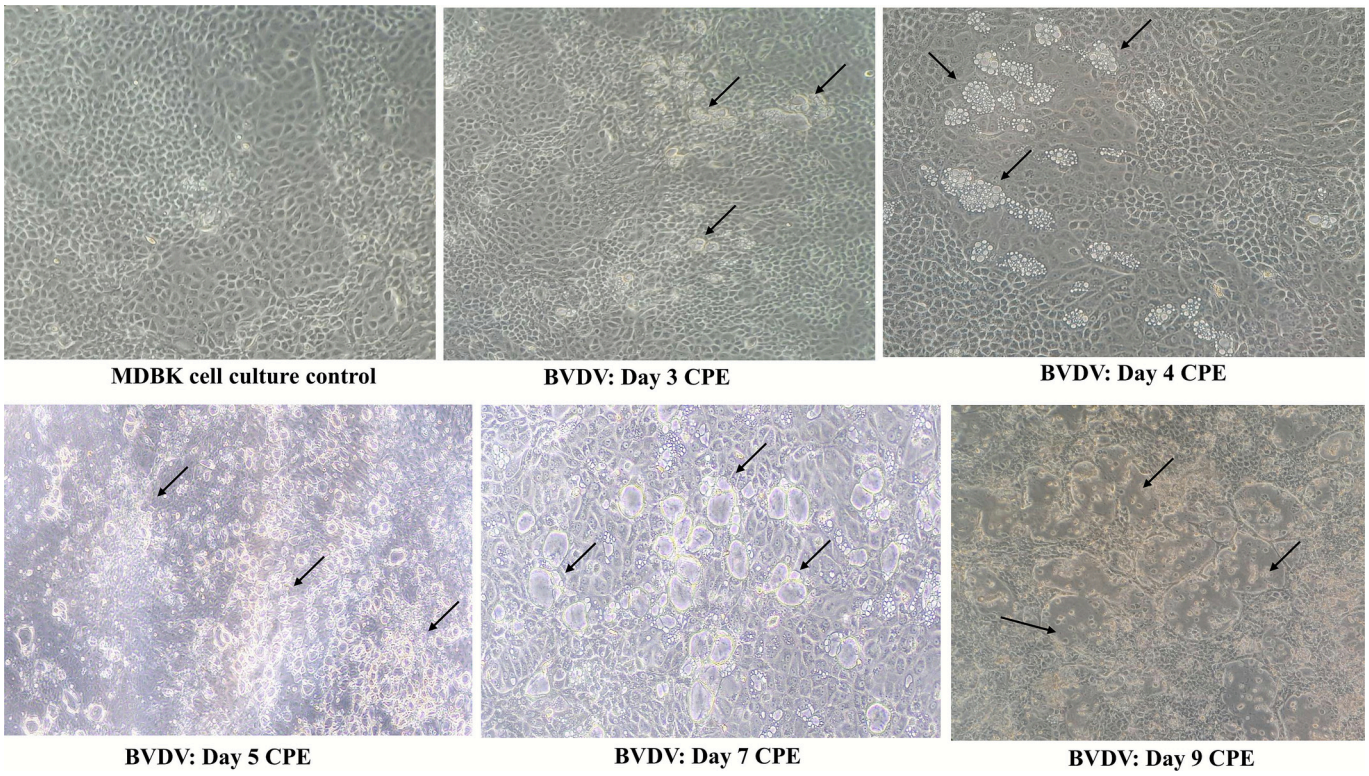

Fig. 3. The CPE observed in MDBK cell monolayers following inoculation with an RT-qPCR–positive BVDV blood sample showed a sequential progression of cytopathic alterations. At day 3 post-infection (hpi), MDBK monolayers exhibited initial cytoplasmic vacuolation. By day 4, vacuolation became more prominent with occasional cell fusion. On days 5 and 7 hpi, extensive vacuolation, cell fusion, and widespread infection of the MDBK monolayer were evident. By day 9 hpi, the monolayer was largely destroyed, characterized by cell shrinkage, rounding, sheet-like detachment, and floating death cells. Arrows indicate representative vacuolized cells and indicating cytopathic biotype of BVDV.

with previous BVDV exposure (seropositivity) **(S.** Table 5**)**. In the multivariable logistic regression analysis, only the two factors "crowded stocking density" (OR: 2.9, 95% CI: 1.50–5.74, $p < 0.00$) and "no use of dedicated shoes and clothing for farm workers" (OR: 5.7, 95% CI: 1.17–27.76, $p < 0.03$) **(**Table 5**)**, were significantly associated with the seropositivity.

For active infection (viremia), 11 variables were identified as potential risk factors ($p \leq 0.20$) in chi-square and univariable logistic regression analyses (**S. Table 6**). In the multivariable logistic regression model, artificial insemination (AI) performed by an "AI technician" was associated with a 10.4 times higher risk (95% CI: 1.90–57.14, $p < 0.00$) compared with AI performed by the dairy farm owner or veterinarian. Additionally, "daily visiting of neighboring farms" by the farm owner or worker was found to be a significant risk factor (OR: 3.1, 95% CI: 1.60–16.31, $p < 0.01$) (Table 5**)**.

4. Discussion

BVDV infection has become a global concern due to its endemic nature and considerable economic impact. It is classified as a notifiable animal disease by the World Organisation for Animal Health (WOAH) and attracts significant attention from livestock producers, researchers, and policymakers. This study investigated the prevalence of BVDV seropositivity and active infection status in cattle herds in Bangladesh. To the best of our knowledge, it is the first study to provide detailed characterization of circulating BVDV species, biotypes, sub-genotypes, and the nature of infection in terms of TI and PI in Bangladesh. Despite being one of the predominant viruses affecting cattle herds, there is no BVDV vaccination program or official control policy in Bangladesh. Understanding the current infection status, viral characteristics, genetic diversity of circulating strains, and associated risk factors in dairy herds is essential for identifying sources and routes of transmission, as well as for designing effective surveillance systems, strategic control measures, and eradication policies.

The study revealed a herd-level seroprevalence of 83.3% and an animal-level seroprevalence of 15.3%, indicating that BVDV is likely endemic among dairy herds in Bangladesh. As BVDV vaccination is not yet administered in Bangladesh, the detection of BVDV antibodies serves as evidence of natural exposure to the virus. However, the herd-level seroprevalence of BVDV varies considerably across geographical locations. Exceptionally high herd-level seroprevalence (58% -100%) of BVDV has been documented in several Asian countries including China, Iran, Kazakhstan, South Korea and Thailand (Scharnböck et al., 2018; Deng et al., 2020; Su et al., 2023). The high herd-level seropositivity observed in this study reflects the widespread distribution of BVDV and represents its significant impact on cattle populations. Similarly, at the animal level, BVDV seroprevalence has been reported to vary widely across regions, ranging from 7.8% to 93.4% in Asia, Europe, North and South America, and Africa (Uddin et al., 2017; Scharnböck et al., 2018; Su et al., 2023). Variation in BVDV seroprevalence may reflect differences in animal demographic factors, immune status, and prior exposure, as well as farm-level factors such as management, poor biosecurity, herd size, and animal movement (Houe, 1995). Moreover, the diagnostic approach, sampling strategy, and current endemic status can also influence apparent prevalence, indicating the need to consider both herd- and animal-level data in epidemiological assessments and control planning.

In this study, RT-qPCR analysis revealed active BVDV infection (viremia) in 79.2% of herds and approximately 11% of individual animals, indicating notable ongoing viral circulation within cattle populations. The herd-level active infection rate observed is consistent with reports from several countries, including China, Iran, and Turkey, where

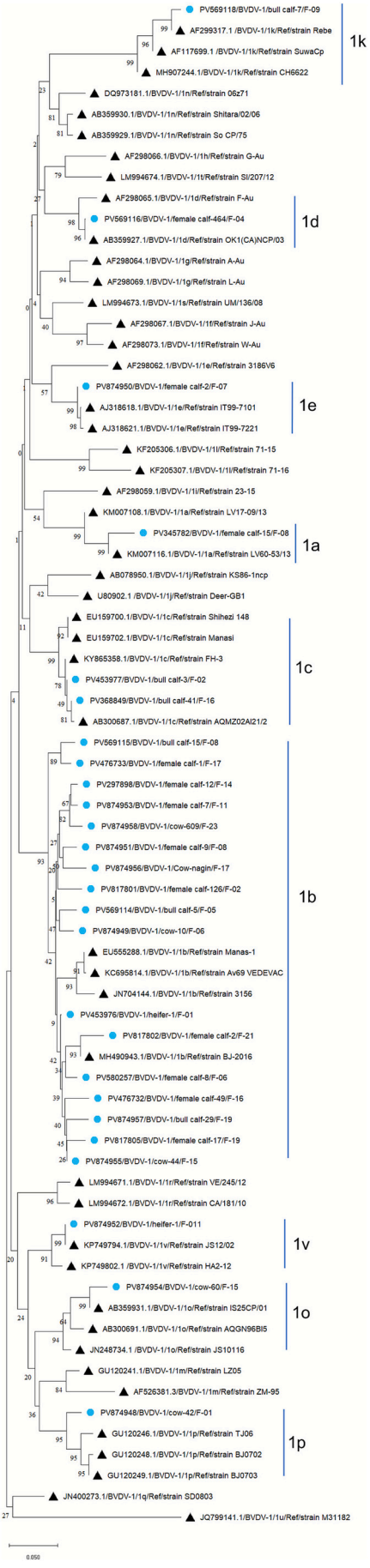

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Fig. 4. Phylogenetic analysis was performed based on the 5′-UTR gene (293 bp) of BVDV-1 (*P. bovis*). The phylogenetic tree was constructed using nucleotide sequences obtained from field samples, along with reference sequences of representative subgenotypes retrieved from the GenBank database (**Supplementary table 1**). Cyan solid circles indicate the subgenotypes identified in this study from different cattle, whereas black triangles represent reference subgenotypes. The tree was generated using the neighbor-joining method in MEGA version 11.0, with 1000 bootstrap replicates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

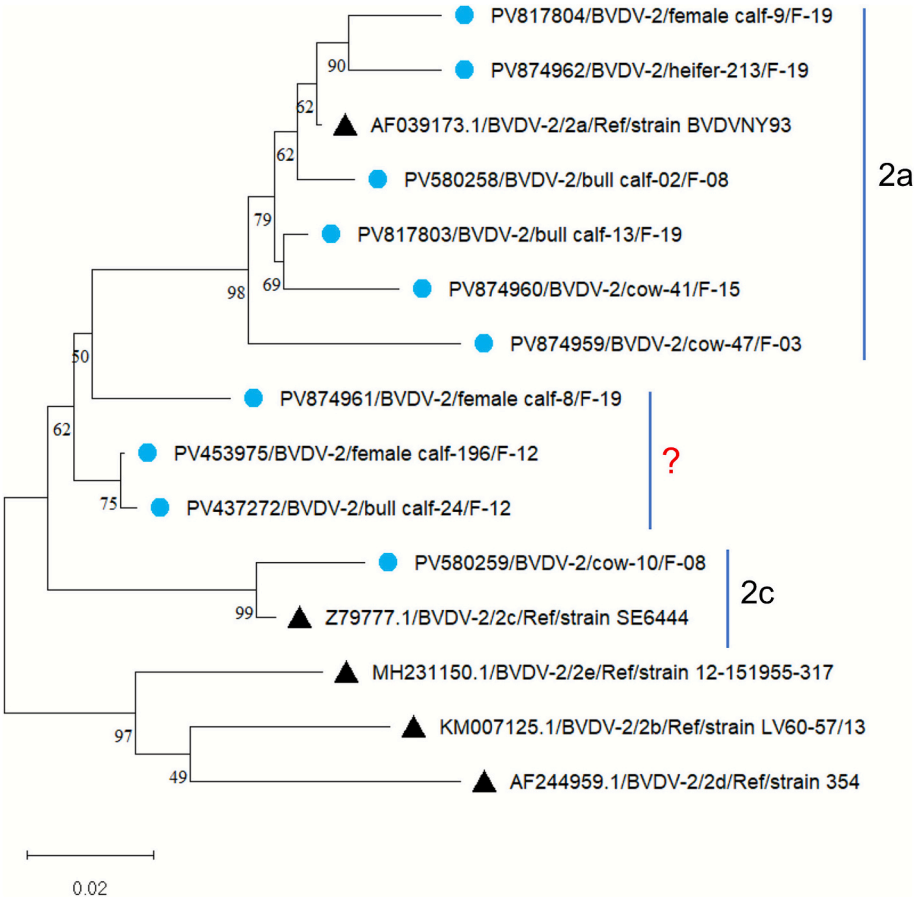

Fig. 5. Phylogenetic analysis was performed based on the 5′-UTR gene (293 bp) of BVDV-2 (*P. tauri*). The phylogenetic tree was created using nucleotide sequences obtained from field samples, along with reference sequences of representative subgenotypes retrieved from the GenBank database (S. Table 1). Cyan solid circles indicate the subgenotypes identified in this study from different cattle, whereas black triangles represent reference subgenotypes. The tree was generated using the neighbor-joining method in MEGA version 11.0, with 1000 bootstrap replicates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

rates range from 66.7% to 82.4% (Scharnböck et al., 2018; Deng et al., 2020; Su et al., 2023). On the other hand, animal-level prevalences ranging from 11.7% to 17.2% have been reported in several Asian countries, including Indonesia, Iran, Iraq, and South Korea (Su et al., 2023), which is comparable to the current findings from Bangladesh. In contrast, lower prevalence rates of 1.7%, 1.9%, and 53.9% have been reported in Nepal, India, and Japan, respectively (Goto et al., 2021; Su et al., 2023). These discrepancies in active infection rates among countries may be attributed to differences in geolocation, study design, detection methods, and population sampling strategies (Uddin et al., 2017). Additionally, variations in climatic conditions, farming practices, biosecurity measures, unregulated cattle trading, and the absence of national BVDV control programs contribute to virus persistence and ongoing circulation within cattle populations. Therefore, reducing the prevalence of active BVDV infection in endemic regions requires immediate rigorous biosecurity practices and continuous strategic control and surveillance programs. Early detection using sensitive molecular tests and removal of PI animals, combined with strategic vaccination, are crucial measures to achieve long-term control.

BVDV was historically classified into two major genotypes based on 5′ untranslated region (5′ UTR) sequences: BVDV-1 (*Pestivirus A*) and BVDV-2 (*Pestivirus B*) (Becher et al., 1997). An additional atypical 'HoBi'-like pestivirus, designated as BVDV-3 (*Pestivirus H*), was identified, which is closely related to bovine pestiviruses at both genetic and antigenic levels (Schirrmeier et al., 2004). However, in the most recent classification by the ICTV (ICTV, 2020), the BVDV genotypes were reclassified under a revised nomenclature: BVDV-1 as *Pestivirus bovis*, BVDV-2 as *Pestivirus tauri*, and 'HoBi'-like pestivirus as *Pestivirus brazilense*. Notably, the designation 'BVDV-3' no longer exists under the current pestivirus nomenclature.

In our study, according to the pestivirus species scheme, *P. bovis* (BVDV-1) was the predominant species, accounting for 70.8% of cases, followed by *P. tauri* (BVDV-2) at 20.8% and HoBi-like pestivirus (*P. brazilense*) at 16.7%. Molecular evidence from several countries reported that BVDV-1 is the predominant species associated with active infections in cattle populations globally (Rana et al., 2025). In Asia, a high prevalence of BVDV-1 has been documented in countries such as China, South Korea, Japan, and Taiwan (Deng et al., 2020; Yeşilbağ et al., 2017; Lin et al., 2025). Similarly, in Europe, BVDV-1 has been reported as the dominant genotype in France, Italy, Germany, the United

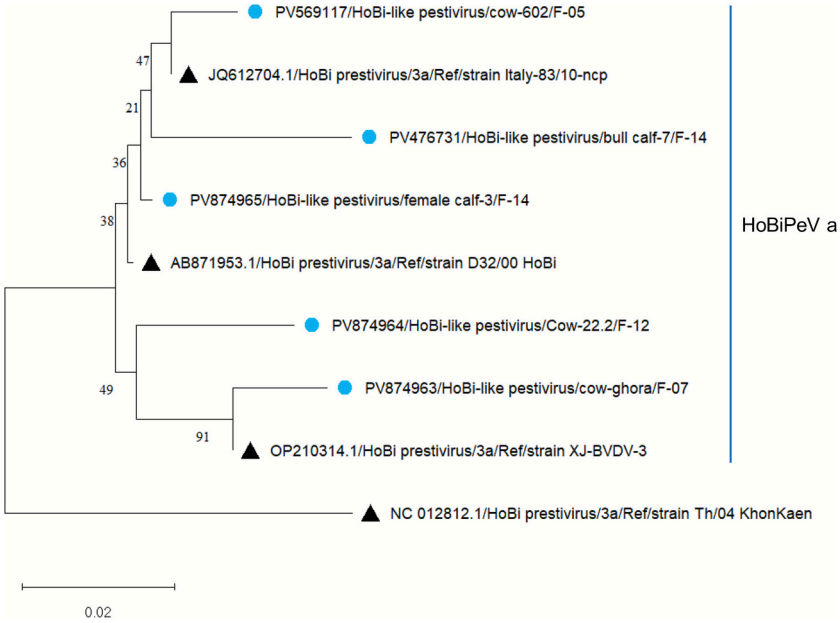

Fig. 6. Phylogenetic analysis was performed based on the 5′-UTR gene (293 bp) of HoBi like pestivirus (*P. brazilences*). The phylogenetic tree was created using nucleotide sequences obtained from field samples, along with reference sequences of representative subgenotypes retrieved from the GenBank database (S. Table 1). Cyan solid circles indicate the subgenotypes identified in this study from different cattle, whereas black triangles represent reference subgenotypes. The tree was generated using the neighbor-joining method in MEGA version 11.0, with 1000 bootstrap replicates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Multivariable logistic regression model assessing various animal-level demographic risk factors independently associated with previous exposure (detection of BVDV antibodies) and active BVDV infection in cattle across different dairy farms.

Outcome variable	Explanatory variable	Description	OR (95% CI)	*p* Value
Presence of BVDV antibody	History of clinical diseases in last 3 months	yes	2.4 (1.35–4.28)	*0.003
		no	1	Reference
	Sex	Female	3.0 (1.04–8.73)	*0.043
		Male	1	Reference
Presence of active BVDV infection	Animal category	Cow	1	Reference
		Heifer	2.3 (1.04–4.90)	*0.038
		Calves	6.2 (1.61–23.45)	*0.008
	Growth rate	Good	1	Reference
		Moderate	1.6 (0.32–7.48)	0.580
		Poor or stunted	3.0 (0.99–3.95)	*0.052

Abbreviation: OR, odds ratio; CI: confidence interval; statistically significant *$p < 0.05$.

Table 5
Multivariable logistic regression model assessing various herd factors and management practices independently associated with the presence of previous exposure (detection of BVDV antibody) and active BVDV infection in cattle across dairy herds.

Outcome variable	Explanatory variable	Description	OR (95% CI)	*p* -value
Presence of BVDV antibody	Stocking density	High (Overcrowded)	1	Reference
		Medium (Crowded)	2.9 (1.50–5.74)	*0.002
		Low (Sufficient space)	2.3 (0.92–5.51)	0.075
	Use of dedicated shoes and cloth for farm worker	Yes	1	Reference
		No	5.7 (1.17–27.76)	*0.031
Presence of active BVDV infection	Person performed artificial insemination (AI)	AI technician	10.4 (1.90–57.14)	*0.009
		Farm owner	1	Reference
		Veterinarian	empty	empty
	Frequency of visiting neighboring farm by farm owner or worker	Daily	3.1 (1.60–16.31)	*0.017
		Weekly	2.4 (0.49–11.92)	0.274
		Monthly	1	Reference
		Yearly	omitted	omitted
		No	2.6 (0.80–15.87)	0.096

Abbreviation: OR, odds ratio; CI: confidence interval; statistically significant *$p < 0.05$.

Kingdom, Spain, Austria, Slovakia, and Denmark (Rivas et al., 2022; Yeşilbağ et al., 2017). In North and South America, BVDV-1 circulation has been widely observed in the United States and Mexico (Kim, 2009; Yeşilbağ et al., 2017), as well as in Argentina, Brazil, and Chile (Yeşilbağ et al., 2017; Vilcek et al., 2004). Reports from Africa have identified BVDV-1 in countries such as South Africa, Tunisia, and Egypt (Yeşilbağ et al., 2017). In Oceania, its presence has been confirmed in Australian and New Zealand dairy herds (Dunowska et al., 2024). Cumulatively, these findings represent the global predominance of BVDV-1 across multiple continents, reinforcing its burden and epidemiological importance in bovine populations. Moreover, BVDV-2 is regarded as a hypervirulent species due to its severe pathological manifestations, including hemorrhagic disorders, lymphopenia, and acute enteritis (Yeşilbağ et al., 2017). This genotype has been reported to circulate dominantly in several countries across the world, including China, India, Russia, Germany, the United States of America, Argentina, and Brazil (Rivas et al., 2022; Deng et al., 2020; Yeşilbağ et al., 2017; Vilcek et al., 2004). Notably, no previous studies have documented evidence of either

BVDV-1 or BVDV-2 in dairy herds within Bangladesh. The present study is, therefore, the first to identify and characterize the BVDV species circulating in the country's cattle populations, filling a critical gap in regional disease surveillance and providing essential epidemiological insights for targeted control strategies in Bangladesh. Notably, Haider et al. (2014) previously reported the presence of HoBi-like pestivirus in cattle populations in Bangladesh. The detection of this atypical pestivirus species in the present study is consistent with these findings.

In addition to species characterization, the present study elucidated the circulating sub-genotypes of BVDV within the sampled population. In phylogenetic analysis, among the 26 BVDV-1 isolates, nine distinct sub-genotypes were identified, of which BVDV-1b was the predominant local type, accounting for 41.5% of all BVDV-1 detections. The identified sub-genotypes have also been reported in bovine populations from multiple geographic regions worldwide. Notably, countries such as China, Russia, France, Italy, Germany, the United Kingdom, Spain, Austria, Slovakia, Denmark, Argentina, Brazil, and Chile have documented wide genetic diversity in BVDV sub-genotypes, indicating the remarkable mutation, evolution, and global dissemination potential of BVDV (Rivas et al., 2022; Yeşilbağ et al., 2017; Vilcek et al., 2004). In the case of BVDV-2, a majority of isolates belonged to the BVDV-2a sub-genotype, with only a single isolate identified as BVDV-2c. Notably, three BVDV-2 sequences formed a distinct phylogenetic cluster that did not align to any of the currently reported sub-genotypes (BVDV-2a to BVDV-2e) and therefore remains unclassified. These sequences may represent a potential novel sub-genotype, tentatively designated as BVDV-2f, pending confirmation through the analysis of additional field isolates. Continued molecular surveillance and the analysis of robust sequence data are essential to validate this classification and to improve our understanding of emerging strains or genotypes. The detection of extensive genetic diversity of BVDV in Bangladesh suggests potential introductions of new strains through the movement of viremic or PI animals, likely facilitated by unrestricted cattle trade within local markets or cattle smuggling across porous borders with neighboring countries such as India and Myanmar, where pre-movement diagnosis is not currently practiced (Gongal et al., 2022). However, the specific pathways of introduction were not directly assessed in this study. The introduction of a new strain can exert selective pressure on the circulating viral population, leading to changes in the virus's genetic makeup over time and the potential emergence of hypervirulent strains (Deng et al., 2020; Lin et al., 2025). To date, there are no BVDV vaccines available in Bangladesh; therefore, it is of the utmost importance to elucidate the circulating species and strains, which is a prerequisite for the rational design and development of an effective vaccine. The high mutation rate of BVDV, resulting in antigenic drift, supports the ongoing evolutionary dynamics of BVDV and facilitates the emergence of antigenically distinct new strains, posing formidable challenges to vaccine development and its efficacy (Deng et al., 2020). Consequently, existing BVDV vaccines used in other countries may fail to elicit robust, cross-protective immunity against newly evolved genotypes or strains (Rana et al., 2025). We recommend large-scale molecular and antigenic characterization of field isolates as a critical prerequisite for the development of region- or country-specific vaccines capable of providing long-lasting, broad-spectrum herd immunity.

We identified three calves (0.8%) as PI, while 38 cattle (10.2%) were confirmed as TI based on two consecutive RT-qPCR tests conducted 21 days apart. Notably, PI animals are immunocompetent and discharge significant amounts of active virus throughout their lives, making them a key source of transmission (Houe, 1995). The prolonged presence of PI animals within a herd increases the risk of continuous viral shedding and transmission to susceptible cattle. In many well-structured dairy production systems in developed countries, the PI status of calves is routinely monitored immediately after birth using ear notch samples collected during ear tagging (Rivas et al., 2022). Screening and prompt removal of PI animals from all herds are indispensable decisions for the successful implementation of BVDV control and eradication programs.

Based on the activity of BVDV on MDBK cultured cells, 65.9% of field isolates were identified as the non-cytopathic biotype, whereas only six isolates (14.6%) were classified as the cytopathic biotype. According to Ridpath et al. (2006), the non-cytopathic type of BVDV is predominant in bovine herds, while the cytopathic biotype is less frequent and typically associated with acute clinical disease. Although the cytopathology of BVDV does not directly correlate with its virulence determinants, both biotypes can induce clinical disease affecting multiple organ systems in the host (Tautz et al., 2015). Notably, the non-cytopathic biotype is only capable of establishing persistent infections when transmitted *in utero* (Ridpath et al., 2006), a biological feature that emphasizes its epidemiological significance and that challenges all control strategies. Cytopathic BVDV arises from mutations in the non-cytopathic virus, often involving insertions of sequences into the NS2–3 region, which preclude persistent infection (Vilcek et al., 2004). The characteristic cytopathic effects observed here, including cytoplasmic vacuolation with large, encapsulated vacuoles that fused to form even larger vacuoles, cell rounding, detachment, and eventual cell death, were closely consistent with those described in a previous study by Birk et al. (2008). Acquisition of cytopathic properties results in hypervirulent causes of cellular destruction, caspase activation, and apoptosis, as demonstrated *in vitro*, indicating its role in acute disease progression (Ridpath et al., 2006). Notably, in this study eight RT-qPCR–positive isolates failed to propagate in the MDBK cell line and therefore could not be biotyped, likely due to low viral load, loss of infectivity during storage, or the presence of non-viable viral particles.

In this study animal demographic factors including female sex (cows and heifers) and a history of clinical disease within the past three months were associated with BVDV seropositivity in cattle herds. Although both male and female animals are equally susceptible to BVDV infection (Wilson et al., 2016), several previous studies have also reported significant differences in seropositivity between sexes (Bello et al., 2016; Demil et al., 2021). Differences in sampling proportions and herd management practices may account for the relatively higher seropositivity observed in female cattle. Female animals, such as heifers and dairy cows, are typically retained in dairy herds for extended periods for milk production and reproduction purposes, thereby increasing their cumulative exposure risk to BVDV (Demil et al., 2021). In contrast, male calves are often sold at an early age or within a short period for meat production, limiting their duration of exposure to the virus. The seropositivity observed in animals with a recent history of clinical disease (within the past three months) likely reflects prior exposure to BVDV and the subsequent development of specific antibodies (Ridpath, 2010).

Besides specific animal categories, animals exhibiting poor or stunted growth showed significantly higher odds of active BVDV infection. It could be due to young animals being more susceptible and immunologically naive as well as having limited prior exposure to BVDV, making them more vulnerable to primary infection (Lanyon et al., 2014). Additionally, the presence of PI or TI animals may facilitate early-life exposure through direct contact with susceptible animals. The significant association between poor or stunted growth and active BVDV infection is a well-documented impact of the virus (Ridpath et al., 2006; Wilson et al., 2016). BVDV can adversely affect growth by damaging the enteric system, reducing feed conversion efficiency, and causing immunosuppression, thereby making the animals susceptible to secondary infections. Notably, the identification of younger groups and growth-impaired cattle as high-risk categories suggests the need for targeted surveillance and early screening in these populations as part of a cost-effective approach to BVDV control in endemic regions.

Among the various herd- and management-related risk factors, high stocking density and the absence of dedicated shoes and clothing for farm workers were significantly associated with higher odds of BVDV seropositivity in cattle herds. Overcrowding in cattle herds increases direct contact between naïve and PI or TI animals, facilitates the transmission of BVDV, and provides ideal conditions for becoming infected (Demil et al., 2021). Furthermore, overcrowded herd

environments often exacerbate stress, which can impair immune responses and increase susceptibility to infection (Wilson et al., 2016). Besides this, a failure to use dedicated shoes or protective footwear and clothing for farm workers is considered a critical biosecurity breach. Farm personnel may act as mechanical vectors, often carrying infectious components such as saliva, nasal discharge, urine, feces, uterine discharge, and aborted materials from BVDV-infected animals to susceptible ones (Evans et al., 2019).

In this study, artificial insemination (AI) performed by non-veterinarian AI technicians, as well as the daily visiting of neighboring farms by farm owners or workers, were significantly associated with higher odds of active BVDV infection in dairy herds. Technicians who provide AI services to multiple farms in a short time frame in BVDV endemic areas without appropriate hygienic practice may inadvertently act as mechanical carriers, transferring infectious material between herds (Nilnont et al., 2016). However, this study did not directly measure virus transmission *via* AI, and the observed association should be interpreted as a risk indicator rather than proof of causality. Since BVDV can be transmitted through semen from BVDV carrier bulls, the risk is markedly increased when natural breeding occurs or semen is collected from non-tested bulls or handled in non-sterile conditions (Nilnont et al., 2016). Moreover, in the context of Bangladesh, PI or TI animals remain undetected; the daily visiting or movements between neighboring farms by farm owners or workers represent a major biosecurity breach that increases the chance of indirect transmission through contaminated footwear, clothing, or farm equipment (Nilnont et al., 2016). In BVDV-prevalent regions, where viremic animals may remain undetected or isolated, even minimum contact with contaminated environments can be sufficient to introduce BVDV into susceptible herds. Therefore, assessing potential risk factors and tracing hidden sources of BVDV in dairy cattle herds are crucial for successful herd management, sustainable production, and profitable farming. Since apparent prevalence can underestimate or overestimate the true infection rate, future studies should calculate true prevalence to provide a more accurate estimate of BVDV infection in cattle. Additionally, whole-genome sequencing is recommended to achieve deeper insights into phylogenomics, evolutionary source and transmission dynamics. Further large-scale molecular surveillance is warranted to comprehensively assess the BVDV status across the country and region.

5. Conclusions

BVDV is widespread in dairy herds in Bangladesh, indicating both previous exposure and ongoing active infections. The detection of different species and diverse sub-genotypes of BVDV addresses a critical gap in understanding the local molecular epidemiology. Notably, BVDV-1 and BVDV-2 were detected for the first time in Bangladesh, with BVDV-1b and BVDV-2a identified as the dominant sub-genotypes in commercial dairy herds. The detection of PI animals within herds highlights their role as continuous sources of viral transmission, predictors of epidemic trends, and ongoing challenges for prevention and control efforts. Furthermore, *in vitro* isolation and characterization of circulating wild-type cytopathic and non-cytopathic biotypes enhance understanding of viral pathobiology and their potential to cause MD in dairy herds. Additionally, identification of animal demographic and herd-level management risk factors provides critical evidence to guide the implementation of stronger, targeted prevention and control strategies. Overall, this study provides an initial foundation for broader-scale surveillance of BVDV in Bangladesh. Future research should focus on assessing cross-species transmission dynamics in mixed farming systems and conducting Knowledge, Attitudes, and Practices (KAP) surveys among dairy farmers and veterinarians to strengthen national BVDV control efforts.

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CRediT authorship contribution statement

Eaftekhar Ahmed Rana: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Md Saiful Islam:** Methodology, Data curation. **Belayet Hossain:** Visualization, Software, Methodology. **Abdul Ahad:** Supervision, Investigation. **David J. Hampson:** Writing – review & editing. **Sam Abraham:** Writing – review & editing. **Subir Sarker:** Writing – review & editing, Validation, Supervision, Conceptualization. **Jully Gogoi-Tiwari:** Writing – review & editing, Validation, Supervision, Conceptualization. **Jasim M. Uddin:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no financial interests and personal relationships that might have influenced the findings presented in this article.

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