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Prevalence and genetic characterization of bovine viral diarrhea virus in dairy cattle in northern China

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Abstract

Background Bovine viral diarrhea virus (BVDV) is one of the major viral pathogens responsible for respiratory disease complexes in cattle and other ruminants; it has spread worldwide and poses a significant threat to the cattle industry. To understand the prevalence and genetic diversity of BVDV in northern China, this study conducted an epidemiological survey of BVDV in dairy cows across 13 provinces in northern China from June 2022 to June 2024. A total of 2,199 nasal swab samples were analyzed by RT-PCR.

Results The results revealed an overall positive rate of 6.05% for BVDV, with values of 6.47% from June 2022 to June 2023 and 5.59% from July 2023 to June 2024. Notably, the positive rate varied by region, with the highest prevalence in Shandong (9.62%) and the lowest in Hebei (1.61%). Phylogenetic analysis of 53 positive samples revealed that all belonged to BVDV-1, with the predominant sub-genotypes being 1a (47.17%), 1 m (28.30%), and 1c (9.43%). No BVDV-2 or BVDV-3 was detected, indicating that BVDV-1a is the most prevalent strain in northern China. This study also highlighted the genetic diversity of BVDV, with nucleotide homology among the sub-genotypes ranging from 70.2 to 92.1%.

Conclusions An epidemiological survey of BVDV conducted in 13 provinces (regions) in northern China between 2022 and 2024 revealed a positive rate of 6.05%. The prevalent genotype identified was BVDV-1, with the predominant sub-genotypes being BVDV-1a, BVDV-1 m, and BVDV-1c. The findings of this study provide new evidence for the molecular epidemiology and genetic evolution of BVDV transmission in northern China, laying a foundational basis for the development of vaccination and control strategies against BVDV.

Keywords Bovine viral diarrhea, Prevalence characterization, Genetic diversity, Dairy cattle, North China

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Background

Bovine viral diarrhea virus (BVDV) is a prototypic member of the genus Pestivirus in the family Flaviviridae and is a single-stranded positive-sense RNA virus. It belongs to the same genus as classical swine fever virus (CSFV, Pestivirus C) and border disease virus (BDV, Pestivirus D), and there is a cross-reaction in serology [1]. BVDV has a wide host range, infecting not only cattle but also a variety of domestic and wild animals, including pigs, sheep, deer, and camels [2, 3]. The main clinical symptoms following BVDV infection include fever, mucosal erosion and ulceration, leukopenia, diarrhea, and coughing. Pregnant cows may suffer from abortion or deliver abnormal fetuses [4].

The complete genome of BVDV is approximately 12.3–12.5 kb in length, comprising a single open reading frame (ORF) that encodes multiple proteins, flanked by 5' and 3' untranslated regions (UTRs) at both ends [5]. The ORF encodes a polyprotein of approximately 4,000 amino acids, which is processed and cleaved by both viral and host proteases into four structural proteins (capsid, Erns, E1, and E2) and eight nonstructural proteins (Npro, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) [6]. According to their cytopathic effects, viruses can be categorized into two biological types: cytopathic and noncytopathic. Additionally, BVDV exhibits significant genetic polymorphism and antigenic variability [7]. This variability has led to the classification of BVDV into three genotypes: BVDV-1, BVDV-2, and BVDV-3 [8]. BVDV-3, also known as the "HoBi-like" virus, was recently discovered in South America, Europe, and Asia [9]. Through phylogenetic analysis of specific genomic regions, such as the 5' UTR, Npro, and E2, BVDV can be further subdivided into various subgenotypes [10]. Currently, 24 sub-genotypes of BVDV-1 (BVDV-1a to -1x) and 5 sub-genotypes of BVDV-2 (BVDV-2a to -2e) have been described [11, 12].

With the development of import and export trade in animals and animal products (serum, embryo, semen, etc.), countries with rapid growth in animal husbandry have become the worst affected areas for BVDV. Moreover, the global seroprevalence of BVDV has exceeded 50%. The expansion of the range of susceptible hosts has also contributed to the accelerated global spread of the virus [13]. Specifically, the seroprevalence rates are reported as follows: 73% in Asia, 46% in Europe, 78% in Oceania, 74% in Africa, 67% in South America, and 75% in North America [14]. A meta-analysis on BVDV in China revealed that from 2003 to 2018, the seroprevalence of BVDV was 57%, while the positivity rate for virological testing was 27.1%. The analysis also indicated an increasing trend in BVDV seroprevalence [15]. BVDV can be detected by both antibody and antigen tests. Antibody positivity indicates that the animal is either in the process of infection or experiencing transient infection, but early diagnosis cannot be made. Additionally, antibody tests cannot detect cattle that are persistently infected with BVDV due to immunological tolerance. The nucleotide sequence homology between BVDV and CSFV is about 66%, with amino acid homology being about 85%. In current animal production, the situation of mixed infections with BVDV and CSFV is quite complex. Therefore, the cross-reactivity between the antibodies of the two viruses affects the accuracy of the antibody detection technology [16]. Antigen positivity indicates that BVDV is spreading in cattle herds. RT-PCR detection of the BVDV antigen is more sensitive than other antigen detection methods and has been widely used in the diagnosis of BVDV [17]. Previously, research on BVDV was mainly conducted in eastern and western China or other limited regions. A national epidemiological survey is mainly carried out to determine the rate of positive anti-BVDV antibody results [18–20]. Surveys conducted in China from 2010 to 2013 and in 2017 revealed a significant increase in the positive ratio of BVDV antibodies after 2010, which may be associated with the promotion of large-scale production models [21, 22]. Moreover, several studies have identified 11 sub-genotypes of BVDV-1 (1a, 1b, 1c, 1d, 1 m, 1o, 1p, 1q, 1u, 1v, 1w) and 4 sub-genotypes of BVDV-2 (2a–2d) in China so far. HoBi-like viruses have also been detected in many cases in some regions of China [15]. These findings indicate the wide genetic diversity of BVDVs in China.

Currently, there are few national epidemiological investigations on BVDV antigens in China. The dairy regions of China are relatively scattered, with an extensive distribution covering all provinces; however, the overall situation is characterized as "a majority in the north and a relative scarcity in the south" [23]. Owing to the diverse natural resources and socioeconomic environments in various regions, the layout of dairy cattle production is constantly changing. Therefore, this study conducted the first analysis of the antigen positivity rate and sub-genotype prevalence of BVDV across 13 provinces in northern China. We also carried out a genetic evolution analysis to further elucidate the genetic diversity of BVDV in these regions. The findings of this study provide an important scientific basis for enhanced control of BVDV circulation in bovine populations. These findings will be essential in designing effective prevention and control measures to reduce the negative impact of this disease on the cattle industry. Meanwhile, the research results will also provide new data and perspectives for global BVDV research.

Results

Detection of BVDV in northern China

Between 2022 and 2024, a comprehensive study was conducted in which 2,199 nasal swab samples from across 13 provinces in northern China were collected and tested. The analysis of these samples revealed that 133 out of 2,199 samples (6.05%) tested positive for BVDV (Table 1). From June 2022 to June 2023, the rate of nucleic acid positivity for BVDV was 6.47%. From July 2023 to June 2024, the rate of nucleic acid positivity for BVDV was 5.59%. The results indicate a decrease in the positive rate over the two periods. Shandong Province presented the highest rate at 9.62% (10/104), surpassing Ningxia's rate of 9.52% (20/210), followed by Shanxi at 8.18% (13/159), Henan at 7.83% (13/166), Qinghai at 7.78% (7/90), Gansu at 7.45% (12/161), Inner Mongolia at 5.91% (13/220), Heilongjiang at 5.47% (7/128), Xinjiang at 5.38% (5/93), Jilin at 4.83% (20/414), Liaoning at 3.6% (4/111), Shaanxi at 3.2% (7/219), and Hebei at 1.61% (2/124). These results further indicate the potential threat of BVDV to the cattle industry.

Phylogenetic analysis

In this study, to reveal the genetic diversity of BVDV in northern China, 5' UTR sequencing was performed on samples that tested positive for RT-PCR. The sequencing results were aligned with reference strains (Table 2) using the BLAST tool, and a phylogenetic analysis was carried out (Fig. 1).

The results showed that a total of 53 sequences were successfully sequenced (Table 3), with all strains identified as BVDV-1 type. The phylogenetic tree, constructed using both sequenced sequences and reference strains, revealed that these sequences were grouped into seven distinct sub-genotypes: 1a, 1c, 1d, 1 m, 1o, 1q, and 1v. Notably, the 1a sub-genotype cluster accounted for the largest proportion, with 25 sequences (47.17%). The 1 m sub-genotype cluster followed with 15 sequences (28.30%). Sub-genotypes 1c and 1d contained 5 sequences each, representing 9.43% of the total. Sub-genotypes 1d and 1o had three sequences each, amounting to 5.66% of the total, whereas sub-genotypes 1q and 1v were less common, with only one sequence each, accounting for 1.89% of the total.

Further analysis of the regional distributions revealed the geographical distribution characteristics of various sub-genotypes of BVDV-1. Among them, sub-genotype 1a was found to be the most prevalent, with a detection rate of 84.62% (11/13) across the widest distribution, including provinces such as Shaanxi, Qinghai, Xinjiang, Heilongjiang, Shandong, Henan, Liaoning, Jilin, Inner Mongolia, Shanxi, and Ningxia. The high proportion and extensive distribution of the sub-genotype 1a reflect its adaptive advantages in the northern region. BVDV-1 m was detected in 6 provinces (46.15%, 6/13), including Inner Mongolia ($n=3$), Shandong ($n=8$), Henan ($n=1$), Gansu ($n=2$), Qinghai ($n=2$), and Hebei ($n=1$). The BVDV-1c sub-genotype was distributed in four provinces (30.77%): Shandong ($n=1$), Ningxia ($n=1$), Gansu ($n=1$), and Shanxi ($n=1$), whereas the BVDV-1d sub-genotype (15.38%) was identified in Shandong and Ningxia, highlighting the varied distribution patterns of BVDV-1 sub-genotypes across the region. In this study, the BVDV-1o, 1q, and 1v sub-genotypes were each detected in only a single province within the northern region. Specifically, BVDV-1o was detected in Ningxia ($n=3$), BVDV-1q ($n=1$) in Henan, and BVDV-1v ($n=1$) in Shanxi. These data provide valuable insights into the geographical distribution of various BVDV sub-genotypes in northern

Table 1 The BVDV prevalence of 13 provinces in northern China

Province	2022.06-2023.06			2023.07-2024.06			Provincial positive rate(%)
	No. sample collected	No. positive sample	Positive Rate(%)	No. sample collected	No. positive sample	Positive Rate(%)	
Liaoning	52	3	5.77	59	1	1.69	3.60
Ningxia	75	13	17.33	135	7	5.19	9.52
Hebei	70	1	1.43	54	1	1.85	1.61
Shaanxi	140	3	2.14	79	4	5.06	3.20
Shandong	41	5	12.2	63	5	7.94	9.62
Gansu	106	8	7.55	55	4	7.27	7.45
Heilongjiang	84	5	5.95	44	2	4.55	5.47
Jilin	257	3	1.17	157	17	10.83	4.83
Qinghai	60	4	6.67	30	3	10	7.78
Xinjiang	53	3	5.66	40	2	5	5.38
Shanxi	56	9	16.07	103	4	3.88	8.18
Henan	50	8	16	116	5	4.31	7.83
Inner Mongolia	100	9	9	120	4	3.33	5.91
Total	1144	74	6.47	1055	59	5.59	6.05

Table 2 List of reference strains for phylogenetic comparison with isolates

Pestivirus pecies	Subtype	Strain	Origin	Location	Collection year	5'UTR Accession number
BVDV-1	1a	NADL	Cattle	USA	1963	AJ133739
BVDV-1	1a	Oregon C24 V	Cattle	USA	1998	AF091605.1
BVDV-1	1a	BJ-2013	Cattle	China	2013	MH490942.1
BVDV-1	1a	SWU-Z6	fecal	China	2016	MF693403
BVDV-1	1b	BJ-2016	Cattle	China	2016	MH490943.1
BVDV-1	1b	CP7	Bos	Germany	1996	U63479.1
BVDV-1	1b	AV69	Cattle	USA	2011	KC695814
BVDV-1	1b	3156	Cattle	China	2011	JN704144
BVDV-1	1c	Crookwell	Cattle	Australia	1989	JQ743606.1
BVDV-1	1c	MF-1	Cattle	China	2016	KY865369
BVDV-1	1c	Bega-like	Bovine	Australia	2012	KF896608
BVDV-1	1c	NM2103	Cattle	China	2021	ON337882
BVDV-1	1d	10-JJ-SKR	Cattle	South Korea	2010	KC757383
BVDV-1	1d	SWU-DJ2	yak	China	2015	MF166858
BVDV-1	1d	F	-	-	-	AF298065.1
BVDV-1	1e	68 – 1	Cattle	UK	2008	MW250802
BVDV-1	1e	S03-1175	Cattle	USA	2003	MW655631
BVDV-1	1e	SLO/2407/2006	Cattle	Slovenia	2006	KX577637
BVDV-1	1f	192-KW/17	Cattle	Poland	2019	MK381368.1
BVDV-1	1f	210-GK/18	Cattle	Poland	2018	MK381386
BVDV-1	1 g	48/08	Cattle	Poland	2008	JN715004.1
BVDV-1	1 g	10/08	Cattle	Poland	2008	JN715004.1
BVDV-1	1 h	BG9a02	Bovine	Italy	2002	MG434576.1
BVDV-1	1 h	CR1a99	Bovine	Italy	1999	MG434575
BVDV-1	1i	ACM/BR/2016	Cattle	Brazil	2017	KX857724
BVDV-1	1i	CA2006	Bovine	USA	2006	MK775204
BVDV-1	1j	A469	Bovine	Chile	2014	GU987129.1
BVDV-1	1j	2/Vr/95	Bovine	Italy	2000	AJ293594.1
BVDV-1	1j	KS 86 – 1 ncp	Cattle	Japan	2002	AB078950
BVDV-1	1k	R3230-95	Cattle	Switzerland	1995	MW655628
BVDV-1	1k	TO/197/11	Cattle	Italy	2011	MW054935
BVDV-1	1 L	71 – 15	Bos taurus	France	2005	KF205306
BVDV-1	1 L	71 – 16	Bos taurus	France	2008	KF205307.1
BVDV-1	1 m	SD-15	Cattle	China	2015	KR866116
BVDV-1	1 m	CHN/HB-01/2017	bovine	China	2017	ON901784
BVDV-1	1n	Shitara/02/06	Cattle	Japan	2006	LC089876
BVDV-1	1o	HY-3	Cattle	China	2016	KY865366.1
BVDV-1	1o	BVDV-NM2312	Cattle	China	2023	PP992316
BVDV-1	1p	TJ06	Cattle	China	2006	GU120246.1
BVDV-1	1p	BJ0702	Cattle	China	2007	GU120248.1
BVDV-1	1p	BJ0703	Cattle	China	2007	GU120249.1
BVDV-1	1q	Camel-6	Camel	China	2010	KC695810
BVDV-1	1q	SD0803	pig	China	2008	JN400273
BVDV-1	1r	VE/245/12	-	Italy	2014	LM994671
BVDV-1	1r	CA/181/10	-	Italy	2015	LM994672
BVDV-1	1t	SI/207/12	-	Italy	2014	LM994674
BVDV-1	1v	EN-6	Cattle	China	2017	MN417813.1
BVDV-1	1v	HB-03	Cattle	China	2018	ON901785.1
BVDV-1	1v	GA190608	Cattle	China	2019	MT933204.1
BVDV-2	2a	HLJ-10	Cattle	China	2011	JF714967
BVDV-2	2b	SD1301	Cattle	China	2012	KJ000672
BVDV-2	2c	SH2210-23	Cattle	Germany	2010	HG426494
BVDV-3	3	XJ-BVDV-3	bovine	China	2022	OP210314.1

Table 2 (continued)

Pestivirus pecies	Subtype	Strain	Origin	Location	Collection year	5'UTR Accession number
BVDV-3	3	Th/04 KhonKaen	bovine	Thailand	2004	NC_012812
CSFV	CSFV	Shimen	swine	China	1999	AF092448.2
CSFV	CSFV	SXCDK	swine	China	2009	GQ923951
BDV	BDV	JSLS12-01	Ovis aries	China	2012	KC963426.1
BDV	BDV	X818	Sheep	Germany	1997	NC_003679.1

China, which may help to elucidate the epidemiological characteristics of BVDV.

The nucleotide (nt) homology percentages in the 5' UTR region between the tested strains and the reference strains available in the GenBank database were as follows: BVDV-1a, 79.7% (NM1 2022, SWU-Z6)–96.4% (SHX1 2022, BJ-2013); BVDV-1c, 69.7% (SDQD1 2023, Crookwell)–95.4% (GS2 2022, NM2103); BVDV-1d, 82.3% (NX1 2022, F)–97.8% (SDQD2 2023, 10JJ-SKR); BVDV-1m, 82.0% (NM1/2 2023, SD-15)–95.4% (HN2 2022, CHN/HB-01/2017); BVDV-1o, 85.8% (NX8 2022, BVDV-NM2312)–88.2% (NX6 2022, HY-3); BVDV-1q, 91.0% (HN1 2022, SD0803)–94.6% (HN1 2022, Camel-6); and BVDV-1v, 78.8% (SXDT1 2023, GA190608)–94.3% (SXDT1 2023, EN-6) (Fig. 2). Sequence identity within the 5' UTR regions among the various sub-genotypes of the sequenced sequences ranged from 70.2 to 92.1%. The largest and smallest differences in sequence homology were both observed between BVDV-1a and BVDV-1c. The largest difference was found between a BVDV-1a sub-genotype from Inner Mongolia in 2022 and a BVDV-1c sub-genotype from Shandong Province in 2023, with a sequence homology of 70.2%. The smallest difference was observed between the BVDV-1a from Shaanxi in 2022 and the BVDV-1c sub-genotype from Gansu in the same year, with a sequence homology of 92.1%.

Discussion

According to statistics, the average annual economic loss per cow caused by BVDV is approximately $46.5 [24]. Dairy cattle suffer greater economic losses ($24.85) than that of beef cattle. Data from Europe indicate that farms in regions where BVDV is endemic suffer economic losses ranging from $6.46 to $87 per cow per year [24]. In recent years, China's dairy farming industry has developed rapidly, becoming the world's third-largest milk producer [23]. The increased scale of animal production and the movement of animals from one place to another have led to a sharp increase in BVDV infections [20]. Therefore, closely monitoring the epidemic characteristics of BVDV, clarifying the variation patterns of epidemic strains, and reassessing the BVDV situation in China's dominant dairy farming areas are crucial for controlling BVDV infections.

In this study, primers targeting the 5' UTR region were used to detect BVDV-1, BVDV-2, and BVDV-3 by RT-PCR in clinical nasal swab samples from dairy cattle collected from 13 provinces in northern China between June 2022 and June 2024 (Table 1). Then, some of the samples that tested positive were selected for 5' UTR sequencing, and the 53 obtained 5' UTR sequences were analyzed through the construction of a phylogenetic tree. The findings of this study revealed that the positivity rate of BVDV RNA in northern China dairy cattle from 2022 to 2024 was 6.05%. Previous studies reported that the positivity rate of BVDV RNA in Chinese dairy cattle was 27.1% [22]. In 2019, Deng et al. detected the prevalence of BVDV RNA in bulk tank milk (BTM) samples in northern China to be 47.3% by qRT-PCR [20]. In comparison, the positive rate of BVDV RNA in the present research was significantly lower, with considerable variation among provinces (regions), ranging from 1.61 to 9.62%.

Previous research has focused primarily on clinical samples such as serum, stool, and BTM, with detection methods predominantly involving RT-PCR and qRT-PCR. The variation in sample sources has led to variable results in the epidemiological investigation of BVDV [25, 26]. It is noteworthy that this study found the positive rates of BVDV infection, from highest to lowest, were as follows: Shandong, Ningxia, Shanxi, Henan, Qinghai, Gansu, Inner Mongolia, Heilongjiang, Xinjiang, Jilin, Liaoning, Shaanxi, and Hebei. In particular, the provinces of Shandong (9.62%), Shanxi (8.18%), and Henan (7.83%) in northern China have a high proportion of cattle at risk of BVDV infection. Our findings are consistent with the analysis of China's dairy farming production layout, which shows a trend of gradual concentration in the northern regions of China, particularly in the provinces of Shandong and Henan, with Henan Province experiencing the fastest growth rate.

The population of dairy cattle in northern China has been steadily growing and is gradually narrowing the gap with the production areas of Northeast China and Inner Mongolia. Conversely, the five provinces of Xinjiang, Hebei, Heilongjiang, Shaanxi, and Shanxi all show a downward trend, with Xinjiang experiencing the most significant decline. A key factor contributing to the long-term presence of BVDV in cattle farms is the existence of Persistently Infected (PI) animals. PI refers to pathogens that remain in the host for an extended period without being eliminated, with incubation periods lasting for

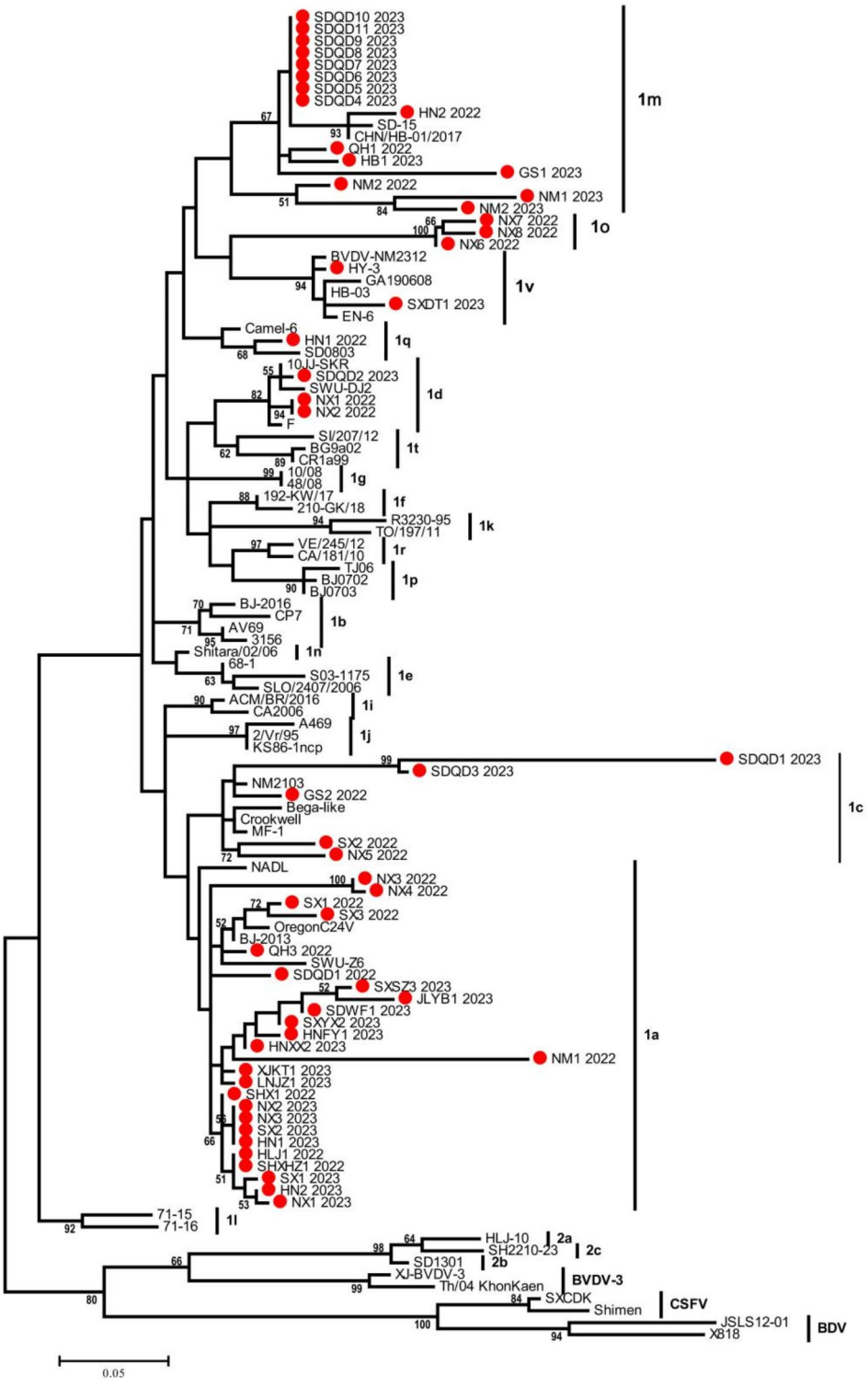

Fig. 1 Phylogenetic analysis based on 5′UTR sequence. Phylogenetic tree analysis of the 5′UTR gene(289 bp) was created using the nucleotide sequences of selected BVDV-1 samples from representative subgenotypes in this study. The tree was constructed by the maximum-likelihood method with 1000 bootstrap replicates using MEGA 6.0 software. All of the reference sequences used in this study were obtained from the GenBank database. Red solid circles indicate the strains identified in this study

Table 3 List of field isolates used in the study

isolate	Year of islolation	Sample	Origin	Genotype	5'UTR Accession number
NM1 2022	2022	Nasal swab	Inner Mongolia	1a	PP816783
NM2 2022	2022	Nasal swab	Inner Mongolia	1 m	PP816784
NM1 2023	2023	Nasal swab	Inner Mongolia	1 m	PP816785
NM2 2023	2023	Nasal swab	Inner Mongolia	1 m	PP816786
SDWF1 2023	2023	Nasal swab	Shandong	1a	PP816787
SDQD1 2022	2022	Nasal swab	Shandong	1a	PP816788
SDQD1 2023	2023	Nasal swab	Shandong	1c	PP816789
SDQD2 2023	2023	Nasal swab	Shandong	1d	PP816790
SDQD3 2023	2023	Nasal swab	Shandong	1c	PP816791
SDQD4 2023	2023	Nasal swab	Shandong	1 m	PP816792
SDQD5 2023	2023	Nasal swab	Shandong	1 m	PP816793
SDQD6 2023	2023	Nasal swab	Shandong	1 m	PP816794
SDQD7 2023	2023	Nasal swab	Shandong	1 m	PP816795
SDQD8 2023	2023	Nasal swab	Shandong	1 m	PP816796
SDQD9 2023	2023	Nasal swab	Shandong	1 m	PP816797
SDQD10 2023	2023	Nasal swab	Shandong	1 m	PP816798
SDQD11 2023	2023	Nasal swab	Shandong	1 m	PP816799
HNFY1 2023	2023	Nasal swab	Henan	1a	PP816800
HNXX2 2023	2023	Nasal swab	Henan	1a	PP816801
HN1 2022	2022	Nasal swab	Henan	1q	PP816802
HN2 2022	2022	Nasal swab	Henan	1 m	PP816803
HN1 2023	2023	Nasal swab	Henan	1a	PP816804
HN2 2023	2023	Nasal swab	Henan	1a	PP816805
SXDT1 2023	2023	Nasal swab	Shanxi	1v	PP816806
SXYX2 2023	2023	Nasal swab	Shanxi	1a	PP816807
SXSZ3 2023	2023	Nasal swab	Shanxi	1a	PP816808
SX1 2022	2022	Nasal swab	Shanxi	1a	PP816809
SX2 2022	2022	Nasal swab	Shanxi	1c	PP816810
SX3 2022	2022	Nasal swab	Shanxi	1a	PP816811
SX1 2023	2023	Nasal swab	Shanxi	1a	PP816812
SX2 2023	2023	Nasal swab	Shanxi	1a	PP816813
NX1 2023	2023	Nasal swab	Ningxia	1a	PP816814
NX2 2023	2023	Nasal swab	Ningxia	1a	PP816815
NX3 2023	2023	Nasal swab	Ningxia	1a	PP816816
NX1 2022	2022	Nasal swab	Ningxia	1d	PP816817
NX2 2022	2022	Nasal swab	Ningxia	1d	PP816818
NX3 2022	2022	Nasal swab	Ningxia	1a	PP816819
NX4 2022	2022	Nasal swab	Ningxia	1a	PP816820
NX5 2022	2022	Nasal swab	Ningxia	1c	PP816821
NX6 2022	2022	Nasal swab	Ningxia	1o	PP816822
NX7 2022	2022	Nasal swab	Ningxia	1o	PP816823
NX8 2022	2022	Nasal swab	Ningxia	1o	PP816824
GS2 2022	2022	Nasal swab	Gansu	1c	PP816826
GS1 2023	2023	Nasal swab	Gansu	1 m	PP816827
QH1 2022	2022	Nasal swab	Qinghai	1 m	PP816828
QH3 2022	2022	Nasal swab	Qinghai	1a	PP816830
HLJ1 2022	2022	Nasal swab	Heilongjiang	1a	PP816831
SHXHZ1 2022	2022	Nasal swab	Shaanxi	1a	PP816832
SHX1 2022	2022	Nasal swab	Shaanxi	1a	PP816833
XJKT1 2023	2023	Nasal swab	Xinjiang	1a	PP816834
HB1 2023	2023	Nasal swab	Hebei	1 m	PP816835
LNJZ1 2023	2023	Nasal swab	Liaoning	1a	PP816836
JLYB1 2023	2023	Nasal swab	Jinlin	1a	PP816837

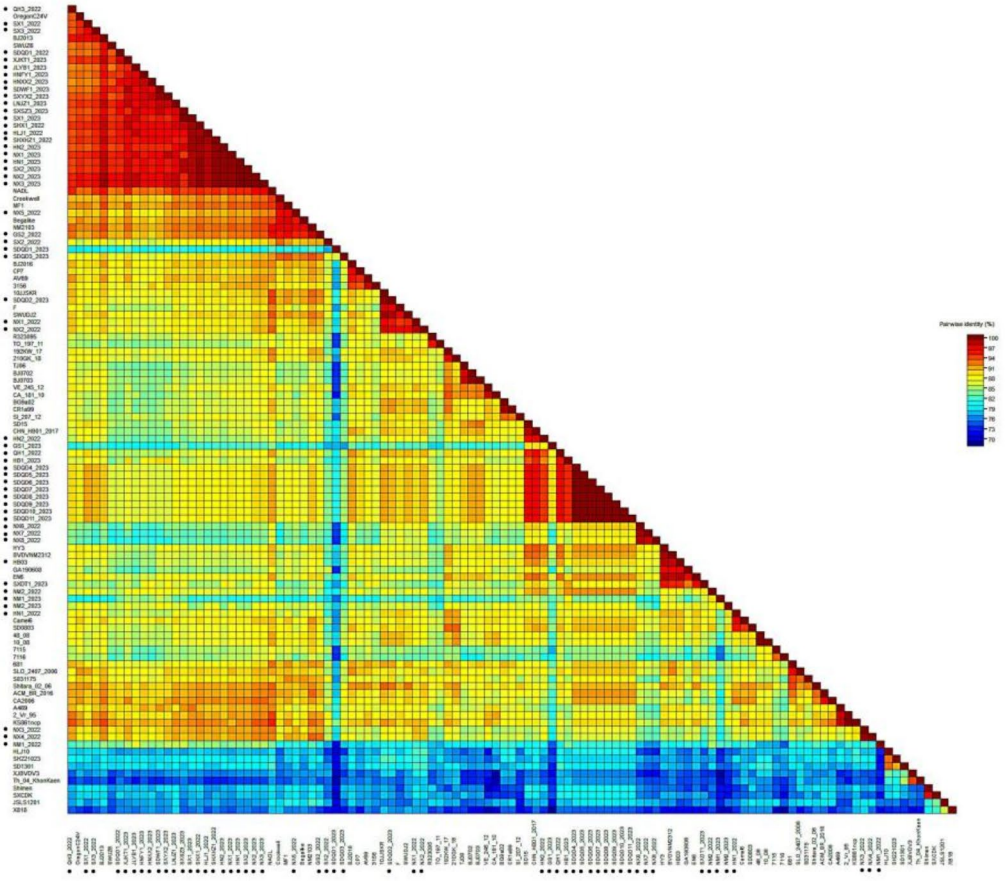

Fig. 2 The pairwise identity score matrix was generated by alignment 306 bp fragment of the 5'UTR gene for 53 BVDV sequencing sequences and 58 BVDV reference strains retrieved from the GenBank database

several months to years; some viruses can even be carried for a lifetime [17]. Clinical symptoms in these animals are often subtle, and the pathogen may not exhibit continuous proliferation. As a result, PI cattle serve as a primary source of BVDV in dairy farms. However, epidemiological studies on the PI of BVDV in China are still relatively sparse. Therefore, further screening of BVDV PIs within these herds is essential for assessing the impact of persistent infections on the prevalence of BVDV. According to previous reports, we found that a key factor contributing to the high risk of BVDV transmission in these regions may be the rapid promotion of large-scale dairy farming, which has resulted in the management equipment and disease prevention facilities not being upgraded in line with their growth. Dairy farms are under considerable pressure to handle fecal sewage treatment, which reduces the efficiency of the production environment and increases the spread of BVDV [27, 28].

We also conducted a study on the genetic diversity of BVDV prevalence in dairy cattle in northern China from 2022 to 2024. In this study, the analysis of the 5' UTR sequence revealed that BVDV-1a, 1c, 1d, 1 m, 1o, 1q, and 1v were the prevalent subgenotypes in northern dairy cattle from 2022 to 2024, whereas BVDV-2 and BVDV-3 were not detected. It is important to note that this study utilized a pair of universal primers for the simultaneous detection of BVDV-1 and BVDV-2. Therefore, there may be undetected cases of BVDV-2 in samples that failed sequencing. A review of reports from the past five years revealed that BVDV-1a, 1c, and BVDV-2 were widespread in dairy cattle in eastern China, whereas BVDV-1a, 1 m, 1q, and BVDV-2 were prevalent in the western dairy farms [17–19]. In addition, the newly discovered sub-genotype BVDV-1v has been detected in Shandong, Inner Mongolia, and Hubei, whereas BVDV-1w has only been reported in Tianjin [12, 29]. In our study, the detection frequency (84.62%) and local prevalence of the BVDV-1a sub-genotype occupied a dominant position, with a very obvious epidemiological advantage. The next most common sub-genotypes were BVDV-1 m (46.15%)

and BVDV-1c (30.77%). Therefore, based on the analysis of this study and the reports on BVDV in China over the past five years, it can be concluded that BVDV-1a, 1c, and 1 m are the dominant sub-genotypes in dairy cattle. This finding is consistent with the results of recent epidemiological surveys of BVDV in Chinese cattle herds [30].

The BVDV-1b sub-genotype was first isolated in China from farms that imported European cattle in 1980. Since then, some research data have shown that sub-genotype 1b is one of the major circulating genetic sub-genotypes in China [31, 32]. However, recent reports indicate that the dominant strains of BVDV in Chinese cattle herds have shifted, with BVDV-1a, 1c, and 1 m emerging as the commonly reported predominant strains. This change may be attributed to immune selection pressures caused by natural infections and the widespread vaccination in recent years [33]. The homology analysis of this study showed that the nucleotide similarity between the sequencing sequences of different sub-genotypes ranged from 70.2 to 92.1%. These observed variations in positive rates across different provinces may reflect the complex genetic diversity of BVDV in China, which can influence the prevalence of dominant epidemic strains. This diversity underscores the importance of understanding the regional epidemiology of BVDV for effective vaccination and control strategies [34]. The nucleotide similarity between different sub-genotypes and reference sequences ranged from 69.7 to 97.8%, which could be related to widespread trade contacts and the introduction into cattle herds. Currently, Australia is the primary source country for Holstein cattle in China [35]. Reports indicate that BVDV-1c is the predominant genotype in Australia [36]. In this study, 5 samples of BVDV-1c were detected, showing a homology range of 69.7–92.9% with Australian strains. Notably, the GS2 2022 strain identified in Gansu Province in 2022 exhibited a high homology of 92.9% with the Bega-like reference strain from Australia. In China, the distribution pattern of units that have adopted imported dairy cattle significantly influences the genetic evolution of BVDV. The overall genetic quality of dairy cattle in China is not high, mostly consisting of imported high-yielding cows and low-generation improved breeds of local dairy cattle. These imported cattle often originate from diverse regions globally, potentially introducing various strains of the virus. The main regions that import dairy cattle are found in Inner Mongolia, Shandong, Guizhou, Ningxia, and other places. The introduction of a new genotype of BVDV will impact the existing viral population, resulting in changes in the virus's genetic makeup over time. This dynamic is particularly significant in areas where imported dairy cattle are concentrated, highlighting the interconnectedness of the livestock trade and viral evolution. Therefore, identifying potential sources of BVDV transmission in dairy cattle is an increasingly important issue that deserves attention.

Conclusion

In conclusion, this study has updated the epidemiological diversity and identified the major sub-genotypes of BVDV in China over the past two years through regional surveys, filling the research gap in the molecular epidemiology of BVDV in the key traditional dairy farming areas of northern China. It helps to enhance our understanding of the variability and genotype distribution of BVDV in China, providing a theoretical basis for identifying transmission sources and predicting epidemic trends. As a global infectious disease, BVDV is still not cured and is prevented mainly through vaccination strategies. The long-term pressure of immune selection caused by vaccination may significantly influence the prevalence of BVDV. Vaccination without PI-culling is unable to prevent losses from BVDV infection. Therefore, conducting epidemiological studies on the prevalence of PI cattle in Chinese herds is necessary for the effective control and prevention of BVDV.

Methods

Clinical sample collection

Between June 2022 and June 2024, a total of 2,199 nasal swab samples were collected from 37 dairy farms across 13 provinces in northern China (including Shandong, Henan, Shanxi, Ningxia, Gansu, Qinghai, Heilongjiang, Shaanxi, Xinjiang, Hebei, Liaoning, Jilin, and the Inner Mongolia Autonomous Region), using a simple random sampling method. During the routine screening, most nasal swab samples were collected from clinically healthy animals. In this study, clinically healthy samples are defined as cattle that do not exhibit any symptoms of disease and are in good physical condition, and these animals have not been vaccinated against BVDV to ensure the purity of the samples. A small number of samples were obtained from cattle that died due to disease or diarrhea; these samples are used only for sequencing and are not included in the calculation of prevalence. Disposable sterile cotton swabs were used to sample at a depth of 10 cm inside the bovine nasal cavity. The details of the sampled animals are summarized in Table 1. The collected samples were stored in sterile plastic tubes containing 1 mL of PBS and transported to the laboratory under cold chain conditions, and frozen at -80 ℃ for future use. Prior to viral isolation and RT-PCR testing, the samples were vortexed for 30 s and then centrifuged at 8,000 r/min for 5 min to collect the supernatant.

RT-PCR

Take 1 mL of PBS buffer and resuspend the collected nasal swab sample in it. RNA was extracted from the

sample using the TIANLONG virus nucleic acid extraction kit. The RNA sample was dissolved in 80 μL of RNase-free water and stored at -80 ℃ until use. Based on the multiple alignment of the 5' UTR of the BVDV 1/2/3 reference sequence, two pairs of primers were designed for BVDV 1/2 and BVDV 3. The primers used were as follows: BVDV 1/2-TF: 5'-GAAGGCCGAAAAGAGG CTA-3'; BVDV 3-TF: 5'-ACTAGTGGTAGCAGTGAG CTCCT-3'; BVDV 1/2/3-TR: 5'-CTCCATGTGCCAT GTACAGC-3'. The sizes of the PCR products for the 5' UTR of BVDV 1/2 and BVDV 3 were 306 bp and 255 bp, respectively. Using the HiScript II One Step RT-PCR Kit (Vazyme, China), RNA was used as a template, and BVDV 1/2 and BVDV 3 universal primers were used for reverse transcription and amplification of the BVDV 5' UTR region. All PCRs were performed in a 50 μL volume containing 25 μL of 2× One Step Mix, 1 pg-1 μg of total RNA, 2 μL of gene-specific primer forward (10 μM), 2 μL of gene-specific primer reverse (10 μM), and RNase-free ddH2O to 50 μL. The PCR amplification products were stored at 4 °C or directly detected by electrophoresis on 1.5% agarose gel. All samples were tested with RT-PCR and all positive samples were subsequently sent for further analysis.

Sequence determination and systematic evolutionary analysis

The construction of the pMD19-T vector was carried out for 113 RT-PCR positive samples. All successfully constructed products (53 in total) were subjected to PCR amplification and electrophoretic analysis on a 1.5% agarose gel. Subsequently, sequencing was conducted by Qingke Biological's Qingdao Branch using Sanger sequencing technology. All sequencing reactions were completed by performing forward and reverse sequencing on both strands using the two pairs of primers designed above. During the sequencing process, the amplified fragments displaying double peaks were cloned and inserted into sequencing vectors. Positive clones were then selected, identified, and sequenced. The ClustalW algorithm from the Molecular Evolutionary Genetics Analysis software package, version 6.0 (MEGA 6.0), was used to align the sequences obtained from this study with the reference sequences of BVDV, and a phylogenetic tree for the 5' UTR region was constructed using the maximum likelihood method. In the phylogenetic tree, the evolutionary distances were computed using the Tamura-Nei parameter model. The robustness of the phylogenetic analysis and the significance of the branching order were assessed through 1000 bootstrap replicates. Reference sequences of the BVDV-1, BVDV-2, and BVDV-3 isolates were obtained from the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/genbank) for comparison (see Table 2). The 53 5' UTR sequences obtained in this study were submitted to GenBank and assigned an accession number (see Table 3). The percentage of sequence identity between BVDV isolates was calculated using the identity matrix function in BioEdit v.7.2.5 software.

Abbreviations

PCR	Polymerase Chain Reaction
RT-PCR	Reverse transcription–polymerase chain reaction
qRT-PCR	Quantitative reverse transcription–polymerase chain reaction

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Not applicable.

Author contributions

XYY, LY, WFX and SJL conceived and designed the experiments. XYY performed the experiments. XYY, LY wrote the manuscript and analyzed the data. CTY, DQQ, SXX contributed to the experiments work. JW, HT revised the manuscript.XLH contributed to the useful discussion. SJL, WFX finalized the manuscript. All authors read and approved the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Nasal swab sampling was conducted as part of disease diagnosis in accordance with the Domestic Animal Infectious Disease Control Law in China. The study obtained informed consent from the farm owners prior to sample collection. The materials used in this study comprised field samples collected during the clinical examination of animals, and these animals were not involved in any experimental studies.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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