

ORIGINAL RESEARCH



Genome Characterization and Phylogenetic Analysis of Bovine Hepacivirus in Inner Mongolia, Northeastern China

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Abstract

Objective: Bovine hepacivirus (BovHepV) is a new member of the genus *Hepacivirus* in the family *Flaviviridae*, which has been detected in cattle in more than seven countries. The purpose of this study was to identify and genetically characterize BovHepV in cattle in Inner Mongolia, northeastern (NE) China.

Methods: A total of 116 serum samples from cattle were collected from HulunBuir in Inner Mongolia from April to May, 2021, and were divided into three pools for metagenomic sequencing. The samples were verified with semi-nested RT-PCR with primers based on the BovHepV sequences obtained from metagenomic sequencing. The complete genomes of BovHepV were amplified, and were used for genome characterization and phylogenetic analysis.

Results: BovHepV was detected in two pools through metagenomic sequencing. Five BovHepV positive samples were identified in Yakeshi of HulunBuir, thus indicating a prevalence of 8.8% (5/57). Two 8840 nucleotide long BovHepV strains YKS01/02 were amplified from the positive samples and showed 79.3%–91.9% nucleotide sequence identity with the discovered BovHepV strains. Phylogenetic analysis classified the YKS01/02 strains into BovHepV subtype G group.

Conclusion: This study reports the first identification of BovHepV in cattle in northeastern China, and expands the known geographical distribution and genetic diversity of BovHepV in the country.

Key words: bovine hepacivirus, phylogenetic analysis, metagenomic sequencing, northeastern China, cattle

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INTRODUCTION

The genus *Hepacivirus*, belonging to the family *Flaviviridae*, comprises 14 genetically diverse viral species (*Hepacivirus* A–N) that infect humans and a wide variety of animals, including rodents, bats, shrews, dogs, equines, cattle, monkeys, and even birds and aquatic animals [1–10]. Bovine hepacivirus (BovHepV) is a newly identified hepatitis C virus (HCV)-like virus identified in cattle [11,12]. Like other hepaciviruses,

BovHepV has a single-stranded positive-sense RNA genome ~8.8 kb nucleotides in length, which encodes a single polyprotein and can be cleaved into structural proteins (Core, E1, and E2) and non-structural proteins (p7, NS2, NS3, NS4a, NS4b, NS5a, and NS5b) by proteases from the host and virus [6,13].

BovHepV was first discovered in German dairy cow serum through high-throughput sequencing technology in 2015 and was thereafter denoted as the hepacivirus

N species. The virus has been detected in more than seven countries in five continents, with viral RNA prevalence ranging from 0.6% to 14.8% [6,11–17]. In China, four BovHepV subtypes (three in genotype 1 and one in genotype 2) have been confirmed in the Guangdong, Yunnan, Jiangsu, and Sichuan Provinces, thus suggesting a complex genetic diversity and an extensive geographic distribution of the virus [11,12,14,18–20]. Notably, BovHepV has been detected in ticks collected from cattle in Guangdong Province, thus indicating that ticks may play an important role in the transmission of the virus.

To date, the public health risk of BovHepV remains unclear, and the potential zoonotic transmission risk also must be confirmed. Therefore, the distribution of the virus worldwide is necessary to aid in monitoring high-risk groups of people (e.g., cattle husbandry or slaughter workers) in BovHepV positive areas. To our knowledge, no epidemiologic study on BovHepV has been conducted in northeastern (NE) China. In this study, we identified and genetically characterized BovHepV in cattle in HulunBuir, Inner Mongolia, NE China, thereby expanding the known geographical distribution and genetic diversity of BovHepV in the country, and providing useful information regarding the prevalence and genetic characteristics of the virus.

METHODS

Sample collection

Hulunbuir (115°31′–126°04′ E and 47°05′–53°20′ N), located in the Inner Mongolia Autonomous Region in northeastern China, is in the boundary zone of Mongolia, Russia, and China. This area is well known for its prairie and animal husbandry and has a population of more than 1 million cattle and 8 million sheep (<http://tjj.hlbe.gov.cn/index/acticle/show/id/643.html>). In this study, blood samples of free-range cattle were collected from Ergun, Genhe, and Yakeshi in HulunBuir from April to May, 2021. Serum was separated from cattle blood by centrifugation at 500 rpm/min for 10 min, then stored at –80°C until use.

RNA library construction and metagenomic sequencing

A total of 116 free-range cattle serum samples were collected from HulunBuir in Inner Mongolia, NE China. The samples were divided equally into three pools, which were used for RNA library construction. After being digested with micrococcal nuclease (NEB, USA) at 37°C for 2 hours, the pooled serum samples were used for RNA extraction with a TIANampVirus RNA Kit (TIANGEN, China) according to the manufacturer's instructions and sent for metagenomic sequencing. Illumina sequencing and library construction were performed at Tanpu Biological Technology Co., LTD (Shanghai, China). Briefly, the RNA from each pool was used for library preparation with a NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, USA)

according to the manufacturer's instructions. After adapter ligation, ten cycles of PCR amplification were performed for sequencing target enrichment. The libraries were pooled at equal molar ratios, denatured and diluted to optimal concentrations, and sequenced with an Illumina NovaSeq 6000 System.

Transcriptome analysis and virus discovery

Downstream bioinformatics analyses of raw data were conducted as previously described [21]. Briefly, after trimming and removal of low quality reads, the paired-read reads were further purified by removal of ribosomal RNA, host contamination, and bacteria sequences with the BBDmap program (<https://github.com/BioInfoTools/bbmap>). The sequences were then assembled into contigs with SPAdes v3.14.1 (<https://github.com/ablab/spades>) and SOAPdenovo v2.04 (<https://github.com/aquaskyline/SOAPdenovo-Trans>) [22,23]. After comparison against the sequences in the non-redundant nucleotide (nt) and protein (nr) databases downloaded from GenBank with BLAST+ v2.10.0, the assembled contigs were further filtered to remove host and bacterial sequences. The relative abundance of the identified viruses was determined by mapping the reads back to the assembled contigs in Bowtie2 v2.3.3.1.

Viral detection and complete genome amplification

To verify the results of metagenomic sequencing, we used semi-nested RT-PCR with primers based on the BovHepV sequences obtained from metagenomic sequencing to identify the virus in all 116 samples. Viral RNA in serum samples was extracted with a TIANampVirus RNA kit (TIANGEN, China) and reverse transcribed with a PrimeScript 1st Strand cDNA Synthesis kit (TaKaRa, Japan) in accordance with the manufacturer's instructions. The specific primers of F (5′-GGCTCACCYTCACATGATCCCT-3′) and R1 (5′-ACCAAGGACATGATTCGCAAA-3′) for the first round, and F and R2 (5′-TGGAACCATT CGGCTTAACACT-3′) for the second round, were used for BovHepV detection. The PCR amplification system was as follows. Briefly, the 25-μL PCR reaction mixture comprised 12.5 μL of Premix Taq (TaKaRa), 1 μL each of F and R1/R2 primers, 1 μL of template cDNA, and 9.5 μL ddH₂O. The amplification parameters were 94°C for 5 min; 35 cycles at 94°C for 30 s, 50°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 10 min. To avoid cross-contamination, we carried out the following steps, as previously described [24]: (i) physical separation of the pre- and post-PCR areas, (ii) cleaning of work surfaces and equipment with 1% (vol/vol) sodium hypochlorite solution, (iii) decontamination of work surfaces and equipment with UV exposure, and (iv) analysis of negative controls to verify cross-contamination. Moreover, semi-nested PCR was also used to amplify the complete genome sequences of BovHepV from positive samples with the primers in Table S1.

Sequence comparison and phylogenetic analysis

The open reading frames were predicted with ORFfinder (<https://www.ncbi.nlm.nih.gov/orffinder>) and annotated as previously described [12]. BovHepV sequences were retrieved from the GenBank database; the downloaded nucleotide and amino acid sequences were aligned with ClustalW; and sequence identity was calculated in the MegAlign program within DNASTarV7.1. The phylogenetic relationships were estimated with the maximum likelihood method in MEGA version 7.0 [25] with default settings. We conducted a bootstrapping analysis of 1000 replicates. The bootstrap values >70% were considered significant and are shown in the trees.

Recombination analysis

Recombination events of BovHepV were detected with the RDP4 software package, which integrates seven recombination detection methods [26]. The analyses were performed with default settings, and recombination events were required to satisfy the following two conditions, according to the RDP manual's suggestions: (i) at least two methods showing a P value <0.05; and (ii) RDP recombination consensus score (RDPRCS) >0.60. If the recombination event met the first condition, but the RDPRCS was between 0.4 and 0.6, a possible recombination event was considered. Otherwise, the event was considered untenable. Additionally, to further confirm the recombination events, we analyzed the aligned BovHepV sequences with Simplot version 3.5.1 [19].

Ethical approval

This study was approved by the Animal Administration and Ethics Committee of the First Hospital of Jilin University. All cattle from which serum samples were collected were handled humanely, and all procedures were performed strictly in accordance with the requirements of the Animal Ethics Procedures and Guidelines of the People's Republic of China.

RESULTS

Sample collection and BovHepV identification

A total of 116 free-range cattle serum samples were collected from Yakeshi (57), Ergun (40), and Genhe (19) in HulunBuir in April and May, 2021 (Fig 1). After metagenomic sequencing, two pooled BovHepV positive samples were sequenced. A total of 1259 reads were annotated as sequences of BovHepV/JS/02, with a mean depth of 91.8× (Fig 2), and showed a nucleotide sequence identity of 91.9% and an amino acid sequence identity of 98.1%. To verify the results of metagenomic sequencing, we analyzed the virus in all 116 samples with semi-nested RT-PCR with primers based on the BovHepV sequences obtained from metagenomic sequencing. Five serum samples from Yakeshi were found to be BovHepV positive, thus indicating a prevalence of 8.8% (Fig 1). Given the high nucleotide identity (100%) of the sequences obtained from the five samples with the Sanger sequencing method, we amplified the complete genome sequences from only two samples

Prevalence of BovHepV

Ergun: 0% (n=40)

Genhe: 0% (n=19)

Yakeshi: 8.8% (n=57)

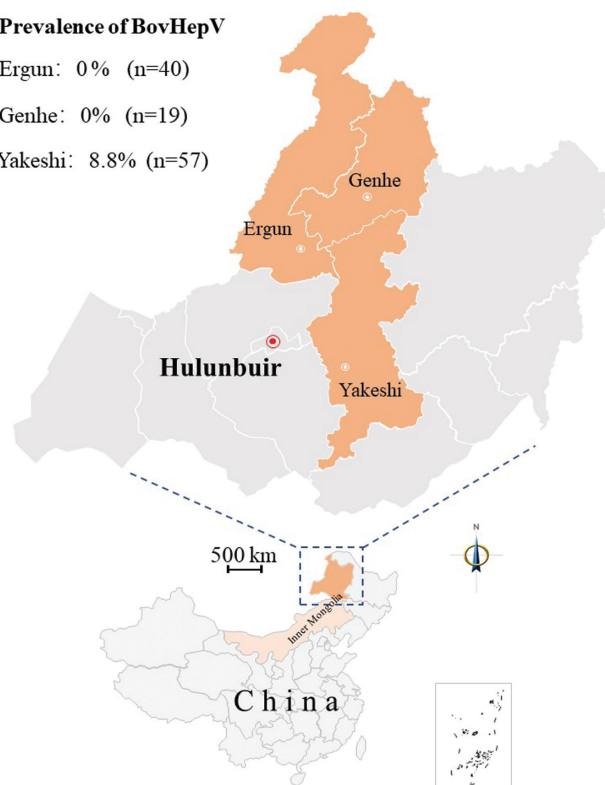


FIGURE 1 | Cattle serum sampling sites for the study in Hulunbuir, NE China. The orange shadowed areas represent the sampling sites.

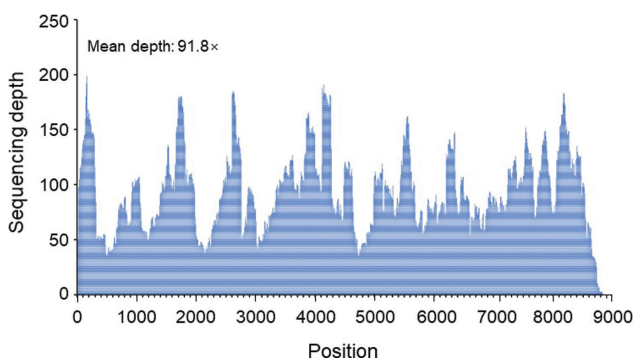


FIGURE 2 | Mapped read count plot of the sequenced BovHepV genome. The histograms show the depth of coverage for each base of the viral genome. The mean depth of the virus genome was 91.8×.

with the designed primers (Table S1) through semi-nested RT-PCR. The assembled BovHepV sequences were designated YKS01 and YKS02, with GenBank accession numbers of OM131409 and OM131410, respectively.

Viral genome analysis

The genomes of the BovHepV/YKS01/02 strains were 8840 nucleotides long and had an 8340-nucleotide-long polyprotein gene, with a genome organization of 5' UTR-core-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-3' UTR (Fig 3A). Similarity analysis indicated that the BovHepV/YKS01/02 strains shared 100% sequence identity at the

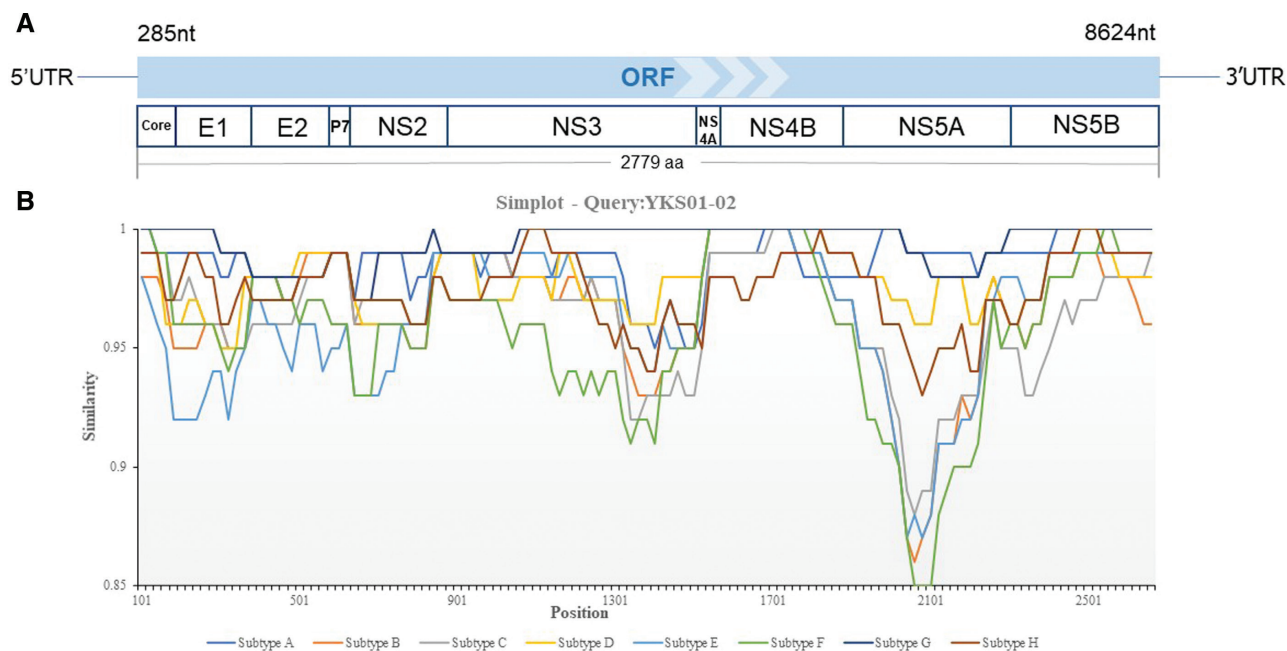


FIGURE 3 | Genomic characterization and SimPlot analysis of BovHepV, on the basis of amino acids of the polyprotein. A. Genome organization of BovHepV identified in this study. B. Simplot analysis of BovHepV subtypes A–H. The identified strains BovHepV/YKS01/02 were used as the query strains, and the analysis was calculated in Simplot version 3.5.1 with a sliding window of 200 and a step size of 20 residues. Different colors indicate different subtypes of BovHepV. The strain names and GenBank accession numbers of the BovHepV strains are listed in Table S2.

nucleotide level, and showed a range of 79.3%–91.9% nucleotide sequence identity and 92.5%–98.1% amino acid sequence identity with the discovered BovHepV sequences (Table 1). Moreover, the identified strains had a nucleotide identity of 91.5%–91.9% with subtype G strains isolated from Jiangsu Province, China, but had a lower nucleotide identity of 79.3%–84.7% with other subtype strains from Ghana, Germany, Brazil, and Guangdong Province in China (Table 1). On the basis of the criterion in which a novel subtype of HCV can be confirmed if it shows a nucleotide sequence identity of <85% with other strains [27], we concluded that the identified strains belonged to subtype G BovHepV. SimPlot analysis revealed the polyprotein amino acid similarity trends among the eight BovHepV subtypes (Fig 3). Notably, the sequences of the strains identified in this study had a lower amino acid similarity in NS5A protein, showing a low amino acid similarity of 81%–90% with B, C, E, and F BovHepV subtypes (Fig 3B).

Phylogenetic analysis and recombination analysis

The phylogenetic analysis results based on the amino acid and nucleotide sequences of the complete polyprotein of BovHepV subtypes A–H, including the sequences newly identified in this study, were similar in topology (Fig 4). The BovHepV strains were clearly separated into eight branches. The newly identified strains corresponded to the BovHepV subtype G isolates but were located in a different branch (Fig 4). On the basis of the different topology and nucleotide sequence identities (91.5–91.9%), we classified the novel isolates as subtype G2 to distinguish them from other subtype G strains discovered in Jiangsu Province, China. The results

of phylogenetic analysis were consistent with the identity analysis shown in Table 1. Moreover, recombination analysis based on RDP4 showed no potential recombination events among YKS01/02 strains and other BovHepV strains.

DISCUSSION

BovHepV, a novel hepacivirus first identified in Ghana and Germany in 2015, has been identified in at least seven countries in five continents [6,11,13–18,20], and has been classified into two genotypes and eight subtypes (belonging to genotype 1) [12], thus suggesting that the virus may have a wide geographical distribution and high genetic diversity. In China, BovHepV has been detected in southern and southwestern provinces, including Guangdong, Jiangsu, Yunnan, and Sichuan Province. To date, no epidemiologic study on BovHepV has been conducted in north or NE China, which includes many livestock farming provinces (e.g., Inner Mongolia, Jilin, and Heilongjiang Province). In this study, we identified subtype G BovHepV in HulunBuir, Inner Mongolia, NE China (Figs 1 and 4), thus providing the first evidence of the presence of BovHepV in cattle in NE China, and expanding the known viral distribution area in the country.

At least four BovHepV subtypes (three in genotype 1 and one in genotype 2) are circulating in China [11,12,14,18,20]. Notably, other countries with a high prevalence of BovHepV, such as Germany and Italy, have also been shown to have multiple genotypes or subtypes co-circulating, thus suggesting a complex global geographic distribution of BovHepV genotypes and subtypes. Although

TABLE 1 | Sequence identity of the BovHepV polyprotein gene at the nucleotide (upper right) and amino acid (lower left) levels, calculated with the MegAlign program in DNASTar V7.1. The compared strains are indicated by accession number and country, and different colors indicate different subtypes/genotypes of the BovHepV strains.

Strains	Subtype A						Subtype B			Subtype C			Subtype D			Subtype E			Subtype F			Subtype G			Subtype H		
	A1	A2	A3	A4	A5	A6	B1	B2	B3	C1	C2	D1	D2	E1	E2	F1	G1	G2	G3	G4	G5	H1	H2	H3			
A1 KP641123_BovHepV_B1Ger2013	93.3	93.8	91	93.6	90.8	80.1	79.8	79.9	80.3	80.4	82.4	82.5	79.5	79.5	80.1	84.7	84.7	84.4	84.5	84.5	84.5	81.9	81.9	81.9			
A2 KP641124_BovHepV_209Ger2014	97.9	93.1	91.6	93.5	90.9	79.9	79.8	80	80.5	80.7	82.5	82.5	79.5	79.5	80.1	84.7	84.7	84.5	84.5	84.5	84.5	81.8	81.8	81.8			
A3 KP641125_BovHepV_379Ger2014	98.2	98.1	91.4	93.6	91	80.2	79.8	80	80.3	80.5	82.5	82.8	79.5	79.5	80.1	84.6	84.6	84.3	84.3	84.1	84	81.7	81.7	81.7			
A4 KP641126_BovHepV_438Ger2014	97.5	97.4	97.4	91.7	90.5	79.6	79.7	80.1	80.2	80.3	82.2	82.3	78.9	78.9	79.6	84.2	84.2	84.3	83.9	83.9	81.8	81.8	81.8	81.8			
A5 KP641127_BovHepV_463Ger2014	98.1	98.1	98	97.5	90.9	80	79.9	80.2	80.3	80.6	82.5	82.9	79.6	79.6	80.3	84.3	84.3	84.4	84.5	84.5	81.9	81.9	81.9	81.9			
A6 MH027953_BovHepV_BH204/16-6	97.3	97.4	97.2	97.1	97.6	79.5	79.7	79.8	79.8	80.1	82.3	82.5	79.5	79.5	79.6	84.2	84.2	84	83.9	83.8	81.7	81.7	81.7	81.7			
B1 KP265950_BovHepV_GHC100	92.9	93.1	93.3	92.7	93	93	90.4	90.9	82.7	82.8	80.7	80.5	82	81.6	81.6	80.1	80.1	80.2	80	79.9	80.1	80.1	80.1	80.1			
B2 KP265948_BovHepV_GHC85	93.1	93.3	93.4	92.8	93.2	93.1	98.7	92	82.4	82.7	80.2	80.5	81.7	81.7	81.1	79.8	79.8	80	79.8	79.8	79.8	79.8	79.8	79.8			
B3 KP265943_BovHepV_GHC25	93	93	93.1	92.8	93.1	93	98.7	99	82.7	82.9	80.4	80.7	81.7	81.7	81.4	79.9	79.9	80.3	80	79.9	80.1	80.1	80.1	80.1			
C1 KP265946_BovHepV_GHC52	92.3	92.4	92.5	92.3	92.3	92	95.5	95.5	95.6	98.4	80.9	80.9	81.5	81.4	81.3	80.5	80.5	79.8	79.9	79.9	79.7	79.7	79.7	79.7			
C2 KP265947_BovHepV_GHC55	93	93.1	93.2	92.9	93.1	92.7	96.2	96.3	98.8	81.1	81.1	81.6	81.6	81.5	81.5	80.7	80.7	80	80.1	80.1	79.9	79.9	79.9	79.9			
D1 MG781018_Hepacivirus_N_BR_MA236B017	93.8	94.1	94.4	93.9	94.3	93.5	93.3	93.1	92.7	93.4	93.9	80.2	80.2	80	80	82	82	82.2	82.1	82.1	84.5	84.5	84.5	84.5			
D2 MG781019_Hepacivirus_N_BR_RN034B019	93.6	93.9	94.3	93.8	93.7	94	93.5	93.4	93.2	92.5	93.2	98.1	80.7	80.7	80	82.5	82.5	82.4	82.4	82.4	84.7	84.7	84.7	84.7			
E1 MG257793_BovHepV_GD/01	92.1	92.1	92.2	91.7	92.1	92	95.4	95.3	95.3	94.4	94.8	92.7	92.6	99.8	81	79.3	79.3	79.8	79.3	79.3	80.6	80.6	80.6	80.6			
E2 MG257794_BovHepV_GD/02	92.1	92.3	91.7	92.2	92.1	95.4	95.3	95.4	94.5	94.9	92.7	92.6	100	81	81	79.3	79.3	79.8	79.2	79.2	80.5	80.5	80.5	80.5			
F1 MH027948_BovHepV_BH18116-20	92	92.1	92.3	92	92.2	92.2	95.2	94.8	94.8	94.4	95	92.6	92.9	94.3	94.4	79.8	79.8	79.4	79.7	79.7	79.6	79.6	79.6	79.6			
G1 OM131409 Bovine hepacivirus YKS01	95.6	96	96.1	95.9	96	96.1	93.6	93.8	93.5	92.9	93.5	94.9	94.9	92.5	92.7	100	91.9	91.5	91.5	91.5	81.7	81.7	81.7	81.7			
G2 OM131410 Bovine hepacivirus YKS02	95.6	96	96.1	95.9	96	96.1	93.6	93.8	93.5	92.9	93.5	94.9	94.9	92.5	92.7	100	91.9	91.5	91.5	91.5	81.7	81.7	81.7	81.7			
G3 MN266283_BovHepV_JS02	95.3	95.6	95.7	95.6	95.7	95.8	93.4	93.4	93.2	92.7	93.2	94.7	94.7	92.5	92.5	97.9	97.9	92.8	92.7	92.7	81.6	81.6	81.6	81.6			
G4 MN266284_BovHepV_JS05	95.4	95.6	95.6	95.5	95.7	95.6	92.9	93.1	92.8	92.4	92.9	94.5	94.4	92.3	92.2	98.1	98.1	98	99.9	81.3	81.3	81.3	81.3	81.3			
G5 MN266285_BovHepV_JS06	95.3	95.5	95.5	95.3	95.6	95.5	92.8	93	92.7	92.4	92.9	94.4	94.3	92.3	92.1	97.9	97.9	97.9	99.8	81.3	81.3	81.3	81.3	81.3			
H1 MZ540979_BovHepV_GDZJ02-2	94	94.1	94.4	94	93.9	94.1	92.8	92.9	92.7	92.1	92.8	95.6	95.5	92	92.2	94.6	94.6	94.6	94.2	94.1	100	100	100	100			
H2 MZ221927_BovHepV_GDZJ02-1	94	94.1	94.4	94	93.9	94.1	92.8	92.9	92.7	92.1	92.8	95.6	95.5	92	92.2	94.6	94.6	94.6	94.2	94.1	100	100	100	100			
H3 MZ540980_BovHepV_GDZJ02-3	94	94.1	94.4	94	93.9	94.1	92.8	92.9	92.7	92.1	92.8	95.6	95.5	92	92.2	94.6	94.6	94.6	94.2	94.1	100	100	100	100			

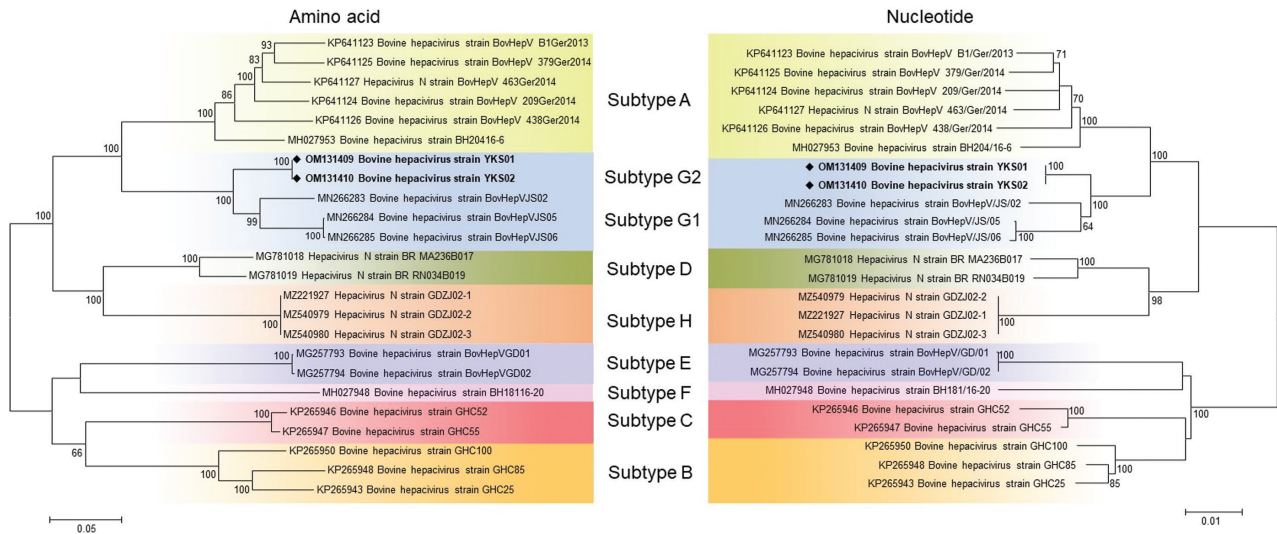


FIGURE 4 | Phylogenetic analysis based on the amino acid and nucleotide sequences of the complete polyprotein of BovHepV subtypes A–H, including the newly identified sequences in this study. The trees were constructed with the maximum likelihood method in MEGA 7.0 with default settings. A bootstrapping analysis of 1000 replicates was conducted, and bootstrap values >70% were considered significant and are shown in the trees. GenBank accession numbers are shown, followed by the names of the viral strains. Different colors indicate different subtypes of BovHepV, and a black rhombus represents the BovHepV identified in this study.

the novel BovHepV strains YKS01/02 were classified into subtype G, their different topology and low nucleotide sequence identity (91.5%–91.9%) from those of other subtype G strains further indicated the genomic diversity of BovHepV within the same subtype.

Except for the 5' UTR, which has been described to be conserved among BovHepV strains [11], our study indicated that the Core, p7, NS4A, NS4B, and NS5B proteins among BovHepV subtypes showed an amino acid identity of >95% (Fig 3), thereby suggesting that these protein encoding genes are relatively conserved and can be used as the target region for BovHepV nucleic acid detection. In contrast, NS5A protein showed relative low identity among genotypes, and is recommended to be used as a target protein gene for genotyping of BovHepV.

Because BovHepV is a novel hepacivirus species identified in recent years, the functions and effects of its proteins in pathogenesis remain unclear; however, mechanistic studies on HCV have provided a reference for studies on BovHepV. For instance, studies have confirmed that NS5A of HCV is a multifunctional protein associated with viral RNA replication and host cellular signaling pathway regulation [28–30]. The targeted inhibitors of NS5A have been verified to have good efficacy in the treatment of HCV [31].

Many epidemiological aspects require further investigation in these newly identified HCV-like viruses. First, more epidemiological studies should be conducted on different cattle species and in more countries practicing cattle husbandry. Second, whether BovHepV can cause hepatitis or other diseases in cattle must be determined, because HCV is a human pathogen that can cause human hepatitis and liver failure. Of note, the BovHepV RNA positive cattle in our study did not show any clinical symptoms. Moreover,

determining whether BovHepV is a zoonotic pathogen will be important. To evaluate the pathogenicity of BovHepV in humans, serological or molecular biological detection should be performed in cattle husbandry and slaughterhouse workers in BovHepV positive areas. Finally, because BovHepV strains have been detected in blood-sucking ticks collected from cattle [12], we suggest that more questing ticks in BovHepV prevalent areas should be collected and screened for the virus. The vector competence of ticks in transmitting BovHepV also should be validated, if possible. Remarkably, the presence of BovHepV has been confirmed in commercial fetal bovine serum [11]. Because bovine serum is one of the most widely used animal-derived biological reagents for cell culture, its viral contamination may have severe adverse effects on experimental results. Therefore, BovHepV must be detected in donor cattle, and positive groups and must be eliminated. In addition, commercial BovHepV positive fetal bovine serum should not be used in cell culturing.

This study has several limitations. First, limited bovine serum samples and collection sites were used in this study; therefore, the results may not be indicative of the prevalence and distribution of BovHepV in NE China. Moreover, we did not attempt to isolate the virus from positive samples, thus limiting further study on the virulence and pathogenicity of the virus. Finally, ticks in the surveyed sites including blood-sucking ticks from cattle, whereas questing ticks were not collected for BovHepV detection. The role of ticks in the transmission of BovHepV in northeastern China remains unclear.

CONCLUSION

In conclusion, BovHepV RNA was first detected in cattle in HulunBuir, Inner Mongolia, NE China, and a prevalence of 8.8% was found at the collection site. The findings

expand the known geographical distribution and genetic diversity of BovHepV in China, and provide useful information regarding the prevalence and genetic characteristics of the virus.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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