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Genomic characterization of two Chinese isolates of *Porcine respiratory and reproductive syndrome virus**

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Summary. The genomes of two isolates of *Porcine respiratory and reproductive syndrome virus* (PRRSV) from China, designated HB-1(sh)/2002 and HB-2(sh)/2002, were sequenced and analyzed. The size of the genomes of HB-1(sh)/2002 and HB-2(sh)/2002 were 15,411 and 15,373 nucleotides respectively, excluding the poly(A) tails. Comparative analysis with the genomic sequences of another Chinese isolate (BJ-4) and North American (VR2332) and European (Lelystad virus, LV) viruses revealed that HB-1(sh)/2002 shared 89.8% identity with BJ-4 and VR2332, but only 54.7% with LV; while HB-2(sh)/2002 shared 89.4% and 89.5% identity with BJ-4 and VR2332 respectively and 54.3% with LV, indicating that the two new Chinese isolates were related to the North American PRRSV genotype. Phylogenetic analysis based on the nucleotide sequence of the structural protein ORF's showed that the two new Chinese isolates belong to same genetic subgroup. HB-2(sh)/2002 additionally exhibited variations in the NSP2 nonstructural protein encoded by ORF1 and the structural protein GP3 encoded by ORF3 in comparison with other North American PRRSV isolates, namely a 12 amino acids deletion in Nsp2 and one amino acid deletion in GP3 were found in HB-2(sh)/2002. Therefore, HB-2(sh)/2002 was a novel strain with unique deletions.

Introduction

Porcine reproductive and respiratory syndrome (PRRS) was first recognized as a 'mystery swine disease' in the United States in 1987 [4, 14]. Since then, PRRS has become one of the most economically important diseases of swine worldwide

*The nucleotide sequences reported in this paper were deposited into the GenBank database with the accession numbers AY150312 and AY262352.

[6, 8, 9, 24]. PRRS virus (PRRSV), together with *Lactate dehydrogenase-elevating virus* (LDV), *Equine arteritis virus* (EAV) and *Simian hemorrhagic fever virus* (SHFV), is a member of the family *Arteriviridae* in the order of *Nidovirales* [5]. The genome of PRRSV is a single-stranded, non-segmented, positive-sense, polyadenylated RNA of approximately 15 kb in length that contains nine open reading frames (ORFs) [7, 15, 16, 3]. Sequence comparison of PRRSV isolates from different geographical regions indicated that there are two major genotypes represented by North American prototype VR2332 strain and European prototype Lelystad virus (LV), respectively [16–18]. Significant sequence differences were found among isolates of the same genotype [12], particularly in the Nsp1β and Nsp2 regions within ORF1a, ORF3 and ORF5 which encode components of the viral RNA polymerase, GP3 (a membrane associated protein) and envelope (E) protein, respectively [1, 2, 13, 16, 17, 19, 3, 24].

In China, a PRRS outbreak occurred in the end of 1995 and the disease has been one of the most significant problems for swine production, resulting in great economic losses each year. Persistent PRRSV infection, detected by respiratory symptoms and a high herd seropositive rate, is prevalent in most intensive pig farms in China. PRRSV was first isolated in China in 1996, however, the genomic characteristics of Chinese PRRSV isolates in comparison with North American and European isolates have not been comprehensively studied. In particular, it is important to determine the full-length genomic sequence of Chinese PRRSV isolates to investigate and understand the genetic diversity between PRRSV isolates in China and those from North America and Europe. In this paper, we report the complete genomic sequences of two Chinese isolates, HB-1(sh)/2002 and HB-2(sh)/2002, and compare them with the genomic sequences of another Chinese isolate (BJ-4) and the prototype strains of the North American and European genotypes.

Materials and methods

Virus isolates and cells

HB-1(sh)/2002 and HB-2(sh)/2002 were isolated from the sera of piglets from nurseries of two swine farms in Heibei province of China in December 2001. HB-1(sh)/2002 was from a farm which had an outbreak of PRRS in 1999. Subsequently, the chronic form of PRRS has persisted in this farm; nursery piglets continuously exhibit mild respiratory symptoms and the seropositive rate for PRRSV antibody (IDEXX ELISA kit) is approximately 40% in the nursery herd. HB-2(sh)/2002 was from a newly-built farm where an acute PRRS outbreak occurred in the middle of October 2001 during which sixty percent of pregnant sows had abortion during the last trimester of gestation and nursery herds presented with severe respiratory symptoms; the seropositive rate for PRRSV antibody was 80% in nursery piglets after the outbreak. No vaccination had been carried out on either farm. For virus isolation, serum specimens were collected and used to inoculate porcine alveolar macrophages (PAM's) prepared from the lungs of fifty-day SPF piglets. The PAM's were passaged twice [RPMI1640 medium supplemented with 10% fetal bovine serum (Hyclone)] prior to inoculation. Obvious cytopathic effects (CPE) were observed by 48 h post-inoculation and the presence of propagation was confirmed by RT-PCR using two pairs of primers [5′ AAT

GGC CAG CCA GTC AAT CA 3′ and 5′ GAA TCA GGC GCA CTG TAT GA 3′ (primers which amplify a conserved sequence within ORF7 of both European and American strains) and 5′ AAA AGT ACA GCT CCG ATG 3′ and 5′ GAA TCA GGC GCA CTG TAT GA (primers which are only able to amplify sequence within ORF7 from European strains)] and immunofluoresence using monoclonal antibody to PRRSV (provided by Iowa State University). The isolates were passaged twice on PAM's to produce the stocks used in this study.

Preparation of viral RNA

On day 3 post-inoculation when ∼95% of the PAM's in culture exhibited CPE, the cells subjected to one round of freeze-thawing at −20 °C prior to extraction of total cell RNA using LS TRIzol Regent(Invitrogen). Isolated total cell RNA was dissolved in Rnase-free water and stored in −80 °C.

Reverse transcription and polymerase chain reaction (RT-PCR) amplification

Oligonucleotide primers to amplify regions of the genomes of HB-1(sh)/2002 and HB-2(sh)/2002 were designed based on the genomic sequences of the VR2332, LV and BJ-4 strains (available from GenBank). These primers together with the lengths, sequence compositions and relative positions within the HB-1(sh)/2002 and HB-2(sh)/2002 genomes of the regions amplified are given in Table 1. For first strand cDNA synthesis, total infected cell RNA extracted from 250 μl of an infected cell culture (∼10^6 cells) was incubated at 90 °C for 5 min in a solution containing 1.25 mM of the appropriate reverse primer. After chilling, 0.5 mM of each dNTP and 10 units of AMV reverse transcriptase (Promega) were added to a total reaction volume of 20 μl containing 10 mM DTT, 50 mM Tris-HCl (pH 8.3), 75 mM KCl, and 3 mM $MgCl_2$. The reaction was incubated for 2 h at 42 °C followed by heat inactivation at 94 °C for 5 min. 5 μl of the products of the first-strand cDNA reaction was used as a template for PCR, which was performed for a total of 30 cycles using the hot start method in the presence of 0.5 mM each of forward and reverse primer (25 pmol/μl) and final concentrations of 0.2 μM of each dNTP, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl (pH 8.8), 2 mM $MgSO_4$, 0.1% Triton X-100 and 2.5 units Taq Polymerase. The amplification reaction protocol was as follows: denaturation at 94 °C for 40 sec, annealing at 53 °C to 58 °C for 40 sec and extension at 72 °C for 1 min per kb pair. PCR products were purified using QIAquick gel extraction kit (QIAquick) and cloned using the pGEM-T easy Vector System I (Promega) according to manufacturer's instructions. For each region amplified, three independent RT-PCR's and clonings were performed.

Amplification and cloning of 5′NCR of genome

A 5′ Full RACE Kit (TaKaRa)was employed to amplify the 5′ noncoding region (NCR) of the two virus isolates. Five primers were designed based on the genome sequence of BJ-4 and VR2332. The 5′ phosphorylated primer 5′ CAA AAG GGC AAA AGT 3′ was used as reverse primer to synthesize the first-strand cDNA. Then the RNA in DNA–RNA hybrid was degraded by treatment with RNase H, the single strand cDNA was circularized with T4 RNA ligase, and a nested inverse PCR was used to amplify the 5′NCR (primers 5′ AAT GCA CGT GGC AAC GTC CA 3′ and 5′ CTG CTG GCT TTC TGC GAT CT 3′ were used in the first reaction and primers 5′ ATG GTT AAA GGG GTG GAG AG 3′ and 5′ GTC GCT GGT ACC CCA TTG TC 3′ were used in the second reaction). As above, after purification the PCR products were cloned using pGEM-T easy Vector System I (Promega).

Table 1. Primers used for amplification of gene fragments of PRRSV HB-1(sh)/2002 and HB-2(sh)/2002

Fragment[1]	Location[1]	Length[1]	Upstream primer[2]	Downstream primer[2]
5′NCR	–	719	–	caa aag ggc aaa agt[a]
1st [b]	–	689	aatgcacgtggcaacgtcca	ctgctggctttctgcgatct
2nd [b]	–	345	atggttaaaggggtggagag	gtcgctggtaccccattgtc
A	30–780(1)	751(1)	ggc att tgt att gtc agg agc	cct ttc ggc cac ata cat gac
	30–781(2)	752(2)		
BC	711–2326(1)	1616	ccc ctt tga gtg tgc tat gg	tca ggg gga ccg agt tgt tg
	712–2327(2)			
D	2231–3192(1)	962(1)	cgc atc aga caa ccg aac aa(1)	agg gct ggt gtc att tag ta(1)
	2204–3234(2)	1031(2)	aat gtt gtg ctt cct ggg gtt ga(2)	tcg atg atg gct tga gct ga(2)
E	3044–3911(1)	868(1)	gcc cct gga ttt gtc tgc tt(1)	aca ggg aga tgg gag acg ag
	2945–3876(2)	932(2)	cga cac aaa ttt cag cag gt(2)	
F	3831–4657(1)	827	tcg gac ggt aaa aag aaa ag	cgg ctt ggt tgg ggt cat aa
	3796–4622(2)			
GH(1)	4562–6210(1)	1649(1)	aac cct ctg aaa aac cca tc(1)	agc aag caa ggc aca aag at(1)
G(2)	4527–5390(2)	864(2)	aac cct ctg aaa aac cca tc(2)	gcc aaa gag aaacca aca ac(2)
H(2)	5295–6175(2)	881(2)	tgcacccccctcaccatcctа(2)	agc aag caa ggc aca aag at(2)
I	6131–7016(1)	886	cgc tcg gtg atg tga aga tt	gcc acg gta tca gca aaa gc
	6096–6981(2)			
J(1)	6935–7795(1)	861(1)	gaa ttg aac ttg ctc agt tgg	agg gtg aag gtc cgg ttg tg
K(1)	7732–8603(1)	872(1)	gcg gct tgg ttg tta ctg ag(1)	gct ctg aat gtc ctt ggt tg(1)
JK(2)	6900–8568(2)	1669(2)	gaa ttg aac ttg ctc agt tgg(2)	gct ctg aat gtc ctt ggt tg(2)
L	8557–9430(1)	874	tgg ctg gaa taa atg gga ac	tta ctc gcc ttc atg tga ta
	8522–9395(2)			
MN	9318–10925(1)	1608	gca ata aca gac tcg cca tc	aat ggc gcg gag aga atc ta
	9283–10890(2)			
OP	10819–12512(1)	1694	tcg tgc tgg ata gaa ata ac	tga gcc acc aca tcc aaa ct
	10784–12477(2)	1694		
ORF2–3	12058–13531(1)	1474(1)	tag gat cca att gca atg aaa tgg	gag gaa ttc aga aga atg taa ata
	12023–13493(2)	1471(2)		
ORF4	12903–13810(1)	908(1)	ggc aag tct ctt tgg tgc	gcg gtc aag aat tcc tcc aac ata
	12868–13772(2)	905(2)		
ORF5	13744–14425(1)	682	agc ctg tct ttt tgc cat tct	ctt ttg tgg agc cgt gct atc
	13706–14387(2)			
ORF6–3′NCR(1)	14346–15447(1)	1102(1)	acc cct ata acc aga gtt tca(1)	Oligo(dT)36A(1)
ORF6(2)	14308–14896(2)	589(2)	acc cct ata acc aga gtt tca(2)	cat ccc cct tct ttc tct tct(2)
ORF7–3′NCR(2)	14715–15398(2)	696(2)	gcaaatgataaccacgcatttgtc(2)	ctcgaggcatgc(dT)22 (2)

[a]Reverse transcription primer used in 5′ full RACE

[b]Nested primers used to amplify 5′NCR by 5′ full RACE

[1]The location and length of each RT-PCR fragment with respect to the HB-1(sh)/2002 genome (1) and HB-2(sh)/2002 genome (2) are given

[2]Primers were used for RT-PCR amplification from both isolates with the exception of those designated (1) and (2) which were used in amplification from HB-1(sh)/2002 and HB-2(sh)/2002 isolates, respectively

Determination and analysis of nucleotide sequence

Automated sequencing reactions utilizing the BigDye terminator v2.0 kit (ABI) were resolved on a ABI PRISM 377-96 sequencer at Shanghai Sangon Bioengineering Ltd. Genomic analyses were conducted using computer software including the DNASTAR package (DNASTAR Inc.), DNAMAN (University of California). For each genomic region amplified, three clones from each of the three independent amplifications were sequenced in both direction; thus nine determinations of the sequence of each region were performed. The PRRSV strains and GenBank accession numbers of the sequences of these strains used for comparative analysis included BJ-4 (AF331831) [26], VR-2332 (U87392) [17], 16244B(NC_001961) [1], P129(AF494042), PA8(AF176348) [24], SP(AF184212), NVSL 97-7985 IA 1-4-2(AF325691), RespPRRS MLV(AF066183) [17], MLV RespPRRS/Repro (AF159149) [1] and Lelystad virus (M96262) [15].

Results and discussion

Genome organization for the HB-1(sh)/2002 and HB-2(sh)/2002 viruses

The sequences of RT-PCR generated cDNA fragments from the HB-1(sh)/2002 and HB-2(sh)/2002 viruses were assembled into consecutive sequence of 15,411 and 15,373 nucleotides respectively, excluding the poly (A) tails. The genome organizations of these viruses were similar to those of other PRRSV isolates. The 5′-noncoding region (NCR) was found to be 189 nucleotides in length for HB-1(sh)/2002, and 190 nucleotides for HB-2(sh)/2002 while the 3′-NCR for both viruses was 151 nucleotides long. The genomes of both viruses contained nine ORFs, two nonstructural protein ORF's (ORF1a and ORF1b) and seven ORF's encoding structural proteins (ORF2a, ORF2b to ORF7).

Sequence comparison of the HB-1(sh)/2002 and HB-2(sh)/2002 with other PRRSV isolates

The sequences of HB-1(sh)/2002 and HB-2(sh)/2002 were compared with those available for other PRRSV isolates, including five North American strains (VR2332, 16244B, PA8, NVSL, and P129), one European strain (LV), three vaccine strains (RespPRRSMLV, RespPRRS/Repro, and SP) and one Chinese isolate (BJ-4).

Genomic sequence: The genomic sequences of HB-1(sh)/2002 and HB-2(sh)/2002 shared only 54.7% and 54.3% nucleotide identity with LV, but higher identity (88.7% $\sim$ 92.0%) with North American isolates including VR2332, 16244B, PA8, NVSL, SP, RespPRRS MLV, MLV RespPRRS/Repro and P129. The sequence identity between HB-1(sh)/2002 and HB-2(sh)/2002 was 93%.

5′NCR: The 5′NCR of HB-1(sh)/2002 (189 nucleotides in length) shared 84.0% nucleotide identity with BJ-4 and VR2332 and 44.1% identity with LV, whereas the 5′NCR of HB-2(sh)/2002 (190 nucleotides in length) had a nucleotide identity of 93.1% with BJ-4 and VR-2332 and 48.1% with LV. The nucleotide identity of the 5′-NCR between HB-1(sh)/2002 and HB-2(sh)/2002 was 95.2%. The 5′NCR's

of these two viruses were characterized by a "T" deletion at the exact 5′ nucleotide, an "A" deletion at position 119 of the HB-1(sh)/2002 5′NCR (accounting for the one nt difference in length between the 5′NCR's of these two viruses), and a "C" insertion which occurs at position 129 of the HB-1(sh)/2002 and at position 130 of the HB-2(sh)/2002 5′NCR. Like previously reported PRRSV sequences [18], a conserved 12 nt motif at the 5′ end of the genome, strings of 8, 11 and 9 conserved nucleotides and a TTAACC motif are present in the 5′NCR of both HB-1(sh)/2002 and HB-2(sh)/2002.

ORF1a and ORF1b: ORF1a of PRRSV encodes a polyprotein predicted to be cleaved at 8 sites, producing 9 final products composed of Nsp1α, Nsp1β, and Nsp2-8, that are involved in viral RNA replication [10, 11, 20, 22, 27]. Our analysis results revealed that the ORF1a of HB-1(sh)/2002 and HB-2(sh)/2002 encoded polyproteins of 2503 and 2491 amino acids, respectively. The deduced amino acid sequence of Nsp1α was relatively conserved among the three Chinese isolates (HB-1(sh)/2002, HB-2(sh)/2002 and BJ-4; Table 2), while Nsp1β was one of the most variable regions within ORF1a. Nsp1β of HB-1(sh)/2002 shared 86.6% and 85.7% amino acid identity with VR2332 and BJ-4 but only 37.1% with LV; Nsp1β of HB-2(sh)/2002 shared 85.3% and 84.3% amino acid identity with VR2332 and BJ-4, respectively and 36.6% with LV. There was 85.7% amino acid identity between Nsp1β of HB-1(sh)/2002 and HB-2(sh)/2002. Within Nsp2, which is the most variable of the ORF1a proteolytic products, HB-1(sh)/2002 shared only 77.9% and 77.3% amino acid identity with VR2332 and BJ-4 and 26.4% with LV; HB-2(sh)/2002 had 78.7% and 78.3% amino acid identity with VR2332 and BJ-4 and 28.0% with LV; the amino acid identity of Nsp2 between HB-1(sh)/2002 and HB-2(sh)/2002 was 84.8% respectively. Interestingly, a 12 amino acid deletion was discovered within Nsp2 of HB-2(sh)/2002 (Fig. 1). This is the first finding of a deletion within Nsp2 of PRRSV, although a 36 amino acid insertion has been reported in the Nsp2 of SP [19]. Therefore, our results show that the Nsp2 of a wild type PRRSV isolates is able to tolerate deletions. Nsp3–8 of HB-1(sh)/2002 and HB-2(sh)/2002 were relatively conserved, sharing 89.2% $\sim$ 97.8% amino acid identity with VR2332 and BJ-4, but only 43.5% $\sim$ 75.0% with LV. ORF1b of HB-1(sh)/2002 and HB-2(sh)/2002 was found to encode a 1460 amino acid polyprotein with three predicated cleavage sites, producing 4 final products, Nsp9–12 [22]. As shown in Table 2, Nsp9–12 of HB-1(sh)/2002 and HB-2(sh)/2002 shared higher degree of amino acid identity with VR2332 and BJ-4.

The frameshifting region between ORF1a and ORF1b: The frameshifting region between ORF1a and ORF1b is located between nt 7680–7767 in the HB-1(sh)/2002 genome and between nt 7645–7732 of the HB 2(sh)/2002 genome. In both viruses, the sequence forming the stem-loop structure downstream of the slippery sequence at which frameshifting occurs has only three nucleotide changes as compared with VR2332 and BJ-4.

Structural protein genes: The genomic region encompassing ORF2a-7 is approximately 3 kb in length, covering the 3′ end of the genome and encoding all the structural proteins of PRRSV. Comparison of deduced amino acid identity of

Table 2. Amino acid identity (%) between the deduced amino acid sequences of the nonstructural and structural proteins of HB-1(sh)/2002 and HB-2(sh)/2002 with VR2332, BJ-4 and LV

ORF	Cleavage product	BJ-4[1]	VR2332[1]	LV[1]
1a	Nsp1α	94.0*	94.0*	66.3*
		97.0**	97.0**	66.9**
	Nsp1β	85.7	86.6	37.1
		84.3	85.3	36.6
	Nsp2	77.3	77.9	26.4
		78.3	78.7	28.0
	Nsp3	93.7	93.7	56.9
		95.3	95.3	57.1
	Nsp4	95.1	95.1	61.1
		96.1	96.1	62.1
	Nsp5	92.4	91.8	71.8
		93.5	92.9	70.6
	Nsp6	93.8	93.8	75.0
		93.8	93.8	75.0
	Nsp7	90.7	91.1	43.5
		89.2	90.3	46.1
	Nsp8	97.8	97.8	66.7
		97.8	97.8	66.7
1b	Nsp9	97.7	98.0	74.3
		97.4	97.7	74.6
	Nsp10	95.7	95.7	63.5
		96.1	95.9	64.2
	Nsp11	95.1	94.2	77.6
		95.1	94.2	75.8
	Nsp12	95.4	95.4	38.0
		94.8	94.8	37.3
Structural proteins	GP2a	93.0	93.8	61.0
		91.0	91.8	57.8
	ORF2b	89.0	90.4	67.1
		91.8	93.2	68.6
	GP3	89.4	89.0	56.9
		88.9	88.5	56.5
	GP4	89.3	89.9	69.1
		88.8	88.8	70.2
	GP5	87.0	88.5	56.6
		87.5	88.5	57.4
	M	97.7	97.7	79.8
		97.7	97.7	78.6
	N	96.7	96.7	65.0
		95.1	95.1	63.2

[1]In each box, the upper figure is the percentage identity with HB-1(sh)/2001 (*) and the lower figure is the percentage identity with HB-2(sh)/2002 (**)

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VR2332 Nsp2  APRRKVGSDCGSPVSLGGDVPNSWEDLAVSSPFDLPTPPEPATPSSELVIVSSPQCIFRPATPLS      519
BJ-4   Nsp2  ----------------------------------------L-----------------------      519
HB-1   Nsp2  ------R-G-----LM-DN---GS---T-GG-LNF---S--M--M--P-LTPAL-RVPKLM---D      519
HB-2   Nsp2  ------R--P-KSIL.............--GG-LNFS--S-LV--LG-P-LMPAS-HVS--V----      507
LV     Nsp2  PSDPMKENMLN-REDEPL-LSQPAPASTTTLVREQTPDNPGSDAGALP-T-REFVPTGPILCHVE      431

VR2332 ORF3  IYEPGRSLWCRIGYDRCGEDDHDELGFMIPPGLSSEGHLTGVYAWLAFLSFSYTAQFHPEIFGIG      130
BJ-4   ORF3  -----------------E----------V----------S-----------------------      130
HB-1   ORF3  -----K-------H-----N--------V----------S-----------------------      130
HB-2   ORF3  V------------H-------------V-----.-----S-----------------------      129
LV     ORF3  RL----NM--K--H---E-R-----LMS--S-YDNLK.-E-Y----------A------L----      129
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Fig. 1. Alignment of the partial deduced amino acid for of the Nsp2 and GP3 genes of the HB-1(sh)/2002, HB-2(sh)/2002, VR2332, BJ-4 and LV strains of PRRSV. Conserved residues are indicated by dashes; deleted amino acids by dots. A reference residue number within each gene is given

ORF's 2a-7 of HB-1(sh)/2002 and HB-2(sh)/2002 with those of VR2332, BJ-4 and LV is summarized in Table 2. The most pronounced variability was found within ORF5. Additionally, a "Ser" in position 100 of GP3 encoded by ORF3 of other PRRSV isolates was deleted in HB-2(sh)/2002 (Fig. 1).

3′NCR: Like all North American isolates of PRRSV, the 3′-NCR of HB-1(sh)/2002 and HB-2(sh)/2002 was found to be 151 nucleotides long, differing from the 3′NCR of LV which is only 114 nucleotides in length. The 3′NCR of HB-1(sh)/2002 contains a deletion and an insertion in the 3′NCR conserved motif together with two point mutations (a deletion outside of this motifs conserves the total length of the 3′NCR of this virus).

Analysis of amino acids predicated to be involved in pathogenesis

By comparing the deduced amino acids sequence of pathogenic strain 16244B with those of VR2332 and MLV RespPRRS/Repro, R. Allende et al. reported five amino acid mutations in the structural protein coding regions and four amino acid mutations in nonstructural protein coding regions respectively that might be responsible for the attenuated phenotype of the vaccine strains [2]. When the corresponding nine amino acids in HB-1(sh)/2002 and HB-2(sh)/2002 were compared with those of PRRSV strains from China and USA (BJ-4, VR2332, 16244B and RespPRRS/Repro), the residues were identical with those found in wild type strains (16244B and VR2332), with exceptions of K151 in GP5 of HB-1(sh)/2002 and Q952 within Nsp10 and Q13 within GP5 of HB-2(sh)/2002, and different from those in the RespPRRS/Repro vaccine strain (the nine residues in BJ-4 were identical to those found in the vaccine strain except for E952 within Nsp2).

Phylogenetic analysis

To understand the genetic relationship of HB-1(sh)/2002 and HB-2(sh)/2002 with other PRRSV isolates, a phylogenetic tree based on sequences of the seven

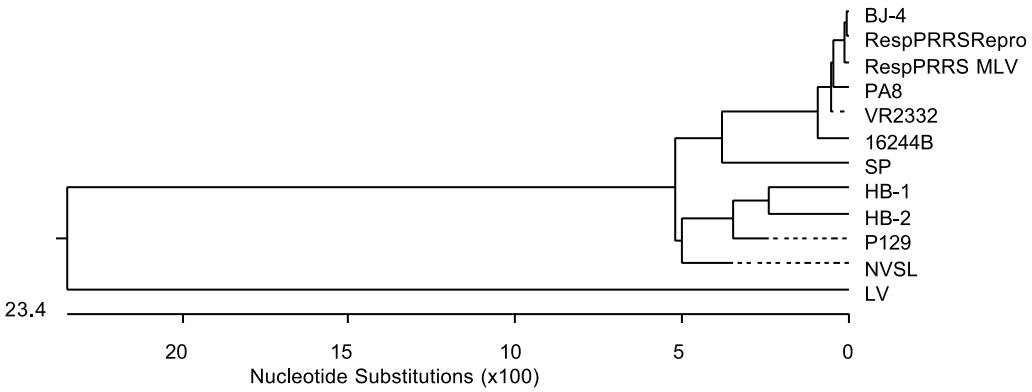

Fig. 2. Phylogenetic analysis of PRRSV isolates. The phylogenetic tree was constructed using the the Clustal W method (MegAlign 5.00, DNASTAR Inc.) based on the deduced amino acid sequence of the seven structural protein genes of these isolates

structural protein genes of ten isolates. As shown in Fig. 2, all three Chinese viruses were in the North American genotype with HB-1(sh)/2002 and HB-2(sh)/2002 forming a subgroup and the BJ-4 isolate belonging to another subgroup closely related to the vaccine strain RespPRRS/Repro, suggesting that this strain may be evolved from a revertant of the vaccine virus.

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