Pathogenic Pseudorabies Virus, China, 2012

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In 2012, an unprecedented large-scale outbreak of disease in pigs in China caused great economic losses to the swine industry. Isolates from pseudorabies virus epidemics in swine herds were characterized. Evidence confirmed that the pathogenic pseudorabies virus was the etiologic agent of this epidemic.

Pseudorabies virus (PRV), also called Aujeszky disease virus or suid herpesvirus type 1, is a member of the *Alphaherpesvirinae* subfamily within the family *Herpesviridae*. This pathogen has major economic consequences in pig husbandry (*1–3*). The PRV genome is a double-stranded linear DNA molecule ≈143 kb long and contains at least 72 genes (*1*,*4*). PRV can infect many kinds of mammals, including ruminants, carnivores, and rodents (*2*,*3*,*5*). However, pigs have been confirmed to be the primary hosts and reservoir of this virus (*6–8*). PRV infection is characterized by nervous system disorders and death in newborn piglets, respiratory disorders in older pigs, and reproductive failure in sows (*7*,*8*). Like other α herpesviruses, PRV infection can be a lifelong latent infection in the peripheral nervous systems of infected pigs, and these latently infected pigs can infect others under certain conditions (*7–9*). In this way, PRV causes devastating disease in pigs and economic losses worldwide.

Vaccination of pigs with attenuated live or inactivated vaccines is widely performed to reduce the huge economic losses caused by PRV infection (*10–12*). Although vaccination confers protection against disease, it does not prevent infection from a wild-type strain. Thus, both the virus in the vaccine and the super-virulent wild-type strain can establish latency within the same animal (*13–15*).

We report an outbreak of PRV infection that devastated the swine-producing regions of China in 2012. We systematically investigated the outbreak to identify the causative agent.

The Study

In January 2012, a previously unknown severe disease was observed in pigs on several farms in northern and eastern China. In Shandong Province, >80,000 pigs were infected. The affected pigs had high fever (≥40.5°C), anorexia, coughing, respiratory distress, conjunctival serous and mucinous secretion, and posterior paralysis. The disease was first observed in older pigs and spread within 2–3 days to younger pigs. Duration of disease was 5–7 days. Rate of illness reached 50%, and mortality was 3%–5%. Most pig deaths were recorded on the third day after monitoring began. Abortion was observed in ≈35% of sows that were 70–90 days pregnant. Viscera (e.g., lung, kidney, heart, liver, and spleen) and serum samples were collected from dead pigs from different provinces. Pathologic examination showed the most striking gross lesions were consolidated in the lungs (Figure 1, panel A), with edema and hemorrhage (Figure 1, panel B). In addition, foci of yellow-white necrosis were observed in the kidneys of some dead pigs (Figure 1, panel C).

To gain insight into the etiologic agent of the disease, we conducted extensive and systematic diagnostic testing, including PCR, ELISA, viral isolation, immunohistochemical staining, and standard bacteriologic culture, to evaluate the specimens. Marc-145 cells, inoculated with various tissue homogenates, showed cytopathic effects. A specific PRV monoclonal antibody was used, and immunopositive cells were observed in infected tissue (online Technical Appendix Figure 1, panels A, B, wwwnc.cdc.gov/EID/article/20/1/13-0531-Techapp1.pdf). The PCR for inocula samples showed that many glycoprotein (g) genes of PRV could be amplified by using the primers specific to the unique gene fragments (online Technical Appendix Figure 2). The PRV gE-ELISA assays (IDEXX Laboratories, Westbrook, ME, USA) indicated that serum samples from the sick pigs contained antibodies against wild-type, virulent PRV glycoprotein E but not against the vaccine strain (Table). All these results indicated that PRV was the causative agent of this disease. Our results also ruled out other suspected agents, such as classical swine fever, African swine fever, porcine reproductive and respiratory syndrome virus, and some bacterial infections. The 3 isolates found here are referred to as NVDC-PRV-BJ, NVDC-PRV-HEB, and NVDC-PRV-SD, according to the provinces from which they were isolated.

The pigs vaccinated with attenuated live PRV vaccines still showed clinical signs of PRV during the outbreak. To confirm the presence of PRV in these herds, 15 pigs were vaccinated with the current vaccine strain and then challenged

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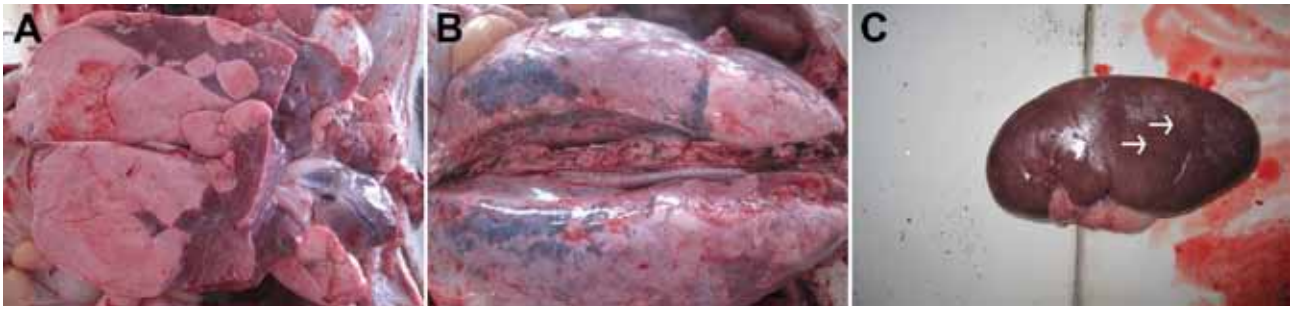

Figure 1. Necropsy specimens from pigs infected with pseudorabies virus. A) Pulmonary consolidation in the lung. B) Edema and hemorrhage of lung. C) Kidney with many yellow-white necrotic spots (arrows).

with the NVDC-PRV-SD strain 21 days after vaccination. The results indicated that the vaccinated pigs had not been given completely effective protection against infection and exhibited obvious clinical signs of disease similar to the typical symptoms observed in the field, suggesting that the virulence of the newly isolated PRV strains had changed. This virulence may have caused the deaths of infected pigs.

To better understand the genetic relationship of the 3 PRV isolates found here to other PRV isolates, we amplified and sequenced the 15 major genes of the 3 isolates (online Technical Appendix Table 1). Compared with other PRV isolates (online Technical Appendix Table 2), there was a 21-nt insertion from nucleotide positions 185–205 in the gC gene, similar to SA215, BJ, DG, Ea, Fa, and P-PrV strains (Figure 2, panel A), which shared 100% nt identity with each other. The gD gene, like isolates Ea, Fa, SA215, and Yangsan, had 6 nt at positions 801–806 and shared 99.4%–99.8% identity with each other (Figure 2, panel B). There were 2 insertions of 6 discontinuous nucleotides each at positions 138–140 and 1472–1474 in the gE gene (Figure 2, panel C). These had 99.9%–100% identity with each other. The rest of the genes from the 3 isolates had no nucleotide insertions or deletion in common with the other PRV isolates. We further analyzed the relationship of these 3 isolates with other PRV isolates using a phylogenetic tree based on the gE gene; the 3 isolates formed a tightly clustered branch and were very closely related to other isolates from Asia (online Technical Appendix Figure 3).

Conclusions

We describe and analyzed a major outbreak of PRV in pigs in China. In these herds, all pigs had been vaccinated against PRV 3 times a year, at approximately the same time as each other. The disease spread to >6 provinces

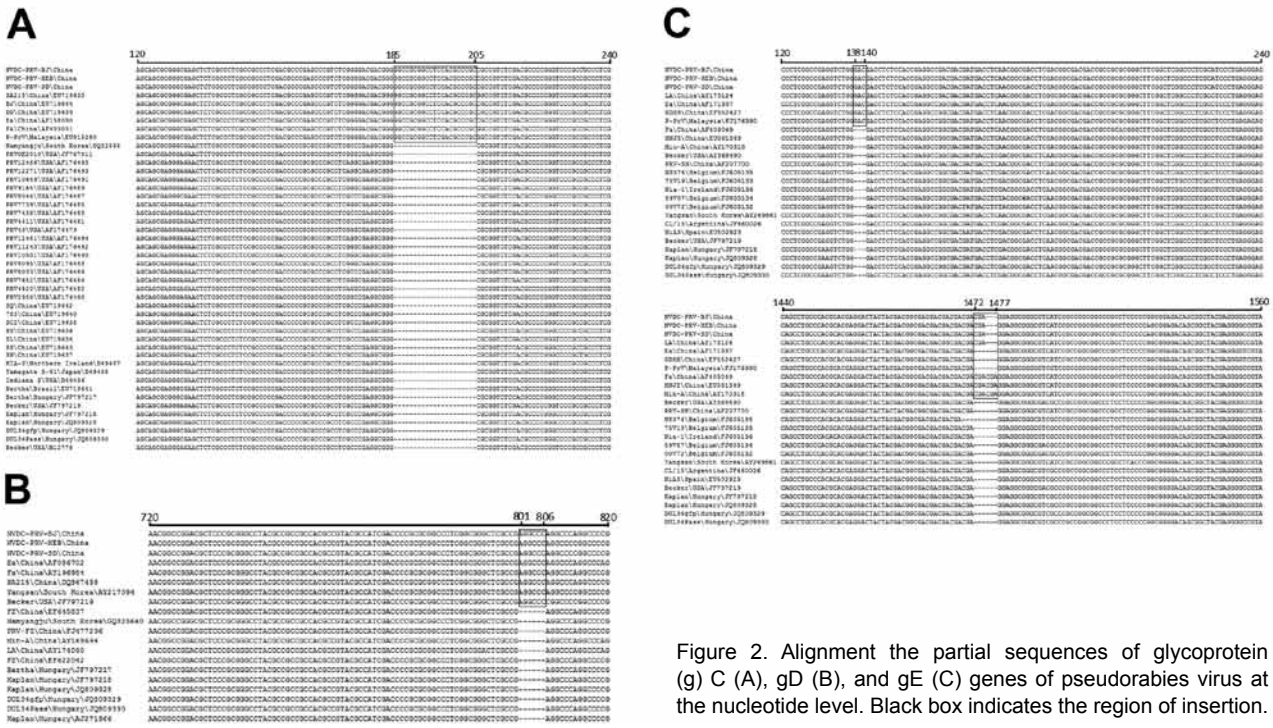

Figure 2. Alignment the partial sequences of glycoprotein (g) C (A), gD (B), and gE (C) genes of pseudorabies virus at the nucleotide level. Black box indicates the region of insertion.

Table. Antibodies to PRV gE in serum from PRV-infected pigs from different provinces, China, 2012

Sample origin province	Collection date	gE ELISA S/N ratio*
Shandong	2012 Jan	0.115
Beijing	2012 Feb	0.240
Hebei	2012 Feb	0.169
Tianjin	2012 Feb	0.171
Liaoning	2012 Mar	0.168
		0.274
		0.168
		0.173
		0.177
		0.179
		0.168
		0.158
		0.143

*S/N ratio was calculated as a ratio of the absorbance of a well with serum to the absorbance of a control well without serum. Serum with an S/N ratio of ≤0.60 was considered positive. PRV, pseudorabies virus; g, glycoprotein.

(including autonomous cities and regions) and caused considerable economic losses among local pig farms.

PRV has been recognized as a source of infection for pigs and continues to circulate globally among herds (*9*). In particular, pigs receiving attenuated live PRV vaccines showed typical clinical symptoms, which suggest that these isolates might have evolved new types of pathogenicity.

Even though the PRV isolates showed nucleotide insertions in the gC, gD, and gE genes, the molecular mechanisms underlying their high pathogenesis have yet to be elucidated. The origin of these lethal isolates within China is still obscure, although phylogenetic trees based on the gE gene here indicated that the 3 isolates are more closely related to the Asia PRV isolates, especially the China isolates, than to isolates from other countries. Because the virulence and origin of PRV is thought to be associated with multiple factors, whether such insertions are related to the virulence of PRV remains an issue and requires further investigation.

In summary, our study indicates that an outbreak of disease in pigs in China, which was of unprecedented scale, was caused by PRV infection. Other pathogens were ruled out. Our findings highlight the need to prevent and control the spread of this virus.

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Technical Appendix

Technical Appendix Table 1. PRV different target genes and primers in this study*

Reference PRV Kaplan strain target gene nucleotide length, bp	Primer sequences (5′→3′)	Amplicon, bp
gE	Forward primer (gE1F): ATGCGGCCCTTTCTGCTGCGCG	611
gD	Reverse primer (gE611R): ACAAAGAACACGGCCCGCGG	493
TK	Forward primer (gE592F): CCGCGGGCCGTGTTCTTTGT	568
gH	Reverse primer (gE1084R): CGTGGCCGTTGTGGGTCAT	634
gL	Forward primer (gE1066F): ATGACCCACAACGGCCACGTC	589
gM	Reverse primer (gE1734R): TTAAGCGGGGCGGGCATTCAACAGG	566
gN	Forward primer (gD1F): ATGCTGCTCGCAGCGCTATTGG	534
gB	Reverse primer (gD634R): TGAAGCTCTCGTCGTTCCAGC	658
gC	Forward primer (gD614F): GCTGGAACGACGAGAGCTTCA	474
gG	Reverse primer (gD1203R): CTACGGACCGGGCTGCGCTTTTA	773
gI	Forward primer (TK1F): ATGCGCATCCTCCGGATCTACCTC	471
gK	Reverse primer (TK566R): ACCAGCATGGCGTAGACGTTGC	574
PK	Forward primer (TK545F): GCAACGTCTACGCCATGCTGGT	628
11K	Reverse primer (TK1079R): TTTATTGGGATGACATACACATAGC	297
28K	Forward primer (gH1F): ATGCCCGCGTCGTCCGTGCGCCT	717
	Reverse primer (gH658R): AGCCGAGCGTGTACACGTGGAAG	732
	Forward primer (gH636F): CTTCCACGTGTACACGCTCGGCT	598
	Reverse primer (gH1310R): GAGAGGACGAGCAGGCTGAA	670
	Forward primer (gH1288F): TTCAGCCTGCTCGACGTCCTCTC	717
	Reverse primer (gH2061R): TCACGACCTGGCGTTTATTAACC	745
	Forward primer (gL1F): TTACTCGGCGGGGGGCCCTCCAT	728
	Reverse primer (gL471R): ATGTCGCCGCTCGTGGCGGTGCT	791
	Forward primer (gM1F): TTATTCAAAGCCGAGGTTCTCG	576
	Reverse primer (gM574R): CGTCGTGACGAACGTCGTCCT	547
	Forward primer (gM554F): AGGACGACGTTCGTCACGACG	939
	Reverse primer (gM1182R): ATGTGCGGGCCGCGCAACGCCGA	551
	Forward primer (gN1F): ATGGTCTCCTCCGCCGGGCTCT	666
	Reverse primer (gN297R): TTATACGCGCCGCCCCGCGG	297
	Forward primer (gB1F): CTACAGGGCGTCGGGGTCCTC	771
	Reverse primer (gB717R): GAGGACTACAACTACGTGCGCAT	
	Forward primer (gB695F): ATGCGCACGTAGTTGTAGTCCTC	
	Reverse primer (gB1427R): GCTACAACAGCACGCACGTGCT	

Forward primer (gB1406F): AGCACGTGCGTGCTGTTGTAGC
Reverse primer (gB2095R): GTGCGTCTCCAAGGCCGAGTACGT
Forward primer (gB2072F): ACGTACTCGGCCTTGGAGACGCAC
Reverse primer (gB2742R): ATGCCCGCTGGTGGCGGTCTTTG
Forward primer (gC1F): ATGGCCTCGCTCGCGCGTGCG
Reverse primer (gC717R): GAACTCGGGCTGGCTGTACAGGA
Forward primer (gC695F): TCCTGTACAGCCAGCCCGAGTTC
Reverse primer (gC1440R): TCACGGCCCCGCCCGGCGGTAGTA
Forward primer (gG1F): ATGAAGTGGGCAACGTGGATC
Reverse primer (gG728R): TCGTAGTAGTCGGCGTAGTCGTC
Forward primer (gG706F): GACGACTACGCCGACTACTACGA
Reverse primer (gG1497R): TCAGGCGGAGGCCACGTGGCGGT
Forward primer (gI1F): ATGATGATGGTGGCGCGCGACGT
Reverse primer (gI576R): CTGTCGTGCCACGATCCGACGA
Forward primer (gI554F): TCGTCGGATCGTGGCACGACAG
Reverse primer (gI1101R): TTATTGTTCCTCTGCGATGGTG
Forward primer (gK1F): TCATCCAAATATGGTAATGTGCCG
Reverse primer (gK939R): ATGCTCCTCGGCGGGCGCCCGCT
Forward primer (PK1F): ATGTTGGCGATGTGGAGATGG
Reverse primer (PK551 R): CTGTACAGATCGCACCGAAAGT
Forward primer (PK530F): ACTTTCGGTGCGATCTGTACAG
Reverse primer (PK1196R): TTATACGGTCCACATTCCAAAG
Forward primer (11K1F): ATGGACACGTTCGACCCCAGC
Reverse primer (11K297R): CTACACGTGCCGGGCGATGAT
Forward primer (28K1F): ATGGGGGTGACGGCCATCACCGT
Reverse primer (28K771R): CTAGGAGATGGTACATCGCG

*PRV, pseudorabies virus; g, glycoprotein.

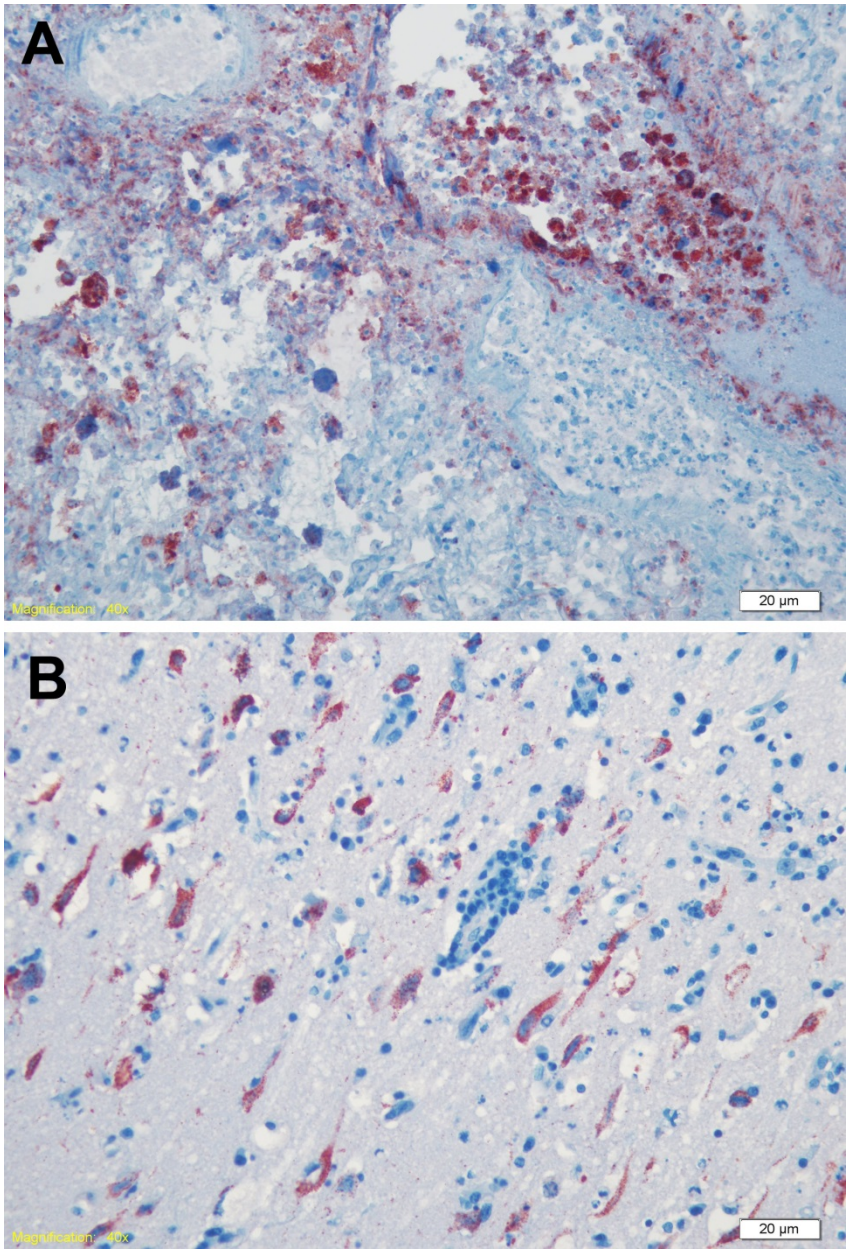
Technical Appendix Table 2. PRV sequences obtained from GenBank used in this study*

Sequence coding for	Strain	GenBank accession no.	Origin
gC	Namyangju	GQ325659	South Korea
gD	PRV OK2010	JF767011	USA
gE	PRV12486	AF176495	USA
	PRV12271	AF176493	USA
	PRV10649	AF176491	USA
	PRV9164	AF176489	USA
	PRV8044	AF176487	USA
	PRV7739	AF176485	USA
	PRV7438	AF176483	USA
	PRV4411	AF176481	USA
	PRV43	AF176479	USA
	PRV12481	AF176494	USA
	PRV11243	AF176492	USA
	PRV10501	AF176490	USA
	PRV8095	AF176488	USA
	PRV8033	AF176486	USA
	PRV7652	AF176484	USA
	PRV4520	AF176482	USA
	PRV2908	AF176480	USA
	Ea	AF158090	China
	Fa	AF403051	China
	P-PrV	EU915280	Malaysia
	BJ	EU719644	China
	SQ	EU719642	China
	783	EU719640	China
	SCZ	EU719638	China
	HS	EU719636	China
	SL	EU719634	China
	SS	EU719643	China
	Bartha	EU719641	Brazil
	DG	EU719639	China
	SN	EU719637	China
	SA215	EU719635	China
	NIA3	D49437	Northern Ireland
	Yamagata S-81	D49435	Japan
	Indiana S	D49436	USA
	Bartha	JF797217	Hungary
	Kaplan	JF797218	Hungary
	Becker	JF797219	USA
	Kaplan	JQ809328	Hungary

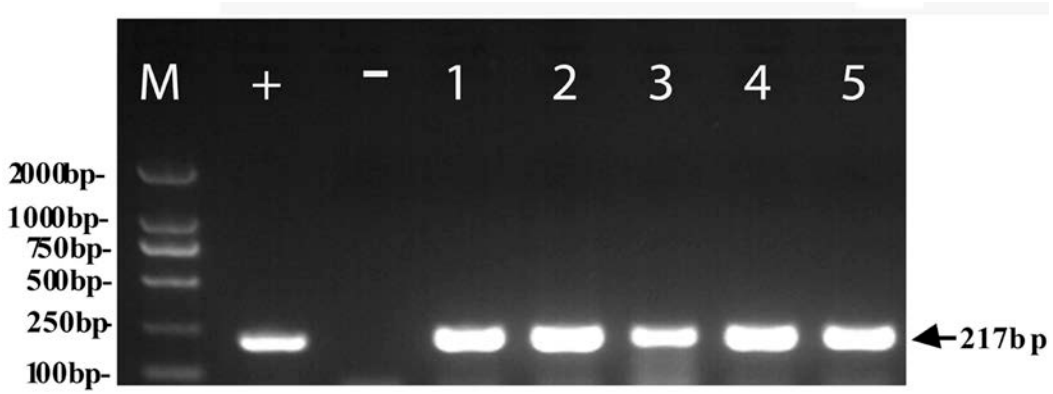
Sequence coding for	Strain	GenBank accession no.	Origin
	DUL34 gfp	JQ809329	Hungary
	DUL34Pass	JQ809330	Hungary
	Becker	M12778	USA
	Namyangju	GQ325660	South Korea
	PRV-FZ	FJ477296	China
	Yangsan	AY217094	Korea
	Min-A	AY169694	China
	LA	AY174090	China
	FZ	EF645837	China
	FZ	EF622042	China
	Ea	AF086702	China
	Fa	AY196984	China
	SA215	DQ367438	China
	Bartha	JF797217	Hungary
	Kaplan	JF797218	Hungary
	Becker	JF797219	USA
	Kaplan	JQ809328	Hungary
	DUL34 gfp	JQ809329	Hungary
	DUL34Pass	JQ809330	Hungary
	Kaplan	AJ271966	Hungary
	Yangsan	AY249861	South Korea
	CL/15	JF460016	Argentina
	HNJZ	EU561349	China
	Becker	AY368490	USA
	PRV-SH	AF207700	China
	Ea	AF171937	China
	SA	GU262988	China
	NS374	FJ605135	Belgium
	Nia-1	FJ605136	Belgium
	89V87	FJ605134	Belgium
	00V72	FJ605132	Belgium
	75V19	FJ605139	Belgium
	LA	AY173124	China
	Fa	AF403049	China
	P-PrV	FJ176390	Malaysia
	NiA3	EU502923	Spain
	GDSH	EF552427	China
	Min-A	AY170318	China
	Kaplan	JF797218	Hungary
	Becker	JF797219	USA
	Kaplan	JQ809328	Hungary

Sequence coding for	Strain	GenBank accession no.	Origin
	DUL34 gfp	JQ809329	Hungary
	DUL34Pass	JQ809330	Hungary

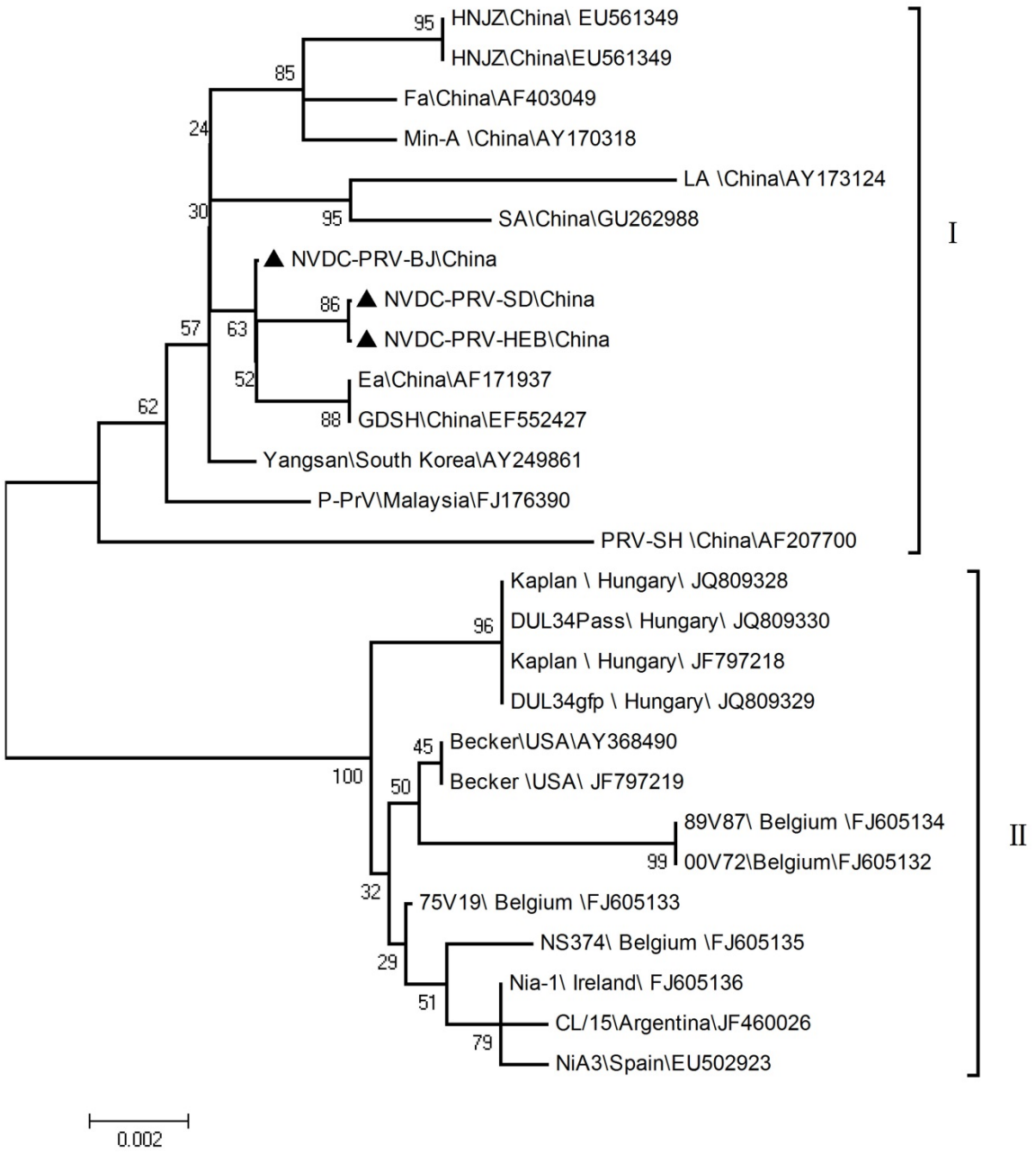
*PRV, pseudorabies virus; g, glycoprotein.



Technical Appendix Figure 1. Immunohistochemical staining of lung (A) and brain (B) specimens of clinically sick pigs. Original magnification ×100.



Technical Appendix Figure 2. PCR of infected tissues with specific primers for pseudorabies virus glycoprotein D (gD)–specific primers (forward primer 5′-CGGAGGACGAGCTGGGGCT-3′; reverse primer 5′-ACGTCCACGCCCCGCTTGAAGC-3′), with a fragment size of 217 bp. +: positive control; –, negative control; 1, 2, 3, 4, and 5: supernatants from the homogenized tissues of lungs, heart, tonsils, lymph nodes and serum, respectively. Arrow indicates the target fragment, 217 kb.



Technical Appendix Figure 3. Phylogenetic trees of the glycoprotein (g) E sequence. Unrooted trees constructed for gE sequences by using Mega v. 4.0 (www.megasoftware.net). The phylogenetic trees indicate the relationship of NVDC-PRV-BJ, NVDC-PRV-HEB, and NVDC-PRV-SD to other PRV isolates. These 3 isolates are indicated in the black triangle. Scale bar indicates number of substitutions per site.