

Isolation of Porcine Epidemic Diarrhea Virus during Outbreaks in South Korea, 2013–2014

Hee-Chun Chung,¹ Van Giap Nguyen,¹ Hyoung-Joon Moon, Jee-Hoon Lee, Seong-Jun Park, Ga-Eun Lee, Hye-Kwon Kim, You-Shun Noh, Chan-Hee Lee, Dane Goede, Bong Kyun Park

Author affiliations: Seoul National University, Seoul, South Korea (H.-C. Chung, J.-H. Lee, G.-E. Lee, Y.-S. Noh, C.-H. Lee, B.K. Park); Vietnam National University of Agriculture, Hanoi, Vietnam (V.G. Nguyen); Green Cross Veterinary Products, Yongin, South Korea (H.-J. Moon); National Forensic Service, Chilgok, South Korea (S.-J. Park); Institute for Basic Science, Daejeon, South Korea (H.-K. Kim); Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea (H.-K. Kim); University of Minnesota, St. Paul, Minnesota, USA (D. Goede)

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To the Editor: Porcine epidemic diarrhea (PED) is an acute infectious diarrhea caused by the PED virus (PEDV), which belongs to the order *Nidovirales*, family *Coronaviridae*, genus *Alphacoronavirus* (*1*). The virus is transmitted mainly through fecal–oral routes and infects all age groups of pigs; the most severe form of disease occurs in suckling piglets (*1*). PEDV was first reported in South Korea in 1992 (*2*), with the occurrence of an outbreak, and has since circulated with considerable genetic diversity (*1,3*). During 2013, PED outbreaks reoccurred in South Korea; however, the emerging PEDVs in these outbreaks were not variants of previous Korean isolates or attenuated vaccine strains (*4,5*). We report on a field isolate of a novel emerging PEDV and the isolate's genetic relationship with other PEDV strains.

During October 2013–June 2014, dead piglets and fecal swabs from 9 provinces of South Korea were sent to the Department of Veterinary Medicine Virology Laboratory at Seoul National University to confirm diagnoses of enteric viral diseases. All samples (30 intestine samples of dead piglets and 16 fecal swabs) were found to be PEDV positive. Attempts to isolate the field strains of PEDV on Vero cell lines followed a previously described protocol with modifications (*6*). An overnight monolayer of Vero cells (80%–100% confluence) was washed twice with 1× phosphate-buffered saline before homogenized samples (0.02 μm filtered) were inoculated with 10% suspension. After 30 min absorption at 37°C with 5% CO₂, maintenance medium (Dulbecco's Modified Eagle Medium supplemented

with trypsin [10 μg/mL], yeast extract (0.04%), tryptose phosphate broth (0.6%), and Antibiotic-Antimycotic 100 (4 μL/mL; Gibco, Thermo Fisher Scientific, Grand Island, NY, USA) were added at a ratio of 1:10. The inoculated cells were cultured for 3–4 days at 37°C in 5% CO₂ atmosphere and were blindly passaged 5 times. One field strain of PEDV (named BM1) was successfully adapted for growth on Vero cells. This virus was isolated from a 60-sow farm (identified as BM farm) that had not vaccinated its animals against PEDV. Pigs of all ages from the farm showed clinical symptoms of diarrhea, and death occurred for 100% of suckling piglets and 10% of sows. Examination at necropsy revealed that the dead piglets from BM farm were covered with brown blotches of dried diarrheal feces and their stomachs were filled with undigested milk. Thin, translucent small intestines that contained yellow fluid were also observed (online Technical Appendix Figure 1, <http://wwwnc.cdc.gov/EID/article/21/12/15-0437-Techapp.pdf>). The BM1 PEDV field isolate induced cytopathic effects of rounded shape (online Technical Appendix Figure 2, panel A) within 48 hours at passage 10. The presence of PEDV in the cell culture was confirmed by immunofluorescence assay (VDPro PEDV FA Reagent kit, MEDIAN Diagnostics, Gangwon-do, South Korea), which showed the specific fluorescence signal (online Technical Appendix Figure 2, panel B). In addition to evidence by microscopic observation, real-time reverse transcription PCR showed that the quantity of viral RNA increased incrementally as the number of passages increased: from 30,325 copies/μL (cycle threshold 16.11) at passage 2 to 418,000 copies/μL (cycle threshold 13.77) at passage 10. Infective titers of the BM1 isolate increased from 10^{4.7} 50% tissue culture infectious doses/mL at passage 2 to 10^{7.9} 50% tissue culture infectious doses/mL at passage 10 (online Technical Appendix; online Technical Appendix Table 2).

The complete S gene of BM1 (GenBank accession no. KP861982) was sequenced for genetic characterization; the gene was 4,161-nt long and encoded 1,386 aa. The spike protein of the BM1 isolate showed substitutions at neutralizing SS6 epitope from LQDGVVKI (7) to SQSGQVKI but identity at the SS2 (7) and 2C10 (8) neutralizing epitopes. The genetic relationship of the BM1 isolate with other PEDVs in the world was inferred from a codon-based alignment of 409 sequences of the complete S gene (online Technical Appendix Table 3). The maximum-likelihood phylogenetic tree was constructed by using the FastTree program (*9*), with the general time reversible nucleotide substitution model. The phylogeny constructed on the basis of the complete S gene (Figure) showed that the BM1 isolate belongs to subgroup 2a, genogroup 2 of PEDV. This isolate clustered closely with emergent PEDV strains in the United States (online Technical Appendix Figure 3), showing 99.2%–99.7% identity with PEDVs of North American

¹These authors contributed equally to this article.

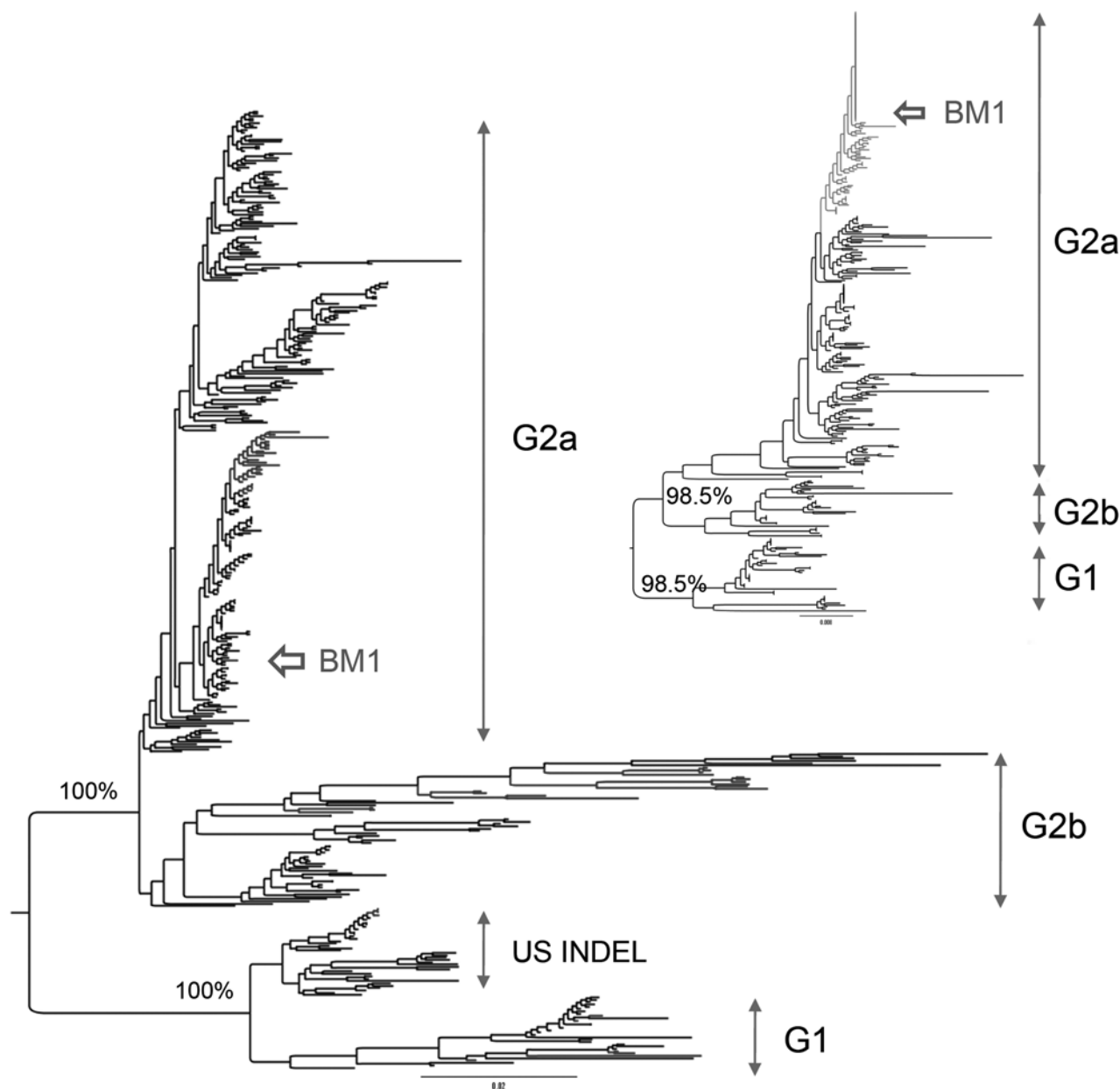


Figure. Maximum-likelihood phylogenetic tree of porcine epidemic diarrhea virus from piglet, South Korea, 2013–2014, constructed on the basis of codon alignment of complete S genes. Inset shows a phylogenetic tree inferred from the complete N genes. Genogroups are shown to the right of each tree. US INDEL is a prototype strain of porcine epidemic diarrhea virus that has insertions and deletions (INDELs) in the spike gene. Scale bars indicate nucleotide substitutions per site. A color version of this figure is available online (<http://wwwnc.cdc.gov/eid/article/21/2/15-0437-F.htm>).

strains (10). This observation was repeated by the phylogenetic inference of the complete N gene (Figure; online Technical Appendix Table 4 and Figure 4). The branching pattern (Figure) clearly showed that BM1 is genetically less related (92.9–93.4% identity) to the live vaccine strains that are derived from genogroup 1 and used currently to prevent PEDV infections in South Korea.

In summary, we isolated the BM1 strain (GenBank accession no. KP861982) in South Korea from a sample from a suckling pig with severe diarrhea; the pig came from a farm that had not vaccinated its pigs against PEDV. The strain was adapted and grew to high titers on Vero cells. The isolate belongs to genogroup 2 and genetically clustered with emerging PEDVs of North American

strains but was loosely related to genogroup 1, the basis of the vaccine used for inoculation against Korean PEDV strains. This isolate may need further evaluation as a candidate for a vaccine to prevent reemerging PEDVs in South Korea.

Acknowledgments

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Address for correspondence: Bong Kyun Park, Department of Veterinary Medicine Virology Laboratory, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul 151-742, South Korea; email address: parkx026@snu.ac.kr

Tickborne Lymphadenopathy Complicated by Acute Myopericarditis, Spain

José Tiago Silva, Francisco López-Medrano, Mario Fernández-Ruiz, Elena Resino Foz, Aránzazu Portillo, José A. Oteo, José María Aguado

Author affiliations: Instituto de Investigación Hospital “12 de Octubre” (+12), Madrid, Spain (J.T. Silva, F. López-Medrano, M. Fernández-Ruiz, E.R. Foz, J.M. Aguado); Centro de Investigación Biomédica de La Rioja, Logroño, Spain (A. Portillo, J.A. Oteo)

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To the Editor: *Dermacentor*-borne necrosis erythema lymphadenopathy/tickborne lymphadenopathy (DEBONEL/TIBOLA) is an apparently benign, self-limiting rickettsial disease transmitted by *Dermacentor* ticks (1,2). *Rickettsia slovaca* was the first etiologic agent isolated, but other species, such as *R. raoultii* and *Candidatus R. rioja*, also might be involved (3–6). If the scalp is affected, a larger number of agents (including *Francisella tularensis*, *Bartonella henselae*, *R. massiliae*, *R. sibirica mongolitimonae*, and *Borrelia burgdorferi*) should be considered within the differential diagnosis of a similar syndrome recently named scalp eschar associated with neck lymphadenopathy after a tick bite (SENLAT) (7). Nevertheless, in Spain, only *R. slovaca*, *Candidatus R. rioja*, and *F. tularensis* are known to cause DEBONEL/TIBOLA/SENLAT (4,6). This entity is considered an emerging rickettsiosis in Europe; cases have been reported from Italy, France, Hungary, Germany, and Portugal (8).

We recently saw a patient in whom acute myopericarditis developed after he was bitten by a large tick on the scalp and showed clinical signs of DEBONEL/TIBOLA/SENLAT, most likely attributable to *R. slovaca* or *Candidatus R. rioja* infection. The patient, a previously healthy 28-year-old man, went on a day-long hiking trip to the northern mountains of Madrid (central Spain; mean altitude 1,300 m) on November 2, 2014. Three days later, he noticed a mild ache on the occipital area of his scalp and found an attached tick that he removed with his fingers. A week later, he sought care from an infectious disease specialist because of itchy discomfort at the area of the tick bite.

Examination revealed an erythematous and elevated punctiform lesion with mild fluctuation in the occipital region accompanied by tender, small lymph node enlargement of both occipital lymphatic chains (Figure). No widespread rash was present. DEBONEL/TIBOLA/SENLAT was diagnosed, and doxycycline (100 mg every 12 hours) was initiated. IgG titer against spotted fever group *Rickettsia*

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Technical Appendix

Methods, Tables, and Figures Related to Phylogenetic Characterization of Porcine Epidemic Diarrhea Virus Isolate

RNA Extraction and RT-PCR

Total RNA was extracted by using RNA mini kit (Qiagen, Valencia, CA, USA), following the manufacturer's instructions. The RNA was then converted into cDNA with the use of random hexamers and commercial M-MLV reverse transcription kit (Invitrogen, Carlsbad, CA, USA), following manufacturer's protocol. Finally, PCR reactions were performed with pathogen-specific primers by using AccuPower ProFi Taq PCR PreMix (Bioneer Ltd, Daejeon, South Korea). The specific primers for detecting porcine epidemic diarrhea virus (PEDV) were PEDV-420F [5'- TAATTGCCCTTTCACCTTGC -3'] and PEDV-420R [5'- TCAACATATGCAGCCTGCTC -3'], which amplified the 420-bp region of the conserved spike coding protein 2 portion (S2) gene (1,2). The thermal profile was initial denaturation at 94°C for 5 min, followed by 38 cycles of 94°C for 30 s, 56°C for 45 s, 72°C for 45 s, and a final extension at 72°C for 7min.

Real-time Quantitative Reverse Transcription PCR

Real-time reverse transcription PCR (Maxima SYBR Green kit, Thermo Fisher Scientific Inc., Grand Island, NY, USA) was performed to quantify the viral RNA of each passage. The PEDV-specific primers were designed as PEDV-QF [5'- TCGCTCTGTGGCAGATCTAGTC-3'] and PEDV-QR [5'- GCGTCAACAACGCCAGGTA -3']. Each of the expression levels of target genes was automatically calculated as C_T values by comparing standard curve values of the PEDV through real-time quantitative reverse transcription PCR (StepOnePlus, Applied Biosystems, Foster City, CA, USA).

Sequencing of Spike Protein and Nucleoprotein-Coding Genes

In this study, a new set of specific primers was designed on the basis of the reference strain of USA/Colorado/2013, KF272920 to amplify complete S and N genes of PEDV. Technical Appendix Table 1 shows the sequences of primers and the annealing temperature of PCR.

Gel Extraction and TA Cloning

The specific PCR bands, which were purified by using the QIAquick Gel Extraction Kit (Qiagen, Düsseldorf, Germany), were used for cloning with the TA cloning kit (Topcloner TA kit, Enzymomics, Daejeon, South Korea) and subsequently transformed into competent *Escherichia coli* cells (DH5 α). The purified DNAs were sequenced by using Macrogen sequencer (Seoul, South Korea) in both directions to verify the accuracy of the sequence (3).

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Technical Appendix Table 1. Sequences of primers and the annealing temperature of PCR.

Target gene	Primer name	Sequence (5'-3')	Product size (bp)	Annealing temperature (°C)	Position
S	FuS1F	TCTTCTGGCGTAATTCCACA	937	53.3	20420–20439
	FuS1R	ATACATTGGCRGCRTAACCA			
S	FuS2F	GYGTTGCGACAARRTGTTAC	993	50.3	21205–21224
	FuS2R	CAAARGMMGCAGAGACAGTA			
S	SF1	TCATCCATTAGTGATGTTGTGTTA	1666	52.9	20527–20550
	SR1	GCCGCAGAGACAGTAATTAACA			
S	SF2	GTGTTCTCAGGTTGCTTTTGACCT	1593	59.0	22049–22072
	SR2	AAAGACTCAGCAAGCAATTGCTGG			
S	SF3	GTACAGTGCCTCTCATAGGTGG	1422	53.9	23489–23512
	SR3	TCTAATTGGAACACTACATTGAGCTC			
N	NF1	TTATGGCTTCTGTCAGTTTT	1544	57.4	26377–26396
	NF2	AGATGAAAAGGTACTGCGTTCC			

Technical Appendix Table 2. Titration of BM1 PEDV isolate from passage 1 to passage 10, South Korea, 2013–2014*

BM1 sample parameter	Intestine†	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Cytopathic effect	ND	ND	+	+	+	+	+	+	+	+	+
RT-PCR	+	+	+	+	+	+	+	+	+	+	+
Real-time (copies/ μ l; C_T value)	1,412; <u>17.96</u>	1,905; <u>17.91</u>	30,325; <u>16.11</u>	21,633; <u>16.42</u>	24,727; <u>16.3</u>	7,287; <u>17.39</u>	16,565; <u>16.77</u>	33,905; <u>16.02</u>	80,579; <u>15.24</u>	283,000; <u>13.98</u>	418,000; <u>13.77</u>
Infectious titer (log ₁₀ TCID ₅₀ /ml)	ND	ND	4.7	4.2	5.2	2.7	5.2	5.7	6.2	7.2	7.9

*ND, not determined; P, passage; PEDV, porcine epidemic diarrhea virus; RT-PCR, reverse transcription PCR; +, positive.

†0.02 μ m filtered of 10% intestine homogenized suspension.**Technical Appendix Table 3.** List of complete spike protein-coding sequences used in PEDV study, South Korea, 2013–2014*

GenBank accession no.	Strain name	Country	Genogroup	Reference
Z25483	Br1/87			
AF353511	CV777		1a	(4)
AF500215	Korea			
AY167585	Chinju99			
AY653204	JS20042	China		
DQ462404	DR13	Korea		
DQ862099	DR13	Korea		
DQ985739	LJB/03	China		
EU031893	DX	China		
GU180142	KNU0801	Korea		
GU180143	KNU0802	Korea		
GU180144	KNU0901	Korea		
GU180145	KNU0902	Korea		
GU180146	KNU0903	Korea		
GU180147	KNU0904	Korea		
GU180148	KNU0905	Korea		
AB548618	83P5	Japan		
AB548619	83P5	Japan		
AB548620	83P5	Japan		
AB548621	83P5	Japan		
AB548622	KH	Japan		
AB548623	NK	Japan		
AB548624	MK	Japan		
JN315706	CH/FJND2/2011	China		
JN381492	CH/FJND3/2011	China		
JN543367	CH/FJND1/2011	China		
JN184634	CNU09122201	Korea		
JN184635	CNU09122202	Korea		
JN980698	CHGD01	China		
JQ257005	CH/KF/11	China		
JQ257006	CH/YY/11	China		
JQ257007	CH/ZY/11	China		
JN599150	CV777	China		

GenBank accession no.	Strain name	Country	Genogroup	Reference
JN825706	BJ20112	China		
JN825707	HB20111	China		
JN825708	HB20112	China		
JN825709	HB20113	China		
JN825710	ZJ20111	China		
JN825711	ZJ20112	China		
JN825712	BJ20111	China	2a	(4)
JQ517274	HuN	China		
JQ627653	CH/AY/11	China		
JQ627654	CH/CG/11	China		
JQ638915	CH/GD/2011	China		
JQ638916	CH/HLJHH/2011	China		
JQ638917	CH/SDLY/2011	China		
JQ638918	CH/HBBD/2011	China		
JQ638919	CH/SDQD/2011	China		
JQ638920	CH/JLCC/2011	China		
JQ638921	CH/BJSY/2011	China		
JQ638922	CH/HBQHD/2011	China		
JQ638923	CH/JLGZL/2011	China		
JQ638924	CH/JL/2011	China		
JQ239429	CH1	China		
JQ239430	CH2	China		
JQ239431	CH3	China		
JQ239432	CH4	China		
JQ239433	CH5	China		
JQ239434	CH6	China		
JQ239435	CH7	China		
JQ239436	CH8	China		
JQ979287	CH9FJ	China		
JQ979288	CH13GX	China		
JQ979289	CH17GZ	China		
JQ979290	CH22JS	China		
JQ979291	CH18Hainan	China		
JX018179	CH/GXNN/2012	China		
JX018180	CH/YNKM/2012	China		
JX018181	CH/AHHF/2012	China		
JX018182	CH/AHHF2/2012	China		
JX018183	CH/HBSN/2012	China		
JX070671	CH/FJXM1/2012	China		
JX070672	CH/FJXM2/2012	China		
JX163294	HBMC2012	China		
JX258672	FQ/FJ/2012	China		
JX242454	CHHYC082011			
JX242455	CHHYC102011			
JX242456	CHHYC112011			
JX242457	CHHYC122011			
JX242458	CHLC102011			
JX242459	CHSONGB122011			
JX242460	CHYHC122011			
JX242461	CHZWC122011			
JX242462	CHHKC082011			
JX242463	CHLC122011			
JX242464	CHSHT122011			
JX512907	HLJ2012	China		
JX435298	BJ20113	China		
JX435299	BJ20121	China		
JX435300	BJ20122	China		
JX435301	HB20114	China		
JX435302	HB20121	China		
JX435303	HB20122	China		
JX435304	HB20123	China		
JX435305	HB20124	China		
JX489155	LC	China	2b	(4)
JX524137	ZJCZ4	China	2b	(4)
JX501317	CH/CY/12	China		
JX501318	CH/HBQX/10	China		
JX501319	CH/HBXX2/11	China		
JX501320	CH/HBXX3/11	China		
JX501321	CH/TY/12	China		

GenBank accession no.	Strain name	Country	Genogroup	Reference
JX501322	CH/XC/12	China		
JX501323	CH/XCYL/11	China		
JX647847	GD1	China	2b	(4)
JX560761	SDM	China	1b	(4)
KC196276	CH/ZMDZY/11	China	2a	(4)
KC242897	FJZZ12011	China		
KC242898	HeN1220122	China		
KC242899	HB120122	China		
KC242900	HB220122	China		
KC242901	AH220123	China		
KC242902	HB320123	China		
KC242903	ZJ1720125	China		
KC242904	YN220127	China		
KC242905	FJ520128	China		
KC242906	FJZZ920128	China		
KC242907	JX420129	China		
KC242908	HN420129	China		
KC242909	GD520129	China		
KC242910	GD820129	China		
KC242911	HN6201211	China		
KC242912	HN8201211	China		
KC787536	CHCCC012013	China		
KC787537	CHDLC012013	China		
KC787538	CHGGC112012	China		
KC787539	CHGMB022013	China		
KC787540	CHHGC012013	China		
KC787541	CHLNC012012	China		
KC787542	CHSBC032013	China		
KC787543	CHSTC122012	China		
KC787544	CHYGC122012	China		
KC787545	CHYXC012013	China		
KF384500	CH/GDGZ/2012	China		
KC879275	AS01	Korea		
KC879276	AS02	Korea		
KC879277	AS03	Korea		
KC879278	NJ01	Korea		
KC879279	NJ02	Korea		
KC879280	AD01	Korea		
KC879281	AD02	Korea		
KC879282	AD03	Korea		
KF453510	AH120411	China		
KF453511	GX120308	China		
KF453512	HB121229	China		
KF453514	SH120301	China		
KF453515	SH121208	China		
KF453516	ZJ110618	China		
KF453517	ZJ111113	China		
KC886306	SCL	China		
KF177254	JY5C	China		
KF177255	JY6C	China		
KF177256	JY7C	China		
KF177257	YJ3F	China		
KF177258	YJ7C	China		
KF294254	HZXS1	China		
KF294255	HZXS2	China		
KF294256	GJL	China		
KF468752	MN	USA	2a	(4)
KF468753	IA1	USA	2a	(4)
KF468754	IA2	USA	2a	(4)
KF468755	XS2013	China		
AB857233	GDS01	China		
AB857234	GDS02	China		
AB857235	GDS03	China		
KF546800	GDFS/2011	China		
KF546801	GDQY/2011	China		
KF546802	GDDG/2011	China		
KF546803	GDJM/2012	China		
KF546804	GDZQ/2012	China		
KF601195	GXLZ/2013	China		

GenBank accession no.	Strain name	Country	Genogroup	Reference
KF601196	JXGZ/2013	China		
KF601197	HNCZ/2013	China		
KF601198	JXJA/2013	China		
KF601199	GXHZ/2013	China		
KF601200	GDEP/2013	China		
KF601201	GXNN/2013	China		
KF577730	YNKM/2012	China		
KF577731	GDMZ/2013	China		
KF577732	GDGZ/2013	China		
KC764952	NPPED2008	Thailand		
KC764953	NPPED0108	Thailand		
KC764954	PED0212	Thailand		
KC764955	PED0210	Thailand		
KC764956	SBPED0211	Thailand		
KC764957	SBPED0211	Thailand		
KC764958	SPPED0212	Thailand		
KC764959	SBPED0211	Thailand		
KC764960	SPPED0212	Thailand		
KF724935	45RWVCF0712	Thailand		
KF724936	155ST0412	Thailand		
KF724937	2253AG0211	Thailand		
KF724938	656ST0413	Thailand		
KF738258	GD120330	China		
KF738259	JS130604	China		
KF738260	JX130506	China		
KF738261	SD130502	China		
KF738262	SD130611	China		
KF738263	SH121229	China		
KF738264	ZJ121030	China		
KJ158152	AHM	China		
KF779469	MF3809/2008	Korea		
KF898124	KPEDV9	Korea		
KJ020932	CHYJ130330	China		
KJ196348	SHQP/YM/2013	China		
KJ588062	KUIDLPED2014007	Korea		
KJ588063	KUIDLPED2014002	Korea		
KJ588064	KUIDLPED2014001	Korea		
KJ451036	KNU1301	Korea		
KJ451037	KNU1302	Korea		
KJ451038	KNU1303	Korea		
KJ451039	KNU1304	Korea		
KJ451040	KNU1305	Korea		
KJ451041	KNU1306	Korea		
KJ451042	KNU1307	Korea		
KJ451043	KNU1308	Korea		
KJ451044	KNU1309	Korea		
KJ451046	KNU1311	Korea		
KJ451047	KNU1401	Korea		
KJ451048	KNU1402	Korea		
KJ451045	KNU1310	Korea		
KJ539151	K13JA121	Korea		
KJ539152	K13JA123	Korea		
KJ539153	K13JA114	Korea		
KJ539154	K14JB01	Korea		
KJ646578	FJZP 2013	China		
KJ646579	FJCL 2013	China		
KJ646580	FJFQ 2014	China		
KJ646581	FJFQ1 2012	China		
KJ646582	FJFQ2 2012	China		
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KJ646584	FJLY 2013	China		
KJ646585	FJND 2012	China		
KJ646586	FJND 2013	China		
KJ646587	FJPT 2013	China		
KJ646588	FJQK 2013	China		
KJ646589	FJQZ 2013	China		
KJ646590	FJYX 2013	China		
KJ646591	FJZP 2014	China		
KJ642641	NJ	China		

GenBank accession no.	Strain name	Country	Genogroup	Reference
KJ645635	Indiana12.83/2013	USA		
KJ645636	Iowa28/2013	USA		
KJ645637	Kansas29/2013	USA		
KJ645638	Colorado30/2013	USA		
KJ645639	Texas31/2013	USA		
KJ645640	Oklahoma32/2013	USA		
KJ645641	Indiana34/2013	USA		
KJ645642	Oklahoma35/2013	USA		
KJ645643	Kansas36/2013	USA		
KJ645644	Oklahoma38/2013	USA		
KJ645645	Texas39/2013	USA		
KJ645646	NorthCarolina40/2013	USA		
KJ645647	Minnesota41/2013	USA		
KJ645648	Minnesota42/2013	USA		
KJ645649	Iowa23.57/2013	USA		
KJ645650	Kansas46/2013	USA		
KJ645651	Colorado47/2013	USA		
KJ645652	Minnesota54/2013	USA		
KJ645653	Wisconsin55/2013	USA		
KJ645654	Tennessee56/2013	USA		
KJ645655	Minnesota58/2013	USA		
KJ645656	Minnesota59/2013	USA		
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KJ645658	Minnesota62/2013	USA		
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KJ645660	Minnesota64/2013	USA		
KJ645661	Minnesota65/2013	USA		
KJ645662	NorthCarolina66/2013	USA		
KJ645663	Minnesota67/2013	USA		
KJ645664	Ohio68/2013	USA		
KJ645665	Ohio69/2013	USA		
KJ645666	Iowa70/2013	USA		
KJ645667	Minnesota72/2013	USA		
KJ645668	Minnesota73/2013	USA		
KJ645669	Wisconsin74/2013	USA		
KJ645670	Ohio75/2013	USA		
KJ645671	Minnesota76/2013	USA		
KJ645672	Minnesota77/2013	USA		
KJ645673	Minnesota78/2013	USA		
KJ645674	Minnesota79/2013	USA		
KJ645675	Illinois81/2013	USA		
KJ645676	Minnesota82/2013	USA		
KJ645677	Minnesota83/2013	USA		
KJ645678	Minnesota85/2013	USA		
KJ645679	Minnesota86/2013	USA		
KJ645680	Illinois87/2013	USA		
KJ645681	Minnesota89/2013	USA		
KJ645682	Minnesota90/2013	USA		
KJ645683	NorthCarolina91/2013	USA		
KJ645684	Missouri92/2013	USA		
KJ645685	Missouri93/2013	USA		
KJ645686	Minnesota94/2013	USA		
KJ645687	Minnesota95/2013	USA		
KJ645688	Iowa96/2013	USA		
KJ645689	Illinois97/2013	USA		
KJ645690	Illinois98/2013	USA		
KJ645691	Minnesota100/2013	USA		
KJ645692	Missouri101/2013	USA		
KJ645693	Missouri102/2013	USA		
KJ645694	Iowa103/2013	USA		
KJ645695	Iowa106/2013	USA		
KJ645696	Iowa107/2013	USA		
KJ645697	Texas128/2014	USA		
KJ645698	Ohio120/2014	USA		
KJ645699	Ohio123/2014	USA		
KJ645700	MEX/124/2014	Mexico		
KJ645701	Kansas125/2014	USA		
KJ645702	Ohio126/2014	USA		
KJ645703	Minnesota127/2014	USA		

GenBank accession no.	Strain name	Country	Genogroup	Reference
KJ645704	Minnesota52/2013	USA		
KJ645705	Minnesota61/2013	USA		
KJ645706	Minnesota71/2013	USA		
KJ645707	Minnesota84/2013	USA		
KJ645708	MEX/104/2013	Mexico		
KJ662670	KNU1305	Korea		
KF840537	CH/ZJCX1/2012	China		
KF840538	CH/ZJHY2/2012	China		
KF840539	CH/ZJJSZ/2012	China		
KF840540	CH/ZJJS1Z/2012	China		
KF840541	CH/JXZS3H/2012	China		
KF840542	CH/JXZS2H/2012	China		
KF840543	CH/ZJXS212/2012	China		
KF840544	CH/ZJHZHY6/2013	China		
KF840545	CH/JXJDZF/2012	China		
KF840546	CH/ZJJS2Z/2012	China		
KF840547	CH/JXZS3L	China		
KF840548	CH/JXZS1223L	China		
KF840549	CH/SDZD1/2012	China		
KF840550	CH/SDZD2/2012	China		
KF840551	CH/HuBWHYQ/2012	China		
KF840552	CH/ZJQZ2/2012	China		
KF840553	CH/ZJHZ1C/2012	China		
KF840554	CH/ZJHZ2C/2012	China		
KF840555	CH/JXJDZ1/2012	China		
KF840556	CH/ZJJS4X/2012	China		
KF840561	CH/JSZLS2/2013	China		
KF840562	CH/JSZLN2/2013	China		
KJ741221	KNU14091	Korea		
KJ741222	KNU14092	Korea		
KJ741223	KNU14093	Korea		
KJ741224	KNU14094	Korea		
KJ741225	KNU14095	Korea		
KJ767195	OhioVBS1	USA		
KJ767196	OhioVBS2	USA		
KJ777677	LZW	China		
KJ777678	LZW	China		
KM189366	ON007	Canada		
KM189368	PEI023	Canada		
KM196109	SK030	Canada		
KM196110	2014022	Canada		
KM196111	MB021	Canada		
KM077139	Minnesota188/2014	USA		
KM392224	TC PC22AP10	USA		
KM392226	TC PC168P2	USA		
KM392231	TC PC182P2	USA		
KM392232	TC Iowa106 (PV39)P1	USA		
KM392225	TC PE103(PC21A)P4	USA		
KM392227	TC PC170P2	USA		
KM392228	TCPC173P2	USA		
KM392229	TC PC177P2	USA		
KM392230	TC PC180P2	USA		
KM403155	KNU14061	Korea		
KM403156	KNU14062	Korea		
KM403157	KNU14063	Korea		
KM403158	KNU14064	Korea		
KM108348	KPV1401	Korea		
KM108349	KPV1402	Korea		
KM108350	KPV1403	Korea		
KM108351	KPV1404	Korea		
KM108352	KPV1405	Korea		
KM108353	KPV1406	Korea		
KM242131	CH/GDZQ/2014	China		
KM287429	XJDB2	China		
KM406178	LS1401	China		
KM406179	MY1402	China		
KM406180	DY1403	China		
KM406181	SH1404	China		
KM406182	MX1405	China		

GenBank accession no.	Strain name	Country	Genogroup	Reference
KM406183	PZ1406	China		
KM406184	PJ1407	China		
KP455313	HUAPED45	Vietnam		
KP455314	HUAPED47	Vietnam		
KP455315	HUAPED55	Vietnam		
KP455316	HUAPED58	Vietnam		
KP455317	HUAPED60	Vietnam		
KP455318	HUAPED63	Vietnam		
KP455319	HUAPED67	Vietnam		
KP455320	HUAPED68	Vietnam		
KP861982(PEDV)	BM1	Korea		This study
JQ771753	YS	China		
JQ771752	ZB	China		
JQ771751	SH	China		
KJ399978	OH851	USA		
KC210145		China	2a	
JX088695		China	2a	
KC210147		China	2a	
JQ282909		China	2a	
KC140102		China	2a	
JX188454		China	2b	
JX261936		China	2b	
JX112709		China	2b	
JQ023161	vDR13	Korea	1r	(4)
JN547228		China	1r	
EF185992		China	1a	
GU937797		Korea	1a	
KC109141		China	1b	
KC210146		China	1b	
JQ023162	aDR13	Korea	1b	(4)
KC189944		China	1b	

*Information about strain name and country were extracted from Genbank. For some entries, the corresponding information was not available. Genogroups for some strains were obtained from the cited reference. PEDV, porcine epidemic diarrhea virus.

Technical Appendix Table 4. List of complete nucleoprotein-coding sequences used in PEDV study, South Korea, 2013–2014*

GenBank accession no.	Strain name	Country	Genogroup	Reference
DQ355224		China		
DQ355223		China		
DQ355221		China		
JN601062		China	G2.1	(5)
JN601061		China		
JN601060		China		
JN601059		China	G1	(5)
JN601058		China	G2.3	(5)
JN601057		China	G2.3	(5)
JN601056		China	G1	(5)
JN601055		China	G2.1	(5)
JN601054		China		
JN601053		China	G1	(5)
JN601052		China		
KR003452		Belgium		
KM609213		China		
KM609212		China		
KM609211		China		
KM609210		China		
KM609209		China		
KM609208		China		
KM609207		China		
KM609206		China		
KM609205		China		
KM609204		China		
KM609203		China		
NC	003436			
KM242131	CH/GDZQ/2014	China		
KM403155	KNU14061	Korea		
KF840558		China		
KF840557		China		

GenBank accession no.	Strain name	Country	Genogroup	Reference
KF840556	CH/ZJJS4X/2012	China		
KF840555	CH/JXJDZ1/2012	China		
KF840554	CH/ZJHZ2C/2012	China		
KF840553	CH/ZJHZ1C/2012	China		
KF840552	CH/ZJQZ2/2012	China		
KF840551	CH/HuBWHYQ/2012	China		
KF840550	CH/SDZD2/2012	China		
KF840549	CH/SDZD1/2012	China		
KF840548	CH/JXZS1223L	China		
KF840547	CH/JXZS3L	China		
KF840546	CH/ZJJS2Z/2012	China		
KF840545	CH/JXJDZF/2012	China		
KF840544	CH/ZJHZHY6/2013	China		
KF840543	CH/ZJXS212/2012	China		
KF840542	CH/JXZS2H/2012	China		
KF840541	CH/JXZS3H/2012	China		
KF840540	CH/ZJJS1Z/2012	China		
KF840539	CH/ZJJSZ/2012	China		
KF840538	CH/ZJHY2/2012	China		
KF840537	CH/ZJCX1/2012	China		
KJ196348	SHQP/YM/2013	China		
KM392230	TCPC180P2	USA		
KM392229	TCPC177P2	USA		
KM392228	TCPC173P2	USA		
KM392227	TCPC170P2	USA		
KM392225	TCPE103(PC21A)P4	USA		
KM392232	TCIowa106 (PV39)P1	USA		
KM392231	TCPC182P2	USA		
KM392226	TCPC168P2	USA		
KM392224	TCPC22AP10	USA		
KJ184549		USA		
KJ588064	KUIDLPED2014001	Korea		
KJ588063	KUIDLPED2014002	Korea		
KJ588062	KUIDLPED2014007	Korea		
KM077139	Minnesota188/2014	USA		
KJ960180		Vietnam		
KJ960179		Vietnam		
KJ960178		Vietnam		
KJ777678	LZW	China		
KJ777677	LZW	China		
KJ662670	KNU1305	Korea		
KJ158152	AHM	China		
KJ642642		China		
KJ778616		USA		
KJ778615		USA		
KJ623926		Korea		
KJ646625		China		
KJ646624		China		
KJ646623		China		
KJ646622		China		
KJ646621		China		
KJ646620		China		
KJ646619		China		
KJ646618		China		
KJ646617		China		
KJ646616		China		
KJ646615		China		
KJ646614		China		
KJ646613		China		
KJ646612		China		
KJ646611		China		
KJ646610		China		
KJ408801		USA	G2.1	(5)
KF761675		China		
KJ020932	CHYJ130330	China		
KJ399978	OH851	USA		
KF994808		China		
KF994807		China		
KF994806		China		

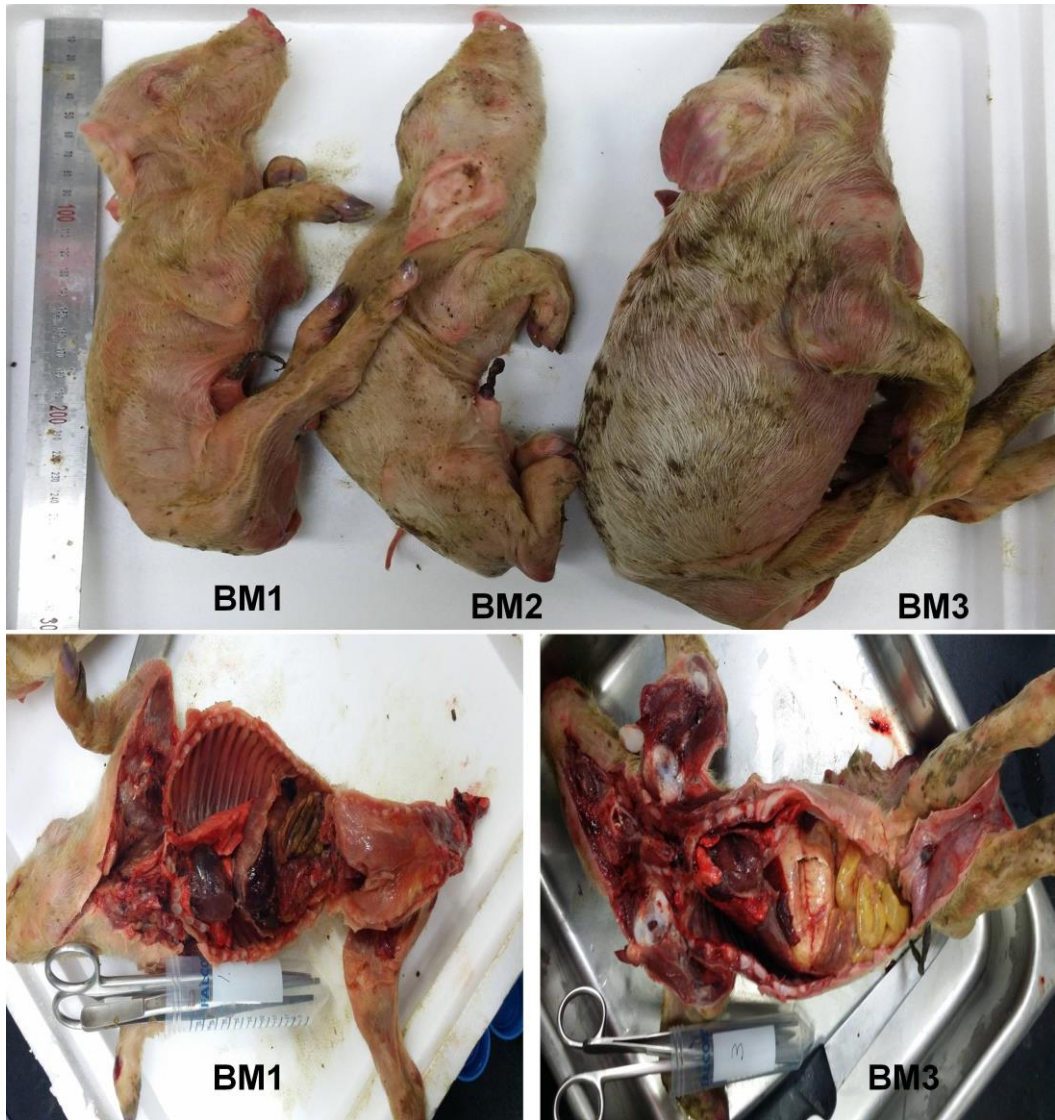
GenBank accession no.	Strain name	Country	Genogroup	Reference
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KF994804		China		
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KF994801		China		
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KF994799		China		
KF994798		China		
KF994797		China		
KF994796		China		
KF994795		China		
KF994794		China		
KF994793		China		
KF994792		China		
KF994791		China		
KF994790		China		
KF994789		China		
KF804028		USA		
KF452323		USA	G2.1	(5)
KF452322		USA	G2.1	(5)
KF468752	MN	USA	2a	(4)
KF468754	IA2	USA	2a	(4)
KF468753	IA1	USA	2a	(4)
KF384500	CH/GDGZ/2012	China		
KF272920		USA	G2.1	(5)
AB618622		Japan	G1	(5)
AB618621		Japan		
AB618620		Japan		
AB618619		Japan	G1	(5)
KC189944		China	1b	(4)
JQ735953		China	G2.1	(5)
JQ743656		China	G2.3	(5)
JQ743655		China	G1	(5)
JQ743654		China	G2.1	(5)
JQ743653		China	G1	(5)
JQ743652		China	G2.1	(5)
JQ743651		China	G1	(5)
JQ743650		China	G2.1	(5)
HQ455346		China		
HQ455345		China	G1	(5)
KC140102		China	2a	(4)
KC109141		China	1b	(4)
JN825712	BJ20111	China	2a	(4)
KC196276	CH/ZMDZY/11	China	2a	(4)
JX560761	SDM	China	1b	(4)
JX647847	GD1	China	2b	(4)
JX524137	ZJCZ4	China	2b	(4)
JX489155	LC	China	2b	(4)
JX406145		China		
JX406144		China		
JX406143		China		
JX406142		China		
JX406141		China		
JX406140		China		
JX406139		China		
JX406138		China		
JX406137		China		
JX406136		China		
JX406135		China		
JX406134		China		
JX512909		China		
JQ723738		China		
JQ723737		China		
JQ723731		China		
JX188454		China	2b	(4)
JX112709		China	2b	(4)
JX088695		China	2a	(4)
JQ023162	aDR13	Korea	1b	(4)
JQ023161	vDR13	Korea	1r	(4)

GenBank accession no.	Strain name	Country	Genogroup	Reference
JQ282909		China	2a	(4)
JN547228		China	1r	(4)
GU937797		Korea	1a	(4)
AY653206		China	G2.1	(5)
AF353511	CV777		1a	(4)
AF237764	Korea		G1	(5)
EF185992	China		1a	(4)
KP691458		China		
KJ526096		China		
KP202365		USA		
KF760557		China		
KP162057		China		
KM887144		China		
KM604665		China		
LM645057		Germany		
KJ645708	MEX/104/2013	Mexico		
KJ645707	Minnesota84/2013	USA		
KJ645706	Minnesota71/2013	USA		
KJ645705	Minnesota61/2013	USA		
KJ645704	Minnesota52/2013	USA		
KJ645703	Minnesota127/2014	USA		
KJ645702	Ohio126/2014	USA		
KJ645701	Kansas125/2014	USA		
KJ645700	MEX/124/2014	Mexico		
KJ645699	Ohio123/2014	USA		
KJ645698	Ohio120/2014	USA		
KJ645697	Texas128/2014	USA		
KJ645696	Iowa107/2013	USA		
KJ645695	Iowa106/2013	USA		
KJ645694	Iowa103/2013	USA		
KJ645693	Missouri102/2013	USA		
KJ645692	Missouri101/2013	USA		
KJ645691	Minnesota100/2013	USA		
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KJ645689	Illinois97/2013	USA		
KJ645688	Iowa96/2013	USA		
KJ645687	Minnesota95/2013	USA		
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KJ645685	Missouri93/2013	USA		
KJ645684	Missouri92/2013	USA		
KJ645683	NorthCarolina91/2013	USA		
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KJ645671	Minnesota76/2013	USA		
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KJ645665	Ohio69/2013	USA		
KJ645664	Ohio68/2013	USA		
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KJ645662	NorthCarolina66/2013	USA		
KJ645661	Minnesota65/2013	USA		
KJ645660	Minnesota64/2013	USA		
KJ645659	Illinois63/2013	USA		
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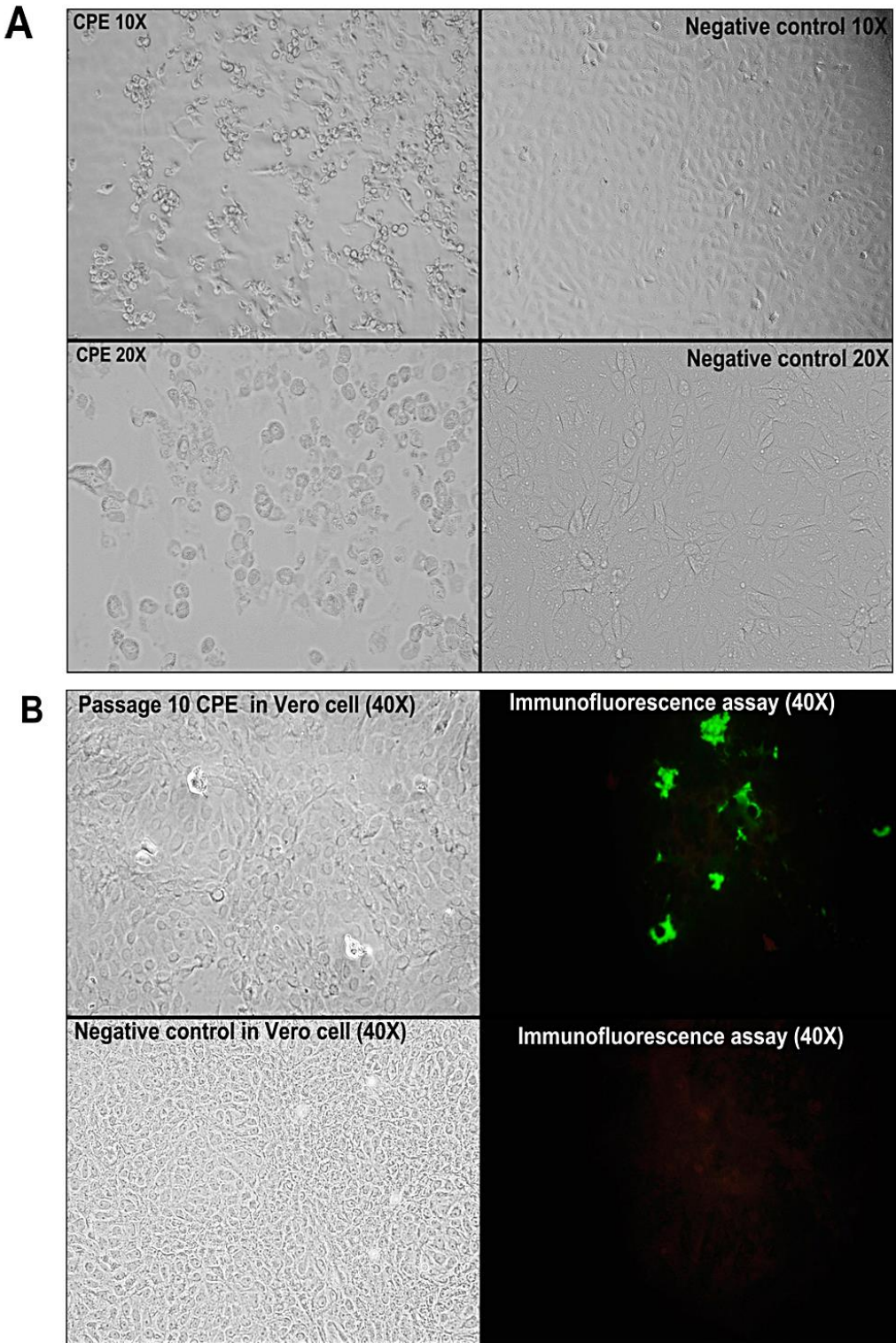
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KJ645649	Iowa23.57/2013	USA		
KJ645648	Minnesota42/2013	USA		
KJ645647	Minnesota41/2013	USA		
KJ645646	NorthCarolina40/2013	USA		
KJ645645	Texas39/2013	USA		
KJ645644	Oklahoma38/2013	USA		
KJ645643	Kansas36/2013	USA		
KJ645642	Oklahoma35/2013	USA		
KJ645641	Indiana34/2013	USA		
KJ645640	Oklahoma32/2013	USA		
KJ645639	Texas31/2013	USA		
KJ645638	Colorado30/2013	USA		
KJ645637	Kansas29/2013	USA		
KJ645636	Iowa28/2013	USA		
KJ645635	Indiana12.83/2013	USA		
KM189367		Canada		
KM052365		USA		
KJ584361		USA		
KF650375		USA	G2.1	(5)
KF650374		USA	G2.1	(5)
KF650373		USA	G2.1	(5)
KF650372		USA	G2.1	(5)
KF650371		USA	G2.1	(5)
KF650370		USA	G2.1	(5)
KF267450		USA	G2.1	(5)
KC210147		China	2a	(4)
KC210146		China	1b	(4)
KC210145		China	2a	(4)
KC243782		China		
JX261936		China	2b	(4)
JQ081280		China		
JQ081279		China		
JQ081278		China		
JQ081277		China		
JQ081276		China		
JQ081275		China		
JQ081274		China		
JQ081273		China		
JN255988		China		
JN255987		China		
JN255986		China		
JN255985		China		
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JN255973		China		
JN173303		China		
JN173302		China		
JN173301		China		
JN173300		China		
JN173299		China		
JN173298		China		
JN173297		China		
JN173296		China		
JN173295		China		
JN173294		China		

GenBank accession no.	Strain name	Country	Genogroup	Reference
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JN173291		China		
JN173290		China		
JN173289		China		
JN173288		China		
JN173287		China		
JN173286		China		
JN173285		China		
JN173284		China		
JN173283		China		
JN173282		China		
JN173281		China		
JN173280		China		
JN173279		China		
JN173278		China		
JN173277		China		
JN173276		China		
JN173275		China		
JN173274		China		
JN173273		China		
JN173272		China		
JN173271		China		
JN173270		China		
JF700126		China		
JF690780		China		
Z14976			G1	(5)
KR270436	BM1	Korea		This study

*Information about strain name and country were extracted from Genbank. For some entries, the corresponding information was not available.
Genogroups for some strains were obtained from the cited reference. PEDV, porcine epidemic diarrhea virus.



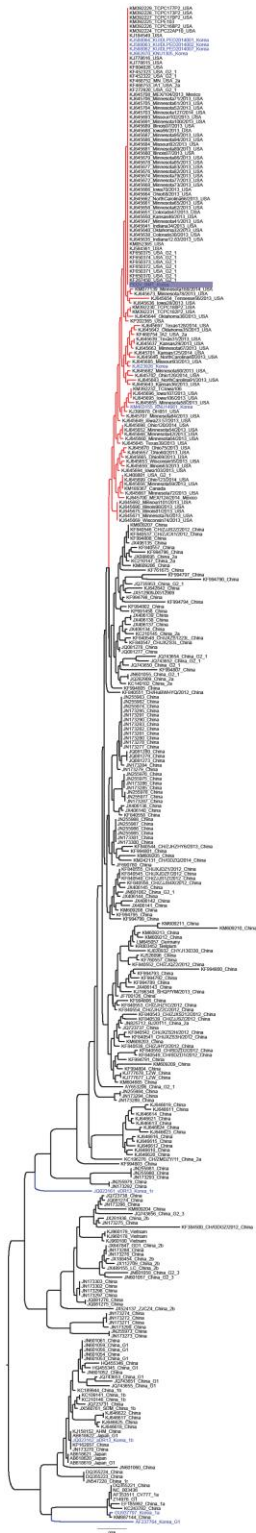
Technical Appendix Figure 1. Gross lesions of dead piglets that had severe diarrhea; pigs came from BM farm.



Technical Appendix Figure 2. Results of microscopic examination and specific IFA staining of Vero cells infected by BM1 isolate of porcine epidemic diarrhea virus at passage level 10. A) Cytopathic effects and B) Immunofluorescence assay.



Technical Appendix Figure 3. Maximum likelihood phylogenetic tree of porcine epidemic diarrhea viruses, constructed on the basis of codon alignment of complete S genes.



Technical Appendix Figure 4. Maximum likelihood phylogenetic tree of porcine epidemic diarrhea viruses, constructed on the basis of codon alignment of complete N genes.