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

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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 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 blottches 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/21/12/150437-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, signal 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 104.7 50% tissue culture infectious doses/mL at passage 2 to 107.9 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 LQDGQVKI (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 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 blottches 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/21/12/150437-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, signal 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 104.7 50% tissue culture infectious doses/mL at passage 2 to 107.9 50% tissue culture infectious doses/mL at passage 10 (online Technical Appendix; online Technical Appendix Table 2).

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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.

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Tickborne Lymphadenopathy Complicated by Acute Myopericarditis, Spain

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To the Editor: Dermacentor-borne necrosis erythema lymphadenopathic 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′- TAATTGCCCTTTCACCTTG –3′] and PEDV-420R [5′- TCAACATATGCGAGGCTC –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′- TCGCTCTGCTGCGAGATCTAGTC-3′] and PEDV-QR [5′- GCGTCAACAGCCAGGTA –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 (DH5α). 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       | ATACATTGGGGRCRATACCA |                 |                           | 21338–21357 |
| S           | FuS2F       | GYGGTGCCGACAARTRGTAC | 993              | 50.3                      | 21205–21224 |
|             | FuS2R       | CAARGMMGCGAGACGAGTA |                 |                           | 22178–22197 |
| S           | SF1         | TCACTCCATTATGATGTTGTTA | 1666             | 52.9                      | 20527–20550 |
|             | SR1         | GGGCGAGAAGCTTATATTAAC |                 |                           | 22169–22192 |
| S           | SF2         | GTTTTCTCGAGTTGCTTTTGA | 1593             | 59.0                      | 22049–22072 |
|             | SR2         | AAAGACTCGAGCAATGGTGTG |                 |                           | 23618–23641 |
| S           | SF3         | GTACAGTGGTCTCTCATAGTTG | 1422             | 53.9                      | 23489–23512 |
|             | SR3         | TCTAATTGGAAACTACATTGCTC |               |                           | 24887–24910 |
| N           | NF1         | TTAAGGCTTCTTCTCAGGTTT | 1544             | 57.4                      | 26377–26396 |
|             | NF2         | AGATGAAAGGTACTGCCTTC |                 |                           | 27899–27920 |

**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 |
|----------------------|-----------|----|----|----|----|----|----|----|----|----|-----|
| Cytotoxic effect     | ND        | +  | +  | +  | +  | +  | +  | +  | +  | +   | +   |
| RT-PCR               | +         | +  | +  | +  | +  | +  | +  | +  | +  | +   | +   |
| Real-time (copies/μl; C<sub>T</sub> value) | 1.412; 17.96 | 1.905; 17.91 | 30.325; 16.11 | 21.633; 16.42 | 24.727; 16.3 | 7.287; 17.39 | 16.565; 16.77 | 33.905; 16.02 | 80.579; 13.98 | 283.000; 13.77 |
| Infectious titer (log<sub>10</sub> TCID<sub>50</sub>/ml) | 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 μm 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      | Korea   | 1a        | (4)       |
| AF353511              | CV777       |         |           |           |
| AF500215              |             |         |           |           |
| AY167585              | Chiju99     |         |           |           |
| AY653204              | JS20042     | Korea   |           |           |
| 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              | CJNU0122201 | Korea   |           |           |
| JN184635              | CJNU0122202 | Korea   |           |           |
| JN800698              | CHGD01      | China   |           |           |
| JQ257005              | CH/KF/11    | China   |           |           |
| JQ257006              | CH/YY/11    | China   |           |           |
| JQ257007              | CH/ZY/11    | China   |           |           |
| JNS59150              | 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/GD/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/HBSC/2012      | China   |           |           |
| JX070671              | CH/FJXM1/2012     | China   |           |           |
| JX070672              | CH/FJXM2/2012     | China   |           |           |
| JX163294              | HBMC2012          | China   |           |           |
| JX258672              | FQ/FJ/2012        | China   |           |           |
| JX242454              | CHHYC082011       | China   |           |           |
| JX242455              | CHHYC102011       | China   |           |           |
| JX242456              | CHHYC122011       | China   |           |           |
| JX242457              | CHHYC122011       | China   |           |           |
| JX242458              | CHLC102011        | China   |           |           |
| JX242459              | CHSONGB122011     | China   |           |           |
| JX242460              | CHYHC122011       | China   |           |           |
| JX242461              | CHZWC122011       | China   |           |           |
| JX242462              | CHHKC082011       | China   |           |           |
| JX242463              | CHLC122011        | China   |           |           |
| JX242464              | CHSHT122011       | China   |           |           |
| JX512907              | HLUJ2012          | 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              | ZJJCZ4            | China   | 2b        | (4)       |
| JX501317              | CH/CY/12          | China   |           |           |
| JX501318              | CH/HBQX/10        | China   |           |           |
| JX501319              | CH/HBXX/11        | China   |           |           |
| JX501320              | CH/HBXX/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              | FJS20128        | China   |           |           |
| KC242906              | FJZZ20128       | China   |           |           |
| KC242907              | JX20129         | China   |           |           |
| KC242908              | HN420129        | China   |           |           |
| KC242909              | GD520129        | China   |           |           |
| KC242910              | GD820129        | China   |           |           |
| KC242911              | HN6201211       | China   |           |           |
| KC242912              | HN8201211       | China   |           |           |
| KC787536              | CHCC012013      | China   |           |           |
| KC787537              | CHDLG012013     | China   |           |           |
| KC787538              | CHGGC112012     | China   |           |           |
| KC787539              | CHGMB022013     | China   |           |           |
| KC787540              | CHHGC012013     | China   |           |           |
| KC787541              | CHLNC012012     | China   |           |           |
| KC787542              | CHSBC032013     | China   |           |           |
| KC787543              | CHSTC12012      | China   |           |           |
| KC787544              | CHYGC12012      | China   |           |           |
| KC787545              | CHYXC012013     | China   |           |           |
| KF384500              | CH/GDGZ2012     | 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              | GDGG/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             | HNZC/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             | GQGZ/2013         | China   |           |           |
| KF779469             | 45RWS/FT0122/12   | Thailand|           |           |
| KF724936             | 155ST0412         | Thailand|           |           |
| KF724937             | 2253AG0211        | Thailand|           |           |
| KF724938             | 656ST0413         | Thailand|           |           |
| KF738258             | SD120330          | China   |           |           |
| KF738259             | JS120604          | China   |           |           |
| KF738260             | JX120506          | China   |           |           |
| KF738261             | SD120502          | China   |           |           |
| KF738262             | SD120611          | China   |           |           |
| KF738263             | SH121229          | China   |           |           |
| KF738264             | ZJ121030          | China   |           |           |
| KF729152             | MF3809/2008       | Korea   |           |           |
| KF9898124            | KPEDV9            | Korea   |           |           |
| KJ020932             | CHYJ130330        | China   |           |           |
| KJ196348             | SHQPYM/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   |           |           |
| KJ451049             | KNU1403           | Korea   |           |           |
| KJ595151             | K13JA121          | Korea   |           |           |
| KJ595152             | K13JA123          | Korea   |           |           |
| KJ595153             | K13JA114          | Korea   |           |           |
| KJ595154             | K14JB01           | Korea   |           |           |
| KJ646578             | FJZP 2013         | China   |           |           |
| KJ646579             | FJCL 2013         | China   |           |           |
| KJ646580             | FJFQ 2014         | China   |           |           |
| KJ646581             | FJFQ1 2012        | China   |           |           |
| KJ646582             | FJFQ2 2012        | China   |           |           |
| KJ646583             | FJLY 2012         | China   |           |           |
| KJ646584             | FJLY 2013         | China   |           |           |
| KJ646585             | FJND 2012         | China   |           |           |
| KJ646586             | FJND 2013         | China   |           |           |
| KJ646587             | FJPT 2013         | China   |           |           |
| KJ646588             | FJKK 2013         | China   |           |           |
| KJ646589             | FJZ 2013          | China   |           |           |
| KJ646590             | FJXY 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             | Kansas43/2013      | 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     |           |           |
| KJ645657             | Ohio60/2013        | USA     |           |           |
| KJ645658             | Minnesota62/2013   | USA     |           |           |
| KJ645659             | Illinois63/2013    | USA     |           |           |
| 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/ZJCHX1/2012            | China       |           |                 |
| KF840538             | CH/ZJHY2/2012             | China       |           |                 |
| KF840539             | CH/ZJJS2/2012             | China       |           |                 |
| KF840540             | CH/ZJJS1Z/2012            | China       |           |                 |
| KF840541             | CH/UXZS3H/2012            | China       |           |                 |
| KF840542             | CH/UXZS2H/2012            | China       |           |                 |
| KF840543             | CH/UXZS12/2012            | China       |           |                 |
| KF840544             | CH/ZJH2HY6/2013           | China       |           |                 |
| KF840545             | CH/UXJIDE/2012            | China       |           |                 |
| KF840546             | CH/ZJJS2Z/2012            | China       |           |                 |
| KF840547             | CH/UXZS3L                 | China       |           |                 |
| KF840548             | CH/UXZS123L               | China       |           |                 |
| KF840549             | CH/SDZD1/2012             | China       |           |                 |
| KF840550             | CH/SDZD2/2012             | China       |           |                 |
| KF840551             | CH/HbBHLY/2012            | China       |           |                 |
| KF840552             | CH/ZJQ22/2012             | China       |           |                 |
| KF840553             | CH/ZJH2/2012              | China       |           |                 |
| KF840554             | CH/ZJHZ2C/2012            | China       |           |                 |
| KF840555             | CH/UXJIDE/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         |           |                 |
| KJ776677             | LZW                       | China       |           |                 |
| KJ776678             | LZW                       | China       |           |                 |
| KM189366             | On007                     | Canada      |           |                 |
| KM189368             | PE1023                    | Canada      |           |                 |
| KM196109             | SK030                     | Canada      |           |                 |
| KM196110             | 2014022                   | Canada      |           |                 |
| KM196111             | MB021                     | Canada      |           |                 |
| KM077139             | Minnesota188/2014          | USA         |           |                 |
| KM392222             | TC PC22AP10                | USA         |           |                 |
| KM392226             | TC PC168P2                 | USA         |           |                 |
| KM392231             | TC PC182P2                 | USA         |           |                 |
| KM392232             | TC Iowa106 (PV39)P1        | USA         |           |                 |
| KM39229              | TC PC103(PC21A)P4          | USA         |           |                 |
| KM39227              | TC PC170P2                 | USA         |           |                 |
| KM39228              | TPC173P2                  | USA         |           |                 |
| KM39229              | TC PC177P2                 | USA         |           |                 |
| KM39230              | 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/GBZQ/2014              | China       |           |                 |
| KM287429             | XJDB2                     | China       |           |                 |
| KM406178             | LS1401                    | China       |           |                 |
| KM406179             | MY1402                    | China       |           |                 |
| KM406180             | DY1403                    | China       |           |                 |
| KM406181             | SH1404                    | China       |           |                 |
| KM406182             | MX1405                    | China       |           |                 |

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| 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)   |           |
| JN189644              | 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.

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**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       | G2.3    | (5)       |           |
| JN601060              | China       | G1      | (5)       |           |
| JN601059              | China       | G2.3    | (5)       |           |
| JN601058              | China       | G1      | (5)       |           |
| JN601057              | China       | G1      | (5)       |           |
| JN601056              | China       | G2.1    | (5)       |           |
| JN601055              | China       | G1      | (5)       |           |
| JN601054              | China       |         |           |           |
| JN601053              | China       |         |           |           |
| 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       |         |           |           |
| NC003436              | CH/GDZQ/2014| China   |           |           |
| KM242131              | KNU14061    | Korea   |           |           |
| KM403155              | KNU14061    | China   |           |           |
| KFB840558              | China       |         |           |           |
| KFB840557              | 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/JXJDZ2/2012     | China   |           |            |
| KF840544              | CH/ZJJHZY6/2013    | 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/ZJJHY2/2012     | China   |           |            |
| KF840537              | CH/ZJCX1/2012      | China   |           |            |
| KJ196348              | SHOP/YM/2013       | China   |           |            |
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| KM392225              | TCP103(PC21A)P4    | USA     |           |            |
| KM392232              | TClowa106 (PV39)P1 | USA     |           |            |
| KM392231              | TCP182P2           | USA     |           |            |
| KM392226              | TCP168P2           | USA     |           |            |
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| KJ588064              | KUIDLPED2014001    | Korea   |           |            |
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| KM077139              | Minnesota188/2014  | USA     |           |            |
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| KJ408801              |                     | China   |           |            |
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| KJ020932              | CHYJ130330          | China   |           |            |
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| KF452322              | USA         | G2.1    | (5)       |           |
| KF468752              | MN          | USA     | 2a        | (4)       |
| KF468754              | IA2         | USA     | 2a        | (4)       |
| KF468753              | IA1         | USA     | 2a        | (4)       |
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| KF272920              | USA         | G2.1    | (5)       |           |
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| AB618620              | Japan       | G1      | (5)       |           |
| AB618619              | Japan       | G1      | (5)       |           |
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| HQ455345              | China       | G1      | (5)       |           |
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| KC109141              | China       | 1b      | (4)       |           |
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| JX088695              | China       | 2a      | (4)       |           |
| JQ023162              | Korea       | 1b      | (4)       |           |
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| JN547228             |             | China   | 1r        | (4)       |
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| AY563206             |             | China   | G2.1      | (5)       |
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| AF237764             | Korea       | G1      |           | (5)       |
| EF185992             | China       | 1a      |           | (4)       |
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| KP202365             | USA         |         |           |           |
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| KP162057             |             | China   |           |           |
| KM887144             |             | China   |           |           |
| KM604665             |             | China   |           |           |
| LM645657             | Germany     |         |           |           |
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| KJ645707             | Minnesota84/2013 | USA |           |           |
| KJ645706             | Minnesota71/2013 | USA |           |           |
| KJ645705             | Minnesota61/2013 | USA |           |           |
| KJ645704             | Minnesota52/2013 | USA |           |           |
| KJ645703             | Minnesota127/2014 | USA |           |           |
| KJ645702             | Ohio126/2014  | USA    |           |           |
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| KJ645697             | Texas128/2014  | USA |           |           |
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| 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 |           |           |
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| KM052365              |                   | USA     |           |           |
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| KF650371              |                   | USA     | G2.1      | (5)       |
| KF650370              |                   | USA     | G2.1      | (5)       |
| KF267450              |                   | USA     | G2.1      | (5)       |
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| JX261936              | China             |         | 2b        | (4)       |
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| JF6907980             |             | China   |           |           |
| Z14976                |             |         |           |           |
| KR270436              | BM1         | Korea   | G1        | 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.