**Pathogenic Pseudorabies Virus, China, 2012**

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

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

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

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

**The Study**

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

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

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

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Pathogenic Pseudorabies Virus, China

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

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

Conclusions

We describe and analyzed a major outbreak of PRV in pigs in China. In these herds, all pigs had been vaccinated against PRV 3 times a year, at approximately the same time as each other. The disease spread to >6 provinces with the NVDC-PRV-SD strain 21 days after vaccination.

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

Figure 2. Alignment the partial sequences of glycoprotein (g) C (A), gD (B), and gE (C) genes of pseudorabies virus at the nucleotide level. Black box indicates the region of insertion.
Her research interests are epidemiology and pathologic analyses of infectious disease.

DISPATCHES

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

| Sample origin province | Collection date | gE ELISA S/N ratio* |
|-----------------------|-----------------|---------------------|
| Shandong              | 2012 Jan        | 0.115               |
| Beijing               | 2012 Feb        | 0.240               |
| Hebei                 | 2012 Feb        | 0.169               |
| Tianjin               | 2012 Feb        | 0.171               |
| Liaoning              | 2012 Mar        | 0.168               |

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

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

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

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## Technical Appendix

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

| Reference PRV Kaplan strain target gene | Primer sequences (5′→3′) | Amplicon, bp |
|----------------------------------------|--------------------------|-------------|
| gE                                     | Forward primer (gE1F): ATGCGGCCCTTTCTGCTGCGCG 611 |
| gD                                     | Reverse primer (gE611R): ACAAAGAACACGGCDCGCCGG 493 |
| TK                                     | Forward primer (gE592F): CGCGACCGCGTCGGCTGCTG 568 |
| gH                                     | Reverse primer (gE1084R): CGTGGCGCGTTGAGGCTCACT 634 |
| gL                                     | Forward primer (gE1066F): ATGACCCACAACGGGCACGTC 589 |
| gM                                     | Reverse primer (gE1734R): TTAAGCGGGGCAGGGCAGGGCT 566 |
| gN                                     | Forward primer (gD1F): ATGCTGCTCCAGGGCAGGATTGG 534 |
| gB                                     | Reverse primer (gD634R): TGAAGCTCTCGTGTTCCAGC 658 |
| gC                                     | Forward primer (gD614F): GCTGGAACGGAGCGAGCTTCA 474 |
| gG                                     | Reverse primer (gD1203R): CTACGGGACCAGGGGTCTTTTA 773 |
| gi                                     | Forward primer (TK1F): ATGCGCATCCTCCAGGATCTACCTC 471 |
| gK                                     | Reverse primer (TK566R): ACCAGCATGCGCTAGGCTTGC 574 |
| PK                                     | Forward primer (TK545F): GCAACGTCTACGCACAGGTGTT 628 |
| 11K                                    | Reverse primer (TK1079R): TTTATGGGATGACATACAGATC 297 |
| 28K                                    | Reverse primer (gH1F): ATGCCCGCGTCGTCCGGCGCT 717 |
|                                        | Forward primer (gH658R): AGCGGACCTTACACGGAGGGAAG 732 |
|                                        | Forward primer (gH636F): CTTCCACTCTCCAGGATCTACCTC 598 |
|                                        | Reverse primer (gH1310R): GAGAAGCGACAGCGACCTGAA 670 |
|                                        | Forward primer (gH1288F): TTACGCCTGTCCAGCGTCTTCTC 717 |
|                                        | Reverse primer (gH2061R): TCACGACTTGCGGTCTTTATACCC 745 |
|                                        | Forward primer (gL1F): TTACTCGGGGGGGGGCGGCTTCA 728 |
|                                        | Reverse primer (gL471R): ATGTCGCGCGCTTACGGCGGTGCT 791 |
|                                        | Forward primer (gL1F): TTACGGAACGGAGGATGACGTTCTC 576 |
|                                        | Reverse primer (gL574R): CGTCGTAACGCTAGGCTCT 457 |
|                                        | Forward primer (gL554F): AGGACGACGTTCGTCGACGAG 939 |
|                                        | Reverse primer (gM1182R): ATGTCGCGCGCGCGCGCGCGCG 551 |
|                                        | Forward primer (gN1F): ATGTCGCTTACGTGGCGGTGCTCT 666 |
|                                        | Reverse primer (gN297R): TTATACGCGCCGGCGCAGGC 297 |
|                                        | Forward primer (gB1F): CTACAGGCGTGCCCAGCAGACG 771 |
|                                        | Reverse primer (gB717R): GAGGACTCAAATCAGTGCGCAT 297 |
|                                        | Forward primer (gB695F): ATGCACGCGCAATGCGAGCTCT 297 |
|                                        | Reverse primer (gB1427R): GCTACCAACAGCGACGCAGCT 551 |
| Primer            | Oligonucleotide Sequence |
|-------------------|--------------------------|
| Forward primer (gB1406F) | AGCACGTGCGTGCTGTTGTAGC   |
| Reverse primer (gB2095R) | GTGCCTCTCCACAGCGAGCATCTG |
| Forward primer (gB2072F) | ACGTACTCGGCCTTGGAGACGCAC |
| Reverse primer (gB2742R) | ATGCCCTCGCTGCAGCGGTTCG   |
| Forward primer (gC1F) | ATGGCCTCGCTCGCGCGTGCG     |
| Reverse primer (gC717R) | GAACTCGGGCTGGCTGTACAGGA  |
| Forward primer (gC695F) | TCCTGTACAGCAGCCGGAGTTTC  |
| Reverse primer (gC1440R) | TCACGCCCCGCCCGCCCGGATAGTA  |
| Forward primer (gG1F) | ATGAGTGCGAAGGCGATGTC     |
| Reverse primer (gG728R) | TCGATAGTCTGGGCCTAGTGTC   |
| Forward primer (gG706F) | GACGACTAGCGCCGACTACAGA  |
| Reverse primer (gG1497R) | TCAGGCGAGCCACAGTGCCGTT |
| Forward primer (gI1F) | ATGATGATGGTGGCGCGGTACGT  |
| Reverse primer (gI576R) | TGGTCGGATCGTTGGGACAGCA   |
| Forward primer (gI554F) | TGGTCGGATCGTTGGGACAGCA   |
| Reverse primer (gI1101R) | TTATTGTTCCTCAGTGGTG    |
| Forward primer (gK1F) | TCATCCAAATATGGAATGTGCGG  |
| Reverse primer (gK939R) | GTGCCCTCGGCCGGGCTCCGCT  |
| Forward primer (PK1F) | ATGTTGCCGTATGAGTAGAGG    |
| Reverse primer (PK551 R) | CTGACGATGCACGGGCAAGGT    |
| Forward primer (PK530F) | ACTTTCCGTGCATCTGTACAG    |
| Reverse primer (PK1196R) | TTATACGGGTCCACATTCAAGAG |
| Forward primer (11K1F) | ATGGACAGCTGCCACCCAGC     |
| Reverse primer (11K297R) | CTACACGTGCCGCGGAGTATGAT |
| Forward primer (28K1F) | ATGGGTTGACGCGGTCACCCGT  |
| Reverse primer (28K771R) | CTAGGAGATGGTTACATCCCG   |

*PRV, pseudorabies virus; g, glycoprotein.
Technical Appendix Table 2. PRV sequences obtained from GenBank used in this study*

| Sequence coding for | Strain          | GenBank accession no. | Origin       |
|---------------------|-----------------|-----------------------|--------------|
| gC                  | Namyangju       | GQ325659              | South Korea  |
| gD                  | PRV OK2010      | JF767011              | USA          |
| gE                  | PRV12466        | AF176495              | USA          |
|                     | PRV12271        | AF176493              | USA          |
|                     | PRV10649        | AF176489              | USA          |
|                     | PRV9164         | AF176489              | USA          |
|                     | PRV8044         | AF176487              | USA          |
|                     | PRV7739         | AF176485              | USA          |
|                     | PRV7438         | AF176483              | USA          |
|                     | PRV4411         | AF176481              | USA          |
|                     | PRV43           | AF176479              | USA          |
|                     | PRV12481        | AF176494              | USA          |
|                     | PRV11243        | AF176492              | USA          |
|                     | PRV10501        | AF176490              | USA          |
|                     | PRV8095         | AF176488              | USA          |
|                     | PRV8033         | AF176486              | USA          |
|                     | PRV7652         | AF176484              | USA          |
|                     | PRV4520         | AF176482              | USA          |
|                     | PRV2908         | AF176480              | USA          |
|                     | Ea              | AF158090              | China        |
|                     | Fa              | AF403051              | China        |
|                     | P-PrV           | EU915280              | Malaysia     |
|                     | BJ              | EU719644              | China        |
|                     | SQ              | EU719642              | China        |
|                     | 783             | EU719640              | China        |
|                     | SCZ             | EU719638              | China        |
|                     | HS              | EU719636              | China        |
|                     | SL              | EU719634              | China        |
|                     | SS              | EU719643              | China        |
|                     | Bartha          | EU719641              | Brazil       |
|                     | DG              | EU719639              | China        |
|                     | SN              | EU719637              | China        |
|                     | SA215           | EU719635              | China        |
|                     | NIA3            | D49437                | Northern Ireland |
|                     | Yamagata S-81  | D49435                | Japan        |
|                     | Indiana S       | D49436                | USA          |
|                     | Bartha          | JF797217              | Hungary      |
|                     | Kaplan          | JF797218              | Hungary      |
|                     | Becker          | JF797219              | USA          |
|                     | Kaplan          | JQ809328              | Hungary      |
| Sequence coding for | Strain   | GenBank accession no. | Origin    |
|---------------------|----------|-----------------------|-----------|
| DUL34 gfp           | JQ809329 | Hungary               |
| DUL34Pass           | JQ809330 | Hungary               |
| Becker              | M12778   | USA                   |
| Namyangju           | GQ325660 | South Korea           |
| PRV-FZ              | FJ477296 | China                 |
| Yangsan             | AY217094 | Korea                 |
| Min-A               | AY169694 | China                 |
| LA                  | AY174090 | China                 |
| FZ                  | EF645837 | China                 |
| FZ                  | EF622042 | China                 |
| Ea                  | AF086702 | China                 |
| Fa                  | AY196984 | China                 |
| SA215               | DQ367438 | China                 |
| Bartha              | JF797217 | Hungary               |
| Kaplan              | JF797218 | Hungary               |
| Becker              | JF797219 | USA                   |
| Kaplan              | JQ809328 | Hungary               |
| DUL34 gfp           | JQ809329 | Hungary               |
| DUL34Pass           | JQ809330 | Hungary               |
| Kaplan              | AJ271966 | Hungary               |
| Yangsan             | AY249861 | South Korea           |
| CL/15               | JF460016 | Argentina             |
| HNJZ                | EU561349 | China                 |
| Becker              | AY368490 | USA                   |
| PRV-SH              | AF207700 | China                 |
| Ea                  | AF171937 | China                 |
| SA                  | GU262988 | China                 |
| NS374               | FJ605135 | Belgium               |
| Nia-1               | FJ605136 | Belgium               |
| 89V87               | FJ605134 | Belgium               |
| 00V72               | FJ605132 | Belgium               |
| 75V19               | FJ605139 | Belgium               |
| LA                  | AY173124 | China                 |
| Fa                  | AF403049 | China                 |
| P-PrV               | FJ176390 | Malaysia              |
| NIA3                | EU502923 | Spain                 |
| GDSH                | EF552427 | China                 |
| Min-A               | AY170318 | China                 |
| Kaplan              | JF797218 | Hungary               |
| Becker              | JF797219 | USA                   |
| Kaplan              | JQ809328 | Hungary               |
| Sequence coding for | Strain     | GenBank accession no. | Origin  |
|---------------------|------------|-----------------------|---------|
| DUL34 gfp           | JQ809329   | Hungary               |
| DUL34Pass           | JQ809330   | Hungary               |

*PRV, pseudorabies virus; g, glycoprotein.

Technical Appendix Figure 1. Immunohistochemical staining of lung (A) and brain (B) specimens of clinically sick pigs. Original magnification ×100.
Technical Appendix Figure 2. PCR of infected tissues with specific primers for pseudorabies virus glycoprotein D (gD)–specific primers (forward primer 5′-CGGAGGACGAGCTGGGGCT-3′; reverse primer 5′-ACGTCCACGCCCCGGCTTTGAAGC-3′), with a fragment size of 217 bp. +: positive control; −, negative control; 1, 2, 3, 4, and 5: supernatants from the homogenized tissues of lungs, heart, tonsils, lymph nodes and serum, respectively. Arrow indicates the target fragment, 217 kb.
Technical Appendix Figure 3. Phylogenetic trees of the glycoprotein (g) E sequence. Unrooted trees constructed for gE sequences by using Mega v. 4.0 (www.megasoftware.net). The phylogenetic trees indicate the relationship of NVDC-PRV-BJ, NVDC-PRV-HEB, and NVDC-PRV-SD to other PRV isolates. These 3 isolates are indicated in the black triangle. Scale bar indicates number of substitutions per site.