Isolation and genetic characterization of a novel porcine epidemic diarrhea virus obtained from an outbreak in 2013-2014 in Japan


Viral Diseases and Epidemiology Research Division, National Institute of Animal Health, National Agriculture and Food Research Organization.

Swine Enteric Coronavirus Diseases International Meeting, Chicago, IL September 25, 2014
Overview

➢ Summary of Pig industry in Japan
➢ Occurrence of PED in Japan including recent outbreak
➢ Isolation and identification of PEDV isolates
➢ Genetic characterization of isolates
Recent pig population in Japan

In last decade, pig population of Japan hover below the 10 million
Distribution of pig population

Top 3 Prefecture (Number of head) in 2014

Heads
- >500,000
- 100,000-<500,000
- 10,000-<100,000
- <10,000

Kagoshima, Miyazaki, Chiba
Distribution of pig population

Top 3 Prefecture (Number of head) in 2014

Heads
- >500,000
- 100,000-<500,000
- 10,000-<100,000
- <10,000
Occurrence of PED in Japan

PED is one of the notifiable diseases under the Act on Domestic Animal Infectious Diseases Control (after 1997).

PED has been recognized from 1980’s, but most of the cases are sporadic with the exception of an outbreak in 1996.

In 2013, a large-scale outbreak was confirmed again after the absence of 7 years.
## Occurrence of PED in Japan (1982-2012)*

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Area</th>
<th>Northern part</th>
<th>Southern part</th>
<th>No. of affected farms/heads</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982</td>
<td>Feb.-Mar.</td>
<td>Tohoku</td>
<td></td>
<td></td>
<td>4</td>
<td>All ages</td>
</tr>
<tr>
<td></td>
<td>Apr.</td>
<td>Tohoku</td>
<td></td>
<td></td>
<td>5/2,756</td>
<td>Dead:179 (piglets)</td>
</tr>
<tr>
<td></td>
<td>Nov.</td>
<td>Shikoku</td>
<td></td>
<td></td>
<td>42.5% of farms</td>
<td>All ages</td>
</tr>
<tr>
<td>1983</td>
<td>Jan.</td>
<td>Hokkaido</td>
<td></td>
<td></td>
<td>1</td>
<td>All ages without fattening pigs</td>
</tr>
<tr>
<td></td>
<td>Mar.</td>
<td>Hokkaido</td>
<td></td>
<td></td>
<td>14/2,103</td>
<td>All ages</td>
</tr>
<tr>
<td></td>
<td>Oct.</td>
<td>Kyushu</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Oct.</td>
<td>Shikoku</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1984</td>
<td>Mar.</td>
<td>Kanto</td>
<td></td>
<td></td>
<td>1/202</td>
<td>Dead:10 (Suckling pigs, Sow)</td>
</tr>
<tr>
<td>1993</td>
<td>Apr.</td>
<td>Hokkaido</td>
<td></td>
<td></td>
<td>1/2,075</td>
<td>All ages, Dead:158 (piglets)</td>
</tr>
<tr>
<td>1994</td>
<td>Jan.</td>
<td>Kyushu</td>
<td></td>
<td></td>
<td>Several</td>
<td>Dead: thousands of Suckling pigs, Sow</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>Kinki</td>
<td></td>
<td></td>
<td>3/1,384</td>
<td>Suckling pigs only, Dead:545</td>
</tr>
<tr>
<td>1995</td>
<td>Feb.</td>
<td>Kanto</td>
<td></td>
<td></td>
<td>1/approx. 600</td>
<td>All ages, Dead: 20-30</td>
</tr>
<tr>
<td>1996</td>
<td>Feb.-Aug.</td>
<td>Hokkaido, Tohoku, Kinki Kyushu (9 prefectures)</td>
<td>102/approx. 80,000</td>
<td>Dead: 39,539 (piglets)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1997</td>
<td></td>
<td>Kyushu, Kinki</td>
<td></td>
<td></td>
<td>3/185</td>
<td>-</td>
</tr>
<tr>
<td>1998</td>
<td>Apr.</td>
<td>Kinki</td>
<td></td>
<td></td>
<td>1/534</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>June-July</td>
<td>Hokkaido</td>
<td></td>
<td></td>
<td>2/1890</td>
<td>-</td>
</tr>
<tr>
<td>1999</td>
<td>Jan.-Feb.</td>
<td>Kinki</td>
<td></td>
<td></td>
<td>2/812</td>
<td>-</td>
</tr>
<tr>
<td>2001</td>
<td>Jan.-Feb.</td>
<td>Kyushu</td>
<td></td>
<td></td>
<td>2/2218</td>
<td>-</td>
</tr>
<tr>
<td>2006</td>
<td>Feb.</td>
<td>Shikoku</td>
<td></td>
<td></td>
<td>1/3</td>
<td>-</td>
</tr>
</tbody>
</table>

*No outbreaks of PED were reported from 2007 to 2012.*
Neutralizing antibody prevalence rate against PEDV in Japan

By the annual report of Animal Health Division, Food Safety and Consumer Affairs Bureau Ministry of Agriculture, Forestry and Fisheries of Japan
PED case reports by week

1st case
Okinawa

2nd case
Ibaraki

3rd and 4th cases
Kagoshima
Miyazaki

Cases

Hokkaido
Tohoku
Kanto
Chubu
Kinki
Chugoku/Shikoku
Kyusyu/Okinawa
Occurrence of PED in Japan 2013-2014

<table>
<thead>
<tr>
<th>Region</th>
<th>Cases</th>
<th>Dead pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hokkaido</td>
<td>23</td>
<td>14275</td>
</tr>
<tr>
<td>Tohoku</td>
<td>81</td>
<td>56397</td>
</tr>
<tr>
<td>Kanto</td>
<td>225</td>
<td>127934</td>
</tr>
<tr>
<td>Chubu</td>
<td>140</td>
<td>45578</td>
</tr>
<tr>
<td>Kinki</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chugoku/Shikoku</td>
<td>19</td>
<td>5863</td>
</tr>
<tr>
<td>Kyusyu/Okinawa</td>
<td>329</td>
<td>122895</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>817</strong></td>
<td><strong>372942</strong></td>
</tr>
</tbody>
</table>

Affected prefectures: 38/47 (80.9%)
Affected farms: 817/5,570 (14.7%)
Affected pigs: approx. 1,227,000/9,685,000 (12.7%)
Dead pigs: approx. 373,000/9,685,000 (3.9%)
(Almost all piglets: 27% of affected pigs were died)

From October 1, 2013 to Aug. 31, 2014
phylogenetic analysis of the spike (S1) region
- nucleotide sequences -

North American Type
Japan (2013-2014)
USA (2013-2014)
Korea (2013-2014)
China (2011-2013)

Japanese Classical (90’s) & Vaccine strains

INDELS Type

Japanese Classical (80’s) & Vaccine strains
phylogenetic analysis of the spike (S1) region
- amino acid sequences -

North American Type
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USA (2013-2014)
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China (2011-2013)

Japanese Classical (90’s) & Vaccine strains
INDELS Type

Japanese Classical (80’s) & Vaccine strains
### Sequence analysis on S genes

<table>
<thead>
<tr>
<th></th>
<th>Global emerging PEDV strains*</th>
<th>Japanese Current strain</th>
<th>Japanese variant†</th>
<th>Japanese Classical strain**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese Current strain</td>
<td>99.5%-99.9%</td>
<td>99.3%-99.9%</td>
<td>-</td>
<td>93.2%-94.9%</td>
</tr>
<tr>
<td>Japanese variant†</td>
<td>99.4%-99.9%</td>
<td>96.3%-96.6%</td>
<td>99.7%</td>
<td>93.1%-96.2%</td>
</tr>
</tbody>
</table>

*Global emerging PEDV strain are US strain (isolate IA1: KF468753) and Korea strain (isolate KNU-1305: KJ451040)*

**Japanese classical field strains are NK(AB548623), KH (AB548622), MK (AB548624), and 83-P5 (AB548618)

† 99.4%-99.9% homology among Japanese variants and US variant strain OH851 (KJ399978) and Chinese strain CH/HBQX/10 (JX501318)
Alignment of the N-terminus residues of the S protein

Insertion and deletion sites were conserved

Alignment of the deduced amino acid sequences corresponding to the first 180 N-terminus residues of the S protein of the current Japanese field PEDV strains with those of the US (OH851 and IA1), Chinese (CH/HBQX/10 and AH2012) and the prototype CV777 strains. Dots indicate the amino acids that are identical to those in the strain OH851. Dashes indicate the deleted sequences. Deletions and insertions are boxed and shaded.
Virus isolation

Cell: Vero cells
Medium: Eagle’s MEM/TPB containing 5-10 ug/mL trypsin

A: CPE (syncytium)
B: IFA (low mag.)
C: IFA (high mag.)
D: mock
Growth kinetics of isolate in 2013

Log TCID₅₀/0.1mL

hours post infection

Intracellular virus

Extracellular virus

CPE
Antigenic comparison among PEDV strains

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>CV777</th>
<th>Japanese Classical strain</th>
<th>Japanese Current strain</th>
<th>Japanese variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔCV777</td>
<td>2560*</td>
<td>1280</td>
<td>1280</td>
<td>1280</td>
</tr>
<tr>
<td>ΔJapanese Classical strain</td>
<td>640</td>
<td>640</td>
<td>640</td>
<td>1280</td>
</tr>
<tr>
<td>ΔJapanese Current strain</td>
<td>2560</td>
<td>2560</td>
<td>2560</td>
<td>2560</td>
</tr>
<tr>
<td>ΔJapanese variant</td>
<td>2560</td>
<td>640</td>
<td>1280</td>
<td>2560</td>
</tr>
</tbody>
</table>

*: IFA antibody titer was expressed as the reciprocal of the highest dilution of serum exhibited specific fluorescence
Conclusion

✓ The Outbreak of PED was occurred in Japan after the absence of 7 years.

✓ The recent Japanese PEDV were genetically close to the viruses isolated in East Asia and the US in 2013.

✓ INDELS Type were also detected from affected pigs

✓ PEDV isolated from affected pigs in 2013-2014 were antigenically similar to each other, and moreover to the CV777 and Japanese classical strain.

✓ The PED outbreak in 2013-2014 in Japan might have been caused by invasion of exotic virus, not by domestic virus.
Thank you very much for your attention