A Serological Comparison of 4 Japanese Isolates of Porcine Enteroviruses with the International Reference Strains

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ABSTRACT. The reference strains of four serotypes (J6, J8, J9 and J10) of porcine enterovirus isolated in Japan were compared with the international reference strains of 11 serotypes (W1 to W11) by cross neutralization tests. No cross reactions were observed between the two groups, although there were minor one-way crosses between W10 and J10, W11 and J9, and W4 and J9. This leads to our conclusion that all 4 Japanese serotypes can be newly added to 11 international serotypes. J9 virus produced type 1 of CPE (CPE I), J6 and J8 did CPE II, and J10 did CPE III. J10 grew in Vero, HeLa cells, and the primate cells, but J6, J8 and J9 did not. — KEY WORDS: CPE type, porcine enterovirus, serological classification.

Porcine enteroviruses (PEV) have been classified into 11 serotypes [6] based on a minimum relation of 5% of the homologous serum titre [3], and CPE-types by the morphology of infected cell culture [11]. Japanese isolates have been classified independently into 10 serotypes, by 3 groups of investigators [4, 5, 8 and Dr. Yasuo Minura's personal communication in National Institute of Animal Health of Japan]. Morimoto et al. [8] proved that reference strains of J1, 2, 7, 3, 5 and 4 relate to prototype strain of W1, 2, 3, 5, 6 and 8, respectively crossing more than 5% of the homologous serum titers [7]. However, 4 Japanese serotypes J6, 8, 9 and 10 have not yet been investigated in their relation to the international serotypes.

In the report the results of cross neutralization among Japanese isolates of 4 serotypes which are unknown in relation with international serotypes and prototype strains of 11 international serotypes are described. The CPE type and growth characteristics in different cell lines of Japanese 10 serotype viruses are also described.

MATERIALS AND METHODS

Cells: Pig kidney cell lines (IB-RS-2) were used for propagation of the viruses and neutralization tests. Baby hamster kidney (BHK-21) cells, African green monkey kidney (Vero) cells and human cervical cancer (HeLa) cells were used for examination of virus growth characteristics.

Virus: The viruses examined in this study are listed in Table 1. International and Japanese serotypes are named as W1 to W11 and J1 to J10, respectively. The prototype viruses of W1 to W11 were supplied by the Animal Virus Research Institute, Pirbright, U.K. The reference viruses of J1 to J10 and swine vesicular disease viruses were from the National Institute of Animal Health of Japan.

Purification of virus: Viruses were concentrated by ammonium sulfate precipitation followed by sucrose density gradient purification as previously described [7, 10].
Table 1. International and Japanese reference strains of porcine enteroviruses

<table>
<thead>
<tr>
<th>Serotype</th>
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</thead>
<tbody>
<tr>
<td>International reference strains</td>
</tr>
<tr>
<td>Designation</td>
</tr>
<tr>
<td>W1</td>
</tr>
<tr>
<td>W2</td>
</tr>
<tr>
<td>W3</td>
</tr>
<tr>
<td>W4</td>
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<tr>
<td>W5</td>
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<tr>
<td>W6</td>
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<tr>
<td>W7</td>
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<tr>
<td>W8</td>
</tr>
<tr>
<td>W9</td>
</tr>
<tr>
<td>W10</td>
</tr>
<tr>
<td>W11</td>
</tr>
</tbody>
</table>

SVDV: swine vesicular disease virus

Antiserum: Guinea pig antisera to prototype viruses of W1 to W11 were supplied by the Animal Virus Research Institutes, Pribright, U.K. Antisera to viruses of J1 to J10 were prepared in guinea pigs according to Knowles et al. [7].

Titration and neutralization test: The virus was diluted 10-fold in growth medium using eight tubes per dilution. Of each dilution, 0.1 ml was mixed with 0.5 ml of IB-RS-2 cells suspension containing about 1×10^5 cells, and incubated at 37°C, in a slant state, for 7 days until CPE appeared in the highest dilution. The TCID_{50} titre was calculated by the Kärber’s method.

A 0.1 ml of virus suspension containing 200TCID_{50} was added to 0.1 ml of each two-fold serum dilution, and the mixtures were incubated at 37°C for 1 hr with agitation at 15 to 20 min intervals. Each 0.1 ml of virus-serum mixture was added to 0.5 ml of IB-RS-2 cell suspension containing about 1×10^5 cells in two test tubes. The tubes were then incubated. The serum neutralization titre was expressed as the reciprocal of the dilution, showing a 50% endpoint. For comparison the titre for a strain was expressed as a percentage of the homologous titre. A strain was regarded as a serologically different type from the reference strain when its relative titre was less than 5%.

Examination of behavior of porcine enterovirus on several line cells: All Japanese isolates were inoculated at M.O.I. 0.1 on IB-RS-2, BHK-2, HeLa, and Vero cells sheathed in 24-well-plates, and the appearance of the CPE was observed. The type of CPE was determined on IB-RS-2 cells.

RESULTS

Cross-neutralization test: There were no cross-reactions above 5% of homologous serum titre, except three minor cross-reactions, among Japanese reference strains 4 serotypes and the international serotypes (Table 2). Minor cross-reactions were noted between W10 (LP54) antiserum and J10 (W47H) virus (5%). W11 (UKG 173/74) antiserum and J9 (50L4) virus (8%), and between J9 (50L4) antiserum and W4
Table 2. Cross-neutralization among 4 Japanese strains and world reference strains of porcine enterovirus

<table>
<thead>
<tr>
<th>Viruses</th>
<th>W4 PS36</th>
<th>W7 F43</th>
<th>W9 UKG 410/73</th>
<th>W10 LP54</th>
<th>W11 UKG 173/74</th>
<th>J6 IP1</th>
<th>J8 4cc</th>
<th>J9 50L4</th>
<th>J10 W47H</th>
<th>SVDV J-1/73</th>
</tr>
</thead>
<tbody>
<tr>
<td>W4 PS36</td>
<td>100&lt;sup&gt;a&lt;/sup&gt; (38,400)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>(1,200)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>W7 F43</td>
<td>-</td>
<td>100 (6,400)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>W9 UKG/410/73</td>
<td>-</td>
<td>-</td>
<td>100 (1,200)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>W10 LD54</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100 (400)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>W11 UKG/173/74</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100 (2,400)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J6 IP1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100 (4,800)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J8 4CC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100 (4,800)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J9 50L4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8 (200)</td>
<td>-</td>
<td>-</td>
<td>100 (25,600)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J10 W47H</td>
<td>-</td>
<td>-</td>
<td>5 (20)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100 (38,400)</td>
<td>-</td>
<td>100 (76,800)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Percent reaction of homologous titre.
<sup>b</sup> Reciprocal of 50 percent neutralization endpoint.
<sup>c</sup> Less than 5 percent of homologous titre.

Table 3. Behavior of Japanese strains of porcine enterovirus on various cell lines

<table>
<thead>
<tr>
<th>Serotype Designation</th>
<th>CPE type on IB-RS-2</th>
<th>CPE on IB-RS-2</th>
<th>CPE on BHK-21</th>
<th>CPE on Vero&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CPE on HeLa&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>J1 (SF12)</td>
<td>I</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J2 (SFK10)</td>
<td>I</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J3 (SF1)</td>
<td>I</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J4 (SF16)</td>
<td>II</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J5 (SFG12)</td>
<td>I</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J6 (IP1)</td>
<td>II</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J7 (SPG30)</td>
<td>I</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J8 (4CC)</td>
<td>II</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J9 (50L4)</td>
<td>I</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J10 (W47H)</td>
<td>III</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>FVDV(J-1/73)</td>
<td>III</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup> Originated from primates.

(PS36) virus (5%). This each minor cross reaction was in a one-way and no reverse cross was observed. Although the data were not shown, there were no cross-reactions above 5% of homologous titres among Japanese serotypes J6, J8, J9, and J10, and other international serotypes (W1, W2, W3, W5, W6, and W8), respectively. This indicates that J6, J8, J9, and J10 are different from the 11 known serotypes.

**CPE type:** All porcine enteroviruses of 10 serotypes isolated in Japan multiplied in
IB-RS-2 cells and exhibited one of three types of CPE (I, II, and III) [2, 11, 12] (Table 3). J9 showed CPE I (Fig 1A), which was characterized by round, refracting cells grouped into foci. J6 and J8 produced CPE II (Fig 1B and 1C), which was characterized by refracting cells with evident and characteristic cytoplasmic prominences. It is interesting that J10 exhibited a characteristic of CPE III (Fig 1D), which appeared disseminated with round refracting cells and was same as the type SVDV showed [1].

Behavior on cell lines: The BHK-21, HeLa and Vero cell lines showed a varied susceptibility to Japanese isolates (Table 3). J9 which showed CPE I did not grow in BHK-21, Vero and HeLa cells. Only J10 multiplied in the three line cells of non-porcine origin, and exhibited a characteristic of CPE III on them as well as on IB-RS-2 cells. J6 (CPE II) grew in BHK-21 cells but J8 (CPE II) did not.

DISCUSSION

Porcine enteroviruses isolated in the U.S.A., U.K. and in Japan were classified into 8 serotypes by Dunne et al. [2]. After that, Knowles et al. [6] proposed three new serotypes, 9, 10, and 11 under the criterion used by Dunne et al. [2] which was a minimum reaction of 5% of homologous serum titre.

In this paper, the relations of 4 Japanese isolates of PEV, such as J6, 8, 9, 10, which have been independently classified, and whose relationship to the international serotypes have not been clarified, to the international serotypes were examined for the first time by neutralization tests. The differences between 4 Japanese serotypes (J6, 8, 9, 10) and 11 international serotypes were clearly demonstrated, although minor cross-reactions were noted between W10 (LP54) antiserum and J10 (W47H) virus
(5%), between W11 (UKG 173/74) antiserum and J9 (50L4) virus (8%), and between J9 (50L4) antiserum and W4 (PS36) virus (5%). There were no cross-reaction in the reverse relationship, respectively. J10 and W10, J9 and W11, and J9 and W4 are thought to be related closer to each other than to the other serotypes. Further studies are necessary to clarify them.

CPE types were found to be a useful aid in the identification of isolates by examining CPE type of the new isolates and other Japanese porcine enteroviruses (Table 3). Porcine enterovirus showed one of three CPE types on IB-RS-2 cells as noted [7]. J6 and J8 showed a characterstic of CPE II, and J9 exhibited that of CPE I. It is notable that J10 produced CPE III the same as SVDV did [1]. CPE type is correlated to serotype. Particular groups of serotypes produced one of three types of CPE.

In behavior on cell lines, J6, 8 with CPE II, and J9 with CPE I did not grow in Vero and HeLa originated from primates. J10 with CPE III grew in IB-RS-2 and Vero and HeLa cells. More number of isolates of the same serotype as J10 are necessary to examine to have the same host range as J10 (W47H).

In conclusion, it was suggested that the serotypes of 4 Japanese isolates (J6, 8, 9 and 10) were new ones different from 11 international serotypes. However, in order to determine the 4 Japanese isolates to be new serotypes, further studies are needed to compare the 4 Japanese isolates with more isolates over the world such as 26 new isolates of different serotypes from 11 known international serotypes as described by Knowles et al. [6].

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要約

我が国で分離された豚エンテロウイルス4株の国際基準株との血清学的比較：本多英一・木俣 新・服部達・熊谷哲夫・津田知幸1)・徳井忠史1)（東京農工大学農学部畜医学科家畜微生物学講座、1)家畜衛生試験場）

——我が国で分離され日本における血清型が決められている豚エンテロウイルス4株（J6, J8, J9, J10）と国際基準株（W1—W11）との交叉中和試験を行った結果、これら4株はそれぞれ国際基準株11血清型とは異なる血清型であることがわかった。このことは国際基準血清型11型に新たに4型を追加する必要があることを示している。またJ9はCPEI型を、J6とJ8はCPEII型を、J10はCPEIII型を示した。