Epidemiology of Seneca Valley Virus: Identification and Characterization of Isolates from Pigs in the United States

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ABSTRACT
Between 1888 and 2005, 12 picornavirus-like viruses were isolated from pigs showing a variety of clinical symptoms in various locations in the United States. Virus neutralization tests using a specific antisera revealed one of the isolates showed them to be antigenically related. Six of these isolates were subjected to a pan-picornavirus RT-PCR which employed primer targets were to the 3' end of the genome. Sequence analysis of the resulting amplicons revealed all the virus isolates to be closely related to each other and to a newly described picornavirus, Seneca valley virus (SVV). SVV-001 had originally been isolated from cell culture media and was presumed to have been introduced via bovine serum of porcine tissue trypan during the cultivation of the cells. SVV is a new picornavirus species and most closely related to the cardioviruses, however, it has a number of genome features which have led to the proposal that it be classified in a new picornavirus genus. Based upon the known sequence of SVV, two additional genome regions (VP1 and 2C) of the porcine isolates of SVV were amplified by RT-PCR and their sequence determined. Relationships between the porcine isolates of SVV were shown in all three genome regions studied. Regression analysis indicated that SVV may have been recently introduced into pigs in the USA. Attempts to infect pigs with two of the isolates failed to demonstrate any specific disease. Serological surveys revealed the presence of specific SVV antibodies in pigs, cattle and mice but not in humans.

INTRODUCTION
Seneca Valley virus-SVV-001 was isolated at Genetic Thryco Inc. (Glenelg, MD) in 2002 from cell culture media. The complete genome sequence was described by Knowles & Hallenbeck (2003) and shown to be related to, but distinct from, the cardioviruses. (Fig. 1) Here we report on the biological and serological characteristics of seven of isolates of SVV made from pigs in various locations in the United States (Michigan, Minnesota, New Jersey, North Dakota, Illinois, Louisiana and California) over a period of 14 years. Our epidemiological analyses reveal the presence of neutralizing antibodies in pigs and other farm animals but not in humans. This information, coupled with the isolation of members of SVV in pigs, suggests that pigs are a natural host for SVV.

MATERIALS & METHODS
RT-PCR & DNA sequencing
Total RNA was extracted from 460 μg/ml ethidium bromide. DNA weight markers (GeneRuler 100 bp DNA Ladder Plus) were run alongside the samples to facilitate product identification and to detect PCR amplification of DNA and primer was achieved enzymatically using ExScript RT-PCR kit (Qiagen). According to the manufacturer’s instructions, PCR amplification were sequenced with the two primer PCR mix six others shown in Table 1 using the DTS Quick Start Kit (Beckman Coulter) according to the manufacturer’s instructions. The sequencing reactions were run on a CEQ8000 Automated Sequencer (Beckman Coulter) according to the manufacturer’s instructions. A pan-picornavirus RT-PCR sequencing method was used to determine the 3’ end of the genome of each virus isolate (Knowles, 2003).

Phylogenetic analysis
Phylogenetic analyses were conducted using MEGA version 3.1 (Kumar et al., 2004). Neighbor-joining trees were constructed using a distance matrix based on the Kimura 2-parameter model of nucleotide substitution. Confidence levels on branches were estimated by bootstrapping (1000 pseudo-replicates).

RELATIONSHIPS BETWEEN SENeca VALLeY VIRUSES
Fig. 3 shows the genetic relationships between SVV-001 and seven of the SVV isolates made from pigs in the USA. Both the 3’ end of the genome and the VP3 encoding region of the earliest SVV isolate, A (NC, 1988), were picked against the date of isolation of each of the later virus isolates.

PREVALENCE OF NEUTRALIZING ANTIBODIES IN FARM ANIMAL POPULATIONS AND OTHER ANIMALS
In order to further study the epidemiology of SVV-001, serum samples were obtained from farm animals including pigs, cows and wild mice. The serum samples were tested individually for the presence of neutralizing antibodies to SVV-001 in a neutralization assay. Of particular interest would be isolates from other countries and non-pig species.

Table 1. Summary of serum samples tested from farm animals in a neutralization assay.

<table>
<thead>
<tr>
<th>Year</th>
<th>Pigs (fom disease-free farm)</th>
<th>Cows</th>
<th>Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>30</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>2000</td>
<td>30</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>1999</td>
<td>35</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

CONCLUSIONS
We have shown considerable sequence identity between SVV-001 and seven viruses isolated at the USDA from extra-farm sources. Additionally, each of these viruses has been shown to be serologically related to each other, as well as to SVV-001. This serological profile of one virus, SVV-001 has been identified to infect pigs. It is clear that SVV-001 is found in pigs, but not in other farm animals or wild mice. The virus may have recently been introduced into this population. It is possible that SVV-001 may infect pigs elsewhere in the world, or perhaps the virus may infect pigs and other farm animals but not in humans. This information, coupled with the isolation of members of SVV in pigs, suggests that pigs are a natural host for SVV.