Survival and infectivity of porcine reproductive and respiratory syndrome virus in swine lagoon effluent

S. A. Dee, B. C. Martinez, C. Clanton

PORCINE reproductive and respiratory syndrome virus (PRRSV) is an enveloped RNA virus that is both heat and pH labile. It is readily inactivated by drying or following contact with a broad range of disinfectants (Benfield and others 1992, Bloemraad and others 1994). In addition, PRRSV survives for extended periods when frozen or if in a moist environment (Benfield and others 1992, Bloemraad and others 1994, Pirtle and Beran 1996). In a study by Pirtle and Beran (1996), a standard concentration of PRRSV (2 x 10^5 TCID50/ml) was added to a sample of water taken from a well and a sample of city water at 1:1 dilution and incubated at 25 to 27°C for 11 days. On each day of the incubation period, an aliquot of inoculated liquid from both sources was collected and tested for the presence of PRRSV. Infectious PRRSV was recovered from well water after nine days and from city water after 11 days.

For handling swine waste, many commercial production systems use high-volume, underslat flushing systems that recirculate lagoon effluent back to the manure pits beneath the animals. With these systems, animals frequently come into contact with effluent from the lagoon as it splashes upward through the slotted concrete flooring (S. Dee, personal observations). The aims of the present study were to determine how long PRRSV could remain viable in swine effluent and to test the infectivity of PRRSV-inoculated effluent in naive pigs.

Effluent was obtained from a single-stage, anaerobic lagoon, with a depth of 6·6 m, that serviced the breeding, gestation, farrowing and nursery facilities of a commercial swine operation located in the mid-west USA. To initiate the study, a 4 litre sample of effluent from the pit of the nursery facility was collected as it flowed into the lagoon basin. Ten 5 ml samples of the effluent were collected and tested for the presence of PRRSV by TaqMan PCR (Perkin-Elmer Applied Biosystems) and virus isolation at the Minnesota Veterinary Diagnostic Laboratory (Bautista and others 1993, Molitor and others 1997). All samples were negative by both tests. Four 20 ml aliquots of effluent were then collected from the original sample, and a 2 ml aliquot of PRRSV strain MN 30-100 (Bierk and others 2001) was added to each one. The concentration of virus present in each 22 ml aliquot of effluent was determined to be 2 x 10^5 TCID50/ml. Two of the four aliquots were held at 4°C and the other two were held at 20°C.

For a positive control, a 2 ml aliquot of PRRSV MN 30-100 was added to 20 ml of minimum essential medium (MEM) and incubated at 4°C and 20°C. For a negative control, a sham-inoculated effluent (20 ml MEM plus 2 ml effluent) was stored at the same temperatures. At 24-hour intervals on days 1 to 12 after inoculation, a 1 ml sample of each of the four aliquots was collected using separate syringes, and the samples were placed in sterile 3 ml plastic tubes. These samples and the remaining 10 ml of the four PRRSV aliquots were frozen at -70°C.

Following the completion of the 12-day sampling period, all samples were tested by PCR and virus isolation. Samples found to be PCR positive and virus isolation negative were tested for the presence of infectious PRRSV using a swine bioassay (Swenson and others 1994). They were centrifuged at 4200 g for 20 minutes, filtered through a 0·3 µm filter (Millipore) and supplemented with 0·05 mg gentamycin sulphate. Sterile cotton swabs were immersed in the inoculum and cultured aerobically and anaerobically at 37°C overnight to confirm freedom from bacterial contamination. These samples (2 ml aliquots) were then injected intramuscularly into PRRSV-naïve pigs and blood samples taken daily for a 14-day period. Throughout the bioassay, all pigs were housed and cared for according to the guidelines of the University of Minnesota Institutional Animal Care and Use Committee.

To test whether PRRSV-contaminated effluent was infectious for swine, 10 three-week-old PRRSV-naïve pigs were housed in separate rooms at the Swine Disease Eradication Center (SDEC) research farm. Upon arrival, the pigs had a blood test and their naive status was confirmed by an ELISA (Idexx) (Snyder and others 1995). Eight pigs were designated as principal pigs, while the other two served as negative controls. During the exposure period, each pig was individually housed in a metal cage with wire mesh flooring that was 0·5 m above the floor of the nursery pen.

Using a manually operated insecticide sprayer (Chapin Manufacturing), each principal pig was exposed to a 20 ml aliquot of effluent that had been processed and inoculated with 2 ml PRRSV strain MN 30-100 (total concentration 2 x 10^5 TCID50) as previously described. The nozzle of the sprayer was adjusted to deliver the inoculated effluent as a fine mist. To simulate the upward displacement of effluent during the flushing process observed on commercial farms, the sprayer was placed under the cage and the contents were discharged vertically so that the misted effluent contacted the flooring of the cage and the principal pigs. The negative control pigs were exposed to the sham-inoculated effluent via the same procedure. The ventral surface, limbs and facial regions of all pigs, as well as the flooring of the cage, were visibly moist after contact with the misted inoculum. One hour after exposure to the inoculated effluent, each pig was returned to its original pen and blood-sampled 14 days later to evaluate its PRRSV status. Samples were tested for the presence of PRRSV antibodies by an ELISA (Idexx) (Snyder and others 1995).

Finally, the effluent was analysed for pH, total solids, total volatile solids, ammoniacal nitrogen, total Kjeldahl nitrogen, chemical oxygen demand, potassium, magnesium, calcium, phosphorus, magnesium and sodium (Anon 1998). The effluent’s characteristics were found to be typical of a high-volume recycle flushing system and are shown in Table 1. The results of the PRRSV tests are summarised in Table 2. At 4°C, all samples (days 1 to 12) from the inoculated effluent in both replicates were PCR positive. Infectious PRRSV was isolated in cell culture from samples collected up to seven days after inoculation in replicates 1, and in samples from day 1 to day 8 after inoculation in replicate 2. A total of eight pigs were tested by swine bioassay (samples from day 9 to day 12) and all were negative for PRRSV. Samples from the positive controls were all PCR positive and virus isolation positive for the 12

TABLE 1: Characteristics of the lagoon effluent used in the study

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Result</th>
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<tbody>
<tr>
<td>pH</td>
<td>8.88</td>
</tr>
<tr>
<td>Total solids (%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Total volatile solids (%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Ammoniacal nitrogen (mg/litre)</td>
<td>470</td>
</tr>
<tr>
<td>Total Kjeldahl nitrogen (mg/litre)</td>
<td>480</td>
</tr>
<tr>
<td>Chemical oxygen demand (mg/litre)</td>
<td>2600</td>
</tr>
<tr>
<td>Potassium (mg/kg)*</td>
<td>26,900</td>
</tr>
<tr>
<td>Phosphorus (mg/kg)*</td>
<td>36,500</td>
</tr>
<tr>
<td>Calcium (mg/kg)*</td>
<td>4550</td>
</tr>
<tr>
<td>Magnesium (mg/kg)*</td>
<td>460</td>
</tr>
<tr>
<td>Sodium (mg/kg)*</td>
<td>60,100</td>
</tr>
</tbody>
</table>

* Calculated on a dry weight basis

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malignant swine (Anas platyrhynchos) could excrete infectious PRRSV and that avian species may play a role in area spread of the virus (Zimmerman and others 1997). While migratory waterfowl such as mallard ducks do indeed nest and feed on swine lagoons (S. Dee, personal observations), infection and shedding of PRRSV by these birds has been impossible to reproduce (Trincado and others 2003). In conclusion, the risk of swine herds becoming infected with PRRSV following contact with contaminated lagoon water appears to be low. Additional laboratory studies involving more animals are needed to develop enough observations to determine the probability of pigs becoming infected following contact with PRRSV–positive effluent. In addition, a survey of effluent samples collected from PRRSV-positive pig farms at both cold and warm temperatures is needed. If viable virus could be recovered under these conditions, the results of the present study would be of greater importance to swine producers and veterinarians.

References


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