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Effect of Challenge Dose and Route on Transmission of Porcine Reproductive and Respiratory Syndrome (PRRS) Virus Infection

Abstract
Porcine reproductive and respiratory syndrome virus (PRRSV) is believed to be highly infectious because of the rapid spread of the virus through populations of domestic swine throughout the world. However, no information is available on the minimum infectious dose of PRRSV. In our experiment, 10 groups of pigs were inoculated with five different quantities of PRRSV via two different routes: intramuscular and intranasal. The presence of virus in serum and early immune response of pigs were monitored for 21 days. We found that 10 or fewer virions were sufficient to achieve infection. No significant difference in the level of immune response of pigs to PRRSV infection was observed among different treatment groups. However, intramuscular exposure appeared to induce a more uniform immune response compared to intranasal exposure. These results confirmed that PRRSV is highly infectious; a fact that should be taken into consideration when designing strategies for control of PRRSV.

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Disciplines
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Effect of Challenge Dose and Route on Transmission of Porcine Reproductive and Respiratory Syndrome (PRRS) Virus Infection

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Summary and Implications
Porcine reproductive and respiratory syndrome virus (PRRSV) is believed to be highly infectious because of the rapid spread of the virus through populations of domestic swine throughout the world. However, no information is available on the minimum infectious dose of PRRSV. In our experiment, 10 groups of pigs were inoculated with five different quantities of PRRSV via two different routes: intramuscular and intranasal. The presence of virus in serum and early immune response of pigs were monitored for 21 days. We found that 10 or fewer virions were sufficient to achieve infection. No significant difference in the level of immune response of pigs to PRRSV infection was observed among different treatment groups. However, intramuscular exposure appeared to induce a more uniform immune response compared to intranasal exposure. These results confirmed that PRRSV is highly infectious; a fact that should be taken into consideration when designing strategies for control of PRRSV.

Introduction
PRRS is a recently identified disease of swine caused by a virus from the arterivirus family (3). Clinical signs of PRRSV infection include infertility, late-term abortions, mummified fetuses and stillborn pigs in breeding stock, and respiratory distress in young pigs (2, 4).

One of the remarkable characteristics of PRRSV has been its rapid spread among swine herds throughout the world (1, 5, 7). From these field observations, it was reasonable to conclude that PRRSV was highly infectious. We carried out this study to measure the degree of infectiousness by evaluating the transmission of PRRSV to swine at different exposure levels and routes.

Materials and Methods
Experimental design
Thirty-six four- to five-week old pigs were obtained from a swine herd free of PRRSV and randomly assigned to 12 groups of three pigs each. Each group of three pigs was housed in one room. Pigs in five groups were intranasally (IN) inoculated with PRRSV at a rate of 10^1, 10^2, 10^3, 10^4, and 10^5 50% fluorescent foci units (FFU_50) per ml. Pigs in another five groups were injected intramuscularly (IM) with virus at the same rates as the IN groups. Finally, two groups served as IM and IN negative controls. Serum was collected from individual pigs periodically over a 21-day period post inoculation (PI) and frozen at -70°C until assayed.

Virus isolation assays
Before performing assays, all serum samples were coded with random numbers to avoid test bias. Virus isolation was attempted on sera collected on days 2, 4, and 7 PI. All serum samples were tested for PRRSV-specific antibody using a commercially available enzyme-linked immunosorbent assay (ELISA).

Porcine alveolar macrophages and the MARC-145 clone of an African Green Monkey kidney cell line were used for virus isolation. Procedures for cell maintenance and virus isolation have been described elsewhere (6). The presence of PRRSV in the culture was confirmed by an immunofluorescence microscopy using PRRSV-specific monoclonal antibody, SDOW17. Animals were considered to be infected when the virus was isolated from serum samples using either cell type.

A commercial PRRS ELISA kit (PRRS:HerdCheck®, Idexx Laboratories, Inc.) was used to detect virus-specific antibody in serum samples. The test was performed and sample-to-positive (S/P) ratios were calculated following the procedures recommended by the manufacturer.

Results and Discussion
Virus isolation results are summarized in Table 1. Regardless of challenge dose of PRRSV, all inoculated pigs, except two pigs inoculated IN with PRRSV at the lowest dose (10^1 FFU_50/ml.), became viremic by day 4 PI. The data indicated that fewer than 10 virions of PRRSV were sufficient to infect pigs. The route of challenge appeared to affect the infection rate only at the lowest exposure dose. For 100% infection rate in case of nasal exposure, pigs required a dose of 10^2 virions.

All pigs in the IM challenge group seroconverted to PRRSV between days 9 to 11 PI. In contrast, pigs in the IN challenge group seroconverted between days 11 and 21 PI (Table 2). No significant difference in the rate or degree of humoral immune response to PRRSV was observed among different treatments (Figure 1). However, IM exposure to
PRRSV appeared to induce a more uniform response than IN inoculation. Collectively, our results showed that PRRSV is highly infectious.

References

Acknowledgment
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Table 1. Effect of challenge dose and route on PRRS virus infection as determined by the detection of viremia.

<table>
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<th>Challenge route</th>
<th>Dose (log_{10})</th>
<th>Days post inoculation</th>
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<td>2</td>
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<tr>
<td>Intranasal</td>
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<tr>
<td></td>
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<td>0</td>
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Table 2. Effect of PRRS virus challenge dose on seroconversion of pigs as determined by ELISA

<table>
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<th>Challenge route</th>
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Figure 1. Early humoral immune response of pigs inoculated with different levels of PRRSV isolate I via intranasal (IN) or intramuscular (IM) routes.