An Evaluation of Transmission of PRRSV and Identification of PRRSV in Mosquitoes

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Porcine reproductive and respiratory syndrome virus (PRRSV) can enter into swine farms by infected pigs and semen[1,2]. Despite strict biosecurity protocols, infection of negative farms still occurs by unknown routes. Mechanical transmission of PRRSV by fomites and needles can occur[3,4]; however, the role of aerosols in transmission of PRRSV is still under debate, and no publication regarding the role of insects in transmission of PRRSV exists.

We have currently established a research farm, and the following studies were conducted in the finishing facility.

Study 1: evaluation of aerosol transmission of PRRSV

The purpose of this study was to determine whether aerosol transmission of PRRSV could occur under field conditions. A total of 210 five-month-old PRRSV-negative pigs were housed in a mechanically ventilated finishing facility consisting of 11 pens. Pen one contained 10 pigs (indirect contact controls). Pen two remained empty, providing a barrier of 2.5 meters from the remaining pigs in pens three to 11. Within pens three through 11, 15 to 16 pigs in each pen were experimentally infected with a field isolate of PRRSV and six to seven pigs in each pen served as direct contact controls. On day five-post inoculation (pi), two trailers (A and B) containing 10 five-week-old PRRSV-naïve sentinel pigs were placed along each side of the building. Trailer A was placed one meter from the exhaust fans on one side of the building, while trailer B was positioned 30 meters from the fans on the other side. The sentinel pigs remained in the trailers for 72 consecutive hours in order to provide continuous exposure to fan exhaust. Following the exposure period, pigs from each trailer were moved to separate buildings located on the same site, 30 and 80 meters respectively, from the infected barn. The PRRSV status of the sentinel groups was monitored for 21 days. Transmission of PRRSV was detected in direct and indirect contact control pigs, but infection was not detected in sentinel pigs. In conclusion, while transmission of PRRSV occurred over a distance of 2.5 meters in the infected animal air space, aerosols emitted from exhaust fans over distances of one to 30 meters did not infect sentinel pigs.

Study 2: Identification of PRRSV in mosquitoes (*Culicidae*)

A diagnostic investigation was made to determine whether PRRSV could be recovered from mosquitoes following exposure to experimentally infected pigs. Approximately 550 mosquitoes were collected on days five, six, seven, nine, 13 and 15 pi of the index pigs in research finishing facility described above. Based on the availability of mosquitoes during each collection day, pools of 10 to 30 mosquitoes were collected in sterile tubes, filled with three ml of minimum essential medium (MEM), and labeled by collection date. Upon arrival to the diagnostic lab, mosquitoes homogenates were tested for evidence of PRRSV nucleic acid by reverse transcriptase polymerase chain reaction (Taqman™PCR). All samples were tested for viable PRRSV by virus isolation (VI) on both MARC-145 cells and porcine alveolar macrophages. PRRSV-positive samples were nucleic acid-sequenced to compare the percent homology of open reading frame (ORF) 5 region of the tested isolate to the same region of the field isolate used for experimental infection in index pigs.

PRRSV-viremia was detected by PCR and VI in 90 to 100 percent and 80 to 86 percent of the index and contact control pigs respectively, on days seven and 14 pi. All tested animals in both groups were ELISA-positive on day 14 pi. All homogenates were VI-negative. The nucleic acid from the PCR-positive homogenate was sequenced and found to be 100 percent homologous to the virus recovered from index and contact control animals on day 14 pi. The PCR-positive mosquito homogenate was tested by swine bioassay. A naïve pig was inoculated with one ml of this sample via the intranasal (0.5ml) and intramuscular (0.5ml) routes. Clinical signs including lethargy and fever (40.6°C) were observed on day 5 pi. PRRSV-viremia was detected by PCR on day 7 pi, and PRRSV-antibodies were detected by ELISA on day 14 pi.

This is the first report documenting the identification of PRRSV in mosquitoes. While homologous infectious PRRSV was transferred from viremic pigs to mosquitoes, these findings do not prove mechanical transmission or vector replication of PRRSV by mosquitoes. Additional studies are planned to characterize the role, if any, of mosquitoes in PRRSV transmission.

References

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