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EVALUATION OF MOSQUITOES AS BIOLOGICAL VECTORS OF PRRSV

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Introduction & Objectives
Potential transmission routes of porcine reproductive and respiratory syndrome virus (PRRSV) that have not been well explored are insects. We have recently demonstrated that mosquitoes (Aedes vexans) can mechanically transmit PRRSV from infected to susceptible pigs under experimental conditions\(^1\). However, duration of viability and site of PRRSV in mosquitoes are currently not understood. Furthermore, it is unknown whether PRRSV is capable of replicating within mosquitoes. In the present study, we attempted to evaluate the potential for mosquitoes (Aedes vexans) to serve as biological vectors of PRRSV. The specific objectives of the study were as follows:

1. **Experiment I**: To determine the duration of PRRSV viability in mosquitoes following feeding on an infected pig, and to document the site of the virus in the mosquitoes.

2. **Experiment II**: To determine whether PRRSV can be transmitted to a susceptible pig by mosquitoes following 7 to 14-day incubation period after feeding on an infected pig.

1. **Experiment I**

   **Materials & Methods**
   Mosquitoes were collected at field by CO\(_2\)-baited light traps. A total of 100 mosquitoes were allowed to feed to repletion on a viremic pig (5-week-old) experimentally infected with PRRSV (MN-30100)\(^2\). To expose mosquitoes to pigs, manual vector transmission protocol was used\(^1\). Following completion of feeding, all blood-fed mosquitoes were placed into humidified incubation cage, and maintained alive under the laboratory conditions (27\(^\circ\) C). A set of 10 mosquitoes were randomly collected at each of the following time: 0 hour (h), 6 h, 12 h, 24 h, 48 h, 72 h, 5 days (d), 7 d, 10 d, and 14 d post-feeding (PF). For assessment of PRRSV on the exterior surface of the mosquitoes, insects of each subset were washed with minimum essential medium (MEM) by vortexing, and the washing fluid was tested by PCR, VI, and swine bioassay. The washed insects were then dissected to remove salivary glands, thorax carcasses, and digestive organs for the PRRSV testing described above.

   **Results & Discussion**
   Infectious PRRSV was detected by PCR and swine bioassay only from the digestive organ homogenates collected at 0 and 6 h PF. In conclusion, infectious PRRSV can survive within the intestinal tract of mosquitoes for up to 6 hours following feeding on an infected pig; however, PRRSV is restricted only to the intestinal tract.

2. **Experiment II**

   **Materials & Methods**
   The same source of mosquitoes described in Experiment I was used. A total of 30 mosquitoes were allowed to feed to repletion on a viremic pig (the donor) inoculated with PRRSV (described in Experiment I). Following the completion of feeding, the blood-fed mosquitoes were maintained alive as described in Experiment I. On each of day 7, 10, and 14 PF, a set of 10 mosquitoes were allowed to feed on a susceptible pig (the recipient) using the manual vector transmission protocol\(^1\). Following feeding on the recipient pig, the insect samples were collected and tested for PRRSV in the same manner as previously described. Following the mosquito-exposure, PRRSV status of the recipient pig was monitored by PCR, VI, and ELISA.

   **Results & Discussion**
   The recipient pig remained PRRSV-negative following the mosquito-exposure. PRRSV was not detected from any mosquito samples collected on days 7, 10, and 14 PF. In conclusion, mosquitoes do not harbor PRRSV to transmit to a susceptible pig following 7 to 14-day incubation period after feeding on an infected pig.

Conclusion & Implications
Our data clearly showed that PRRSV is not capable to replicate and disseminate within mosquitoes (Aedes vexans), indicating that mosquitoes do not serve as biological vectors of PRRSV. The finding that infectious PRRSV can survive within the intestinal tract of mosquitoes for up to 6 hours, along with the ability of mosquitoes to mechanically transmit PRRSV\(^1\), suggests that while mosquitoes may play a role in area-spread of PRRSV during warm weather, they should serve strictly as mechanical vectors.

References