RESEARCH REPORT
SWINE HEALTH

Title: Susceptibility Of Selected Non-Swine Species To Infection With PRRS Virus **- NPB #97-1974**

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Abstract

The objective of this research was to assess the capacity of several animal species, commonly found in or near swine facilities, to serve as hosts of porcine reproductive and respiratory syndrome virus (PRRSV). Dogs, cats, skunks, raccoons, opossums, rats, mice, house sparrows, and starlings were inoculated with PRRSV. Serum samples from the mammals and fecal samples from the birds were collected every three or four days for assay by virus isolation and reverse transcriptase-polymerase chain reaction (RT-PCR) to test for evidence of PRRSV. Tonsil and lymph nodes samples were collected for assay by virus isolation at postmortem from the dogs, cats, skunks, raccoons, and opossums following euthanasia on day 21 PI.

Virus isolation results were negative for serum samples and postmortem tissue samples from dogs, cats, skunks, raccoons, and opossums. Virus isolation results were also negative for serum samples from rats and mice and fecal samples from the house sparrows and starlings. Feces collected from one cage of sparrows were positive by RT-PCR on day 3 PI but negative on other days. Serum samples from one opossum and one raccoon on day 3 PI and another opossum on day 14 PI were RT-PCR positive. Proving that PRRSV can replicate and infect these two species would likely require a more sustained level of detection of PRRSV RNA in serial samples of each animal over a longer period of time. In summary, although the results do not eliminate the possibility that animals other than swine are capable of transmitting PRRSV, they do not support the hypothesis that the animals tested in this experiment are likely hosts or reservoirs of PRRSV.

Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) prevention and control strategies rely on a comprehensive understanding of the ecology of the PRRS virus (PRRSV). In particular, understanding the modes of transmission of PRRSV from one herd to another has direct impact on the development of successful biosecurity

programs and facility designs. Owners of swine herds not infected with PRRSV need to know what precautions to take to maintain their negative status. Owners contemplating depopulation and repopulation with PRRSV-free animals need to know if available biosecurity measures and facilities will be adequate to prevent re-infection. Consequently, the role of non-swine vectors in the transmission of PRRSV has been designated a research priority area of NPPC. Knowing which animals may serve as hosts for the virus is of primary importance in identifying potential modes of transmission.

Only a few species of animals have been investigated as possible hosts of PRRSV. The only animals in which the virus has been shown to replicate are swine and some species of birds. Virus isolation from the feces of birds orally inoculated with PRRSV was attempted on days 0, 7, 10, 12, 14, 21, and 24 PI. PRRSV was recovered from the feces of guinea fowl on days 5 and 12 PI, from chickens on day 5 PI, and from Mallard ducks on nearly all sample days from Day 5 to 24 PI. No virus was isolated from Muscovey ducks. Although the birds did not demonstrate signs of clinical disease, the long period of fecal shedding indicated that the virus replicates in certain avian species (Zimmerman et al., 1997). Isolation attempts of PRRSV from rats and mice captured on a swine farm endemically infected with PRRSV were unsuccessful. Attempts to experimentally infect laboratory mice and rats with PRRSV were also not fruitful. (Hooper et al., 1994).

Objectives

The objective of this research was to assess the capacity of several animal species, commonly found in or near swine facilities, to serve as hosts of PRRSV. This is a necessary first step in determining what role these species might play in the transmission of PRRSV between swine herds.

Procedures

Four animals of each species of dogs, cats, skunks, and raccoons and fifty each of rats and mice were obtained through licensed commercial dealers. Four opossums and 13 house sparrows and five starlings were trapped from the wild. The investigators received the necessary permits from the State of Nebraska prior to procuring the protected animals. Conventional three- to five-week-old pigs were used as control animals.

The same protocol was followed for the dogs, cats, skunks, raccoons, and opossums. Prior to inoculation and sample collection the animals were anesthetized with intramuscular injections. Animals were inoculated with PRRSV by intranasal and intramuscular routes of administration. Serum samples were collected on days 0, 3, 6 or 7, 10 or 11, 13 or 14, and 21 post inoculation (PI). Tonsil and lymph nodes samples were collected at postmortem following euthanasia on day 21 PI. The animals were euthanized by overdose of barbiturates administered intravenously. The mice and rats were given 0.25 ml of the PRRSV solution intraperitoneally. Ten rats and ten mice were anesthetized prior to exsanguination on days 3, 6, 10, 13, and 21 PI. Two groups of house sparrows and one group of starlings were placed in separate cages. The birds were given PRRSV administered in drinking water. On days 0, 3, 7, 11, 14, and 21 PI, feces were collected from trays placed under the birdcages. A pig, serving as a positive control, was inoculated and sampled concurrently with the other animals.

Virus isolation using standard techniques with MARC 145 cells was conducted on serum from the rats and mice, serum and postmortem tissues from the other mammals, and feces from the house sparrows and starlings. Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to determine if viral genetic material (RNA) was present in selected samples.

Results

Although four opossums were inoculated with virus, serum samples were collected from only two of them. The other two opossums died, two and five days after inoculation. Necropsy results suggested both opossums succumbed to widespread infections with *Staphylococcus sp.*

Virus isolation results were negative for serum samples and postmortem tissue samples from dogs, cats, skunks, raccoons, and opossums. Virus isolation results were also negative for serum samples from rats and mice and fecal samples from the house sparrows and starlings. PRRSV was successfully cultured from serum samples collected from positive control pigs on days 3 through day 10 or 14 PI demonstrating the virus inoculum was infectious and the virus isolation procedure was functional.

Feces collected from one cage of sparrows was positive by RT-PCR on day 3 PI but negative on days 0, 7, 11, 14, and 21 PI. The significance of the positive RNA signal associated with the feces of sparrows on day 3 PI, but not later, is not clear. It may indicate that virus replication occurred during a limited time in the host. Alternatively, the positive RT-PCR test may have resulted from excretion of viral RNA present in the inoculum without replication of PRRSV occurring. The lack of positive virus isolation results would support the second scenario. However, it was noted in the trial that most of the bird feces were visibly dried prior to collection and the conditions may not have been conducive to virus survival. Furthermore, although virus has been successfully cultured from feces of other species of birds (Zimmerman et al., 1997), virus did not survive for more than one day in swine feces (Pirtle and Beran, 1996). Finally, inadvertent contamination with PRRSV genetic material resulting in a false positive reaction can not be ruled out at this time.

Day 3 PI serum samples from all mammalian species were tested by RT-PCR to screen for the presence of viral RNA. One raccoon and one opossum were positive, samples from dogs, cats, rats, mice, and skunks were negative. In response to the day 3 PI screening results, further testing was focused on raccoons and opossums. Day 0 through day 21 PI serum samples from the raccoons and opossums were tested by RT-PCR. On these subsequent tests, the screening test results were confirmed for the day 3 PI opossum samples and an additional opossum was found to be positive on day 14 PI. In contrast, all of the raccoon samples were negative, including the samples from the raccoon that was previously positive on day 3 PI. Lymph node and tonsil samples collected at necropsy from raccoons and opossums were negative by RT-PCR.

The positive RT-PCR results in the serum of two opossums and one raccoon are not conclusive. Proving that PRRSV can replicate and infect these two species would likely require a more sustained level of detection of PRRSV RNA in serial samples of each animal over a longer period of time. The inability to detect viral RNA when the positive raccoon serum sample was retested could indicate a concentration of virus that is beneath the analytic sensitivity of the test. The lack of positive RT-PCR tests of samples collected earlier from the opossum that was positive on day 14 PI could also be explained by low levels of virus. However, false reactions resulting from contamination or a lack of analytic specificity could also explain the sporadic nature of the results. The corresponding negative virus isolation results suggest that virus did not replicate and produce viremia in those animals. Further work, including serology, is underway to more definitively determine if replication occurred in these species. Presence of specific antibodies at day 21 PI would provide unequivocal evidence of replication of PRRSV in these species. In addition, serology would also be useful to show that these species did not have serum antibodies prior to inoculation and were fully susceptible to infection. In summary, although the results do not eliminate the

possibility that animals other than swine are capable of transmitting PRRSV, they do not support the hypothesis that the animals tested in this experiment are likely hosts or reservoirs of PRRSV.

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