ORIGINAL RESEARCH

Persistent and contact infection in nursery pigs experimentally infected with porcine reproductive and respiratory syndrome (PRRS) virus

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Summary — *The ability of porcine reproductive and respiratory syndrome (PRRS) virus to induce a persistent infection in nursery pigs was demonstrated, and the establishment of contact infection was compared in groups of sentinel pigs placed into contact with infected pigs at different intervals. Sixteen 3-week-old pigs from a farm free of PRRS were divided into four equal groups. Four pigs were moved into an isolation room and inoculated intranasally with PRRS virus (principal group). Thereafter, three sentinel groups of four pigs each were placed into the room in contact with principal pigs, so that the three sentinel groups were placed in contact on days 3, 10, and 24 post-inoculation (PI), respectively. Clinical signs were observed daily, and blood, nasal swabs, and fecal samples were collected from each pig at 2- to 7-day intervals. Clinical signs were not observed in any of the pigs. Viremia was evident in principal pigs from day 3 up to day 35 PI. In the sentinel groups, the duration of viremia varied among groups: pigs placed in contact later in the course of the experiment had a shorter viremic period. Viremia was not detected in two of four pigs of sentinel group 3. Duration of virus shedding through nasal secretion and feces was similar to the duration of viremia for pigs in each group, but virus recovery from nasal and fecal samples was inconsistent compared to virus recovered from the blood. PRRS virus antibody was detected by indirect-fluorescent antibody (IFA) assay in every pig soon after the onset of viremia, and the viremia was maintained at high antibody levels. We discuss the variables in the carrier status and suggest different management practices that may reduce the opportunity for pig-to-pig transmission of PRRS virus on endemically infected farms.*

Porcine reproductive and respiratory syndrome (PRRS) virus has recently been identified as an important pathogen in the swine of North America and Europe.[1-5] The virus causes reproductive failure in pregnant sows and high mortality associated with respiratory disease in young pigs. Serologic studies have indicated that PRRS virus infection is highly prevalent in pigs on United States swine farms and has occurred since at least 1986.[6] In a recent serologic study of 2787 selected sera, 35.1% were positive, and 56.3% of the herds, had st least one pig seropositive to PRRS virus.[7] Although the number of herds with the acute form of PRRS appears to be decreasing, PRRS is now endemic in many herds. Endemic PRRS is manifested as increased mortality and poor performance in nursery pigs, with active virus spreading mainly in the nurseries.[8]

At present, we don't fully understand how the virus is transmitted to and maintained in the nurseries of an infected herd. We conducted the present study to determine:

- whether PRRS virus can persist in pigs;
- how long PRRS virus can persist in pigs;
- how long infected pigs can shed virus; and
- whether sentinel pigs placed in contact with infected pigs can be infected and can shed virus.

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Methods

Experimental design

Sixteen seronegative 3-week-old pigs were selected from a University of Minnesota herd that was seronegative for PRRS virus. Four pigs (principal group) were moved into an isolation room with a cement floor and were inoculated intranasally with a common strain of PRRS virus isolate [$10^{4.5}$ tissue culture infective $dose_{50}$ ($TCID_{50}$)/mL]. PRRS MN-1b, inoculated in this experiment, was first isolated from a herd with a clinical history typical of PRRS virus infection.[2] PRRS MN-1b virus has been used in our previous experiments at the University of Minnesota, and has also been used as seed virus for indirect-fluorescent antibody (IFA) test procedures. The IFA test has been performed on over 5000 field serum samples during the last 3 years.

Thereafter, three sentinel groups of four pigs each were placed into the room in contact with principal pigs, so that:

- sentinel group 1 was placed in contact on day 3 post-inoculation (PI);
- sentinel group 2 was placed in contact on day 10 PI; and
- sentinel group 3 was placed in contact on day 24 PI.

All sentinel pigs remained in the room in contact with principal pigs until they were slaughtered, so that:

- principal pigs were slaughtered on day 56 PI,
- sentinel group 1 pigs were slaughtered on day 32 post-contact (PC);
- sentinel group 2 pigs were slaughtered on day 32 PC; and
- sentinel group 3 pigs were slaughtered on day 21 PC.

The room was cleaned daily with a low-pressure hose using hot water. Clinical signs were observed daily, and blood, nasal swabs, and fecal samples were collected from all pigs.

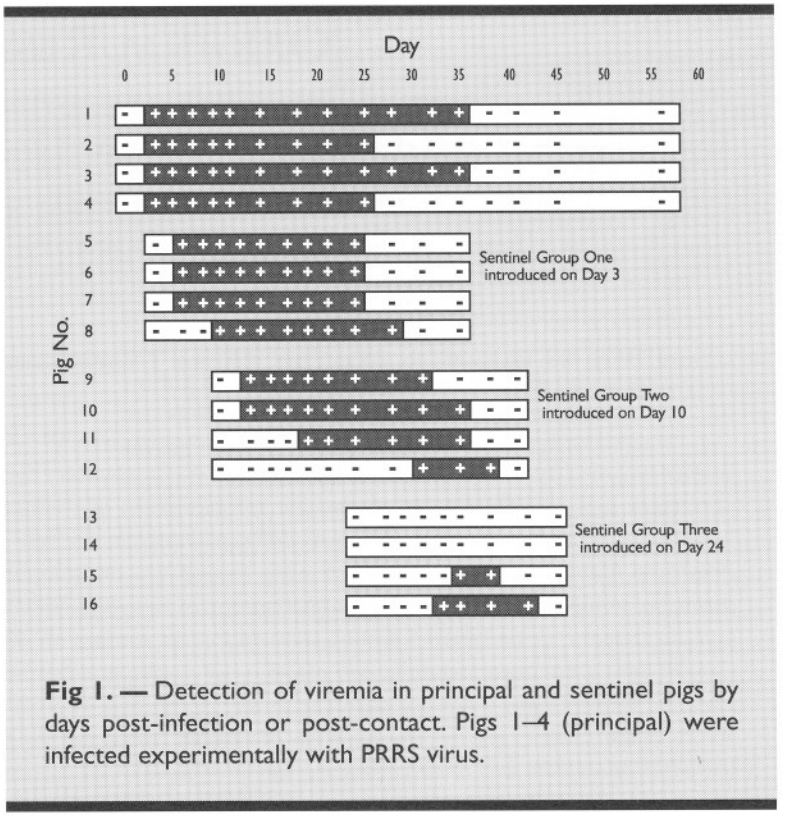

Fig 1. — Detection of viremia in principal and sentinel pigs by days post-infection or post-contact. Pigs 1–4 (principal) were infected experimentally with PRRS virus.

Sample process and laboratory examination

We collected blood samples in vacutainers that contained sodium heparin and centrifuged them at 1000 rpm for 10 minutes. We used the plasma to assess viremia. We collected nasal swabs from both nostrils using predampened swabs in tubes containing 1 mL of Roswell Park Memorial Institute (RPMI) medium. We vortexed the swabs, squeezed them into the tube, and discarded them. We collected approximately 0.5 g of feces from each pig in a tube that contained 2 mL of RPMI medium. We centrifuged the tubes containing swab extracts or feces at 5000 rpm for 20 minutes, and then stored each supernatant at -70°C until we examined it for virus.

Virus isolation

We isolated the PRRS virus using swine alveolar macrophages (SAM). We prepared SAM cells and propagated virus on SAM cells using methods previously described.[2] We used RPMI medium containing 7% fetal bovine serum, 0.15% sodium bicarbonate, and antibiotics. Serum was also collected from the blood for detection of PRRS antibody using the IFA assay, as previously described.[6]

Results

Clinical signs were not observed in any of the pigs. (Clinical signs are often mild or nonexistent in controlled experiments.) Viremia appeared to endure longer in principal pigs than in sentinels (Fig 1). In sentinel groups, the pattern of viremia varied among groups: the longer the interval between inoculation in principals and contact for sentinel pigs, the shorter the duration during which we could detect viremia. We detected viremia in:

- principal pigs for a mean of 28.0 days;
- sentinel group 1 pigs for a mean of 19.0 days;
- sentinel group 2 pigs for a mean of 16.7 days; and
- sentinel group 3 pigs for a mean of 3.5 days.

We did not detect viremia in two of four pigs in sentinel group 3 during the 21-day observation period. We recovered virus from one of four fecal samples and one of four nasal swabs of principal pigs collected up to 35 and 38 days PI, respectively (Fig 2). Virus isolations from nasal swabs and feces were not consistent with those from blood samples. Virus was isolated from 10 of 156 nasal swab samples and in 55 of 154 fecal samples. Antibody response measured by IFA was detected in sera as early as 7 days PI in principal pigs and 9 days PC in sentinel pigs (Fig 3).

Discussion

In this study, we demonstrated that PRRS virus infection persists for up to 35 days and that infection can be transmitted to sentinel pigs housed with infected pigs. Our study attempted to mimic typical pig movement into nurseries on swine farms. We observed an obvious difference in the duration of viremia and virus excretion among groups of sentinel pigs. The incidence of virus infection was the lowest in sentinel group 3, suggesting that virus shedding from pigs of the previous groups was not sufficient to infect them all.

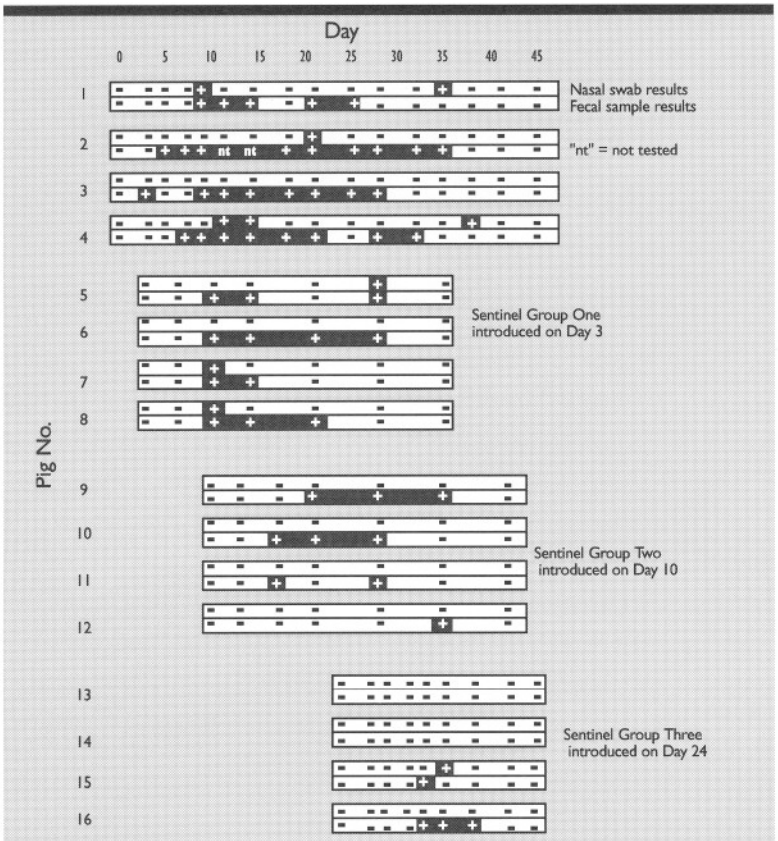

Fig 2. — Detection of virus from nasal swab and feces of principal and sentinel pigs by days post-infection or post-contact. For each pig, nasal swab test results are on the top half of the bar; feces test results are on the bottom half.

However, in two of the four pigs placed in contact 24 days PI (sentinel group 3) we could not detect evidence of infection, suggesting that either the amount of virus in the pen was decreased or that these two pigs were too old to be infected (sentinel group 3 pigs were 21 days older than sentinel group 1 pigs on day of contact). Certainly, older nursery pigs in the field are less likely to benefit from the protection of passive immunity. Thus, older nursery pigs in the field may actually be *more* susceptible to infection. At this time, we do not understand enough about the protective properties of passive immunity to PRRS virus to be able to confidently extrapolate our experimental findings to the field.

Viremia began later and lasted a shorter time in some pigs. This could be because they received a low dose of virus, although the possibility of changes in viral properties cannot be dismissed.

From these results, we surmise that pig-to-pig infection within a pen could be reduced by changing management practices on swine farms. Any practice that hampers the transmission of PRRS virus in the nursery will reduce concentration of the virus. Producers may be able to reduce the transmission of virus from pigs in a previous group by:

- batch farrowing/weaning at intervals of at least 3 weeks; or

Various factors in the host and virus undoubtedly influence the duration of viremia. Age of pigs at the time of PRRS virus infection appears to be one of the factors. Viremia is reported to endure for shorter periods (9 days PI) in sows,[9] probably because they have better immune capability than younger pigs. Although virus recovery from nasal swabs and feces was not coincident with plasma samples, the duration of virus excretion and the viremic period appeared to be similar. From these results, it appears that younger pigs can act as carriers of PRRS virus infection for at least 35 days.

We did not expect virus to be shed in the feces of infected pigs for such a long period. At present, we do not know how well virus replicates in the gastrointestinal tract. Nevertheless, virus was recovered more commonly from feces than from nasal swabs. This may indicate that PRRS virus is transmitted more commonly via the fecal-oral route than through nose-to-nose contact.

We expected viremia to develop and pigs in each group to seroconvert in an "all-or-nothing" fashion.

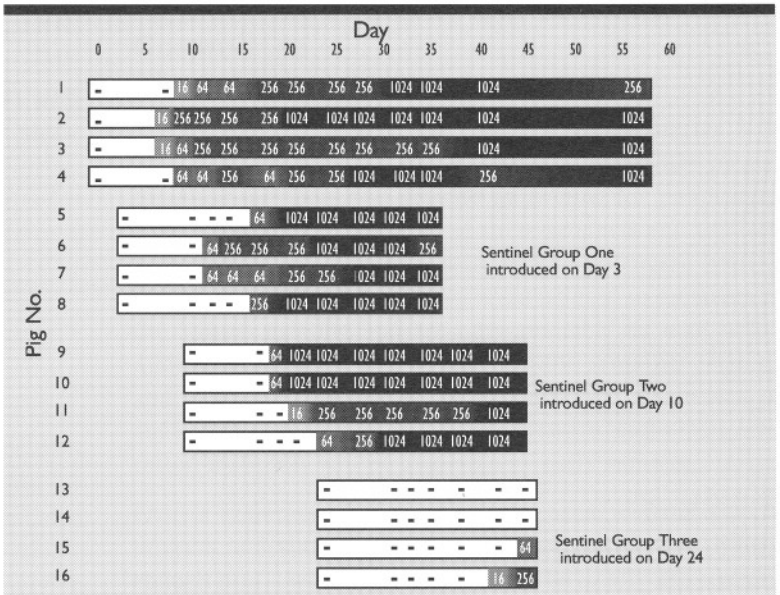

Fig 3. — Detection of antibody to PRRS virus in principal and sentinel pigs at different intervals by an indirect fluorescent antibody test. "-" = IFA titer < 1 : 16

- waiting 3 weeks or more between groups entering a continuous-flow nursery facility.

Floor types that reduce contact with feces will also reduce the opportunity for infection by fecal-oral route. We speculate that routine practice of these methods would eventually break the chain of infection in the nursery and may induce spontaneous elimination from an infected herd.

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