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Brief Communication

Evidence for local spread of porcine reproductive and respiratory syndrome virus

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Summary

This report presents evidence from a field investigation indicating that indirect area spread of porcine reproductive and respiratory syndrome virus occurred among swine farms in north-central Iowa. The mode of transmission was not determined.

Keywords: swine, porcine reproductive and respiratory syndrome virus, indirect transmission, biosecurity

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Field reports have suggested that transmission of porcine reproductive and respiratory syndrome virus (PRRSV) may occur by direct contact (pig-to-pig and via virus-contaminated semen)[1-3] and by indirect contact (ie, fomites, biological or mechanical vectors, and aerosol transmission).[4-7] Experimental studies have confirmed the role of direct transmission in the spread of PRRSV from infected to naive swine by way of pig contact[8-10] and insemination of virus-contaminated semen,[11-13] while results from indirect transmission studies have not been so straightforward.[9,14-16] If indirect spread of PRRSV among herds does occur, pork producers may need to redesign biosecurity strategies to account for this threat. This report presents evidence from a field investigation indicating that indirect area spread of PRRSV occurred among swine herds in north-central Iowa.

Materials and methods

As part of an ongoing surveillance program at the National Animal Disease Center for suspected virus-induced reproductive diseases, the authors of this report were contacted by the attending veterinarians for a number of herds experiencing epidemics of maternal reproductive failure beginning in late July 1998. Reproductive failure was characterized by late-term abortions (90 to 110 days of gestation), weak-born pigs (pigs born alive that appeared normal but were listless, unthrifty, and weak, and frequently died within hours of birth), stillborn pigs, and litters that were composed primarily of fetuses that had died late in gestation.

Diagnostic samples (serum from aborting sows, and presuckle serum and lung lavage from weak-born pigs) were collected within 96 hours of the first recognized abortions in a herd. Samples were collected from seven herds during a 2-week period, and identification of the herds as A, B, C, D, E, F, and G was based on the order in which the samples were collected and received. Six of the seven farms were located relatively close to each other, and one (Farm D) was located about 33 km from the nearest of the other herds (Figure 1). Producers from each farm were interviewed as part of the investigation.

As a routine part of the surveillance program, samples are tested for cytopathic agents, as previously described.[17] When PRRSV is isolated, a randomly selected isolate from each PRRSV-positive herd is evaluated by restriction fragment length polymorphism (RFLP) analysis. The method of performing the RFLP test and summarizing the resulting digestion pattern as a three-digit code has been described in detail elsewhere.[18] To provide additional information of potential epidemiological importance, nucleotide sequence analysis of open reading frame (ORF) 5 of the PRRSV genome is conducted when needed.[18]

Results

The PRRSV was isolated from at least one pig from each of the seven herds, with a range of one to four pigs per herd, and a total of 25 isolates from seven herds (Table 1). No other cytopathic agents were isolated, and a diagnosis of PRRSV-induced reproductive failure was made, based on clinical signs and isolation of virus from congenitally-infected weak-born pigs.

On each of six of the farms (Farms A, B, C, D, E, and G), the randomly-selected PRRSV isolate had a 1-4-1 RFLP pattern, whereas the isolate from the remaining farm (Farm F) had a 1-7-1 pattern (Table 1). When the remaining isolates from each of the seven herds were tested, all isolates from Herds A, B, C, D, E, and G (n=19, including the original six isolates) had a 1-4-1 pattern, as did most of the isolates from Herd F, namely, from the serum and lung lavage samples from pig F-2 and from the lung lavage samples from pigs F-3 and F-4. However, isolates from the serum sample that was initially tested from Herd F and the lung lavage sample from pig F-1 both had a 1-7-1 pattern (Table 1).

Nucleotide sequence analysis was completed for all 25 PRRSV isolates. Sequence analysis of isolates from lung lavage and serum samples from pigs F-1 and F-2 of Herd F revealed a minor degree of genetic diversity. For pig F-1, sequences differed by one nucleotide, resulting in one predicted amino acid difference (Pro-15 for the lung lavage isolate and Leu-15 for the serum isolate). For pig F-2, sequences differed by three nucleotides resulting in three predicted amino acid differences (Leu-15, Ser-33, and Ala-63 for the lung lavage isolate and Pro-15, Asn-33, and Val-63 for the serum isolate).

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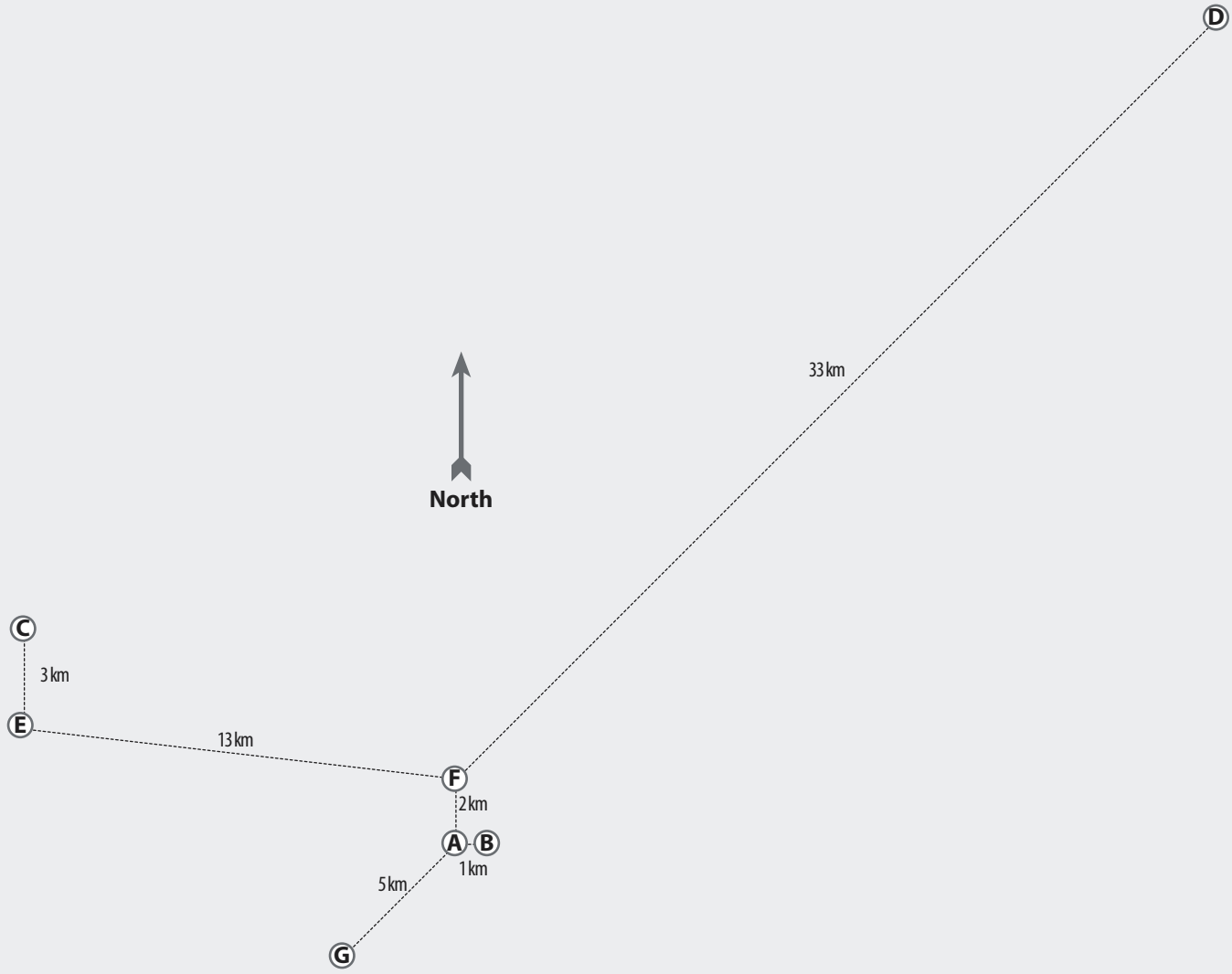
Figure 1: Relative location of seven farms, identified by letters A through G, on which clinical signs (reproductive failure in sows and gilts) compatible with porcine reproductive and respiratory syndrome (PRRS) developed within a 2-week period. The PRRS virus was isolated from at least one pig from each herd.



Interviews with the producers indicated that they did not share equipment, have a common feed source, or attend livestock shows where swine could have commingled. Dead animal disposal consisted of on-site incineration on three farms, burial on two farms, and the same commercial service for two farms (Farms D and E). Except for Farm D, the attending veterinarians had not been on these farms for at least the previous 6 months.

The seven herds ranged in size from 50 to 500 sows, used single-site production, and were farrow-to-finish (Herds A, B, C, E, F, and G) or farrow-to-wean herds (Herd D). Herd D, the largest herd (about 500 sows), farrowed sows weekly and received replacement gilts from several sources, and artificial insemination was the predominate method of breeding. In the six other herds, a batch-farrowing system was used, replacement gilts were developed internally, and natural service was the method of breeding. For these herds, replacement boars were purchased and introduced directly into the herds every 9 to 18 months. No single source of replacement boars was consistently used. No new boars had been purchased by any of these farms for at least 6 months.

Animals at Farm D were housed in total confinement buildings, while the remaining herds were housed in open-front buildings, and occasionally gestating sows were kept on pasture. For at least several months prior to the epidemics, the only clinical sign observed in any of the sow herds was anorexia during the first part of July in Farm B's gestating sows housed on pasture. In the past, Herds A, B, D, E, F, and G had received either a live PRRSV vaccine, an autogenous killed PRRSV vaccine, or both. No vaccine was being used at the time of the epidemics. The only commonality among these herds was that a group of sows and gilts was scheduled to farrow about August 1.

The meteorological data for several months preceding the epizootics was typical for north-central Iowa. For example, in June and July, daytime temperatures average 28 and 30°C and nighttime temperatures average 15 and 17°C, respectively. Most days have some amount of wind, generally from a southerly direction, and the relative humidity is variable.

Discussion

Although our previous experiences suggest that it is uncommon to have the same

RFLP pattern in PRRSV isolates from different herds, we have observed this before for farms that were located relatively close to each other and for herds that shared a common source of replacement animals.[19] In those cases, we suspected that the PRRSV isolates might have been related and analyzed the nucleotide sequence of the most variable portion of the viral genome, ORF 5, a structural gene consisting of 603 nucleotides. Minor variation was found in nucleotide sequence among isolates from the same herd (1 to 5 nucleotides, 0.16 to 0.8 % difference) and more variation among isolates from different herds (5 to 52 nucleotides, 0.8 to 8.6 % difference), suggesting that the isolates from different herds were not closely related.[19] These observations are supported by a recent study that analyzed the ORF 5 nucleotide sequence for PRRSV isolates recovered from 48 herds in Illinois and Iowa.[20] The authors concluded that the diversity in nucleotide sequence among the herds was due in part to the importation of virus into the herd via replacement animals or the use of contaminated semen and was not due to indirect area spread of virus. This genetic diversity also may be attributed to the development of viral quasispecies within the same pig,[21] that may result in the emergence of new strains in a herd.

In the cases described in this report, sequence differences among isolates, when present, were generally no more extensive than those between isolates from different samples (serum or lung lavage) from the same pig. This observation of genetically distinct virus concurrently present in one pig supports a previous report of genetically distinct virus circulating in the same pig at different times.[21]

Porcine reproductive and respiratory syndrome virus, like other RNA viruses, has a higher mutation rate than DNA viruses, which have a mismatch repair mechanism.[22] The high degree of homology among PRRSV isolates from Farms A, B, C, D, E, and G is striking, considering that the PRRSV ORF 5 nucleotide sequence is regarded as the most variable structural gene,[23-30] that recombination may play a role in PRRSV genetic diversity,[31] and that variability in the ORF 5 sequence is reported among herds.[19,20] The viruses in these herds apparently had a very recent common ancestor, since little if any mutation was detected in ORF 5. Genetic analysis of viral isolates and herd histories suggest that area spread of PRRSV occurred among Farms A, B, C, D, E, and G, unassociated with any direct transmission by swine or indirect transmission by humans or fomites. This putative area spread of PRRSV may have been due to air-borne transmission[4-7] or mammalian, avian,[32] or insect vectors[33] (biological or mechanical).

Several other farrow-to-finish herds of similar size in the area were reported to have been clinically affected; however, we did not receive samples from these herds. The health status for many of the swine herds in the local area was unknown, since their style of production had switched from farrow-to-finish to contract finishing, and these units were not under the care of the referring veterinarians. Moreover, reproductive failure, the predominate clinical sign observed in these epidemics, could not have been observed in finishing units. Although the clinical onset of the epidemics is known, the time when these herds became infected with PRRSV is not known, and it was not possible to evaluate the effects of any weather pattern on area spread.

There are three possible explanations for the observed nucleotide sequence differences among all Farm F viral isolates and between Farm F virus and the viral isolates from the other farms. The first possibility is that the Farm F virus was not related to the

Table 1: Restriction fragment length polymorphism (RFLP) pattern and open reading frame (ORF) 5 nucleotide sequence homology for isolates of porcine reproductive and respiratory syndrome virus (PRRSV) recovered from serum or lung lavage samples from seven swine herds located within a 40-km^2 area

Pig[1]	Sample[2]	RFLP[3]	Nucleotide homology[4]
A-1	S	1-4-1	100
A-2	S	1-4-1	100
A-3	S	1-4-1	100
A-4	S	1-4-1	100
B-1	S	1-4-1	100
B-2	S	1-4-1	99.7
B-3	S	1-4-1	99.8
C-1	S	1-4-1	99.8
C-2	S	1-4-1	99.8
C-3	S	1-4-1	100
D-1	S	1-4-1	100
E-1	S	1-4-1	100
E-2	S	1-4-1	100
E-3	S	1-4-1	100
E-4	S	1-4-1	100
F-1	S	1-7-1	98.7
F-1	LV	1-7-1	98.8
F-2	S	1-4-1	98.8
F-2	LV	1-4-1	99.0
F-3	LV	1-4-1	99.5
F-4	LV	1-4-1	99.5
G-1	LV	1-4-1	100
G-2	S	1-4-1	100
G-2	LV	1-4-1	100
G-3	LV	1-4-1	100

[1] Pigs identified by herd letter (A, B, C, D, E, F, and G) and number.
[2] Type of sample: S = serum, LV = lung lavage.
[3] Three-digit code for RFLP analysis.
[4] Percent homology between ORF 5 nucleotide sequence for each PRRSV isolate and consensus ORF 5 nucleotide sequence for all 25 PRRSV isolates.

virus identified on the other farms, and the genetic differences among virus isolates tested from the four Farm F pigs reflect on-farm mutations of an endemic PRRSV infection that developed into a clinical event about the time that the other herds developed clinical disease. The second possibility is that the Farm F virus was related to the virus identified on the other farms. The genetic differences among the viral isolates from the four Farm F pigs and between these isolates and the isolates from other farms suggest that when the virus entered Herd F, it underwent more rapid mutation than the same virus in Herds A, B, C, D, E, and G. The third possibility is that the Farm F virus sequence differences reflect the introduction of the area-spread virus into a herd that had an endemic PRRSV infection, and the genetic divergence may represent the progeny of two virus strains undergoing recombination and mutation events. Further investigations are needed to validate any of these hypotheses.

Implications

- This report supports the hypothesis that PRRSV area spread may occur; however, the method of virus transmission and the risk of PRRSV area spread to a naive herd are unknown.
- Pork producers may need to consider the potential risk of PRRSV area spread when making plans for locating and building new facilities.

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