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BRIEF COMMUNICATIONS

Endemic porcine reproductive and respiratory syndrome virus infection of nursery pigs in two swine herds without current reproductive failure

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An outbreak of porcine reproductive and respiratory syndrome (PRRS), formerly called swine infertility and reproductive syndrome or mystery swine disease, occurred on 18 farms in Wabash County, Indiana, in the spring of 1989.[2,7] An extensive diagnostic investigation conducted at Purdue Animal Disease Diagnostic Laboratory (ADDL) and diagnostic testing at a number of other laboratories did not result in a conclusive etiologic diagnosis.[2,6,7] In July 1991, a novel virus (Lelystad virus) was isolated at the Central Veterinary Institute in Lelystad, The Netherlands, from pigs and sows on Dutch farms that were experiencing PRRS-like outbreaks.[8] Disease typical of PRRS was reproduced by inoculation of pregnant sows and specific-pathogen-free pigs with Lelystad virus.[4,5] Frozen convalescent sera collected from affected farms during the 1989 PRRS outbreak in Wabash County were among those forwarded by The ADDL to the Central Veterinary Institute in July 1991. Antibodies that reacted with Lelystad virus in an immunoperoxidase monolayer assay were detected in some of those sera.[8]

Within 3 months of the onset of the 1989 epizootic of PRRS on Wabash County farms, reproductive performance and suckling pig performance returned to pre-PRRS levels; however, poor performance of nursery and/or grower pigs continued on some farms. An enzootic infection with the PRRS virus in the nursery and/or grower pigs was suspected on these farms. In December 1991, 2 of these farms were selected for further investigation. The goal was to determine whether clinically ill nursery pigs were infected with the PRRS virus. On each farm, the owner and/or herd manager helped select 8 "typical" acutely affected nursery pigs. Selected pigs had been clinically ill for <24 hours and were 6-8 weeks old. Heparinized blood samples were collected from all pigs, and 4 pigs were euthanized and complete necropsy examinations were performed. PRRS virus isolation in primary porcine pulmonary macrophage cultures was completed on washed buffy coat cells from all pigs and from lung and spleen from pigs that were necropsied. Microscopic examination of tissues and laboratory tests for common viral and bacterial pathogens were completed on selected tissues for all pigs.

Farm 1 was a 500-sow farrow-to-finish farm in which all animals were housed in enclosed environmentally regulated buildings. Far-rowing rooms were managed all-in/all-out, and nursery, growing, and finishing rooms were managed as continuous flow. Pigs were weaned into nursery rooms at 3-4 weeks of age and moved from nursery rooms to growing rooms at 9-10 weeks of age. In April 1989, there was an acute outbreak of PRRS. Many sows farrowed prematurely (days 107-111 of gestation), and most litters were composed of weak live-born pigs, dead pigs. in various stages of in utero autolysis, and occasional mummified pigs. The mortality rate was high in suckling and weanling pigs. Reproductive performance and mortality in suckling pigs returned to pre-PRRS levels within 3 months; however, mortality in nursery pigs remained elevated. Nursery mortality averaged 3.1% for the 11 months before the PRRS outbreak, compared with 7.4% for the 34 months (ending January 1992) following the outbreak (Fig. 1). Since the PRRS outbreak, nursery mortality has been cyclic, with 5-6 months of low mortality (3-5%) followed by 5-6 months of higher mortality (7-16%). Highest mortality occurred in winter months. Most pigs died 2-3 weeks after weaning (5-7 weeks of age). Affected pigs initially were lethargic then exhibited anorexia and cyanosis of skin on the extremities within 12-24 hours. On day 2 of the illness, some pigs became dyspneic. Most pigs died 1-3 days after onset of clinical signs. Response to multiple intramuscular injections of broad spectrum antibiotics was poor.

Farm 2 was a 1,000-sow far-row-to-finish farm in which all animals were housed in enclosed environmentally regulated buildings. Management was similar to farm 1. Farrowing rooms were managed all-in/all-out, and nursery, growing, and finishing rooms were managed as continuous flow. An acute outbreak of PRRS occurred in May 1989, and reproductive performance returned to pre-PRRS levels in 3 months. Detailed mortality records were not available, but cyclic elevations in nursery mortality have continued since the PRRS outbreak.

Necropsy findings in all 8 pigs from both farms were typical of septicemic salmonellosis. Pigs were thin, with rough hair coats, and had purple discoloration of skin on the ventral abdomen and extremities. There was mild to moderate icterus and serous atrophy of fat. Lungs failed to collapse completely. Cranial ventral lung lobules (20-50%) were purple or tan, firm, and moist, and airways contained white opaque exudate. The remaining caudal dorsal lobules were mottled purple-pink and were slightly firm, yet resilient, suggesting an interstitial pneumonia. Some livers had 1-2-mm-diameter white foci scattered throughout the parenchyma. Gastrohepatic lymph nodes were enlarged (1.5-2.5 times nor-

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mal), congested, and edematous. Spleens were enlarged (1.5-3 times normal). Petechiae diffusely covered the cortical surfaces of kidneys. Occasional thin strands of fibrin were loosely adherent to abdominal viscera. Inconsistent lesions included fibrinous pericarditis and focal pleuritis in 1 pig, edema in the mesentery of the spiral colon in 1 pig, and multiple tonsillar abscesses in 1 pig.

Microscopic lesions in tissues from all 8 pigs were typical of septicemic salmonellosis and unusually severe. Lesions were purulent bronchopneumonia in the cranial ventral lung lobes, diffuse interstitial pneumonia in the dorsal caudal lung lobes, hepatitis with multifocal necrosis, necrotizing lymphadenitis in mesenteric and gastrohepatic lymph nodes, multifocal renal cortical hemorrhages, and segmental vasculitis with perivascular necrosis and abscesses in the leptomeninges and brains.

Salmonella choleraesuis var. *kunzendorf* was cultured from lungs and other tissues in all 4 pigs from farm 1 and in 3/4 pigs from farm 2. In addition to *S. choleraesuis, Pasteurella multocida* and *Actinobacillus pyogenes* were isolated from the lungs of 1 pig from farm 2.

PRRS virus was isolated from lung and spleen in 4/4 pigs and from buffy coat cells in 2/8 pigs from farm 1. PRRS virus was also isolated from lungs in 3/4 pigs, from spleen in 4/4 pigs, and from washed buffy coat cells in 6/7 pigs from farm 2. All lungs were negative for swine influenza virus by fluorescent antibody test and virus isolation. All lungs, spleens, tonsils, and brains were negative for pseudorabies virus by fluorescent antibody test and virus isolation.

These findings confirm that PRRS virus was present in nursery pigs on 2 farms that were reproductively normal for more than 2.5 years following a classic reproductive outbreak of PRRS. The presence of the virus suggests that there has been endemic PRS virus infection of nursery pigs on these farms since the original PRRS outbreaks.

Because nursery mortality associated with septicemic salmonellosis increased subsequent to the original PRRS outbreaks, PRRS virus infection of nursery pigs probably renders them more susceptible to lethal septicemia caused by *S. choleraesuis.* Other workers have demonstrated a similar role for PRRS virus in enhancing the severity of disease associated with *Streptococcus suis* in 10-day-old pigs.[1] This potential PRRS viral enhancement of bacterial disease is further supported by the unusual features of septicemic salmonellosis on these farms. Microscopic lesions in all pigs were unusually severe, response to multiple parenteral doses of broad spectrum antibiotics was unusually poor, and nearly all clinically ill pigs died, resulting in unusually high *S. choleraesuis*-associated mortality in nursery pigs.

A mechanism for PRRS virus-induced increase in susceptibility to bacterial pathogens might be suppression of macrophage function, i.e., suppression of phagocytosis and/or killing of bacteria. In pigs inoculated with Lelystad virus, virus replication was demonstrated in macrophages within lung and spleen by immunoperoxidase staining.[4] In addition, preliminary studies have demonstrated suppressed pulmonary alveolar macrophage function in PRRS virus-infected pigs.[3,9] More work is needed to understand the significance of PRRS viremia in nursery pigs.

Because the most severe elevations in nursery mortality occurred in winter months, environmental factors, such as lowered ambient temperature, increased range in fluctuation of ambient temperature, lowered ventilation rates, and elevated relative humidity, probably also contributed to the high mortality rate in nursery pigs.

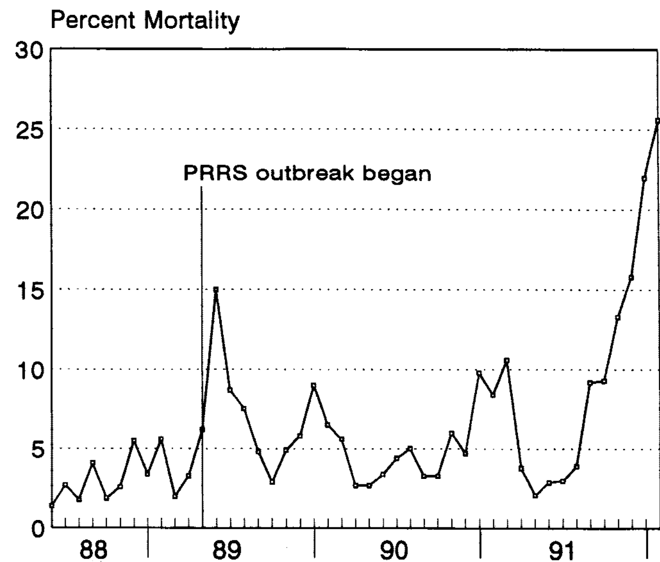

Figure 1. Monthly percent mortality in nursery pigs before and after a porcine reproductive and respiratory syndrome outbreak on farm 1.

No gross or microscopic lesions suggestive of PRRS virus infection were discerned in these pigs, apart from the severe lesions typical of *S. choleraesuis.* Therefore, diagnosis of endemic PRRS virus infection of nursery pigs when bacterial disease is also present probably will need to rely on suggestive clinical history and PRRS virus isolation and not on the presence of PRRS lesions.

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Characterization of field isolates of porcine group C rotaviruses using gene 5 (VP6) and gene 8 (VP7) cDNA probes

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The association of the serogroup (Gp) C rotaviruses with outbreaks of acute gastroenteritis in humans,[2-4,11,12] pigs, [1,8,13,21] and cattle[28] and the detection of antibodies in these species[26] has made these viruses important emerging agents of viral diarrheas. The Gp C viruses have been studied much less extensively than have the ubiquitous Gp A rotaviruses.[20],[23] The recent association of human Gp C rotavirus with a fatal case of enteritis within a family[3] indicates the severity of the disease caused by this virus.

Like Gp A rotaviruses, Gp C rotaviruses are fastidious in their in vitro cell culture requirements. Only 2 strains, the porcine (Cowden) and bovine (Shintoku) rotaviruses, have been successfully propagated in cell cultures.[22,25,28] Therefore, greater efforts have been channeled into other procedures for diagnosing infection. Monoclonal antibodies (Mabs) to VP6 of porcine[14] and human Gp C rotaviruses are now used in enzyme-linked immunosorbent assays (ELISA) to detect Gp C rotaviruses.[5,14]

Recently, the polymerase chain reaction (PCR) technique has been used for detecting Gp C rotaviruses. The genetic diversity of the Gp C rotavirus genes 5 (VP6), 6 (NS34), and 8 (VP7) was analyzed by northern blot and dot blot hybridization techniques, using cDNA probes for these genes from the Cowden strain of Gp C rotavirus.[9,10] The objectives of this study were to apply similar hybridization techniques for detecting porcine Gp C rotaviruses in a diverse pool of field samples using probes made from Cowden Gp C rotavirus genes 5 and 8. We also correlated our findings with the results previously published for some of these samples using the biotin-streptavidin-enhanced ELISA. [15]

The porcine fecal or intestinal samples used in this study contained the following rotaviruses: OSU (Gp A), Ohio (Gp B), and Cowden (Gp C). We also used Gp C rotavirus field samples BS(N608), D(K519), FS(W1970), SB(K249, K556), and MV(Z1858, Z1859) and rotavirus-negative fecal samples (2999 and 21009). Sample sources have been described previously.[15] S1632 was a field sample that tested weakly positive for Gp C rotavirus by polyacrylamide gel electrophoresis (PAGE) and was stored at 4 C for over 6 months. In addition, 8 other diarrheic fecal samples designated as PW2F, PW6A, PW6C, PW7B, PWl7D, PW36A, PW37B, and PW41I were included in this study. They were obtained about 4 years ago from postweaning (PW) pigs at the Ohio Agricultural Research Center Robinson Swine Herd. These samples had been stored at -20 C with intermittent freezing and thawing. These PW samples had been analyzed by PAGE and cell culture immunofluorescence (CCIF). PAGE analyses of these samples revealed only electropherogroup C rotaviruses except for 2 samples, PW6A and PW37B, that also contained Gp A rotaviruses and 1 sample, PW37B, that was negative for both Gp A and C rotaviruses (K. W. Theil, unpublished data). Rotavirus double-stranded (ds) RNA was extracted from the 21 samples and prepared for PAGE analysis as previously described.[7] Double-stranded RNA was suspended in diethyl pyrocarbonate[a] (DEP)-treated sterile distilled water and stored at -20 C. Concentrations of dsRNA were estimated by absorbance at 260 nm. Rotavirus dsRNA was analyzed with 7.5% resolving and 3% stacking gels as previously described.[7]

Partial length gene 5 (PC4- 19) and full length gene 8 (PC8-18) cDNA clones were prepared from the Cowden strain and inserted into a pTZ18R plasmid. The preparation and characterization of these clones have been described previously. [17] Both clones were grown in Luria broth medium, purified, and analyzed by gel electrophoresis using standard methods[24] and radiolabeled with ^{32}P-dCTP by nick translation as described previously. [18]

Northern blot and dot blot hybridizations were carried out at moderate stringency conditions (42 C, 50% formamide and 5 x standard saline citrate [SSC]) as previously described.[9,18,19] For dot blots, the dsRNA samples were denatured by heat and dotted (5-25 µ1) onto nytran membranes.[b] For northern blots, the dsRNA was separated by PAGE and electrotransferred onto nytran membranes. The membranes

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