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The influence of animal age, bacterial co-infection and porcine reproductive and respiratory syndrome virus (PRRSV) isolate pathogenicity on virus load in individual pigs

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, single-stranded positive sense RNA virus belonging to the family *Arterividae*.[1] Since its emergence in the United States during the late 1980s, PRRSV has been one of the most difficult and costly diseases to control in the swine industry.[2] One feature of PRRSV that complicates control through traditional methods is its ability to undergo genetic change through the process of mutation and recombination, resulting in the development of isolates with varying degrees of pathogenicity.[3-6] Variability in the severity of the reproductive and the respiratory forms of PRRS have been reported following experimental inoculation with different isolates.[4-6] Significant differences have been shown in the pathogenicity of nine U.S. PRRSV isolates with regards to clinical disease and lung lesions following experimental infection,[6] while similar results have also been reported between U.S. PRRSV isolates (VR-2431 and VR-2385) and Lelystad (European isolate) PRRSV.[7] In addition, it has also been reported that pregnant gilts exposed to isolates that differ in pathogenicity demonstrated differences in abortion rates, fetal death and neonatal pig health varied, thus leading to the conclusion that the impact of PRRSV-induced reproductive disease may be isolate-dependent.[4]

Besides these reported differences in clinical signs and lesions following PRRSV infection, the concentration of PRRSV (virus load) in individual pigs has also been reported to vary according to isolate virulence. Infection of susceptible pigs with highly virulent isolates resulted in significantly higher virus loads in blood and tissues when compared to pigs infected with avirulent isolates.[8,9] Other factors that may influence virus loads in pigs are bacterial co-infection and animal age. Pathogens such as *Bordetella bronchiseptica and Mycoplasma hyopneumoniae* are commonly found as opportunistic agents in PRRSV infection and have been documented to increase the severity and duration of PRRSV-induced pneumoniae.[10,11] Similarly, younger pigs have been shown to be more vulnerable to PRRSV infection, resulting in higher levels of viremia and virus excretion when compared to older animals.[12]

The influence of all 3 of these variables (animal age, bacterial co-infection and PRRSV isolate pathogenicity) can be observed in today's commercial swine production systems, particularly those which co-mingle pigs from different sources. This method of rearing pigs frequently results in a poor level of health in the wean-to-finish stage brought about by the presence of a mixed infection of PRRSV and opportunistic respiratory bacterial pathogens (Dee S., personal experience). In order to better control post-weaning PRRS, it is critical to understand the impact of these variables on PRRS virus load in nursery and finishing age pigs. Therefore, the objective of this study was to evaluate the role of age, bacterial co-infection and PRRSV isolate pathogenicity on the quantity of PRRSV in individual pigs. The study was based on the hypothesis that PRRS virus loads would be significantly higher in animals of a younger age with a reduced level of health. For the purpose of the study, a reduced level of health was defined as a mixed infection of PRRSV and *Mycoplasma hyopneumoniae* (*M hyo*).

Materials and methods

Virus source

Two PRRS virus isolates were selected for this study based on signs of clinical disease. PRRSV MN-30100, an avirulent isolate, was obtained from a persistently infected sow within a chronically infected breeding herd.[13] The clinical signs observed following infection with this isolate included transient depression, anorexia (24-48 hours) and mild fever of 40-41° C.[13] In contrast, the PRRSV MN-184 isolate was originally obtained from an infected farm experiencing severe reproductive disease and sow mortality in southern Minnesota in 2001.[8] Besides elevated mortality, clinical signs induced by the PRRSV MN-184 isolate included prolonged depression, anorexia and high fever(>42° C).[8]

PRRS virus infection model

Pigs were divided into groups according to age and housed in a separate room in the isolation facility. (2 months: 4-6 pigs/group and 6 months: 4-5 pigs/group). One control pig for each group was housed in a separate room within the isolation facility. On day 0, both 2 and 6-month old pigs were inoculated intranasally with either the aviru-

lent isolate MN-30100 (25-kg, 5 pigs/group; 120-kg, 4 pigs/group) or the virulent isolate MN-184 (25-kg, 6 pigs/group; 120-kg 5 pigs/group). Group size varied between ages and virus types due to space requirements of the isolation facility for different sized pigs and the expected mortality level of the MN-184 virus. All pigs received 2 mL of inoculum at a concentration of 1 x 10^4 $TCID_{50}$/mL. Control pigs were sham-inoculated intranasally with 2 mL of sterile saline. Successful PRRSV inoculation was monitored by serum qualitative polymerase chain reaction (PCR)[a] on days 1-3 post-infection.

PRRSV and Mycoplasma hyopneumoniae co-infection model

Similar to pigs infected with PRRSV alone, pigs co-infected with *M hyo* and PRRSV were divided into groups according to age (25-kg, n=4-6 pigs/group; 120-kg, n=4-5 pigs/group) and housed in the University of Minnesota College of Veterinary Medicine isolation facility. One control pig for each group was housed in a separate room within the isolation facility. On day 0, the pigs were anesthetized using a combination of xylazine hydrochloride[b] (1.5 mg/kg) and a mixture of tileamine and zolazepam hydrochloride[c] (8 mg/kg) administered intramuscularly.[14] While anesthetized, pigs were intubated and inoculated intratrachially with *M hyo* 232 at concentrations of 10 and 25-mL containing 10^5 color-changing units [CCU]/mL to infected 25-kg and 120-kg pigs.[11] Twenty-one days post-*Mycoplasma* inoculation, pigs were intranasally inoculated with either PRRSV MN-30100 or MN-184.[11] Negative control pigs were anesthetized and sham-inoculated with sterile saline. Successful *M hyo* infection was monitored by nasal swab PCR[15] on days 7, 15 and 21 and ELISA antibody test[d] on days 15 and 21 post-*M hyo* inoculation. Successful PRRSV inoculation was monitored by collecting blood samples between days 1-3 post-PRRSV infection and testing by qualitative PCR[a].

Sampling protocol

Following experimental infection, blood, nasal and oropharyngeal samples were collected from each pig on alternating days from 1-21 PI. Blood samples were collected by jugular venipuncture using a sterile vacutainer tube[e]. Nasal samples were collected by inserting a sterile swab[f] 2 cm deep into each of the nares. Oropharyngeal swabs were collected by drawing a similar swab across the hard and soft palates for a 5 second period. All swabs were then placed into sterile plastic tubes[g] containing 2 mL of sterile cell culture fluid. On day 21 post-PRRSV inoculation, pigs were euthanized using an intravenous injection of sodium pentobarbital[h] at a dose of 100 mg/kg. Pigs were necropsied and 1-gram of lung, tonsil, lateral retropharyngeal, tracheobronchial and sternal lymph nodes was collected from each pig. Tracheal swabs were also collected by inserting a sterile swab 5 cm deep into the trachea and drawing the swab upwards, contacting the interior surface of the trachea. Samples were stored individually at -20° C for 1-2 days prior to testing.

TaqMan quantitative PCR

Quantitative assessment of PRRS viral load in all samples was conducted using TaqMan polymerase chain reaction (PCR) at the Minnesota Veterinary Diagnostic Laboratory. This procedure was based on modifications of the TaqMan PCR protocol previously described.[a,16] Data were expressed in units of $TCID_{50}$/mL (serum and swabs) or $TCID_{50}$/g (tissues). A standard curve was developed for the quantitative PCR by making 10-fold dilutions of PRRSV MN-30100 and PRRSV MN-184, with dilutions ranged from 1 x 10^{-6} $TCID_{50}$/mL to 1 x $10^{4.6}$ $TCID_{50}$/mL. Each sample was run in triplicate and the mean RNA concentration calculated.

Data analysis

PRRS virus load in samples collected form live animals were compared across virus isolate, bacterial co-infection and animal age using a generalized analysis of variance (ANOVA) to test for differences between variables.[i] As distributions were often negative, a Kruskal-Wallis one-way ANOVA was used to asses the effects of animal age, bacterial co-infection and isolate pathogenicity on tissue virus load.

Summary of results

In regards to PRRS virus loads in blood, levels were significantly higher in pigs infected with PRRSV MN-184 (p=0.047) and in pigs co-infected with *M hyo*, independent of PRRSV isolate (p=0.022). The impact of animal age on viral load in blood, while not significant, showed a trend toward positively influencing blood samples (p=0.085). When evaluating PRRS virus loads in oropharyngeal swabs, infection with PRRSV MN-184 also demonstrated a trend towards significance (p=0.085) while significant increases were observed in animals co-infected with *M hyo* (p=0.045), independent of PRRSV isolate. Once again, the effects of animal age did not significantly influence virus load (p=0.118) in oropharyngeal samples. Similarly, PRRS virus loads demonstrated a trend towards significance in nasal swab samples (p=0.067) in pigs co-infected with *M hyo*; however, the effect of age (p=0.655) and PRRSV isolate (p=0.192) were not significant.

In regards to PRRS virus loads in tissues, 2-month old animals had significantly higher levels in lymph nodes (p=.026), lung (p=.019) and tracheal swabs (p =.0002) when compared to 6-month old animals, independent of PRRSV isolate. Infection with PRRSV MN-184 resulted in significantly higher virus loads in tonsil samples (p=.0003) whereas pigs co-infected with PRRSV MN-184 and *M hyo* resulted in significantly higher concentrations in tonsil (p=.0003) and lymphoid tissue samples

(p=0.0001). Finally, significant increases were not detected in tissues from pigs infected with PRRSV MN-30100 regardless of *M hyo* co-infection (p >0.23). All samples collected from control pigs remained PCR and ELISA negative throughout the study.

Footnotes

aPerkin-Elmer Applied Biosystems, Foster City, CA

bAnased, Lloyd Laboratories, Shenandoah, IA.

cTelazol, Fort Dodge Anima Health, Fort Dodge, IA.

dDAKO ELISA test, DAKO Laboratories, Denmark.

eVacutainer, Becton Dickinson, Franklin Park, NJ

fDacron swab, Fischer Science Laboratory, Hanover Park, IL

gFalcon tube, Becton Dickinson, Franklin Park, NJ

hBeuthanasia D, Schering Plough, Newark, NJ

iStatistix(r) for Windows, Analytical Software, Tallahassee, FL

References

1. Cavanagh D. Nidovirales: A new order comprising *Coronaviridae* and Arteriviridae. *Arch Virol* 1997; 629-633.
2. Dee SA, Joo HS, Polson DD, et al. Evaluation on the effects of nursery depopulation on the profitability of 34 pig farms. *Vet Rec* 1997; 140:498-500.
3. Chang CC, Yoon KJ, Zimmerman JJ, et al. Evolution of porcine reproductive and respiratory syndrome virus during sequential passage in pigs. *J Virol* 2002:76:4750-4763.
4. Mengeling WL, Vorwald AC, Lager KM et al. Comparison among isolates of porcine reproductive and respiratory syndrome virus for their ability to cause reproductive failure. *Am J Vet Res* 1996; 57:834-839.
5. Murtaugh MP, Yuan S, Faaberg KS. Appearance of novel PRRSV isolates by recombination in the natural environment. *Adv Exp Med Biol* 2001; 494:31-36.
6. Halbur PG, Paul PS, Frey ML, et al. Comparison of the pathogenicity of two U.S. porcine reproductive and respiratory syndrome virus isolates with that of the Lelystad virus. *Vet Pathol* 1995; 32:648-660.
7. Halbur PG, Paul PS, Meng XJ, et al. Comparative pathogenicity of nine U.S. porcine reproductive and respiratory syndrome virus (PRRSV) isolates in a five-week-old cesarean-derived, colostrums-deprived pig model. *J Vet Diag Invest* 1996; 8:11-20.
8. Johnson W, Roof M, Vaughn E, et al. Pathogenic and humoral immune responses to porcine reproductive and respiratory syndrome virus (PRRSV) are related to viral lad in acute infection. *Vet Immunol and Immunopath* 2004; 102:233-247.
9. Xiao Z, Batista L, Dee S, et al. The level of virus-specific T-cell and macrophage recruitment in porcine reproductive and respiratory syndrome virus infection in pigs is independent of virus load. *J Virol 2004*; 78: 5923-5933.
10. Brockmeier SL, Palmer MV, Bolin SR. Effects of intranasal inoculation of porcine reproductive and respiratory syndrome virus, *Bordetella bronchiseptica*, or a combination of both organisms in pigs. *Am J Vet Res* 2000; 61:892-899.
11. Thacker EL, Halbur PG, Ross RF, et al. *Mycoplasma hyopneumoniae* potentiation of porcine reproductive and respiratory syndrome virus-induced pneumoniae. *J Clin Microbiol* 1999; 37:620-627.
12. Van der Linden IFA, Voermans JJM, van der Linde-Bril EM, et al. Virological kinetics and immunological responses to a porcine reproductive and respiratory syndrome virus infection of pigs at different ages. *Vaccine* 2003; 21:1952-1957.
13. Bierk MD, Dee SA, Rossow KD, et al. Transmission of PRRS virus from persistently infected sows to contact controls. *Can J Vet Res* 2001; 65:261-266.
14. Ko JC, Williams BL, Smith VL, et al. Comparison of Telazol, Telazol-ketamine, Telazol-xylazine, and Telazol-ketamine-xylazine as chemical reisolatet and anesthetic induction combination in swine. *Lab Anim Sci* 1993; 43:476-480.
15. Calsamiglia M, Pijoan C, Trigo A. Application of a nested polymerase chain reaction assay to detect *Mycoplasma hyopneumoniae* from nasal swabs. *J Vet Diag Invest* 1999; 11:246-251.
16. Molitor TW, Tune KA, Shin J, et al. Application of TaqMan™ PCR in the detection of porcine reproductive and respiratory syndrome virus, in *Proceedings, Allen D Leman Swine Conf* 1997; 173-175.