Evaluation of the effects of animal age, concurrent bacterial infection, and pathogenicity of porcine reproductive and respiratory syndrome virus on virus concentration in pigs

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Objective—To evaluate the influences of animal age, bacterial coinfection, and porcine reproductive and respiratory syndrome virus (PRRSV) isolate pathogenicity on virus concentration in pigs.

Animals—Twenty-one 2-month-old pigs and eighteen 6-month-old pigs.

Procedure—Pigs were grouped according to age and infected with mildly virulent or virulent isolates of PRRSV. The role of concurrent bacterial infection was assessed by infecting selected pigs with *Mycoplasma hyopneumoniae* 21 days prior to inoculation with PRRSV. On alternating days, blood and swab specimens of nasal secretions and oropharyngeal secretions were collected. On day 21 after inoculation with PRRSV, selected tissues were harvested. Concentrations of PRRSV were determined by use of quantitative real-time PCR and expressed in units of $TCID_{50}$ per milliliter (sera and swab specimens) or $TCID_{50}$ per gram (tissue specimens).

Results—Concentrations of virus were higher in blood and tonsils of pigs infected with virulent PRRSV. Pigs infected with virulent PRRSV and *M hyopneumoniae* had significantly higher concentrations of viral RNA in lymphoid and tonsillar tissue. Coinfection with *M hyopneumoniae* resulted in a higher viral load in oropharyngeal swab specimens and blood samples, independent of virulence of the PRRSV isolate. Two-month-old pigs had significantly higher viral loads in lymph nodes, lungs, and tracheal swab specimens than did 6-month-old pigs, independent of virulence of the PRRSV isolate.

Conclusions and Clinical Relevance—Multiple factors affect PRRSV concentration in pigs, including pathogenicity of the PRRSV isolate, age, and concurrent infection with *M hyopneumoniae*. (*Am J Vet Res* 2006;67:489–493)

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Porcine reproductive and respiratory syndrome virus is an enveloped, single-stranded positive-sense RNA virus belonging to the family *Arteriviridae*.[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 via traditional methods is its ability to undergo genetic change through the processes of mutation and recombination, resulting in development of isolates with various degrees of pathogenicity.[3-6] Variability in severity of the reproductive and respiratory forms of PRRSV infection has been reported in association with different isolates after experimental inoculation.[4-6] Significant differences in pathogenicity were detected among 9 PRRSV isolates from the United States with regard to clinical disease and severity of lung lesions after experimental infection, and similar differences were reported[6,7] between PRRSV isolates (VR-2431 and VR-2385) from the United States and the European (Lelystad) strain of the virus. In addition, pregnant gilts exposed to isolates of differing pathogenicity had differences in abortion rate, fetal death rate, and health of neonatal pigs, leading investigators to conclude that the impact of PRRSV-induced reproductive disease may be isolate dependent.[4]

Besides the reported differences in clinical signs and lesions associated with PRRSV infection, concentrations of PRRSV in individual pigs reportedly vary according to isolate virulence. Infection of susceptible pigs with highly pathogenic isolates results in higher viral concentrations in blood and tissues, compared with concentrations in pigs infected with mildly virulent isolates.[8,9] Other factors that may influence viral concentrations in pigs are concurrent bacterial infection and animal age. Pathogens such as *Bordetella bronchiseptica* and *Mycoplasma hyopneumoniae* are common opportunistic agents; concurrent infection with these pathogens results in porcine respiratory disease complex and increases the severity and duration of PRRSV-induced pneumonia.[10,11] Similarly, younger pigs are more vulnerable to PRRSV infection, develop higher levels of viremia, and excrete more virus, compared with older pigs.[12]

PRRSV	Porcine reproductive and respiratory syndrome virus
RT-PCR	Real-time PCR

Animal age, concurrent bacterial infection, and pathogenicity of the PRRSV isolate affect production in commercial swine systems, particularly those in which pigs from different sources are comingled. This method of rearing pigs frequently results in poor health in pigs in the wean-to-finish stage, a state that may be brought about by mixed infection with PRRSV and opportunistic bacterial pathogens. An understanding of the impact of these variables on PRRS virus concentration in nursery and finishing-age pigs is of great importance. The objective of our study was to evaluate the effects of age, concurrent bacterial infection, and PRRSV isolate pathogenicity on quantities of PRRSV recovered from individual pigs. We hypothesized that concentrations of PRRSV would be significantly higher in younger pigs that had poor health. For purposes of the study, poor health was defined as having mixed infection with PRRSV and *M hyopneumoniae*.

Materials and Methods

Animals and study site—Procedures were conducted at the University of Minnesota College of Veterinary Medicine isolation facility. Facilities included a series of separately ventilated rooms containing slurry pits to prevent cross-contamination of pathogens among rooms. Twenty-one 2-month-old pigs (body weight, 25 kg) and eighteen 6-month-old pigs (120 kg) were purchased from a source known to be free of PRRSV and *M hyopneumoniae* on the basis of a 10-year history of diagnostic testing and the absence of clinical signs of infection with either pathogen. Pigs were transported to the isolation facility and acclimatized for 3 days prior to initiation of the study. Blood and swab specimens of nasal secretions were collected for analysis via RT-PCR assay to verify that pigs were free of PRRSV and *M hyopneumoniae* prior to inclusion in the study.[13] During the study, pigs were housed and cared for according to University of Minnesota Institutional Animal Care and Use Committee guidelines.

Source of virus—Two PRRSV isolates were selected for use on the basis of clinical signs elicited in pigs after experimental intranasal inoculation. An isolate of low pathogenicity (PRRSV MN-30100) was obtained from a persistently infected sow in a commercial production site.[14] Clinical signs observed after intranasal infection with that isolate included transient depression, lack of appetite for 24 to 48 hours, and mild fever (40° to 41°C).[13] In contrast, the highly virulent isolate (PRRSV MN-184) originated at a farm in southern Minnesota affected with severe reproductive disease and high sow mortality rates.[8] In addition to high mortality rates, clinical signs induced by the PRRSV MN-184 isolate after experimental intranasal inoculation included a prolonged period of depression, absence of appetite, and high fever (> 42°C).[8]

PRRSV infection—Pigs were allocated into groups according to age, and groups were housed in separate rooms in the isolation facility. One control pig for each group was housed in a separate room. On day 0, pigs were inoculated intranasally with PRRSV MN-30100 (2-month-old pigs, 5 pigs/group; 6-month-old pigs, 4 pigs/group) or PRRSV MN-184 (2-month-old pigs, 6 pigs/group; 6-month-old pigs, 5 pigs/group). Group size varied between ages and virus types because of space requirements in the isolation facility for differently sized pigs and the anticipated mortality rate in pigs inoculated with the MN-184 strain of virus. Subject pigs received 2 mL of inoculum with a viral concentration of 1×10^4 $TCID_{50}$/mL. Control pigs were sham-inoculated intranasally with 2 mL of sterile saline (0.9% NaCl) solution. Successful PRRSV inoculation was determined by results of serum qualitative RT-PCR[a] on days 1 to 3 after infection.

PRRSV and *M hyopneumoniae* coinfection—Pigs infected with *M hyopneumoniae* and PRRSV were allocated into groups according to age (2-month-old pigs, 6 pigs/group; 6-month-old pigs, 5 pigs/group). One control pig for each group was housed in a separate room. On day 0, pigs were anesthetized with a combination of xylazine hydrochloride[b] (dose, 1.5 mg/kg) and a commercially available mixture of tiletamine and zolazepam hydrochloride[c] (dose, 8 mg/kg) administered IM.[15] After induction, pigs were intubated and inoculated via intratracheal instillation of a solution containing 10^5 color-changing units of *M hyopneumoniae* strain 232/mL in volumes of 10 and 25 mL to 25- and 120-kg pigs, respectively.[11] Twenty-one days after bacterial inoculation, pigs were intranasally inoculated with PRRSV MN-30100 or MN-184 according to a described method.[11] Control pigs were anesthetized and sham-inoculated with sterile saline solution via the same intratracheal procedure. Nasal swab specimens were collected and tested by use of a nested PCR[13] on days 7, 15, and 21 to determine successful infection with *M hyopneumoniae*. Serum samples were collected from each pig to test for serum antibodies against *M hyopneumoniae* by use of an ELISA[d] on days 15 and 21 after bacterial inoculation. Successful PRRSV inoculation was monitored by collection of blood samples on days 1 to 3 after PRRSV infection and by testing via qualitative RT-PCR assay.[16,a]

Sampling protocol—Swab specimens of blood, nasal secretions, and oropharyngeal secretions were collected from each pig on alternating days on days 1 to 21 after the viral inoculation procedures. Blood samples were collected in sterile vacuum tubes[e] via jugular venipuncture. Nasal samples were collected by inserting a sterile swab[f] 2 cm into each of the nares. Oropharyngeal swabs were collected by drawing a similar sterile swab across the hard and soft portions of the palate for 5 seconds. Swabs were placed into sterile plastic tubes[g] containing 2 mL of sterile saline solution. On day 21, post-PRRSV inoculation, pigs were euthanatized with an IV injection of sodium pentobarbital[h] at a dose of 100 mg/kg. During necropsy, 1-g specimens of tissue from the lung, tonsil, lateral retropharyngeal lymph node, tracheobroncheal lymph node, and sternal lymph node were collected. Tracheal swabs were collected by inserting a sterile swab 5 cm into the trachea and drawing the swab upwards so that it contacted the luminal surface of the trachea. Samples were stored individually at –20°C for 1 to 2 days prior to testing.

Quantitative RT-PCR procedure—Quantitative assessment of PRRSV in samples was conducted by use of an RT-PCR kit[a] at the Minnesota Veterinary Diagnostic Laboratory in a procedure modified from a published protocol.[16] Data were expressed as $TCID_{50}$ per milliliter (for sera and swab specimens) or $TCID_{50}$ per gram (for tissues). A standard curve was developed for the quantitative RT-PCR procedure by preparing 10-fold dilutions of PRRSV MN-30100 and PRRSV MN-184, with dilutions ranging from 1×10^{-6} $TCID_{50}$/mL to $1 \times 10^{4.6}$ $TCID_{50}$/mL. Samples were assayed in triplicate, and mean RNA concentrations were calculated from those values.

Statistical analysis—Concentrations of PRRSV in specimens from live animals were compared for the variables of viral isolate, concurrent bacterial infection, and animal age by use of a generalized ANOVA.[i] Because distributions were often negative, a Kruskal-Wallis 1-way ANOVA was used to assess effects of animal age, concurrent bacterial infection, and isolate pathogenicity on viral concentration in tissues obtained at necropsy. For all comparisons, values of $P < 0.05$ were considered significant.

Results

All pigs had negative results of tests for PRRSV and *M hyopneumoniae* prior to initiation of the study. Pigs of all age groups and infection models had clinical signs of viremia 1 to 3 days after PRRSV inoculation. Serum antibodies against *M hyopneumoniae* were detected by use of ELISA[d] by day 21 after bacterial inoculation in all coinfected pigs.

Infection with PRRSV MN-30100—Clinical signs in 2- and 6-month-old pigs infected with PRRSV MN-30100 alone included transient fever (39° to 40°C), inappetance (24 to 48 hours after inoculation), and mild depression. None of those pigs died. In 2-month-old pigs, mean concentrations of PRRSV RNA were 254 $TCID_{50}$/mL (in blood samples) and 8 $TCID_{50}$/mL (in both nasal and oropharyngeal swab specimens). In 6-month-old pigs, mean RNA concentrations were 5 $TCID_{50}$/mL (blood), 4 $TCID_{50}$/mL (nasal swab specimens), and 8 $TCID_{50}$/mL (oropharyngeal swab specimens; **Table 1**). Regarding PRRSV RNA concentrations in tissues, mean values in 2-month-old pigs were 1 $TCID_{50}$/mL (tracheal swab), 3 $TCID_{50}$/mL (tonsil and lymph nodes), and 13 $TCID_{50}$/mL (lung). Mean RNA concentrations in 6-month-old pigs were 1 $TCID_{50}$/mL (tracheal swab, tonsil), 3 $TCID_{50}$/mL (lymph nodes), and 27 $TCID_{50}$/mL (lung; **Table 2**).

Concurrent infection with PRRSV MN-30100 and *M hyopneumoniae*—Clinical signs and death rates in 2- and 6-month-old pigs coinfected with PRRSV MN-30100 and *M hyopneumoniae* were similar across and within age groups, except that a dry cough was observed by day 21 after inoculation of *M hyopneumoniae* and continued through the sampling period. In 2-month-old pigs, mean concentrations of PRRSV RNA were 326 $TCID_{50}$/mL (in blood samples), 6 $TCID_{50}$/mL (in nasal swab specimens), and 36 $TCID_{50}$/mL (in oropharyngeal swab specimens). In 6-month old pigs, mean RNA concentrations were 270 $TCID_{50}$/mL (in blood samples), 20 $TCID_{50}$/mL (in nasal swab specimens), and 23 $TCID_{50}$/mL (in oropharyngeal swab specimens; Table 1). Regarding PRRSV RNA concentrations in tissues, mean values in 2-month-old pigs were 240 $TCID_{50}$/mL (tracheal swab), 49 $TCID_{50}$/mL (tonsil), 210 $TCID_{50}$/mL (lymph nodes), and 196 $TCID_{50}$/mL (lung). Mean RNA concentrations in 6-month-old pigs were 28 $TCID_{50}$/mL (tracheal swab), 12 $TCID_{50}$/mL (tonsil), 20 $TCID_{50}$/mL (lymph nodes), and 36 $TCID_{50}$/mL (lung; Table 2).

Infection with PRRSV MN-184—Clinical signs in pigs infected with PRRSV MN-184 alone were more severe and of longer duration than those observed in pigs infected with PRRSV MN-30100. Clinical signs in pigs infected with PRRSV MN-184 included high rectal temperatures (> 42°C) and profound anorexia and depression throughout the 21-day testing period. One 6-month-old pig died on day 15 after inoculation as a result of respiratory illness. In 2-month-old pigs, mean concentrations of PRRSV RNA were 332 $TCID_{50}$/mL (in blood samples), 5 $TCID_{50}$/mL (in nasal swab specimens), and 24 $TCID_{50}$/mL (in oropharyngeal swab specimens). In 6-month-old pigs, mean RNA concentrations were 472 $TCID_{50}$/mL (blood), 13 $TCID_{50}$/mL (nasal swab specimens), and 58 $TCID_{50}$/mL (oropharyngeal swab specimens; Table 1). Regarding PRRSV RNA concentrations in tissues, mean values in 2-month-old pigs were 12 $TCID_{50}$/mL (tracheal swab), 51 $TCID_{50}$/mL (tonsil), 45 $TCID_{50}$/mL (lymph nodes), and 1150 $TCID_{50}$/mL (lung). Mean RNA concentrations in 6-month old pigs were 76 $TCID_{50}$/mL (tracheal swab), 55 $TCID_{50}$/mL (tonsil), 44 $TCID_{50}$/mL (lymph node), and 480 $TCID_{50}$/mL (lung; Table 2).

Table 1—Concentrations of PRRSV in blood and nasal and oropharyngeal secretions from 2- and 6-month-old pigs infected with various combinations of a mildly virulent strain of PRRSV, a highly virulent strain of PRRSV, and *Mycoplasma hyopneumoniae*.

	Blood*			Nasal secretions			Oropharyngeal secretions		
Group	Peak†	Mean‡	SD§	Peak	Mean	SD	Peak	Mean	SD
PRRSV MN-30100									
2-month-old pigs	4,000	254	297	100	8	8	110	8	11
6-month-old pigs	49	5	7	79	4	8	121	8	13
PRRSV MN-30100 and *M hyopneumoniae*									
2-month-old pigs	3,800	326	422	60	6	9	287	36	63
6-month-old pigs	6,681	270	559	612	20	48	204	23	36
PRRSV MN-184									
2-month-old pigs	5,306	332	485	26	5	6	122	24	21
6-month-old pigs	10,246	472	781	189	13	31	940	58	91
PRRSV MN-184 and *M hyopneumoniae*									
2-month-old pigs	195,241	10,500	13,764	1,838	53	121	26,107	1,200	2,445
6-month-old pigs	13,074	890	1,720	1,180	41	85	607	50	104

PRRSV MN-30100 = Pigs were inoculated with an isolate of mildly virulent virus. PRRSV MN-30100 and *M hyopneumoniae* = Pigs were inoculated with an isolate of mildly virulent virus 21 days after inoculation with *Mycoplasma hyopneumoniae*. PRRSV MN-184 = Pigs were inoculated with an isolate of highly virulent virus. PRRSV MN-184 and *M hyopneumoniae* = Pigs were inoculated with an isolate of highly virulent virus 21 days after inoculation with *M hyopneumoniae*.

*Units reported in units of $TCID_{50}$/mL. †Peak concentration among all pigs in specified group. ‡Mean concentrations for all pigs in specified group during the 21-day testing period. §SD of mean concentrations.

Table 2—Concentrations of PRRSV in tissue samples obtained during necropsy examination from the same pigs as in Table 1.

	Lymph node*			Lung			Tonsil			Tracheal swab¶		
	Peak†	Mean‡	SD§	Peak	Mean	SD	Peak	Mean	SD	Peak	Mean	SD
PRRSV MN 30-100												
2-month-old pigs	9	3	1	50	13	21	10	3	4	1	1	0.2
6-month-old pigs	5	3	1	80	27	35	2	1	1	2	1	1
PRRSV MN-30100 and *M hyopneumoniae*												
2-month-old pigs	806	210	397	770	196	383	134	49	63	378	240	160
6-month-old pigs	90	20	12	110	36	25	32	12	37	42	28	34
PRRSV MN 184												
2-month-old pigs	85	45	28	6,737	1,130	2,748	180	51	63	71	12	29
6-month-old pigs	110	44	46	2,400	480	1,073	154	55	52	380	76	170
PRRSV MN 184 and *M hyopneumoniae*												
2-month-old pigs	89	52	27	1,702	650	878	95	32	37	320	103	131
6-month-old pigs	456	104	197	405	81	181	123	39	51	3	1	2

See Table 1 for key.

Concurrent infection with PRRSV MN-184 and *M hyopneumoniae*—Clinical signs in pigs coinfected with PRRSV MN-184 and *M hyopneumoniae* were severe. In addition to clinical signs observed in coinfected pigs, labored breathing, severe coughing, and gaunt body condition resulting from anorexia were observed, especially in 2-month-old pigs. Pigs often lay in recumbent positions and were nonresponsive to physical stimulation. Sample collection was suspended on postinoculation day 11 in 6-month-old pigs and on days 11 and 19 in 2-month-old pigs because of the severity of clinical signs. One 2-month-old pig was euthanatized on day 3 after inoculation because of the severity of clinical signs, and one 6-month-old pig died on day 9 after inoculation. Mean concentrations of PRRSV RNA in 2-month old pigs were 10,500 $TCID_{50}$/mL (in blood samples), 53 $TCID_{50}$/mL (in nasal swab specimens), and 1,200 $TCID_{50}$/mL (in oropharyngeal secretions). In 6-month-old pigs, mean RNA concentrations were 890 $TCID_{50}$/mL (in blood samples), 41 $TCID_{50}$/mL (in nasal swab specimens), and 50 $TCID_{50}$/mL (in oropharyngeal swab specimens; Table 1). Regarding PRRSV RNA concentrations in tissues, mean values in 2-month-old pigs were 103 $TCID_{50}$/mL (tracheal swab), 32 $TCID_{50}$/mL (tonsil), 52 $TCID_{50}$/mL (lymph nodes), and 650 $TCID_{50}$/mL (lung). Mean RNA concentrations in 6-month old pigs were 1 $TCID_{50}$/mL (tracheal swab), 39 $TCID_{50}$/mL (tonsil), 104 $TCID_{50}$/mL (lymph nodes), and 81 $TCID_{50}$/mL (lung; Table 2).

Statistical analyses indicated that in blood samples, concentrations were significantly higher in pigs infected with PRRSV MN-184 ($P = 0.047$) and in pigs coinfected with *M hyopneumoniae* ($P = 0.022$), regardless of the PRRSV isolate used for infection. Concentrations were significantly ($P = 0.045$) higher in animals coinfected with *M hyopneumoniae*, independent of the PRRSV isolate. Animal age did not significantly ($P = 0.118$) affect virus concentration in oropharyngeal swab specimens.

Regarding concentrations of PRRSV in tissues, 2-month-old pigs had significantly higher concentrations in lymph nodes ($P = 0.026$), lung ($P = 0.019$), and tracheal swab specimens ($P < 0.001$), compared with those in 6-month-old pigs, irrespective of PRRSV isolate. Infection with the PRRSV MN-184 isolate yielded significantly higher concentrations of virus in tonsils ($P < 0.001$), compared with those in pigs infected with PRRSV MN-30100, whereas pigs concurrently infected with PRRSV MN-184 and *M hyopneumoniae* had significantly higher concentrations in tonsils ($P < 0.001$) and lymph nodes ($P < 0.001$). All samples from control pigs had negative results of RT-PCR and ELISA testing throughout the study.

Discussion

Control of PRRSV infection in pigs in the postweaning period is dependent on a proper understanding of the effects of certain factors that are commonly encountered in modern production systems on populations of pigs infected with the virus. Our objectives were to evaluate the roles of animal age, concurrent bacterial infection, and pathogenicity of the PRRSV isolate on viral concentrations in individual pigs. Although these variables have been investigated independently, to the authors' knowledge, studies of their combined effect have not been published. Our study was designed to investigate the hypothesis that concentrations of PRRSV would be significantly higher in younger pigs with a poor health status; for purposes of the study, we defined poor health as the state of concurrent infection with PRRSV and *M hyopneumoniae*.

Our results indicated that the 3 variables evaluated affected PRRSV concentration. Infection with a highly pathogenic isolate (PRRSV MN-184) significantly affected viral concentrations detected in blood and tonsil tissues, whereas concentrations in animals infected with an isolate of lesser pathogenicity (PRRSV MN-30100) were not affected. Age of the animal also played a role, with younger pigs having significantly higher viral concentrations in tissues than older pigs, independent of PRRSV isolate. Concurrent infection with *M hyopneumoniae* yielded significantly higher PRRSV concentrations in blood and oropharyngeal swab specimens, compared with those in animals infected with PRRSV alone,

independent of the isolate. Significantly higher viral concentrations were also observed in lymph nodes and tonsil tissue from pigs coinfected with the highly pathogenic PRRSV isolate and *M hyopneumoniae*.

These findings were in accordance with results of other studies,[11] in which coinfection with PRRSV and *M hyopneumoniae* exacerbated PRRSV-induced disease and resulted in persistent lung lesions. Pigs with concurrent bacterial-viral infections also had higher mean concentrations of virus in lung tissue than pigs infected with only 1 of those 2 pathogens. Our study expanded that line of investigation by evaluating the role of coinfection with *M hyopneumoniae* along with the concurrent influences of PRRSV isolate pathogenicity and animal age. This was performed quantitatively across multiple sampling sites in individual pigs, and data were reported as mean virus concentrations rather than as a disease score. Nonetheless, results indicate that infection with *M hyopneumoniae* in combination with PRRSV infection not only increased the duration and severity of clinical signs but also enhanced the concentrations of viral RNA in various samples.

Our results should be interpreted carefully for several reasons. Only 2 isolates of PRRSV were evaluated; therefore, the applicability of results is limited to pigs infected with PRRSV MN-30100 or PRRSV MN-184. Those isolates were selected on the basis of their representation of a wide spectrum of pathogenicity. Similarly, only a single bacterium was used to assess the effects of concurrent bacterial infection. However, the bacterium was selected on the basis of its association with the porcine respiratory disease complex and on the availability of published data.

The present study was not designed to determine infectious doses of PRRSV. Data were reported as $TCID_{50}$ per milliliter or $TCID_{50}$ per gram on the basis of values extrapolated from standard curves.[17] It is possible that higher concentrations of virus may have been detected if another unit of measure, such as RNA copies/mL, had been used or if tonsil scrapings rather than swabs had been used to assess viral concentrations in the oropharynx. However, because of the assay's commercial availability and the ability to test large volumes of samples and maintain consistency, the quantitative RT-PCR test was used. Results cannot be extrapolated beyond the level of the individual because of the small sample size in each test group. However, this sample size permitted more frequent sampling and, hence, more numerous antemortem (n = 11) and postmortem (6) samples from each pig.

Despite these limitations, results provide preliminary data for concentrations of PRRSV recovered from pigs that differed in health status and age. Future studies are warranted to investigate the use of other diagnostic techniques for quantification of infectious dose at the individual animal level and that replicate the present study with a larger number of animals, with the goal of improving PRRS control at the herd level and eventual eradication of the disease.

a. Perkin-Elmer Applied Biosystems, Foster City, Calif.
b. Anased, Lloyd Laboratories, Shenandoah, Iowa.
c. Telazol, Fort Dodge Animal Health, Fort Dodge, Iowa.
d. DAKO ELISA test, DAKO Laboratories, Denmark.
e. Vacutainer, Becton-Dickinson, Franklin Park, NJ.
f. Dacron swab, Fischer Science Laboratory, Hanover Park, Ill.
g. Falcon tube, Becton-Dickinson, Franklin Park, NJ.
h. Beuthanasia D, Schering Plough, Newark, NJ.
i. Statistix for Windows, Analytical Software, Tallahassee, Fla.

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