Title: Improved vaccines for porcine reproductive and respiratory syndrome
NPB #99-146

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Date Received: 2/3/2000

ABSTRACT

An attenuated-porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) vaccine first became available commercially in 1994. This vaccine, initially marketed under the name RespPRRS® and more recently as RespPRRS/Repro®, and another vaccine, introduced later and marketed under the name PrimePac PRRS®, are now used extensively. Although vaccines are now often included in strategies for the prevention and control of PRRS, numerous field observations and related experimental studies have revealed that they sometimes fail to provide complete protection. The most common theory in regard to this lack of complete protection is genetic variation among strains, namely, between the vaccine strain and the virulent field strain(s) circulating in an affected herd. The hypothesis of this experiment was that a vaccine containing several strains of PRRSV (multi-strain vaccine) might provide better protection than the single-strain vaccines in current use.

The experiment comprised 6 groups with 8 pigs/group. Treatments were the following: Group I, nonvaccinated, nonchallenged (nonchallenged = not exposed to virulent virus); Group II, nonvaccinated, challenged (challenged = exposed to virulent virus); Group III, vaccinated (single-strain vaccine), nonchallenged; Group IV, vaccinated (single-strain vaccine), challenged; Group V, vaccinated (multi-strain vaccine), nonchallenged; and Group VI, vaccinated (multi-strain vaccine), challenged. The single-strain vaccine was RespPRRS/Repro®. The multi-strain vaccine was RespPRRS/Repro® plus 4 additional strains of PRRSV that had been attenuated in our laboratory. The virulent (challenge) strain of PRRSV to which groups II, IV, and VI were exposed was isolated from a severe clinical epidemic of atypical PRRS. It was a

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different strain than any of those in the single-strain and multi-strain vaccines. Pigs were vaccinated when they were 2-3 weeks of age and challenged 4 weeks later. On the basis of 1 or more of the measurements of body temperatures, clinical signs, weight gains, virus isolations, and lung lesions both types of vaccine provided a measurable, but less than full, protection against challenge. For example, the mean extent of lung lesions was reduced from 56% for nonvaccinated pigs of group II to 7% and 11% for pigs of groups IV and VI, respectively. Neither vaccine was clearly superior in regard to effectiveness. Our impression was that protective immunity would have been greater had challenge been delayed for at least several weeks because vaccine virus was still circulating in the blood of vaccinated pigs at the time of challenge. Delayed challenge also might have revealed multi-strain vaccine to be superior in regard to protective immunity in that on the basis of lymph node enlargement pigs appeared to have a more forceful immunologic response to multi-strain vaccine. Two or more strains of PRRSV were often isolated from the blood of pigs vaccinated with the multi-strain vaccine. Irrespective of the type of prior vaccination, virulent virus quickly predominated in the circulation of most challenged pigs.

INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is believed by many to be the most economically important virus-induced disease currently faced by the swine industry. The PRRS virus (PRRSV) can cause clinical disease, potentiate the effects of other pathogens, and interfere (via national embargoes) with free access to international markets.

The relatively recent development and commercial availability of attenuated live-virus vaccines has offered new hope in the battle against PRRS. While the primary purpose of vaccination is to protect against clinical illness should the vaccinate subsequently be exposed to virulent PRRSV, it also has the potential to interfere with the spread of virulent virus by increasing the threshold of infection and decreasing the magnitude and duration of shedding. Unfortunately the level of protective immunity provided by current vaccines has sometimes been unsatisfactory, especially against strains of PRRSV isolated from epidemics of what has been referred to as "atypical" or "acute" PRRS. These so-called vaccine failures have emphasized that if vaccines are to be used as a reliable tool for the control of PRRS under present conditions of commercial swine production, some improvements in either vaccination strategies or in the vaccines themselves are necessary.

Because vaccine failures may be more or less associated with differences among strains of PRRSV, specifically between the attenuated strain used in a vaccine and the virulent strain to which the vaccinates are subsequently exposed, it has been proposed that a multi-strain vaccine might be more efficacious. To test this hypothesis a multi-strain vaccine composed of 5 attenuated strains of PRRSV, namely, RespPRRS/Repro® and 4 additional strains attenuated in our laboratory, was tested and compared to a currently used single-strain vaccine, namely, RespPRRS/Repro®. Various measurements and tests made on pigs after vaccination and after exposure to virulent PRRSV (challenge) were used to determine vaccine efficacy.

OBJECTIVE

To determine if a multi-strain PRRS vaccine would be more effective than a

single-strain vaccine in providing protective immunity against a virulent, heterologous strain of PRRSV.

PROCEDURES

The experimental design comprised 6 groups (I through VI) of 8 pigs/group. The pigs were selected from 8 litters of specific-pathogen-free gilts that were free of antibody for PRRSV and were farrowed in isolation at the National Animal Disease Center. Pigs were weaned and assigned to treatment groups when they were 2 to 3 weeks of age (day 0 of the experiment). Each group comprised 2 pigs each of litters 1 and 8, and 1 pig each of litters 2, 6, and 7. The remaining pig of each group was from litter 3, 4, or 5. In addition to the selection of pigs so that groups would be comprised mostly of an equal number of pigs from each litter, pigs were selected so that the array of body weights and the total of body weights of each group would be about the same at the beginning of the experiment. Each group was kept on raised decks in a separate isolation room under otherwise similar conditions. Groups I and II were not vaccinated. Groups III through VI were vaccinated intramuscularly at day 0 of the experiment with either a single-strain (groups III and IV) or multi-strain (groups V and VI) vaccine. The single-strain vaccine was a commercial vaccine (RespPRRS/Repro®) that had been passaged once in cell culture in our laboratory and adjusted to a titer of 10^6 median cell culture infections doses ($CCID_{50}$)/ml. The multi-strain vaccine was a mixture of the same preparation of RespPRRS/Repro® plus 4 additional strains of PRRSV (1 of which was isolated from a clinically severe epidemic of atypical PRRS) that had been attenuated by 251 cell culture passages in our laboratory. The multi-strain vaccine was adjusted to a titer of 10^6 $CCID_{50}$ of each strain/ml. Groups II, IV, and VI were exposed oronasally to a virulent field strain of PRRSV at day 28 of the experiment. This strain had been isolated from a clinically severe epidemic of atypical PRRS. It had no known strain relationship to any of the strains included in the vaccines. Pigs were weighed on day 0 (just before vaccination), and again on days 7, 14, 21, 28 (just before challenge), 35, and 42. They were usually bled just after they were weighed. The exceptions were: 1) pigs were bled on day 13 instead of day 14; and 2) one-half of the pigs of each group were bled on day 42 when they were euthanized and necropsied, and the remaining pigs of each group were bled on day 43 when they were euthanized and necropsied. This 2-day interval is referred to hereafter as day 42 for the purpose of presenting experimental results. Additional procedures performed at necropsy included: 1) visual estimation of the extent of lung lesions (i.e. percentage of clearly distinguishable consolidation relative to total surface area); 2) lung lavage except for about the 1/3 ventral portion of the right cardiac lobe that was collected for histologic examination (during lavage the cut surface was closed with a hemostat); 3) collection and weighing of 3 selected lymph nodes (sternal and left and right deep cervical).

All pigs were observed for clinical signs at least twice daily. Body temperatures were taken for all pigs just before, and daily for the first 10 days after, vaccination, and just before, and for the first 12 days after, challenge.

Throughout the experiment each group was fed twice daily in an amount that was estimated to be just slightly more, on the basis of the previous feeding, than they would consume by the time of the next feeding. Groups were judged to have normal or reduced feed consumption by comparison with groups I and II following vaccination (days 0 to 28) and group I following challenge (days 28 through 42).

Blood (serum) samples were tested for antibody by enzyme-linked

immunosorbent assay (ELISA). Serum and lung lavage fluids were tested for PRRSV by virus isolation (VI) in cell culture and by the polymerase chain reaction (PCR). For VI, all samples were first tested (screened) by adding a relatively small volume of sample (50 ul) to a 2 cm^2 monolayer of cells. Those negative on the first test were retested by adding a larger volume of sample (500 ul) to a 25 cm^2 monolayer of cells. All samples that were virus-positive by PCR were tested by restriction endonuclease fragment length polymorphism analysis (RFLP) to determine the strain or strains of PRRSV in the sample (each of the stains had a unique RFLP pattern). The 5 attenuated strains of PRRSV are referred to hereafter as strains 1 through 5 with strain 1 representing RespPRRS/Repro®. The virulent challenge strain of PRRSV is referred to hereafter as strain 6. When more than 1 strain was identified in the blood or lavage fluid of a pig the restriction pattern was reported as mixed because it was not possible in some cases to determine with certainty which of the strains contributed to the mixture.

RESULTS

Clinical signs: All groups that were vaccinated or challenged or both had some days during the 42-day interval of the experiment when they consumed less feed than did nontreated pigs during the same interval (Table 1). The only other obvious clinical sign was mild, transient listlessness in some pigs beginning several days after vaccination and mild to marked listlessness in some pigs beginning several days after challenge. The most severely affected pigs after challenge were in nonvaccinated group II. During the immediate 10-day interval following vaccination (days 0 through 10 of the experiment) body temperatures fluctuated widely among pigs (individual temperature not shown) within a group as well as among groups (Fig. 1) without any clearly obvious affect of vaccination, i.e. nonvaccinated groups I and II versus vaccinated groups III through VI. During the immediate 12-day interval following challenge (days 28 through 42 of the experiment) there was a similar fluctuation among body temperatures of pigs within a group and among groups (Fig. 2). However, in this case body temperatures of group II (nonvaccinated, challenged) were generally higher than those of any of the other groups. This difference was especially striking during the interval from 7 through 12 days (the last day temperatures were taken) after challenge. On day 42, i.e. 2 weeks after challenge, the mean body weight of pigs of group I (nontreated) was 56.86 lbs. whereas that of the challenged group II was 51.68 lbs. A slight difference in the mean body weights of these 2 groups before challenge (group I = 40.6, group II = 38.9) appeared to be magnified after challenge. The mean body weights of the vaccinated, and vaccinated and challenged groups, were between these 2 extremes (Fig. 3).

Antibody response: All vaccinated pigs (groups III through VI) developed antibodies detectable by ELISA (S/P ratios >0.4) by 14 days after vaccination. In general the S/P ratios for these groups continued to increase at a lesser rate thereafter irrespective of subsequent challenge, i.e. groups III and V versus groups IV and VI. The S/P ratios for nonvaccinated group II remained at or near 0 until challenged at day 28. The S/P ratios for the nonvaccinated and nonchallenged group I remained at or near 0 throughout the experiment (Fig. 4).

Virus identification: Porcine reproductive and respiratory syndrome virus was identified by both VI and PCR in the sera of all vaccinate pigs (groups III through VI) at day 7, 13, and 21 after vaccination, and in the sera of all pigs of the nonvaccinated and challenged group (group II) at days 7 and 14 (experiment days 35 and 42) after challenge (Table 2), at which time all pigs were euthanized and necropsied. In some

instances virus was identified by PCR, but not by VI. In total, i.e. considering the 205 serum samples obtained from pigs 1 week or more after vaccination or challenge or both, 160 (78%) were positive by VI and 192 (94%) were positive by PCR. Vaccination appeared to somewhat reduce the incidence of VI and PCR positive samples following challenge (group II versus groups IV and VI). The virus was more often identified in lung lavage fluids than in sera when these samples were obtained on the same day. Of the 39 lung lavage fluids obtained at necropsy from pigs that had been vaccinated or challenged or both, 33 (85%) were positive by VI and 38 (97%) were positive by PCR. There were no VI-positive samples that were not also positive by PCR. However, a comparison of the relative sensitivity of VI and PCR is tempered slightly by the fact that 4 sera that were positive by VI but negative by PCR after the first round of testing were retested and found positive by PCR. Of the 193 VI-positive samples (both sera and lung lavage fluids) 136 were positive when initially screened in a relatively small volume whereas the remaining 57 were positive only when tested in a larger volume (see PROCEDURES for additional testing details).

Strain identification: The strain or strains of PRRSV identified in sera and lung lavage fluids was consistent with expectations on the basis of group treatments (Table 3). Only RespPRRS/Repro® was identified in the sera of pigs vaccinated with the single-strain vaccine (groups III and IV) until after challenge (group IV) when, without exception, the challenge strain predominated in both sera and lung lavage fluids. Conversely a mixture of strains was often identified in the sera of pigs vaccinated with the multi-strain vaccine (groups V and VI) until after challenge (group VI). Thereafter the challenge strain usually predominated. However, there were 4 exceptions, namely 1 of the vaccine strains (the same strain in each case) was still identified in the sera of pigs 43, 44, and 47 on day 35, i.e. 7 days after challenge, and in the lung lavage fluid of pig 44 at necropsy 14 days after challenge. In addition, an unusual RFLP pattern was identified for PRRSV isolated from the serum of pig 33 of group V at day 42.

Lung lesions: There were marked differences among the treatment groups in regard to the extent of macroscopically obvious lung lesions (Table 4). At the extremes were group I without any discernible lesions and group II with a mean value for lesions of 56%. Lung lesions were less extensive in pigs of groups IV and VI that were vaccinated 4 weeks before challenge (groups IV and VI) than they were in the nonvaccinated, challenged group II. Microscopic examination of lungs of pigs of group I revealed no pathologic changes. For the other groups changes, which were characteristic of PRRSV-induced pneumonia, ranged from mild and limited for pigs of groups that had only been vaccinated to severe and extensive for pigs that had only been challenged.

Lymph nodes: Compared to nonvaccinated, nonchallenged group I pigs, lymph nodes for pigs of all other groups were enlarged (Table 4). The degree of enlargement (on the basis of relative weights) among groups ranged from about 2 times (group III) to 5 times (group VI) that of the group I.

SUMMARY OF KNOWLEDGE GAINED FROM THIS STUDY THAT IS OF POTENTIAL BENEFIT TO SWINE PRODUCERS

1) Vaccination of 2-3 week old pigs with an attenuated single-strain or multi-strain porcine reproductive and respiratory syndrome virus (PRRSV) vaccine provided measurable protection against subsequent challenge with a highly virulent field strain of PRRSV.

2) The fact that vaccine virus was still circulating in the blood of many pigs at 6 weeks after vaccination suggests that to provide the maximum level of protection vaccine should be administered as long as possible before the likelihood of exposure to virulent PRRSV.

3) Vaccination of pigs with a multi-strain PRRSV vaccine often resulted in a demonstrable multi-strain infection with the potential for recombination and altered strain properties.

4) An unusual RFLP pattern (i.e. different from that of any of the strains administered to pigs) for PRRSV isolated from pig 33 of group V at day 42 raised the possibility that there had been strain recombination in at least one instance. Because of the potential practical significance of this event studies are ongoing. Moreover, depending on future research priorities we may investigate additional isolates from this study by using techniques that are specifically designed to screen for recombinants.

5) Regardless of the strain or strains of vaccine virus circulating in the blood of pigs at the time of challenge, the virulent challenge strain quickly predominated in most pigs thereafter. As a consequence infection of vaccinated pigs with a virulent field strain of PRRSV would likely be detected by diagnostic tests such as RFLP analysis or base sequencing.

6) Selected lymph nodes of pigs that were vaccinated or both vaccinated and challenged were enlarged 2 to about 5 times compared to untreated pigs. Whether this was simply the result of an unusually forceful immune response or reflected an as yet undefined additional effect of PRRSV on lymphoid tissue is unknown. In either case, the degree of enlargement appeared to be related to the type of vaccine in that it was almost twice as much for the multi-strain vaccine than it was for the single-strain vaccine. What this means relative to the overall immune response has yet to be defined. But it might be that a multi-strain vaccine would be contraindicated in the presence of other infections. On the other hand, in keeping with the initial hypothesis of this experiment, a multi-strain vaccine might be more effective than a single-strain vaccine for subsequent protection against virulent PRRSV when used in older pigs such as replacement gilts.

Table I. Days of reduced feed consumption*

	Experiment days†			
Group	0–3	4–19	20–32	33–42
I	0‡	0	0	0
II	0	0	0	5
III	0	4	0	0
IV	0	3	0	2
V	0	4	0	0
VI	0	7	0	3

* By comparison with nonvaccinated groups I and II during the interval of days 0 to 28 and with nonchallenged group I during the interval of days 28 through 42.

† Intervals were selected on the basis of whether 1 or more groups were judged to have had reduced feed consumption during any of the days during the interval.

‡ Number of days group was judged to have reduced feed consumption.

Table 2. Detection of Porcine Reproductive and Respiratory Syndrome Virus by Virus Isolation (VI) and by the Polymerase Chain Reaction (PCR)

	Experiment day*						
Group	7	14	21	28	35	42	42
I	0/8†	0/8	0/8	0/8	0/8 (0/8)	0/8 (0/8)	0/8 (0/8)
II	0/8 (0/2)	0/8 (0/2)	0/8 (0/2)	0/8 (0/3)	8/8 (8/8)	8/8 (8/8)	8/8 (8/8)
III	8/8 (8/8)	8/8 (8/8)	8/8 (8/8)	7/8 (8/8)	0/8 (3/8)	4/8 (7/8)	7/8 (8/8)
IV	8/8 (8/8)	8/8 (8/8)	8/8 (8/8)	6/7‡ (7/7)	5/7 (7/7)	4/7 (7/7)	6/7 (7/7)
V	8/8 (8/8)	8/8 (8/8)	8/8 (8/8)	8/8 (8/8)	0/8 (4/8)	2/8 (8/8)	6/8 (7/8)
VI	8/8 (8/8)	8/8 (8/8)	8/8 (8/8)	7/8 (8/8)	3/8 (8/8)	2/8 (5/8)	6/8 (8/8)

* Columns 1 through 6 (days 7 through 42) contain results with serum samples; The last column (column 7) contains the results with lung lavage samples.
† Numerator = number of samples positive; Denominator = number of samples (pigs) tested. Values outside of the parentheses = results by VI; Values inside the parentheses = the results by PCR. None of the serum samples obtained from group I pigs before day 35 and only a few of the serum samples obtained from group II pigs before day 35 were tested by PCR. None of the serum samples obtained on day 0 were VI-positive.
‡ One pig of this group died of a massive thoracic hemorrhage that was possibly due to trauma caused by bleeding on day 21.

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Table 3. Results of Virus Isolation (VI), Polymerase Chain Reaction (PCR), and Restriction Fragment Length Polymerase (RFLP) Testing of Serum and Lung Lavage Fluid Samples.

		Experiment Day													
		Sera												Lavage	
Group	Pig	7		13		21		28		35		42		42	
I	1	(--)[*]		(--)		(--)		(--)		(--)	(--)	(--)	(--)	(--)	(--)
	2	(--)		(--)		(--)		(--)		(--)	(--)	(--)	(--)	(--)	(--)
	3	(--)		(--)		(--)		(--)		(--)	(--)	(--)	(--)	(--)	(--)
	4	(--)		(--)		(--)		(--)		(--)	(--)	(--)	(--)	(--)	(--)
	5	(--)		(--)		(--)		(--)		(--)	(--)	(--)	(--)	(--)	(--)
	6	(--)		(--)		(--)		(--)		(--)	(--)	(--)	(--)	(--)	(--)
	7	(--)		(--)		(--)		(--)		(--)	(--)	(--)	(--)	(--)	(--)
	8	(--)		(--)		(--)		(--)		(--)	(--)	(--)	(--)	(--)	(--)
II	9	(--)		(--)		(--)		(--)		(+)	(+[6])	(+)	(+[6])	(+)	(+[6])
	10	(--)		(--)		(--)		(--)		(+)	(+[6])	(+)	(+[6])	(+)	(+[6])
	11	(--)		(--)		(--)		(--)		(+)	(+[6])	(+)	(+[6])	(+)	(+[6])
	12	(--)		(--)		(--)		(--)		(+)	(+[6])	(+)	(+[6])	(+)	(+[6])
	13	(--)		(--)		(--)		(--)		(+)	(+[6])	(+)	(+[6])	(+)	(+[6])
	14	(--)		(--)		(--)		(--)	(--)	(+)	(+[6])	(+)	(+[6])	(+)	(+[6])
	15	(--)	(--)	(--)	(--)	(--)	(--)	(--)	(--)	(+)	(+[6])	(+)	(+[6])	(+)	(+[6])
	16	(--)	(--)	(--)	(--)	(--)	(--)	(--)	(--)	(+)	(+[6])	(+)	(+[6])	(+)	(+[6])
III	17	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(--)	(--)	(+)	(+[1])	(+)	(+[1])
	18	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(--)	(+[1])	(--)	(--)	(--)	(+[1])	(+)	(+[1])
	19	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(--)	(--)	(--)	(+[1])	(--)	(+[1])
	20	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(--)	(+[1])	(+)	(+[1])	(+)	(+[1])
	21	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(--)	(--)	(--)	(--)	(+)	(+[m])
	22	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(--)	(+[1])	(+)	(+[1])	(+)	(+[1])
	23	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(--)	(+[1])	(+)	(+[1])	(+)	(+[1])
	24	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(--)	(--)	(--)	(+[1])	(+)	(+[1])
IV	25	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(+)	(+[6])	(+)	(+[6])	(+)	(+[6])
	26	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(--)	(+[1])	(--)	(+[6])	(--)	(+[6])	(+)	(+[6])
	27	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(--)	(+[1])	(+)	(+[6])	(+)	(+[6])	(--)	(+[6])
	28	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(+)	(+[6])	(+)	(+[6])	(+)	(+[6])
	29	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	NA[†]		NA		NA		NA	
	30	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(+)	(+[6])	(+)	(+[6])	(+)	(+[6])
	31	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(--)	(+[6])	(--)	(+[6])	(+)	(+[6])
	32	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(+)	(+[1])	(+)	(+[6])	(--)	(+[6])	(+)	(+[6])
V	33	(+)	(+[3])	(+)	(+[3])	(+)	(+[m])	(+)	(+[m])	(--)	(--)	(--)	(+[‡])	(+)	(+[3])
	34	(+)	(+[m])	(+)	(+[4])	(+)	(+[4])	(+)	(+[4])	(--)	(+[4])	(--)	(+[4])	(+)	(+[4])
	35	(+)	(+[1])	(+)	(+[m])	(+)	(+[1])	(+)	(+[m])	(--)	(+[¶])	(--)	(+[4])	(+)	(+[m])
	36	(+)	(+[m])	(+)	(+[m])	(+)	(+[m])	(+)	(+[m])	(--)	(+[4])	(--)	(+[4])	(+)	(+[m])
	37	(+)	(+[3])	(+)	(+[m])	(+)	(+[3])	(+)	(+[3])	(--)	(--)	(--)	(+[3])	(--)	(+[3])

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	38	(+)	(+1)	(+)	(+^{1})	(+)	(+^{m})	(+)	(+^{1})	(--)	(--)	(+)	(+^{m})	(--)	(--)
	39	(+)	(+^{m})	(+)	(+^{m})	(+)	(+^{4})	(+)	(+^{4})	(--)	(+^{4})	(+)	(+^{4})	(+)	(+^{4})
	40	(+)	(+^{1})	(+)	(+^{m})	(+)	(+^{m})	(+)	(+^{m})	(--)	(--)	(--)	(+^{m})	(+)	(+^{m})
VI	41	(+)	(+^{m})	(+)	(+^{m})	(+)	(+^{m})	(+)	(+^{m})	(+)	(+^{6})	(--)	(+^{6})	(+)	(+^{m})
	42	(+)	(+^{m})	(+)	(+^{4})	(+)	(+^{m})	(+)	(+^{4})	(--)	(+^{6})	(--)	(--)	(+)	(+^{6})
	43	(+)	(+^{m})	(+)	(+^{m})	(+)	(+^{1})	(+)	(+^{m})	(--)	(+^{4})	(+)	(+^{6})	(+)	(+^{6})
	44	(+)	(+^{m})	(+)	(+^{m})	(+)	(+^{m})	(+)	(+^{4})	(--)	(+^{4})	(--)	(--)	(+)	(+^{4})
	45	(+)	(+^{1})	(+)	(+^{m})	(+)	(+^{m})	(+)	(+^{m})	(+)	(+^{6})	(--)	(+^{6})	(--)	(+^{m})
	46	(+)	(+^{m})	(+)	(+^{m})	(+)	(+^{4})	(--)	(+^{4})	(--)	(+^{6})	(--)	(+^{6})	(+)	(+^{6})
	47	(+)	(+^{1})	(+)	(+^{4})	(+)	(+^{m})	(+)	(+^{4})	(--)	(+^{4})	(+)	(+^{6})	(+)	(+^{6})
	48	(+)	(+^{m})	(+)	(+^{m})	(+)	(+^{m})	(+)	(+^{m})	(+)	(+^{6})	(--)	(--)	(--)	(+^{6})

* Within the first or only set of parentheses in each column are the results of VI, and within the second set of parentheses are the results of PCR (+ = positive, -- = negative); The superscript associated with the positive results of PCR indicates the strain of PRRSV identified by PCR. Superscripts 1,2,3,4,5, and 6 = strains 1 (RespPRRS/Repro®), 2, 3, 4, 5, and 6 (virulent challenge strain), respectively. The identification of more that 1 strain in the same sample is indicated by the superscript m. With few exceptions (pigs 15 and 16 on days 7, 13, 21, and 28, and pig 14 on day 28) serum samples obtained from pigs of groups I and II were not tested by PCR until after group II pigs were challenged at day 28. † Pig 29 died as a result of a massive thoracic hemorrhage that was believed to be due to trauma caused by bleeding on day 21. ‡ An unusual RFLP pattern was detected in this sample. ¶ Not enough product to test by RFLP.

Table 4. Lung and Lymph Node Lesions

Group	Lymph node weight (gms)	Lung score
I	3.89*	0†
II	12.33	56
III	7.82	0
IV	14.41	7
V	13.80	1
VI	18.98	11

* Mean weight in grams/pig of selected lymph nodes (sternal, and left and right deep cervical) that were obtained at necropsy at day 42.
† Estimated % of lung consolidation on the basis of visual inspection of the lung surface at the time of necropsy at day 42.

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