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Characterization of the humoral immune response to porcine reproductive and respiratory syndrome (PRRS) virus infection

Kyoung-Jin Yoon, Jeffrey J. Zimmerman, Sabrina L. Swenson,
Michael J. McGinley, Ken A. Eernisse, Andy Brevik,
Lydia L. Rhinehart, Merwin L. Frey, Howard T. Hill,
Kenneth B. Platt

Abstract. The development of the humoral immune response against porcine reproductive and respiratory syndrome (PRRS) virus was monitored by an indirect fluorescent antibody (IFA) test, immunoperoxidase monolayer assay (IPMA), enzyme-linked immunosorbent assay (ELISA), and serum virus neutralization (SVN) test over a 105-day period in 8 pigs experimentally infected with ATCC strain VR-2402. Specific antibodies against PRRS virus were first detected by the IFA test, IPMA, ELISA, and the SVN test 9-11, 5-9, 9-13, and 9-28 days postinoculation (PI), respectively, and reached their maximum values by 4-5, 5-6, 4-6, and 10-11 weeks PI, respectively, thereafter. After reaching maximum value, all assays showed a decline in antibody levels. Assuming a constant rate of antibody decay, it was estimated by regression analysis that the ELISA, IFA, IPMA, and SVN antibody titers would approach the lower limits of detection by approximately days 137, 158, 324, and 356 PI, respectively. In this study, the immunoperoxidase monolayer assay appeared to offer slightly better performance relative to the IFA test, ELISA, and SVN test in terms of earlier detection and slower rate of decline in antibody titers. Western immunoblot analysis revealed that antibody specific for the 15-kD viral protein was present in all pigs by 7 days PI and persisted throughout the 105-day observation period. Initial detection of antibodies to the 19-, 23-, and 26-kD proteins varied among pigs, ranging from 9 to 35 days PI. Thereafter, the antibody responses to these 3 viral proteins of PRRS virus continued to be detected throughout the 105-day study period. These results clearly indicate that the 15-kD protein is the most immunogenic of the 4 viral proteins identified and may provide the antigenic basis for the development of improved diagnostic tests for the detection of PRRS virus antibodies.

Porcine reproductive and respiratory syndrome (PRRS) is a relatively new viral disease of swine. In 1987, PRRS was reported as a new, devastating disease of swine in the United States (Keffaber KK: 1989, Am Assoc Swine Pract Newsl 1[2]:1-9). It continues to be an economically significant health problem in swine-producing regions throughout the world because of losses from respiratory disease in neonates and nursery pigs and reproductive losses in breeding stock.[5,8] In response to the economic importance of PRRS, a significant research effort has been mounted to develop reliable diagnostic tests and effective vaccines.

An enveloped RNA virus was identified as the causative agent for PRRS and provisionally assigned to the genus *Arterivirus* of the family Togaviridae on the basis of morphological and biological characteristics, genomic organization, and the strategy of gene expression.[4,6,10,16,17] Although genomic sequence analysis has suggested that the virus may contain as many as 6 structural proteins,[6,10] only 3 structural proteins with molecular masses of approximately 15, 19, and 26 kD have been consistently demonstrated (Benfield D, Harris L, Nelson E, et al.: 1992, Am Assoc Swine Pract Newsl 4[4]: 19-21).[12] The functions of these proteins have not been completely determined, but current evidence indicates that the 15-kD protein is a nucleocapsid protein, whereas the 19- and 26-kD proteins are presumed to be components of the viral envelope (Benfield D, Harris L, Nelson E, et al.: 1992, Am Assoc Swine Pract Newsl 4[4]: 19-21.[6,10] The immunobiological roles of the viral proteins have not been characterized, although researchers have speculated that the 26-kD protein may be associated with induction of serum neutralizing antibodies against PRRS virus (Choi CS, Gustafson KV, Baustista EM, et al.: 1993, Abstr Conf Res Workers Anim Dis #P69).

From the Department of Microbiology, Immunology and Preventive Medicine (Yoon, Rhinehart, Platt), and the Veterinary Diagnostic Laboratory (Zimmerman, Swenson, Hill), College of Veterinary Medicine, Iowa State University, Ames, IA 50011, the National Veterinary Services Laboratories, USDA, APHIS, VS, Ames, IA 50010 (Eernisse, Frey), and Bayer Corporation, Agriculture Division, Animal Health Products, Shawnee Mission, KS 66201 (McGinley, Brevik).

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The indirect fluorescent antibody (IFA) test,[18] serum virus neutralization (SVN) test,[4,11] immunoperoxidase monolayer assay (IPMA), [17] and enzyme-linked immunosorbent assay (ELISA)[1] have been described for the detection of specific antibodies against PRRS virus. Currently, most North American veterinary diagnostic laboratories are using the IFA test and/or the SVN test to detect PRRS virus-specific antibodies, whereas European laboratories have relied on the IPMA using PRRS virus-infected porcine alveolar macrophages (PAM).[5,20] The recent licensure of a commercial ELISA kit[a] will probably change this picture.

The IFA test and IPMA are thought to be highly specific and sensitive tests.[17,18] Antibodies to PRRS virus are usually detected by these tests between 7 and 15 days after infection. Both the IFA test and the IPMA appear to be accurate for 2-3 months after infection but may lose their ability to detect antibodies against PRRS virus as soon as 3-6 months after exposure to PRRS virus (Frey M, Eernisse K, Landgraf J, et al.: 1992, Am Assoc Swine Pract Newsl 4[4]:31; Ohlinger V, Haas B, Sallmüller A, et al.: 1992, Am Assoc Swine Pract Newsl 4[4]:24).[15]

The ELISA format is also reported to be sensitive and specific.[1] In one study, PRRS virus-specific antibodies could be detected by ELISA as early as 10 days after exposure.[2] The performance of the commercial ELISA has not yet been reported.

The SVN test is also considered to be a specific test, but previous studies have suggested that the SVN test is less sensitive than the IFA test and the IPMA.[4,5,11] Neutralizing antibodies against PRRS virus may develop as late as 1-2 months after infection (Frey M, Eernisse K, Landgraf J, et al.: 1992, Am Assoc Swine Pract Newsl 4[4]:31).[11,15] However, a recent report indicated that the sensitivity of the SVN test could be increased by adding fresh normal swine serum to serum being assayed.[19]

The following study was conducted to characterize the ontogeny of humoral immune response of pigs to PRRS virus and to compare the performance of the IFA test, IPMA, ELISA, and SVN test using sera collected from a homogeneous group of pigs over a 10% day period following nasal inoculation with the ISU-P PRRS virus isolate (ATCC VR-2402). The viral protein specificity of the antibody response of these pigs was determined by western immunoblotting.

Materials and methods

Experimental design. Eight 5-6-wk-old crossbred pigs were obtained from a PRRS-virus-free herd. Pigs were numbered and randomly assigned to 2 separately housed groups of 4 pigs each. All pigs were intranasally inoculated with $10^{4.5}$ $TCID_{50}$ of PRRS virus. Serum samples were collected from each pig prior to inoculation, every other day during the first 15 days postinoculation (PI), and weekly thereafter through day 105. All serum samples were aliquoted and stored at -20 C until assayed for PRRS virus-specific antibodies by the IFA test, IPMA, ELISA, and SVN test. Aliquots of each serum sample were stored at -70 C until assayed for the presence of virus. Prior to performing the serological tests, serum samples were randomized and renumbered. Results from the serological assays were compared by curvilinear regression.[14]

The viral protein specificity of the antibody response was determined by western immunoblotting in 7 pigs using the same serum samples described above. Pigs were selected for the assay based on the time that neutralizing antibodies were first detected, i.e., early responders (3 pigs), in which neutralizing antibodies appeared on days 9 or 11, and late responders (4 pigs), in which neutralizing antibodies were first detected on days 21 or 28 PI. Serum virus neutralizing antibody was not detected in 1 of the 8 pigs until day 15 PI, which prevented its classification as an early or late responder. Consequently, sera from this pig were not assayed by western immunoblotting.

Virus. A cytopathic field isolate of the PRRS virus, designated ISU-P (ATCC VR-2402), was used in the study. The virus was originally isolated from pigs in a herd undergoing an acute outbreak of PRRS using virus-free PAM. The isolate was purified by limiting dilution in PAM culture and was plaque cloned twice in MA104 cells. The virus reproduced clinical disease compatible with PRRS in experimentally infected pigs and was recognized by PRRS-virus-specific monoclonal antibody SDOW17 and polyclonal swine serum raised against PRRS virus ATCC VR-2332. In addition, electron microscopy and western immunoblotting revealed a morphological structure and protein composition similar to that reported by other investigators (Pol J, Wagenaar F: 1992, Am Assoc Swine Pract Newsl 4[4]:29).[4]

Virus isolation. Serum samples were diluted 1:5 in RPMI-1640 medium[a] supplemented with 10% fetal bovine serum (FBS),[a] 10 mM HEPES,[b] and an antibiotic-antimycotic mixture (100 U/ml penicillin, 100 µg/ml streptomycin, 50 µg/ml gentamicin, and 0.25 µg/ml amphotericin B).[b] Diluted samples (0.2 ml) were inoculated onto 24-hr-old PAM cultures prepared in 48-well plates.[c] The inoculated cells were incubated at 37 C and observed for the cytopathic effect (CPE) typical of the virus for up to 7 days PI. The presence of PRRS virus in the cultures showing CPE was confirmed by subinoculation onto MA104 cell monolayers prepared on &chambered glass slides,[d] incubation for 48 hr, and staining with PRRS virus fluorescent monoclonal antibody conjugate (SDOW17).[e] A sample was considered negative after 2 blind passages in PAM.

Indirect fluorescent antibody test. The IFA test was performed using a protocol developed at the National Veterinary Services Laboratories, Ames, IA (Frey M, Eernisse K, Landgraf J, et al.: 1992, Am Assoc Swine Pract Newsl 4[4]:31). The MA104 cells were placed in 8-chambered glass slides and incubated in Dulbecco's modified Eagle's medium (DMEM)[b] supplemented with 10% FBS and the antibiotic-antimycotic mixture for 48-72 hr at 37 C in a humidified 5% CO_2 atmosphere. PRRS-virus-infected MA104 cell monolayers were prepared by inoculating culture medium

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with sufficient PRRS virus to produce 15-20 plaque forming units. Cultures were incubated for 20 hr at 37 C. The monolayers were then fixed by immersion in 100% acetone for 10 min, air dried, and stored at - 70 C until used. Viral-antigen-free cell controls were prepared in an identical manner using uninfected MA104 cells. Sera to be tested were serially diluted 2-fold in 0.01 M phosphate-buffered saline (PBS, pH 7.2), beginning with 1:20 dilution. Individual chambers were inoculated with 50 µl of each serum dilution and incubated at 37 C for 30 min in a humid environment. The preparations were then washed 3 times for 10 min each with PBS. Antigen-antibody reactions were visualized by reacting potential antigen-antibody complexes with optimally diluted goat anti-swine IgG conjugated with fluorescein isothiocyanate[f] for 30 min at 37 C in a humid environment. Indirect fluorescent antibody titers were recorded as the reciprocal of the highest serum dilution in which typical cytoplasmic fluorescence was observed.

Immunoperoxidase monolayer assay. PRRS-virus-infected MA104 cell monolayers were prepared in 96-well microtitration plates[g] by replacing DMEM supplemented with 10% FBS, 10 mM HEPES, and the antibiotic-antimycotic mixture on confluent 1-day-old monolayers with the medium (50 µl/well) containing 10^2 $TCID_{50}$ of virus. After 1 hr of incubation at 37 C, 100 µl of the medium was added to individual wells. Microtitration plates containing both infected and uninfected (control) cell monolayers were maintained at 37 C for 2 days, fixed with cold acetone : methanol (70:30) for 10 min, and stored at -20 C until used. Serum samples were serially diluted 2-fold in 0.1 M Tris-HCl buffer (pH 7.6) beginning at 1:20. Immediately prior to use, fixed cell monolayers were treated with commercially supplied prediluted normal goat serum[f] for 30 min at ambient temperature to block nonspecific binding sites. Subsequently, duplicate 50-µl aliquots of each serum dilution were added to individual wells. The preparations were then incubated at 37 C for 30 min and washed 3 times with Tris-HCl buffer. Antigen-antibody reactions were visualized with a biotin-streptavidin horseradish peroxidase system[13] using the protocol provided by the manufacturer. Fifty microliters of biotinylated goat anti-swine IgG conjugate[f] were added to individual wells and permitted to react with potential antibody-antigen complexes for 30 min at 37 C in a humidified chamber. The plates were washed as described above, and excess buffer was removed. Fifty microliters of streptavidin conjugated to peroxidase[f] was then added to individual wells and incubated at ambient temperature for 30 min. The plates were washed as described above, and antigen-antibody reactions were visualized by adding 100 µl of DAB (diaminobenzidine tetrachloride) substrate[f] to individual wells and incubating for 5-10 min at ambient temperature. The color reaction was stopped by washing with distilled deionized water 3 times. Immunoperoxidase monolayer assay antibody titers were recorded as the reciprocal of the highest serum dilution in which a specific color reaction was observed.

Enzyme-linked immunosorbent assay. The ELISA was performed using a commercial kit (HerdChek®:PRRS)[a] as directed by the manufacturer. All reagents necessary for performing the assay were provided with the kit, and the assay was conducted at ambient temperature. Serum samples were diluted 1:40 in a sample diluent. One hundred microliters of each diluted sample was added to duplicate wells coated with proprietary PRRS viral antigen or normal cell antigen. Reference positive and negative pig sera, prediluted by the manufacturer, were also included in each plate. The plates containing reference and test sera were incubated for 30 min then washed 3-5 times with a phosphate-buffered wash solution containing Tween (300 µl/well). Excess wash solutions were removed, and 100 µl of anti-porcine IgG conjugated with horseradish peroxidase was added into each well. After a 30-min incubation, the conjugate was removed and the plates were rinsed as described above. Potential antigen-antibody reactions were visualized by adding 100 µl of TMB (3,3',5,5'-tetramethylbenzidine) substrate solution and incubating for 15 min. Color reactions were then stopped by adding 100 µl of a stop solution containing hydrofluoric acid into each well. Optical density (OD) of each well was measured at 630 nm wavelength using a computerized microplate reader. The presence or absence of antibody to PRRS was determined by calculating the sample to positive (S/P) ratio:

$$S/P = (\Delta OD \text{ of test serum between viral and control antigen}) \div (\Delta OD \text{ of positive reference between viral and control antigen}).$$

Samples were considered to be positive for PRRS virus antibody if the S/P ratio was greater than 0.4.

Serum virus neutralization test. The SVN test was performed in 96-well microtitration plates using MA104 cells. Serum samples were heat inactivated at 56 C for 40 min prior to performing the test and serially diluted 2-fold in DMEM supplemented with 10% FBS, 10 mM HEPES, and the antibiotic-antimycotic mixture. Each dilution of serum was mixed with an equal volume of PRRS virus containing 10^2 $TCID_{50}$/0.1 ml and incubated at 37 C for 60 min. Two hundred microliters of each mixture was added to a microtitration plate well containing 24-hr-old confluent MA 104 cell monolayers and incubated at 37 C. Monolayers were observed at the end of 5 days, and antibody titers were expressed as the reciprocal of the highest serum dilution in which no CPE was observed. Each sample was run in triplicate.

Preparation of PRRS virus antigens for the western immunoblot assay. Confluent MA104 cell monolayers were infected with PRRS virus at a concentration of 10^4 $TCID_{50}$/ 75-cm^2 flask and incubated for 3-4 days at 37 C. Virus-infected cells were harvested and pelleted by centrifugation at 1,000 x g for 10 min. The cell pellet was resuspended and disrupted in a lysis buffer (pH 8.0; 0.05 M Tris, 0.15 M NaCl, 0.002 M ethylenediaminetetraacetic acid [EDTA], 0.5% deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 1% NP-40, 0.1% sodium azide, 0.1% gelatin, 0.1% bovine serum albumin) at a rate of 1 ml buffer/0.1 ml cell pellet. The lysis buffer also contained protease inhibitors as described previously.[9] The suspension was stirred overnight at 4 C and then centrifuged at 600 x g for 10 min. The supematant was saved as crude viral antigen, aliquoted, and stored at -70 C. Control antigen was prepared in the same manner using uninfected MA 104 cells.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

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Table 1. Serum antibody response* of 8 pigs measured by the indirect fluorescent antibody (IFA) test, the immunoperoxidase monolayer assay (IPMA), the enzyme-linked immunosorbent assay (ELISA), and the serum virus neutralization (SVN) test after intranasal inoculation with PRRS virus isolate ISU-P.

		Days postinoculation										
Test	Titer	0	3	5	7	9	11	13	15	21	28–98†	105
IFA	≥20	0	0	0	0	4	8	8	8	8	8	6
IPMA	≥20	0	0	4	6	8	8	8	8	8	8	8
ELISA	≥0.4‡	0	0	0	0	3	7	8	8	8	8	8
SVN	≥2	0	0	0	0	1	3	3	4	6	8	8

* Number of pigs positive by each test.
† Identical responses from samples collected at 7-day intervals from 28 to 98 days PI.
‡ Presence or absence of specific antibodies was determined by calculating the sample-to-positive (S/P) ratio of optical densities at 1:40 dilution of samples.

(SDS-PAGE). A modified Laemmli procedure[7] was used to separate proteins on a discontinuous slab gel (70 x 80 x 0.75 mm) consisting of a 5% stacking gel and a 14% resolving gel, cross-linked with bis-acrylamide at a ratio of 30:0.8. Proteins were solubilized in sample buffer (pH 6.8) containing 0.0625 M Tris, 2% SDS, 5% 2-mercaptoethanol, 0.05% bromophenol blue, and 10% glycerol at 100 C for 5 min. Ten microliters of each denatured sample (20 μg proteins) and 5 μl of prestained SDS-PAGE molecular standards[h] were loaded in alternate lanes on the gel. The molecular standard contained 6 proteins with molecular masses of approximately 43, 29, 18, 14.3, 6.2, and 3 kD. Electrophoresis was carried out using a vertical minigel apparatus[i] as directed by the manufacturer. All gels were electrophoresed at 100 V (Model 1000/500 power supply)[i] until samples reached the stacking/separating gel interface. Sample separation was then completed by electrophoresis at a constant voltage of 200 V until the dye front reached 0.5 cm from the bottom. This migration limit was imposed on all separations in an attempt to standardize protein migration patterns.

Electrophoretic transfer of proteins. A minitransblot electrophoretic transfer cell[i] was used following the recommended procedure of the manufacturer. Viral and cellular proteins, along with the standard molecular markers separated in gels, were electrophoretically transferred to 0.45-μm nitrocellulose membranes[i] immediately following SDS-PAGE. Transfer was carried out at 4 C for 60 min at 250 mA in transfer buffer (pH 8.3) consisting of 25 mM Tris, 192 mM glycine, and 20% (v/v) methanol.

Western immunoblot assay. Nitrocellulose membranes containing viral and control cellular antigens were blocked overnight at 4 C with 1% gelatin dissolved in Tris-buffered saline (TBS, pH 7.5) containing 500 mM NaCl and 20 mM Tris. The membranes were then washed for 10-15 min in gently agitating TBS and cut into strips containing viral and cellular antigens and molecular standards. Pig serum samples and reference sera were diluted 1:10 and 1:50 in TBS containing 0.05% Tween 20 (TTBS) and 1% gelatin. The source of the positive reference serum was serum collected from 3-mo-old pig approximately 55 days after it had been experimentally infected with PRRS virus isolate ISU-P by nasal inoculation. Normal reference serum was collected from an age-matched pig free of PRRS virus infection. Each diluted serum sample was added to a single membrane strip and incubated for 2 hr at 37 C in a humidified chamber. Following incubation, the membranes were washed 3 times in gently agitating TTBS for 5 min. Antigen-antibody reactions were visualized with optimally diluted goat anti-swine IgG (H + L) labeled with horseradish peroxidase and TMB membrane peroxidase substrate.[f] The color reaction was stopped by 3 brief washes in deionized water. Appearance of virus-specific reactivity was assessed by comparing the antibody responses to viral and to cellular antigens. Approximate molecular masses antigens identified were determined by comparison with the protein standards using linear regression.

Results

All pigs inoculated with PRRS virus remained clinically normal over the course of the study. Viremia was first detected 3-5 days PI and persisted through days 9-15 PI. The antibody response over time, as indicated by the IFA test, IPMA, ELISA, and the SVN test, is summarized in Table 1 and Fig. 1.

PRRS virus specific antibodies were first detected by the IFA test on day 9 PI in 4 of 8 pigs. The remaining 4 pigs seroconverted by day 11. The IFA titers rose to ≥ 640 by 28 days PI in all pigs and then began to decline. Antibody titers in 2 of 8 pigs had fallen below detectable limits by day 105 PI and ranged from 40 to 160 in the remaining 6 pigs.

Specific antibodies were first detected by the IPMA on day 5 PI in 4 of 8 pigs. The remaining 4 pigs seroconverted by day 9 PI. The IPMA antibody titers ranged from 640 to ≥ 1,280 between 28 and 42 days PI. Thereafter, IPMA titers declined slowly, as compared with the IFA titers, ranging from 40 to ≥ 1,280 on day 105 PI.

Antibodies specific for PRRS virus were first detected by the ELISA on day 9 in 3 of 8 pigs. Four more pigs seroconverted on day 11 PI and the remaining pig seroconverted on day 13 PI. Based on the S/P ratio, ELISA antibody titers rose to maximum value (2.0-3.0 S/P ratio) by 28-42 days PI and then began to decline. All 8 pigs remained seropositive through day 105 PI, and the S/P ratios ranged between 0.8 and 1.4.

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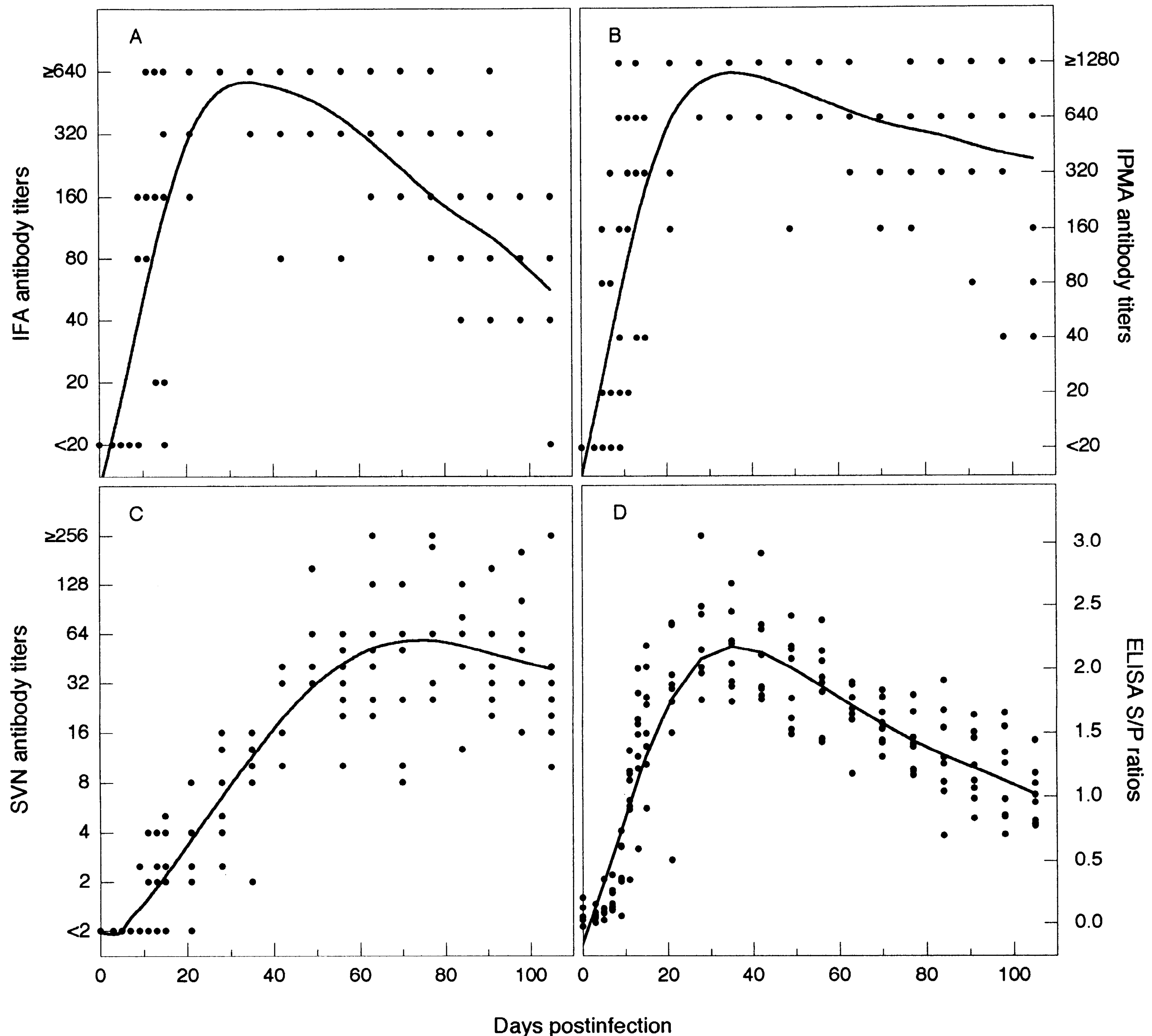

Figure 1. Antibody responses of 8 pigs to PRRS virus infection by the indirect fluorescent antibody test **(A),** the immunoperoxidase monolayer assay **(B),** the serum virus neutralization test **(C),** and the enzyme-linked immunosorbent assay (D). The line on each graph represents curve fitting, and dots show distribution of serologic responses.

The SVN antibodies were slow to appear relative to antibodies detected by the other three tests. Neutralizing antibodies were first detected in 1 pig on day 9, 2 pigs on day 11, and a fourth pig on day 15 PI. The remaining 4 pigs seroconverted by day 28 PI. Neutralizing antibody titers rose slowly for 63-77 days PI in 6 pigs and then began to decline. However, SVN antibodies in 2 of 8 pigs continued to rise through day 105 PI. Maximum SVN antibody titers ranged from 64 to ≥ 256 during the 105-day study period. On day 105 PI, neutralizing antibody titers ranged from 8 to ≥ 256. Assuming a constant rate of antibody decay from peak levels, ELISA, IFA, IPMA, and SVN antibody titers were estimated by regression analysis to drop below the detectable limits of the tests at approximately 137, 158, 324, and 356 days PI, respectively.

Western immunoblot analysis of early and late neutralizing antibody responders is summarized in Table 2 and Fig. 2. Four PRRS viral proteins with molecular masses of approximately 15, 19, 23, and 26 kD were consistently identified by western immunoblot (Fig. 2). Antibodies specific for the 15-kD protein were first detected in all 3 early responder pigs on day 5 PI. In contrast, antibodies to the 19-, 23-, and 26-kD proteins first appeared at 11-15, 15, and 9-11 days PI, respec-

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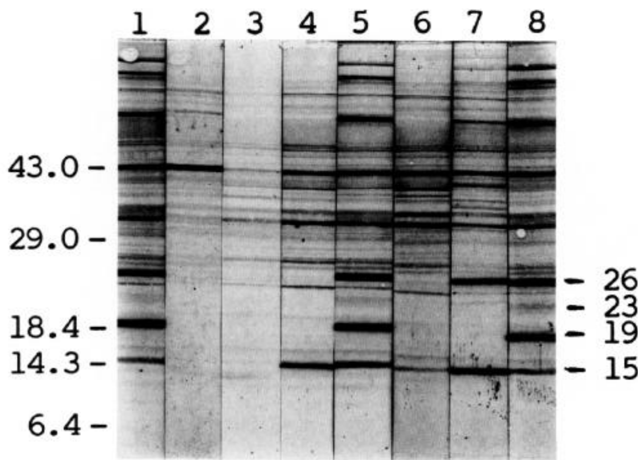

Figure 2. Representative western immunoblot analysis of the antibody response of pigs to PRRS virus infection using pooled sera from 3 early SVN responders, in which neutralizing antibodies were first detected between 9 and 11 days PI, and 4 late SVN responders, in which neutralizing antibodies were initially detected between 21 and 28 days PI. Lane 1: positive reference serum on viral antigen; lane 2: positive reference serum on control antigen; lane 3: early and late responders prior to infection; lane 4: early responders on day 7 PI; lane 5: early responders on day 15 PI; lane 6: late responders on day 7 PI; lane 7: late responders on day 15 PI; lane 8: late responders on day 28 PI.

tively. Following their initial detection, antibodies against specific viral proteins were present through the remainder of the study.

No response to viral protein was detected in the 4 late responders until day 7 PI. Antibody to the 15-kD protein was detected in all 4 pigs on day 7 PI. Antibody specific for the 26-kD protein was first detected at 15-28 days PI. Antibodies specific for the 19- and 23-kD proteins appeared at 21-28 and 28-42 days PI, respectively. As in the early responders, antibodies against specific viral proteins persisted until the end of the study following their initial detection.

Table 3. Chronology* of PRRS viremia, SVN activity, and antibodies specific for viral proteins in 7 pigs.

Pig no.	Detection of viremia: First day	Detection of viremia: Last day	Appearance of SVN activity	Appearance of antibody against viral proteins: 15 kD	19 kD	23 kD	26 kD
1	3	9	9	5	11	15	9
2	3	11	11	5	11	15	11
3	3	11	11	5	15	15	11
4	5	13	21	7	21	28	15
5	5	13	21	7	21	28	15
6	5	15	28	7	28	42	21
7	5	15	28	7	28	35	28

* Number of days after inoculation with PRRS virus.

Viremia, initial detection of neutralizing antibody, and first appearance of antibodies against each PRRS viral protein are given in Table 3 for individual pigs. Viremia was first detected in all early responders on day 3 PI and all late responders on day 5 PI. The mean duration of viremia in early responders was 10.3 days PI, in contrast to 14.0 days PI in late responders. The mean onset of neutralizing antibody in early responders was 10.3 days PI versus 24.5 days PI in late responders. The mean onset of antibodies to the 15-, 19-, 23-, and 26-kD viral proteins as determined by western immunoblot was 5.0, 12.3, 15.0, and 10.3 days PI, respectively, in early responders and 7.0, 24.5, 33.3, and 19.8 days PI, respectively, in the late responders.

Discussion

The objective of this study was to characterize the ontogeny of the humoral immune response in pigs to PRRS virus infection. The antibody response was monitored using 4 serological tests routinely used to detect PRRS-virus-specific antibodies (IFA test, IPMA, ELISA, SVN test). The viral antigen specificity of the

Table 2. Appearance of PRRS virus protein-specific antibodies* in early SVN responders versus late SVN responders as determined by western immunoblot analysis.

SVN response	Viral protein (kD)	Days postinoculation: 0	3	5	7	9	11	15	21	28	35	42–105§
Early responders‡	15	0	0	3	3	3	3	3	3	3	3	3
	19	0	0	0	0	0	2	3	3	3	3	3
	23	0	0	0	0	0	0	3	3	3	3	3
	26	0	0	0	0	1	3	3	3	3	3	3
Late responders§	15	0	0	0	4	4	4	4	4	4	4	4
	19	0	0	0	0	0	0	0	2	4	4	4
	23	0	0	0	0	0	0	0	0	2	3	4
	26	0	0	0	0	0	0	2	3	4	4	4

* Number of pigs with detectable antibody against each viral protein.
† Identical responses from samples collected at 7-day intervals from 42 to 98 days PI.
‡ SVN response first detected on day 9 or 11 postinoculation ($n = 3$).
§ SVN response first detected on day 21 or 28 postinoculation ($n = 4$).

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antibody response was further characterized by western immunoblot analysis.

PRRS virus specific antibodies were first detected 5-9 (IPMA), 9-11 (IFA), 9-13 (ELISA), and 9-28 (SVN) days PI and, assuming a constant antibody decay rate, were predicted to decline to the lower limits of detection by approximately 137 (ELISA), 158 (IFA), 324 (IPMA), and 356 (SVN) days PI. The initial antibody responses of pigs to PRRS virus infection as revealed by the 4 tests (IFA, IPMA, ELISA, SVN) evaluated in this study are in general agreement with the findings of other investigators, who reported that PRRS virus specific antibodies were initially detected by the IFA test, IPMA, and ELISA 7-10 days PI in contrast to detection by the SVN test at days 28-35 PI (Frey M, Eernisse K, Landgraf J, et al.: 1992, Am Assoc Swine Pract Newsl 4[4]:31; Ohlinger V, Haas B, Sallmüller A, et al.: 1992, Am Assoc Swine Pract Newsl 4[4]: 24).[2,11,15,17,18] The results suggest marked differences in the kinetics involved in the 4 tests. The IPMA appeared to provide the best overall performance based on its ability to detect antibodies in both early and late infections and the relatively short turn-around time of the test. However, sensitivity and specificity comparisons must be carried out before concluding which is actually the best test. The antibody response of the experimentally infected pigs as detected by the 4 serological tests may have been different if the pigs were infected at an earlier or later age, were of a different genetic background, or were infected with a different PRRS virus isolate.

PRRS virus consists of at least 3 structural proteins with molecular masses of approximately 15, 19, and 26 kD (Benfield D, Harris L, Nelson E, et al.: 1992, Am Assoc Swine Pract Newsl 4[4]: 19-21).[12] In our study, we consistently detected 15-, 19-, 23-, and 26-kD viral proteins by western immunoblot (Fig. 2). Investigators recently described the detection of 15-, 16-, 19-, 22-, and 26-kD proteins by radioimmunoprecipitation.[3] The detection of an additional protein (16 kD) is consistent with genomic studies of the PRRS virus, indicating that as many as 6 structural proteins may be encoded by the viral genome.[6,10] The failure in our study to detect the 16-kD protein may have been due to conformational changes of this protein brought about by the reducing conditions that the viral preparation was subjected to during processing for western immunoblotting.

In the current study, antibodies specific for the 15-kD protein were detected in all pigs by day 7 PI and persisted through day 105 PI (Table 2). Antibodies to the remaining 3 proteins (19, 23, 26 kD) were not detected until 15 days PI in any early SVN-antibody-producing pigs. Antibodies to these 3 proteins were not detected in any late SVN-antibody-producing pigs until day 42 PI. These results clearly indicate that the 15-kD protein is the most immunogenic of the PRRS virus structural proteins and may provide the antigenic basis for the development of improved diagnostic tests for the detection of PRRS virus specific antibodies.

The data summarized in Tables 2 and 3 also indicate that the 26-kD protein is associated with the induction of neutralizing activity. This conclusion is based on the fact that no SVN activity was detected in any of the 4 late responding pigs until after antibodies to the 26-kD protein were present. The data also indicate that the 15-kD protein is not associated with neutralizing activity; antibody to this protein was present in 4 of 8 pigs for 14-21 days prior to the initial detection of neutralizing antibody. The potential role that antibodies specific for the 19- and 23-kD proteins may play in viral neutralization requires further study.

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Sources and manufacturers

a. IDEXX Laboratories, Westbrook, ME.
b. Sigma Chemical Co., St. Louis, MO.
c. Costar Corp., Cambridge, MA.
d. Nunc, Naperville, IL.
e. Dr. David Benfield, South Dakota State University, Brookings, SD.
f. Kirkegaard and Perry Laboratories, Gaithersburg, MD.
g. Coming, Coming, NY.
h. GIBCO BRL Life Technologies, Gaithersburg, MD.
i. Bio-Rad Laboratories, Richmond, CA.

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