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PRRS virus serum neutralizing antibody as a measure of protection

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Introduction

Extensive research into the etiology, pathogenesis, epidemiology, and control of PRRS virus infection in swine has been carried out during the last 10 years, resulting in a greater understanding of this disease. Despite the significant gains in understanding the disease, there is still a lack of essential information concerning protective immunity. Currently, PRRS vaccines are widely used on farms without much understanding of the mechanism that leads to protection in vaccinated animals.

Pigs develop both humoral and cell-mediated immunity after PRRS virus infection, but the relative importance of each one in protection is not well defined. Concurrent with the viremic period, which lasts for 2–4 weeks, non-neutralizing antibodies develop 7–14 days following infection. Initially, these antibodies are high and last for a relatively short period. Neutralizing antibodies develop 1–2 months following exposure and may last for an extended period. Once pigs develop these neutralizing antibodies, viremia is usually absent.

Even though there appears to be a relationship between the presence of antibodies and the absence of viremia, there have been reports that suggest otherwise due to the unusual persistence of PRRS. In one paper, piglets that were born with the virus, transmitted the virus to commingled sentinels for up to 112 days after birth, and viral RNA was detected for up to 210 days after birth.[1] In another study, PRRS virus could also be isolated from persistently infected pigs with high levels of neutralizing antibodies.[2]

PRRS virus strain variation has been demonstrated under field conditions. In addition, differences in pathogenicity among different PRRS virus strains have been demonstrated under experimental conditions. An important, yet currently unanswered, question is whether cross-protective immunity between virus strains exists on the farm level; relationships between field virus isolates and vaccine virus strains are also important.

In this paper, results of four studies on serum neutralizing (SN) antibody responses in pigs are reported. The goal of the study is to assess whether there is a relationship between protection and the presence of SN antibodies.

Studies

Study 1. Development of SN antibody and viremia

In order to demonstrate the relationship between viremia and SN antibody, three-week-old pigs were inoculated with a ResPRRS virus at day 0, with an experimental killed PRRS virus vaccine at day 35, and with another ResPRRS virus at day 57. Results of viremia and SN antibody response are shown in **Table 1**. Following the first inoculation with ResPRRS vaccine, viremia was evident for up to 24 days post-inoculation (PI). The SN antibody[3] was first detected at 28 days PI and increased to 1:64 follow-

Table 1. Viremia and SN antibody response following inoculation with a PRRS virus vaccine (ResPRRS), an experimental killed vaccine and ResPRRS virus in three-week-old pigs

Pig	Day post inoculation																
No.	0*	3	7	10	14	17	21	24	28	35*	45	53	57*	59	61	63	70
75	NT/-*	+/NT	+/NT	-/NT	+/-	+/NT	+/NT	+/NT	NT/-	-/-	NT/16	NT/16	NT/NT	-/128	-/NT	-/128	NT/64
76	NT/-	+/NT	+/NT	-/NT	+/-	+/NT	-/NT	-/NT	NT/-	-/2	NT/64	NT/64	NT/NT	-/128	-/NT	-/128	NT/128
77	NT/-	+/NT	+/NT	-/NT	+/-	-/NT	-/NT	-/NT	NT/-	-/-	NT/-	NT/2	NT/NT	-/2	-/NT	-/2	NT/-
78	NT/-	+/NT	+/NT	+/NT	+/-	-/NT	-/NT	+/NT	NT/-	-/-	NT/16	NT/32	NT/NT	-/64	-/NT	-/128	NT/32
79	NT/-	+/NT	+/NT	+/NT	+/-	+/NT	+/NT	-/NT	NT/4	-/-	NT/4	NT/8	NT/NT	-/8	-/NT	-/8	NT/8
80	NT/-	+/NT	+/NT	+/NT	-/-	+/NT	+/NT	+/NT	NT/4	-/4	NT/2	NT/8	NT/NT	-/32	-/NT	-/64	NT/32

* Day 0 - ResPRRS was inoculated intramuscularly; Day 35 - A killed PRRS virus vaccine was inoculated intramuscularly; Day 57 - ResPRRS was inoculated intramuscularly
a. Viremia detection/SN antibody titer to MN-1b virus; NT = not tested

ing inoculation with a killed vaccine. After the second inoculation with ResPRRS, SN titers continued to increase, while viremia was absent in all of the pigs. One of the 6 pigs developed a low level of SN titer (1:2). These results indicate that repeated vaccination may cause a continuous increase in SN antibody, with viremia potentially being prevented in pigs with detectable SN antibody.

Study 2. Comparison of SN titers using 3 different PRRS virus strains

Six sera each from 146 farms in the midwestern states were selected randomly from a serum bank. Sera from 27 of 146 farms had been collected prior to June 1994 at which time ResPRRS was not available. The sera were tested for SN antibody titers to a wild type PRRS virus (MN-1b) and two commercial PRRS vaccine viruses. Percents of SN negative samples were fewer with sera collected after June 1994. There was no major difference in SN titers when MN-1b and the two vaccine viruses were used in the SN test. The SN titers of 1:4 or less to MN-1b, vaccine A, and vaccine B viruses were 71.8%, 76.6% and 78.9%, respectively (**Table 2**). These results indicate that PRRS virus strains prevalent in midwestern farms are serologically related to the two vaccine strains.

In another study, a total of 215 adult swine sera from a farm were tested for SN antibody to a farm-specific PRRS virus and two vaccine virus strains. Vaccine A was routinely used on the farm. A wild type PRRS virus was isolated, and RFLP analysis resulted in a 1–4–2 cut pattern. The numbers of sera with the same or different SN titers to each virus are shown in **Table 3**. Differences in SN titer to farm virus vs vaccine A, farm virus vs vaccine B, and vaccine A vs vaccine B were minimal.

Study 3. SN antibody titer changes of sows in an endemically infected farm

Twenty sows on a farm with endemic PRRS virus infection were tested monthly for rises and falls of SN antibody levels during a six-month period, and the results are shown in **Table 4**. No major differences were found in SN titers tested against the farm-specific isolate and the vaccine virus. The 20 sows could be classified into three groups by antibody patterns; 12 sows maintaining low

Table 2. Prevalence of SN antibody titers to PRRS virus MN-1b, vaccine virus A and B in sera of breeding pigs of 146 farms collected before (group 1) and after (group 2) June 1, 1994

PRRS virus	SN titer range	Group 1 sera (27 farms)	Group 2 sera (119 farms)	Total (%)
MN-1b	<2	82 (50.6)[a]	209 (29.6)	291 (33.5)
	1:2-4	57 (35.2)	276 (39.0)	333 (38.3)
	1:8-16	14 (8.6)	137 (19.4)	151 (17.4)
	≥1:32	9 (5.6)	85 (12.0)	94 (10.8)
MLV-A	<2	96 (59.3)	260 (36.8)	356 (41.0)
	1:2-4	46 (28.4)	263 (37.2)	309 (35.6)
	1:8-16	11 (6.8)	133 (18.8)	144 (16.6)
	≥1:32	9 (5.6)	51 (7.2)	60 (6.9)
MLV-B	<2	111 (68.5)	362 (53.8)	473 (56.6)
	1:2-4	24 (14.8)	162 (24.1)	186 (22.3)
	1:8-16	18 (11.1)	89 (13.2)	107 (12.8)
	≥1:32	9 (5.6)	60 (8.9)	69 (8.3)

[a] No. of sera (percents)

Table 3. Differences in SN titers in breeding pigs on a farm between a farm-specific PRRS virus and two vaccine viruses

SN titer difference	Farm virus vs vaccine A	Farm virus vs vaccine B	Vaccine A vs vaccine B
Same titer	53[a]	51	62
2-fold	72	90	79
4-fold	50	53	58
8-fold	28	14	18
16-fold	11	3	6
≥32-fold	1	4	3

[a] No. of sera

Table 4. Changes of SN titers of 20 sows under farm conditions during a six-month period

Sow No.	SN titers to a farm isolate 1217M						SN titers to ResPRRS virus					
	1	2	3	4	5	6	1	2	3	4	5	6
7709	2	2	—	16	4	4	8	—	2	8	8	4
7213	2	NT	2	2	NT	—	8	NT	—	—	NT	—
6403	4	4	—	8	4	16	2	2	4	8	4	8
6561	4	4	2	2	4	—	—	—	2	2	4	16
8378	4	4	—	4	NT	8	4	2	—	4	NT	4
4185	4	—	—	2	4	2	2	—	—	8	8	2
9373	4	4	—	4	4	4	2	2	—	>64	>64	32
9137	4	4	8	4	8	16	4	—	4	4	4	4
9679	4	4	2	2	4	2	16	2	2	2	2	—
9226	8	16	—	8	2	2	4	2	—	4	—	4
7331	8	2	2	2	4	2	2	4	—	—	16	4
8994	32	4	—	—	8	2	16	16	—	—	4	8
9333	8	8	2	16	8	64	8	2	2	4	16	32
5592	8	8	4	4	8	8	8	4	8	8	8	16
5060	32	8	4	4	4	16	16	8	16	32	16	16
8079	32	8	8	8	64	8	2	—	2	4	16	4
9272	>64	32	32	32	32	32	16	16	8	32	32	32
7371	—	2	—	2	4	>64	—	2	—	—	>64	32
7180	8	8	—	64	>64	64	8	2	—	8	16	8
8484	16	—	—	8	>64	32	16	—	—	4	>64	32

Table 5. Viremia and antibody response in cull sows following a challenge with PRRSV

Sow No.	Day 0	Day 13	Day 0	Day 2	Day 4	Day 6	Day 8	Day 11	Day 13
892	-/-[a]	-/-	–	+	–	+/+[b]	+	–	–
801	-/-	-/-	–	–	+	+/-	+	–	–
7016	2/-	2/-	–	–	–	-/-	–	–	–
5445	4/-	2/-	–	–	–	-/-	–	–	–
8641	4/-	16/-	–	–	–	-/-	–	–	–
6508	8/-	32/-	–	–	–	-/-	–	–	–
1688	8/-	32/-	–	–	–	-/-	–	–	–
1349	8/-	32/-	–	–	–	-/-	–	–	–
7849	16/-	32/-	–	–	–	-/-	–	–	–
1095	16/-	4/-	–	–	–	-/-	–	–	–
161	16/-	64/-	–	–	–	-/-	–	–	–
3008	16/-	4/-	–	–	–	-/-	–	–	–
6059	32/-	8/-	–	–	–	-/-	–	–	–
2193	64/-	32/-	–	–	–	-/-	–	–	–

a. Antibody titers by SN/IFA assay
b. PRRS virus detection by isolation/PCR method
Challenge virus was a pool of three wild type isolates from the farm.
SN titers were tested using the pool challenge virus.

antibody levels (SN titers negative to 1:8), five sows maintaining high antibody levels (SN titers 1:8–1:64), and three sows changing from negative or low to high antibody levels. Seroconversion appears to have occurred in at least three of the 20 sows during the six-month period, thus indicating that active infection was being maintained in part of the population.

Study 4. Viremia in sows with different SN antibody levels following a challenge with PRRS virus

Twenty culled sows with various levels of SN antibody (1:2–64) were obtained from a farm with endemic PRRS virus infection. PRRS vaccination (ResPRRS/Repro) was routinely carried out in the farm. Two additional sows were purchased from a PRRS-free farm. All sows were housed in isolation rooms and inoculated intranasally with a pool of three farm-specific PRRS virus isolates. Blood samples were collected from each sow every other day for two weeks, with the resulting samples being examined for viremia and serology. As shown in **Table 5**, PRRS virus was recovered from two SN antibody negative sows between two and eight days post-challenge. However, virus was not recovered from 20 SN antibody positive sows up to 13 days post-challenge. Sera from the 20 antibody-positive sows at day six post-challenge were further tested by PCR, and the results were all negative. These results indicate that sows with detectable SN antibody levels will be protected from viremia.

Discussion

Following an infection in pigs with PRRS virus, it is believed that SN antibody is detectable only after cessation of viremia, and it appears that viremia will not be present following a challenge to pigs with detectable SN titers. Therefore, SN antibody could be used as an indicator for protection, and a standardized SN method could be used to measure the protection of a herd. This would enable producers to determine the probability of a clinical outbreak of PRRS based on the SN antibody profile of the breeding herd.

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