ORIGINAL RESEARCH

Detecting subpopulations after PRRS virus infection in large breeding herds using multiple serologic tests

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Summary

Objective: *To assess the presence of breeding herd subpopulations after infection with porcine reproductive and respiratory syndrome virus (PRRSV).*

Design and procedure: *Ten swine herds were selected based on the inability to control postweaning PRRS problems using nursery depopulation or piglet vaccination. No vaccines had been used in breeding animals. The existence of subpopulations at a point in time and over time was assessed. Cross-sectional serologic profiles of randomly selected sows were collected and samples were analyzed for IgM and IgG indirect-fluorescent antibodies (IFA), and serum neutralization (SN) antibodies at a point in time. Based on the results, sows were classified into one of two subpopulations: 'not infected' (negative on all three tests) or 'acutely infected' (IgM or IgG positive at ≥1:64 and SN negative). The ability of subpopulations to persist over time was also assessed. In conjunction with the previously described subpopulations, another subpopulation, 'antibody decay,' was assessed. This classification was based on evidence of a previous infection, with subsequent antibody decay over time. Two herds from the original 10 were selected and specific sows were serially sampled three times over a 6-month period.*

Results: *Sows were found to be 'not infected' in eight herds (mean = 26%, range = 0%–52%) and 'acutely infected' in five herds (mean = 14%, range = 0%–36%). Further, all three subpopulations persisted over time and sows changed subpopulations, depending on whether viral infection took place.*

Implications: *Breeding herd subpopulations may exist and may be a factor in maintaining persistent viral transmission in chronically infected farms.*

Keywords: *swine, porcine reproductive and respiratory syndrome virus (PRRSV), antibodies, breeding herd*

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Since its inception, nursery depopulation (ND) as a potential method for controlling postweaning porcine reproductive and respiratory syndrome (PRRS) has met with both success and failure.[1] While the majority of the farms that have used ND reported improvements in nursery performance, in certain cases postweaning PRRS problems have persisted due to the inability to control transmission of virus in the breeding herd.[2] While the use of commercially available PRRS vaccines has frequently improved weaned piglet performance, in some cases problems have persisted. Therefore, it appears that preventing the transmission of PRRS virus among adult swine is important to the success of both ND and vaccination regimes, and it becomes critical to determine the factors that predispose viral shedding within the breeding herd.

It has been reported that in a population of sows, up to 15% may remain seronegative to PRRSV 2–3 months after a natural herd infection.[3] Therefore, "subpopulations" of non-infected, potentially naive animals may exist within infected populations. Persistently infected sows have been reported, and virus has been isolated from tonsillar tissue for up to 157 days postinfection (PI).[4] If such animals initiate shedding, naive animals may become infected, resulting in irregular periods of viral circulation and clinical PRRS. If sows become exposed in late gestation, transplacental infection may take place, and piglets may be infected during parturition or during lactation. Subsequent weaning of infected piglets leads to recurrent infection of nursery populations, resulting in repetitive outbreaks of postweaning PRRS. The purpose of this paper is to evaluate whether subpopulations exist, and to assess the potential for viral transmission to occur among subpopulations using existing diagnostic tests.

According to current literature, there are three serologic tests available for detecting antibodies to PRRSV. The indirect fluorescent antibody (IFA) test has been the primary serologic test in the United States.[5] This assay measures IgG and detects the formation of an antibody within 7–10 days PI. Antibodies persist for up to 3–4 months. The serum neutralization (SN) test detects antibodies within 9–28 days PI and these antibodies have been reported to exist for up to 341 days.[6,7] Recently, an IgM IFA test has been described to detect acute PRRSV infection.[8] IgM IFA titers can be detected within 5 days PI; however, the duration of IgM antibody is very short (21–28 days). It is particularly interesting that there is a high percentage (81%) of isolation of PRRSV from IgM-positive samples.[8]

Based on the use of these three tests, we theorized that it may be possible to detect whether different levels of infection to PRRSV exist at a

single point in time, as well as to determine whether subpopulations persist over time, within an infected herd. We attempted to classify sampled animals into one of three subpopulations based on the type of antibodies detected (Table 1). Animals found to be negative on all three tests were classified as 'not infected.' Animals were classified as 'acutely infected' if SN titers were negative but IgM or IgG IFA titers were present at ≥1:64. Animals were placed in the 'antibody decay' category if we observed a reduction in IFA or SN titers over time.

Materials and methods

Experimental design

Part 1: Assessment of subpopulations at a point in time

Ten herds were selected for part 1 of the study. All herds were located in West Central Minnesota or in eastern Kansas. All had similar breeding herd inventories (>1000 sows) and used one-site production. Facilities consisted of total confinement buildings, and sows were individually housed in stalls. All herds had experienced postweaning PRRS problems for up to 1–7 years at the time the study began. Clinically, piglets experienced an increased level of respiratory disease at 1–4 weeks of age. Ocular and nasal discharge, coughing, sneezing, and anorexia were present, as well as an increased incidence of *Streptococcus suis* meningitis and colibacillosis. All herds had failed to successfully control these problems using both nursery depopulation and piglet vaccination. No vaccination of breeding animals had taken place prior to the start of the study.

A cross-sectional serologic profile was selected from randomly selected gestating sows for antibody analysis. At least 18 sows were sampled per farm and samples were collected from all areas of the gestation facility. This sample size was selected to provide an estimate of the prevalence of sows that were negative on all three tests with ±20% accuracy at 95% confidence where the expected true prevalence of seronegative sows was 20%.

Blood samples were collected via jugular venipuncture using an 18-gauge 3.8-cm needle and 12.0-mL syringe. Approximately 8.0 mL of blood was collected. Serum was separated via centrifugation at 2500 rpm for 15 minutes, frozen at –20°C and delivered to the University of Minnesota swine virology laboratory. Samples were analyzed for IgM, IgG, and SN antibodies to PRRSV as previously described.[5,8,9] All testing involved consistent laboratory personnel and repeated testing of randomly selected sera was conducted to assess the accuracy of the results.

Table 1

Classification of sampled animals

Category	IgM	IgG	SN
Not infected	negative	negative	negative
Acutely infected	≥64	≥64	negative
Antibody decay	decay	decay	decay

Part 2: Assessment of subpopulations over time

The potential of subpopulations to exist over a period of time in an infected farm was assessed. It was hypothesized that some animals may remain 'not infected' to PRRSV while others may become 'acutely infected' or exhibit 'antibody decay' over time. In order to test this theory, we decided to serially test selected animals over a 6-month period. Two herds from the previous group of 10 were selected, based on the owners' willingness to participate in further testing. Herd 1 had been infected since 1988, while Herd 2 was originally infected in 1994. A minimum of 15 randomly selected sows were sampled three times with a 3-month interval between tests. Samples were analyzed as previously described. Based on an expected prevalence of 20% 'not infected' animals within the population, this sample size allowed us to be 95% confident with a ±20% accuracy of detecting change in the percent of seronegative animals over time.

Results

Part 1: Assessment of subpopulations at a point in time

A total of 258 breeding females were tested. Subpopulations of 'not infected' sows were detected in eight herds (Table 2). The mean percentage of individual samples with a 'not infected' classification was 26% (65 of 258) with a range of 0%–52%. Subpopulations of 'acutely infected' sows were detected in five herds. The mean percentage of samples with this response profile was 14% (35 of 258) with a range of 0%–36%.

Part 2: Assessment of subpopulations over time

Based on the results from the two herds that underwent repeated sampling (Tables 3 and 4), it appeared that one or more of the following

Table 2

Assessment of subpopulations at a point in time

Farm #	Sows tested	Detected: Not infected[1]	Detected: Acutely infected[2]
1	30	7	4
2	25	13	9
3	20	2	5
4	22	0	0
5	45	15	16
6	40	9	1
7	20	8	0
8	18	7	0
9	20	0	0
10	18	4	0
Total	**258**	**65**	**35**
	Mean Percentage:	26%	14%
	Range:	0–52%	0–36%

1 Not Infected = negative on all 3 tests

2 Acutely Infected = Animals demonstrating one of two patterns: [IgM (≥1:64), IgG (–), SN (–)] or [IgM (–), IgG (≥1:64), SN(–)]

may have taken place:

- animals may exist within an infected population for a period at least 6 months and remain serologically negative according to the tests used in this study;
- 'not infected' animals may become 'acutely infected;' and/or
- previously infected animals may exhibit 'antibody decay' over time.

Discussion

The data from these studies appear to suggest that after natural infection with PRRSV, different serologic profiles may exist. It also appears that persistent viral transmission may take place within a population.

Table 3

Assessment of subpopulations over time—serologic test results for farm 1

Sow #	Dec. 1994 IgM	IgG	SN	Mar. 1995 IgM	IgG	SN	Jul. 1995 IgM	IgG	SN	Category
1	–	–	–	–	–	–	–	–	–	1: Not infected
2	–	–	–	–	–	–	–	–	–	
3	–	–	–	–	–	–	–	–	–	
4	–	–	–	–	256	2	–	16	2	2: Acutely infected
5	–	–	–	–	256	16	–	–	–	
6	–	–	–	64	–	–		NT		
7	–	–	–	64	–	–	–	–	16	
8	–	–	32	–	–	4	–	–	–	3: Antibody decay
9	–	256	16	–	64	2		NT		
10	–	64	8	–	–	4	–	–	–	
11	–	–	16	–	–	–		NT		
12	–	1024	16	–	–	–		NT		
13	–	1024	–	–	–	–		NT		
14	–	256	–	–	16	–	–	–	–	
15	–	–	8	–	–	–	–	–	–	

NT = Not tested (culled)

Table 4

Assessment of subpopulations over time—serologic test results for farm 2

Sow #	Jan. 1995 IgM	IgG	SN	Apr. 1995 IgM	IgG	SN	Jul. 1995 IgM	IgG	SN	Category
1	–	–	–	–	–	–	–	–	–	1: Not infected
2	–	–	–	–	–	–	–	–	–	
3	–	–	–	–	–	–	–	–	–	
4	–	–	–	–	–	–	–	–	–	
5	–	–	–	–	–	–	64	–	–	2: Acutely infected
6	–	–	–	64	–	–	–	–	–	
7	–	–	–	–	–	–	64	–	–	
8	–	–	–	–	64	2	–	16	16	
9	–	16	8	–	–	2	–	–	–	3: Antibody decay
10	–	–	4	–	–	–	–	–	–	
11	–	16	256	–	–	8	–	–	4	
12	–	16	4	–	–	–	–	–	–	
13	–	–	128	–	–	8	–	–	2	
14	–	64	32	–	64	4	–	–	2	
15	–	64	256	–	–	32	–	–	4	
16	–	64	4	–	–	2	–	–	–	

Since control of viral transmission in the breeding herd is critical for the success of ND, these findings may potentially explain why certain strategies targeted to control postweaning PRRS do not always succeed.

While the serologic results appear to indicate that subpopulations do exist and that viral transmission over time does take place within the breeding herds sampled, a few considerations need to be mentioned before any conclusions can be drawn:

- The source of virus may not have been previously infected sows or boars. All herds had experienced chronic PRRS problems in the nursery and active infection was taking place in this phase. Therefore, fomites, employee traffic, and/or an undetermined vector may have been repeatedly introducing virus into the breeding herd.
- The IgM conjugate used in this study may have low specificity. If the use of this product did not result in the specific detection of IgM antibody to PRRSV, we may be incorrect in classifying pigs as 'acutely infected.'
- Pigs classified as 'not infected' may have either been pigs that were actually previously infected but failed to mount an antibody response, or pigs for whom sufficient time had elapsed to allow antibodies to decay to undetectable levels.

While these concerns are indeed valid, the literature appears to support our data.[1,2,9] Several papers have described the serologic profile we observed, which is characteristic for endemically infected herds.[1,2] This profile consists of a low prevalence (<10%) of serologically positive breeding animals and a high prevalence (>50%) of seropositive nursery piglets. If virus was transmitted between these populations on a regular basis, it is questionable whether this pattern would develop so consistently. Secondly, the IgM IFA test used in this study has been previously reported to be highly specific, and all procedures and materials used to analyze IgM antibodies in the present study used the same protocol described in that earlier paper.[9] Finally, it is indeed possible that some of the samples classified as 'not infected' may have been previously exposed but antibodies were not detectable. This study relied strictly on tests that measured the humoral response. Until tests that are capable of measuring the cell-mediated immune response become available, this question may be difficult to answer.

While certain sows in the 'antibody decay' subpopulation did demonstrate a reduction in titer level over time, no relationships can be drawn between their existing immune status and the concurrent decline of antibody. However, the simultaneous presence of all three subpopulations appears to indicate that the spread of virus within chronically infected herds can be limited. Therefore, while PRRSV may be highly infectious, it may not be very contagious. The role of population size or facility design is not known; however, the fact that breeding herd inventories were large and stall housing was employed may have minimized sow interaction and reduced the spread of virus within the population.

If the presence of subpopulations proves to be a significant factor in the control of endemic postweaning PRRS, it becomes critical to develop measures to consistently expose all animals to virus. While previous reports have documented the ability to control viral shedding in

the breeding herd by managing the gilt pool, this strategy only appears to be effective in herds with breeding herd inventories of 500 or fewer sows.[10] Attempts to use this strategy in large herds (>1000 sows) has resulted in acute reproductive outbreaks of PRRS 5–6 months after the ND protocol was completed.[2,11,12] The severity of the clinical signs seen during these episodes appeared to indicate the presence of naive sows within the breeding population.

One potential solution to this problem is a controlled infection of the breeding herd. By purposefully exposing all members of a population to the agent in question, it may be possible to eliminate subpopulations and produce consistent herd immunity. While this practice has been described for the control of transmissible gastroenteritis virus, no such protocol has been reported for PRRS.[13] Although there is no commercially available PRRSV vaccine approved for use in adult swine at this time, the ability to provide consistent exposure to virus via vaccination may enhance future PRRS control programs. This work is currently in progress.[14]

In conclusion, if our interpretation of the serologic data is correct, then breeding herd subpopulations may exist and may be a factor for maintaining viral transmission within problem herds. It is unfortunate that only two of the 10 study herds could be followed over time. This was due to the cost of three serologic assays per animal tested, the inconvenience to the owner and veterinary practitioner, or a farm-specific decision to initiate breeding herd vaccination. Despite this small number of herds, it appears that in this particular case, subpopulations appear to exist in chronically infected herds at a point in time, as well as over time. Whether this exists elsewhere needs to be assessed on an individual farm basis. Finally, this study should be re-evaluated after diagnostic tests that do not rely strictly on detecting a humoral response after PRRSV infection are developed.

Implications

- Subpopulations may be an important factor in enhancing PRRS viral transmission within infected breeding herds.
- The inability to control breeding herd subpopulations may reduce the chances of successfully controlling postweaning PRRS

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