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Cross-sectional and longitudinal studies of PCV2 infection in 4 boar studs

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Introduction

Boar studs have a uniquely influential role in health management in the swine industry. Because individual boar studs typically provide regular shipments of semen to many sow farms, the potential for rapid and widespread dissemination of semen borne pathogens is daunting. In recent years, the threat of dissemination of PRRS from boar studs in the USA has prompted considerable investment in biosecurity at boar studs, and research into optimization of monitoring methods for early detection of PRRS virus in boar stud populations.[1-3]

Clinical syndromes associated with porcine circovirus type 2 (PCV2), most notably Postweaning Multisystemic Wasting Syndrome (PMWS), are the most significant swine health issue to emerge since PRRS in 1987. PMWS was first reported in 1996 in Canada and has since caused major epidemics in many countries.[4,5] The occurrence of this global epidemic is yet to be adequately explained, particularly as the necessary infectious agent (PCV2) is ubiquitous but the disease occurs in only a minority of PCV2 infected pigs.[6] Although the infectivity of PCV2 virus in semen, and natural transmission via semen, remains to be proven, PCV2 nucleic acids have been demonstrated in boar semen in both field and experimental studies.[7-10] A study of 98 year-old boars from 49 farms in Korea found 20% of semen samples were PCR positive. In contrast, a much lower prevalence (3.3%) of PCV2 DNA was found in 903 semen samples from 43 boars at a single stud in Canada, and detection in boars was sporadic.[10] Higher prevalence of positive semen samples was seen in younger boars and the data indicated that both boar age and breed may influence the prevalence of PCV2 in semen.[10]

Currently, the population dynamics of PCV2 infections in swine populations are poorly documented. Without knowledge of the diversity of PCV2 viruses that circulate within swine populations, rational approaches to monitoring and control are not possible. We know PCV2 is shed in semen, but do not understand the dynamics of PCV2 transmission, shedding and immunity as boars are introduced into operating boar studs. The purpose of this study was to obtain detailed information about the epidemiology of PCV2 in four different boar studs with multiple genetic sources. Both cross-sectional and longitudinal studies were performed in all four studs to describe both the prevalence detection of PCV2 nucleic acids in serum and semen, and to analyze the genetic variability of the virus in each population.

Materials and methods

Overview of study herds

The boar studs sampled housed populations of 100 to 400 boars. The number of source farms for the individual studs ranged from two to seven. Each sow farm flow for each source farm was considered a specific source, even if the genetics across sow farms were similar. Genetic makeup of the boars was also recorded as either 'purebred' boars (identified by breed) or 'composite' boars (crossbreeds identified by their predominant breed).

Cross-sectional study

On four different dates within a 5 week period (November to December 2006), serum, blood swab, and semen samples were collected from 60 boars on each of the four boar studs. All boars had entered the main working areas of the studs (i.e. were not in isolation barns). For logistic reasons, convenience sampling was employed rather than formal random sampling, and the first 60 boars brought for collection were sampled. Samples were submitted to the Veterinary Diagnostic Laboratory, University of Minnesota (VDL). The serum, blood swab, and semen samples were tested by individual PCR for PCV2, and DNA sequencing was done on each positive using routine methods employed at the VDL.

Longitudinal study

In each of the four studs, longitudinal sampling of recently introduced boars was initiated at the same time that the cross-sectional sampling was conducted. A target sample size of 30 boars per study was chosen and sampling (serum only) was commenced within three weeks of arrival to the isolation units of the respective studs. The boars were then sampled at 4 week intervals for a further five samplings. After the first sampling (serum only), both semen (as boars were trained to collection dummies) and serum samples were collected at each sampling date. Samples were submitted to the VDL for PCR testing for PCV2 and DNA sequencing of positive samples.

Analysis

Data were maintained in an Excel spreadsheet and imported into commercial statistical software (Statistix 8.0,

Analytical Software, Tallahassee, FL). Descriptive statistics were calculated for key variables and conventional methods (chi-square analysis, one way ANOVA, logistic regression) were used to perform a range of comparative analyses. Analysis of sequence data is not yet completed and the descriptive results only are presented.

Results

Cross-sectional studies

Of the 240 boars sampled, 61 (25.4%, 95% confidence interval 20.3% to 31.2%) were found to be PCR positive for PCV2 on at least one sample. The proportion of positive boars differed significantly among studs (chi-square analysis, $P < 0.05$), with Stud 1 having markedly higher prevalence (38%) than the other 3 studs (17% to 23%). Mean boar age also differed significantly among studs (one-way ANOVA, $P < 0.001$), with the boars in Stud 1 being considerably younger (mean 8.6 months) than the boars in the other 3 studs (20.4 months, **Table 1**). On all studs, multiple strains of PCV2 were present based on predicted RFLP types derived from DNA sequences.

Effect of boar age on likelihood of positive PCR results

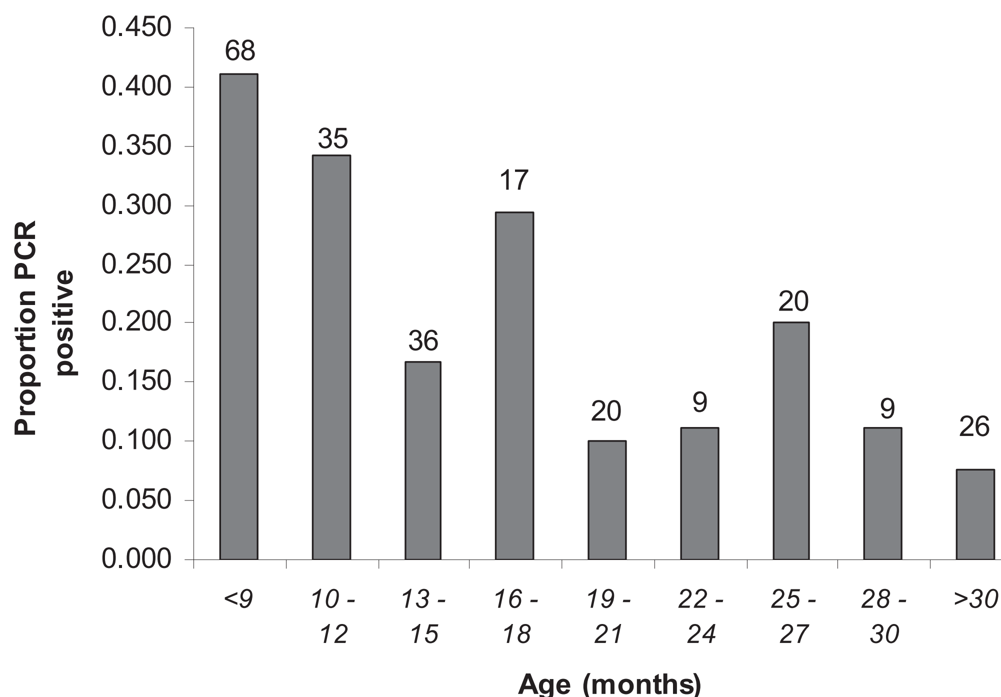
Because of the confounding between boar age and stud, results were compared by boar age in months. When boars were classified as positive if any of the three samples (serum, blood swab or semen) were positive, a clear association between age and risk of positivity is apparent (**Figure 1**).

The trend of higher prevalence of PCR positivity in young boars was evident across all three sample types (**Table 2**). Based on logistic regression analysis of serum data, the odds [Odds ratio (OR) = 0.93, 95% CI 0.88 – 0.98] of a positive PCR result decreased approximately 7% per month of age ($P = 0.01$). Similar results were seen with semen samples (OR = 0.92, 95% CI 0.86 – 0.97)

Table 1: Numbers of boars in each study that were PCR positive on any sample

Stud	Sample n	N (proportion, 95% CI) PCV2 positive	Mean boar age	SD age
1	60	23 (0.38; 0.26 - 0.51)	8.6^{A}	0.8
2	60	14 (0.23; 0.13 – 0.34)	18.6^{B}	9.2
3	60	10 (0.17; 0.07 – 0.26)	22.9^{BC}	10.3
4	60	14 (0.23; 0.13 – 0.34)	19.8^{C}	8.5

Figure 1: Proportion of 240 boars found PCR positive in serum, blood swabs or semen, by age group in months (data labels indicate sample size per age group)



PRRS/PMWS

Table 2: Number (n) and proportion (prop.) of PCR positive serum, swab, and semen samples from boars by age (Year 1 = < 12 months, Year 2 = 13 to 24 months; Year 3 = > 24 months)

		Serum		Swab		Semen	
Age (yr)	***n***	***n pos***	***prop***	***n***	***n pos***	***n***	***n pos***
<1	103	19	0.185	17	0.165	22	0.214
1-2	82	7	0.085	5	0.061	5	0.061
>2	55	3	0.055	4	0.073	4	0.073

PCV2 positivity by breed

Figure 2 summarizes results for the prevalence of PCV2 nucleic acids in blood samples (serum and swab combined) and semen samples by breed. Although there appears to be some variability (e.g., no positive semen samples form 18 Large White boars), differences between breeds did not approach statistical significance for blood samples ($P = 0.20$, chi-square analysis) or semen samples ($P = 0.49$) in univariate analyses. Also, in multivariable logistic regression models including farm, breed and boar age as independent variables, only boar age was significant.

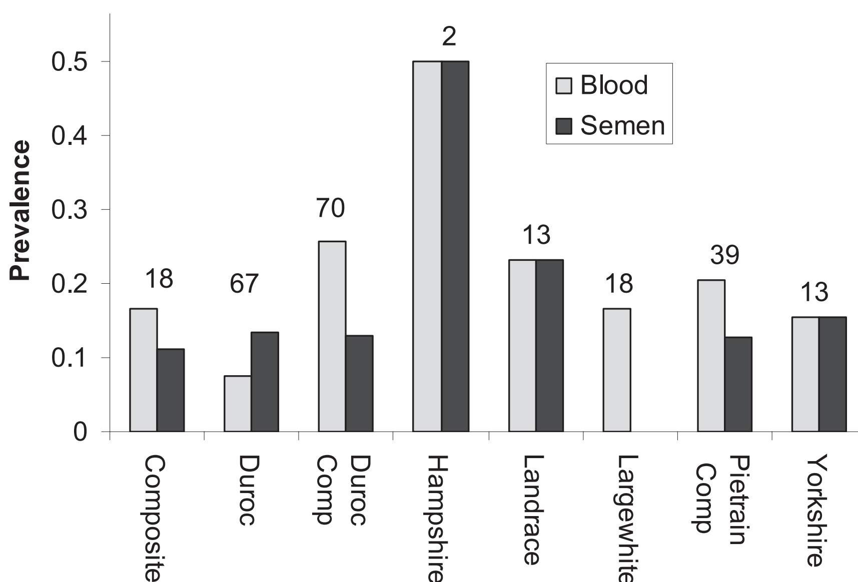
Comparison PCR results of serum, blood swab, and semen samples

Of the 61 boars yielding at least one PCR positive result, the majority (40, 66%) were positive on only one of the three samples. Seventeen (28%) were positive on two samples, and only 4 (7%) were positive for all three samples. The three samples yielded very similar numbers of positive boars (29, 26, and 31 positive boars for serum, swabs and semen respectively), suggesting sensitivity for detecting infected boars of less than 50% for any of the individual samples. Agreement was generally very poor between results of the three samples. Of 53 boars positive on either serum or swab samples, only 12 were positive on both (kappa = 0.36; 95% CI 0.23 – 0.49). Agreement was even poorer between serum and semen samples, with both samples being positive in only 6 of 54 boars that were positive on either test (kappa = .09).

Longitudinal sampling of boars

Samples of serum (483 samples from 95 boars) and semen (322 samples from 82 boars) were collected from boars at monthly intervals. The mean age of boars at the date of first sampling was 8.4 months (range 7 to 11 months). Overall, 34.3% of serum samples and 25.8% of semen samples were PCR positive for PCV2. At the time of first sampling, prevalence of positive serum samples ranged from 23% to 97% of boars positive among the 4 studs ($P < 0.001$). Consistent with the conclusion of the cross-sectional study, prevalence of positive samples tended to decrease over time (**Figure 3**). The apparent increase in prevalence in the later sampling events was largely attributable to results from a single stud. When restricted to boars with complete sample sets (i.e. data available for all 6 sampling events), the odds of serum samples being PCR positive declined by approximately 25% with each month of age (OR = 0.75, $P < 0.001$; 95% CI 0.67 – 0.87). This

Figure 2: Proportion of 240 boars found PCR positive in blood (serum, swab combined), or semen by breed (data labels indicate sample size per age group)



PRRS/PMWS

observation was fairly consistent across all studs, with OR estimates for individual studs ranging from 0.55 to 0.85. Although, this effect appears stronger than that from the cross-sectional study (OR = 0.93), this difference is likely attributable to the much broader age range included in the cross-sectional study (boars up to 37 months of age).

Complete sample sets for serum were obtained from 60 boars. Nine (15%) of the 60 boars were negative on all serum samples, but no boars were positive on all 6 samples (**Figure 4**). The nine consistently negative animals also were uniformly negative on a total of 40 semen samples.

With respect to semen results, complete sets of 5 monthly samples were obtained from 41 boars. Of these, more than half (23, 56%) were negative on all semen samples and 2 boars were positive on every semen sample (**Figure 5**). However, only 9 of these boars were uniformly negative on serum samples (total 54) while 28% of the serum samples of the other semen negative boars were positive.

As seen in the cross-sectional study, breed was not a significant factor influencing the probability of positive PCR results. Also similar to the cross-sectional study, agreement between PCR results of paired serum and semen samples was poor. Concordant results were obtained for

Figure 3: Proportion of serum and semen samples PCR positive for PCV2 by sampling event (months)

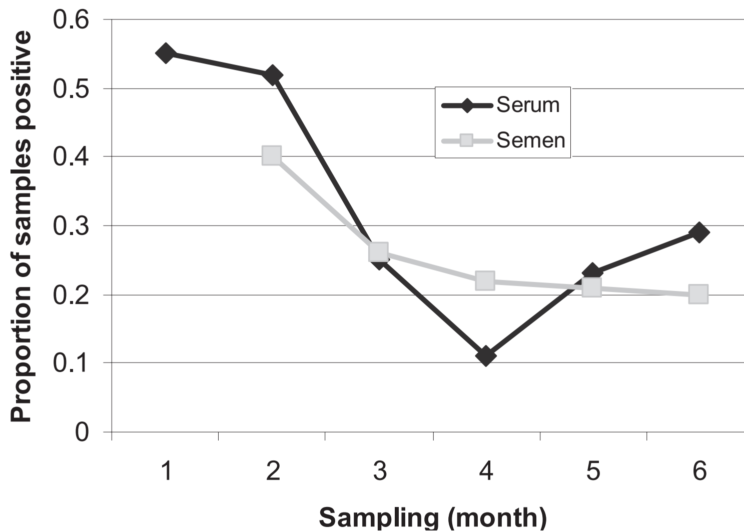
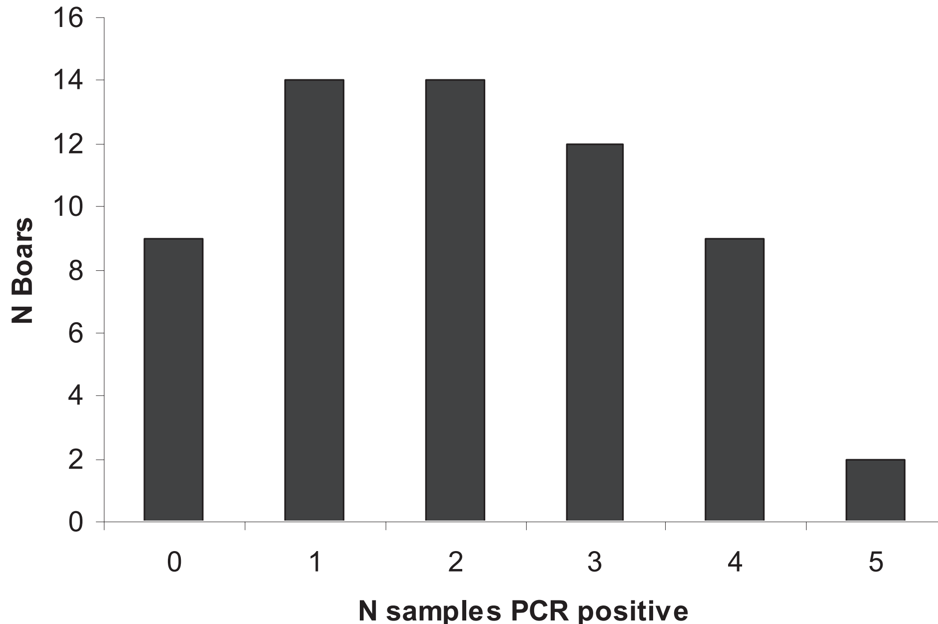
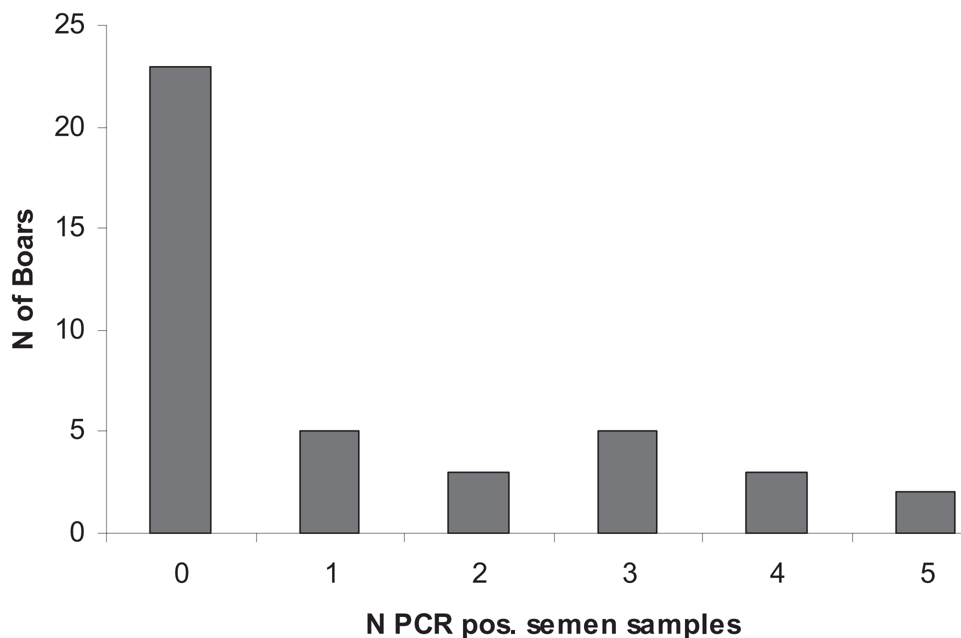


Figure 4: Distribution of 60 boars by number of PCR positive serum samples among 6 samples collected over consecutive months



PRRS/PMWS

Figure 5: Distribution of 41 boars by number of PCR positive semen among 5 samples collected over consecutive months



220 of 320 paired serum and semen samples (68.8%), but agreement beyond chance was poor (kappa = 0.19; 95% CI 0.07 – 0.31). However, odds of a positive semen result were significantly higher in boars with positive serum samples (OR = 2.54; 95% CI 1.48 – 4.32). On the other hand, semen samples paired to 235 negative serum tests were found to be PCR positive on 49 (20.8%) occasions, indicating that the negative predictive value (NPV) of a negative serum test was about 80%. However, given that study was in young introduced boars, higher NPV values would be expected in older boar populations (due to lower prevalence of positive semen). This was borne out by the data from the cross-sectional study in which the estimated NPVs for a negative serum sample were 0.79 for boars up to 12 months of age, 0.93 for boars from 13 to 24 months, and 0.94 for boars over 2 years old. However, even in the oldest group, based on these data about one out of 20 serum negative boars would be expected to be PCR positive in semen.

PRRS/PMWS

Discussion

Our results confirm previous studies indicating that PCV2 is prevalent in boar stud populations and that PCV2 nucleic acids can be commonly detected in semen. A previous study of a single boar stud in Canada indicated that both boar age and breed may influence the likelihood of semen samples being positive for PCV2.[10] We have previously observed from necropsy results that PCV2 is commonly identified in the young boar population, while pilot testing of older boars in studs indicated a relatively low prevalence of positive semen and serum samples (unpublished data). Our data from both the cross sectional and longitudinal sampling of boars across 4 studs indicate a strong negative effect of age on likelihood of PCV2 positive serum and semen samples. Furthermore, the magnitude of this effect appeared to be fairly consistent across all the farms studied. However, unlike the Canadian study,[10] we were not able to identify a significant effect of breed, and positive samples were obtained from all breeds included in the study.

Although the importance of semen as a vehicle for transmission of PCV2 remains unproven, the prevalence of the agent in semen and the severity of the associated diseases invite consideration of how boar studs might manage this issue. Unlike monitoring boar studs for PRRS virus, which is conducted to ensure boar stud populations are maintained free of the virus, PCV2 infection is likely to be endemic in all boar stud populations. The consistently poor agreement between results of PCR tests on paired samples from individual animals indicates that any efforts to monitor PCV2 infection in boar studs will be problematic. Discordant results were seen between concurrent samples from individual boars and across serial samples from boars over time. Given the marked effect of age on likelihood of positive PCR results in all samples, one potential approach to reduce the risk of dissemination of PCV2 to recipient sow farms is to test boars until they are PCR negative and seropositive. However the apparently long duration and often intermittent nature of shedding in semen reported previously[10] and also evident in our study indicate that this would be logistically challenging.

Although analysis of the DNA sequences is incomplete, the observation of multiple "strains" of PCV2 occurring concurrently was made on all 4 studs. This finding is hardly surprising and further analysis of the longitudinal data should yield insight into whether repeated PCV2 detection in boars is predominantly attributable to persistence of infection rather than reinfection with different viruses over time. Although there has been some discussion of the relationship between genetic variants of PCV2 and pathogenicity,[11,12] the role of 'new strains' of PCV2 in explaining the recent epidemic of severe PMWS in the USA remains uncertain. Field observations in Minnesota and other states strongly suggest that strains with a 321 RFLP pattern are predominantly involved in outbreaks of severe disease. If specific variants of PCV2 were confirmed to be more pathogenic, of if introduction of novel strains into a stud resulted in increased shedding, then screening for introduction of new strain sequences might be considered. However this would require thorough documentation of the viruses endemic to a stud and would clearly involve high costs in testing. Such investments may be difficult to justify without clear evidence of the importance of PCV2 transmission in semen.

A more pragmatic approach may be vaccination of boar studs and studies of the efficacy of vaccination in reducing the prevalence of virus in semen are necessary.

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