Associations between genetics, farm characteristics and clinical disease in field outbreaks of porcine reproductive and respiratory syndrome virus

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Abstract

Porcine reproductive and respiratory syndrome (PRRS) is a disease of domestic swine characterized by exceptionally high clinical variability. This study addresses the question of whether clinical variability in PRRS results from (a) genetic variation among viral isolates and/or (b) variation in management practices among farms on which isolates are found. Genetic data (open reading frame 5 gene sequences) and data on farm characteristics and associated clinical disease signs were collected for 62 PRRS virus (PRRSV) field isolates, representing 52 farms. Clinical disease signs were interrelated — confirming that a true reproductive syndrome exists (involving abortions, infertility in sows, deaths of sows and preweaning mortality).

Pairs of farms experiencing deaths in their sow populations also tended to share viral isolates which were more similar to one another than expected by chance alone. This implies that sow death (one of the more-severe manifestations of PRRS) is under genetic influence. Large herd size was a significant risk factor for the death of sows and for respiratory disease in nursery pigs. All-in–all-out management practices in the nursery were protective against reproductive signs in the sow herd. All-in–all-out management practices in the finishing stages of production were protective against respiratory disease in nursery pigs — but were paradoxically associated with an increased risk of infertility in sows. These results suggest that farm-management practices can also influence which PRRS clinical signs are manifested during an outbreak. In general, signs associated with PRRS appear to result from a combination of genetic factors and herd-management characteristics. The relative contributions of these two influences differ depending on the specific clinical sign in question. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is among the most clinically variable diseases of domestic swine. PRRS virus (PRRSV, a member of the *Arteriviridae*; Plagemann and Moennig, 1992) can cause infertility in sows of any age or parity, and abortion at any stage of gestation (Done et al., 1996; Mengeling et al., 1996; Zimmerman et al., 1997). The respiratory form of the infection can affect animals of any age, with clinical signs ranging from mild to severe interstitial pneumonia (often compounded by secondary viral and bacterial infections; Done et al., 1996; Van Reeth, 1997). PRRS can be subclinical, or can be associated with high mortality even in immunologically mature adults (Mengeling et al., 1998).

The reasons for the remarkable clinical variability of PRRS are poorly understood. PRRSV is highly variable genetically (Kapur et al., 1996; Murtaugh et al., 1998). Genetic differences have been documented between PRRSV isolates of different virulence (Meng et al., 1995). Intrinsic differences among viral isolates may therefore account for the observed variability in PRRS clinical severity (Halbur et al., 1996; Mengeling et al., 1998). Alternatively, herd characteristics may play a primary role. Conditions which predispose animals to infection via direct contact (e.g. overcrowding) may facilitate the spread of PRRSV within herds (Albina, 1997; Zimmerman et al., 1997). The secondary pathogens which may interact with PRRS are as varied as the disease itself (e.g. *Streptococcus suis*, *Haemophilus parasuis*, *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, swine influenza virus, porcine respiratory coronavirus; see Done et al., 1996; Van Reeth, 1997); the pathogen load on the farm may therefore affect the manner in which PRRS manifests itself clinically.

Previous studies of these issues have focused on documenting genetic and antigenic differences among PRRSV isolates of different virulence in controlled experimental settings (e.g. Halbur et al., 1996; Mengeling et al., 1996, 1998; Yoon et al., 1997). This study takes a complimentary epidemiologic approach, making use of a large collection of PRRSV field isolates and associated clinical data from natural outbreaks. Specifically, this study identifies statistical associations between genetics, farm characteristics, and clinical disease. In doing so, it tests the following two hypotheses (which are not mutually exclusive): (a) that PRRS clinical variability results from genetic variability among viral isolates; and (b) that PRRS clinical variability results from variability in the management practices of the farms on which PRRSV is found.

This retrospective study used data collected opportunistically by veterinarians; it cannot, therefore, address all of the factors commonly believed to be important in determining the clinical severity of PRRS. For example, this study does not quantify variation among farms with respect to which other pathogens were present. The strength of any statistical associations documented herein may therefore be underestimated, due to an anticipated large amount of unmeasured variation. In this light, the primary goal of this

study is to document basic associations between genetics, herd characteristics and PRRS clinical severity, in the hopes that future studies may measure such associations with greater precision.

2. Materials and methods

Sixty-two PRRSV isolates (representing 52 farms) were obtained from the Illinois Animal Disease Laboratories in Galesburg, IL. These isolates had been submitted for diagnostic testing by veterinarians from Illinois and eastern Iowa between April 1997 and July 1998. Clinical data (including farms of origin and names of submitting veterinarians) accompanied each sample. Epidemiologic data pertaining to each sample were collected through interviews with submitting veterinarians. Veterinarians were contacted by telephone, informed of the purpose of the study, and assured that all information obtained would remain confidential. All telephone interviews were conducted by the same interviewer (TLG) to eliminate error due to inter-observer variation.

Veterinarians were first asked to confirm the locations of the herds from which PRRSV isolates were collected, as well as dates of collection. Veterinarians were then asked to provide information about the clinical disease signs which were present on the farms at the times of sample collection, as well as information about the farms themselves. Epidemiologic variables collected are described in Table 1. Predictor variables (farm characteristics) were chosen either because they were predicted to influence the rate and extent of transmission of PRRSV within herds (CONFINE, HERDSIZE, NURSAIAO, FINAIAO), or because they were predicted to influence the extent and severity of clinical signs once transmission had occurred (VACCINE). Outcome variables were chosen to represent the full spectrum of clinical disease manifestations typical of PRRS outbreaks (Done et al., 1996).

Genetic data consisted of nucleotide sequences of the open reading frame 5 (ORF5) gene of PRRSV. This gene, which codes for an immunologically important membrane-spanning protein, was chosen because of its high variability among field isolates within the United States (Kapur et al., 1996; Murtaugh et al., 1998) and because of documented differences in ORF5 between PRRSV isolates of low and high experimental virulence (Meng et al., 1995). Genetic methodologies included in vitro propagation of viral isolates followed by reverse transcription polymerase chain reaction of ORF5 and automated fluorescent DNA sequencing. For each PRRSV isolate, 603 nucleotide bases of cDNA (reverse-transcribed genomic RNA) were thus sequenced. The genetic techniques used are described in detail elsewhere (Goldberg et al., in press).

Genetic similarity between isolates was computed as the uncorrected pairwise percent nucleotide similarity between ORF5 sequences. Specifically, genetic similarity between all possible pairs of isolates was calculated as the raw percentage of nucleotide bases which the two sequences shared. A matrix of genetic distances was thus obtained, with values in cells ranging from a possible minimum of 0% to a possible maximum of 100%. Genetically identical isolates from the same farm were collapsed to avoid pseudoreplication.

Clinical similarity between isolates was scored as either zero or one for each clinical sign: pairs of isolates received a score of one when the same clinical sign was present

Table 1
Description of variables used in epidemiologic analyses of PRRSV (52 farms, IL, USA; 1997–1998)

Variable name	Type	Description	Direction[f]	n[g]	%[g]
Predictor variables[a]					
CONFINE	Categorical	Pigs kept in partial confinement or total confinement	Positive	52	67
HERDSIZE	Interval	Number of pigs on the farm[c]	Positive	44	–[h]
NURSAIAO	Categorical	All-in–all-out management practiced in the nursery[d]	Negative	45	76
FINAIAO	Categorical	All-in–all-out management practiced in the grow/finisher[d]	Negative	50	55
VACCINE	Categorical	PRRSV vaccine used on the farm[e]	Negative	52	53
Outcome variables[b]					
ABORT	Categorical	Abortions	–	40	38
INFERT	Categorical	Infertility in sows	–	40	45
SOWDEATH	Categorical	Death of sows	–	40	20
PWMORT	Categorical	Preweaning mortality	–	40	45
RESPNURS	Categorical	Respiratory disease in nursery pigs	–	46	74
RESPFIN	Categorical	Respiratory disease in grow/finish pigs	–	49	72
LONGTIME	Categorical	Longer time to market for grow/finish pigs	–	49	60

[a] Predictor variables represent characteristics of the farm at the time the PRRSV sample was collected.

[b] Outcome variables represent clinical disease signs on the farm at the time the PRRSV sample was collected.

[c] Herd size was defined as the number of sows plus the number of pigs marketed per year. Where data on the number of pigs marketed per year was missing, the number of sows was multiplied by 18.27, which was the mean number of pigs marketed per sow in the data set.

[d] All-in–all-out is a management strategy in which animals are moved through the production cycle (farrowing → nursery → finishing stages of production) in cohorts, to reduce disease transmission across age classes. Facilities were considered AIAO only if AIAO was practiced at the level of the room, building or site. AIAO at the level of the pen was considered "continuous flow".

[e] VACCINE represents a PRRSV vaccine having been used on the farm prior to the time of sample collection.

[f] Predicted direction of association of predictor variable with all outcome variables.

[g] n = number of farms reporting; % = percentage of farms practicing the management strategy listed, or experiencing the clinical sign listed (categorical variables).

[h] Interval-level variable; quartile 1 (Q1) = 2741, Q2 = 5024, Q3 = 10 962; minimum 1927; maximum 38 540 pigs.

during their respective outbreaks; otherwise, pairs of isolates received a score of zero. A matrix of clinical similarity was thus obtained, equal in size to the matrix of genetic similarity described above, with values in cells being either zero or one.

Correlations between matrices of genetic similarity and clinical similarity were performed using Mantel tests of matrix correlation (Mantel, 1967), conducted with computer program *The R Package* (Legendre and Vaudour, 1991). A standardized form of the Mantel test statistic (r) was used (Smouse et al., 1986). For each matrix correlation performed, a null distribution of r was created using 10 000 Monte Carlo permutations of the matrices (Hope, 1968). Observed r values were compared to their respective null distributions, and probabilities were calculated as the proportion of null values as extreme or more extreme than the observed value of r.

Clinical disease signs were predicted to be positively intercorrelated, because farms experiencing any PRRS clinical sign should be predisposed to experiencing others. For

this reason, bivariable associations were calculated between clinical disease signs using PROC FREQ of the computer program SAS (SAS Institute, 1989), as an aid to interpreting associations between farm characteristics and clinical disease signs (described below).

Multiple-logistic-regression analyses were performed using PROC LOGISTIC of SAS to investigate the overall and independent contributions of predictor variables (farm characteristics) to each of the seven outcome variables (clinical-disease signs). All predictor variables were initially entered into each regression model, and then were subject to backwards stepwise elimination with a *P*-to-enter and *P*-to-remove of 0.10. Missing-value indicator variables were coded as needed. One-tailed statistical tests were used since directional predictions were made in all instances (Table 1). Results were considered significant at the 0.05 level.

3. Results

All submitting veterinarians were personally contacted by telephone, and epidemiologic data were collected for all 62 isolates included in the study. Interviews with the veterinarians indicated that these farms represented a wide range of farm types, from single-site small family farms to multiple-site large corporate operations. Most farms (71%) were farrow-to-finish facilities; 6% were farrow-to-wean facilities, and 23% were wean-to-finish facilities. Farms varied widely with respect to their herd management practices and the clinical signs associated with their PRRS outbreaks (Table 1).

Complete ORF5 gene sequences were obtained for 55 of the 62 isolates, representing 46 of the 52 farms in the study. Genetic similarity between isolates ranged from 84.9 to 100%, with a mean pairwise difference at the nucleotide level of 1.4%. A detailed description of the genetic structure of these isolates is presented elsewhere (Goldberg et al., in press). The sequences themselves are available through GenBank (accession numbers AF176424-AF176478).

Several significant bivariable statistical associations between clinical disease signs were documented. Abortion was correlated with infertility in sows (OR = 70; $P < 0.001$), death of sows (OR = 19; $P < 0.001$) and preweaning mortality (OR = 27; $P < 0.001$). Similarly, infertility in sows was correlated with both death of sows (OR = 11; $P = 0.003$) and with preweaning mortality (OR = 14; $P < 0.001$). The presence of respiratory disease in finishing pigs was associated with longer time to market for these pigs (OR = 14; $P < 0.001$). Weaker significant associations were also documented between infertility in sows and longer time to market for grow/finish pigs (OR = 4; $P = 0.04$), and between preweaning mortality and longer time to market for grow/finish pigs (OR = 4; $P = 0.04$). Abortion was non-significantly associated with longer time to market (OR = 3; $P = 0.09$). All other associations between clinical disease signs had probabilities ≥ 0.15.

Table 2 presents the results of Mantel tests of matrix correlation used for investigating the relationship between the genetic similarity of PRRSV isolates and the similarity of their associated clinical disease signs. Only SOWDEATH was significantly correlated with genetic similarity between isolates ($r = 0.69$; $P = 0.02$). Pairs of farms experiencing

Table 2
Matrix correlations between PRRSV genetic similarity and similarity of PRRS clinical disease signs (52 farms, IL, USA; 1997–1998)

Clinical disease sign	Mantel test parameters		
	n^a	r^b	P^c
Abortion	42	0.18	0.24
Infertility in sows	42	0.01	0.13
Death in sows	42	0.69	0.02
Preweaning mortality	42	−0.05	0.37
Respiratory disease in:			
Nursery pigs	48	0.17	0.19
Grow/finish pigs	54	−0.05	0.37
Longer time to market for grow/finish pigs	54	−0.07	0.28

[a] Values indicate sizes of matrices, and vary due to missing data.

[b] Values represent the standardized form of the Mantel test statistic proposed by Smouse et al. (1986).

[c] Probabilities are one-tailed when relationships were in the predicted (positive) direction, and two-tailed otherwise.

deaths in their sow populations were therefore more likely to share genetically similar PRRSV isolates than chance alone would predict.

The five predictors (farm characteristics) offered to the logistic multiple regressions (Table 3) accounted for the presence of clinical disease signs in only four cases: abortion, infertility in sows, death of sows, and respiratory disease in nursery pigs. In all cases, the Hosmer–Lemeshow residual test for goodness-of-fit of the logistic regression model was low, with all P values ≥ 0.365 (indicating adequate fit of the logistic regression model to these data).

Total-confinement housing and PRRSV vaccine use were not associated with the presence of any clinical disease sign. However, larger herd size was associated with increased deaths in the sow population and with higher respiratory disease in the nursery. All-in–all-out management practices in the nursery were protective for abortions, infertility, and deaths in the sow population. All-in–all-out management practices in the finishing stages of production were protective for respiratory disease in the nursery. Paradoxically, all-in–all-out management in the finishing stages of production was associated with an increased risk of abortion and infertility in sows.

4. Discussion

Our results confirm previous observations that PRRS is clinically highly variable (Done et al., 1996), and that the virus is genetically highly variable (Kapur et al., 1996; Murtaugh et al., 1998). The clinical disease signs recorded in this study were not, however, independent. Abortions, infertility in sows, death in the sow population and, to a lesser extent, preweaning mortality were all interrelated — confirming that PRRS does indeed cause a syndrome of reproductive signs. Similarly, longer time to market was

Table 3
Logistic multiple regressions of farm characteristics as predictors of PRRSV clinical disease signs (52 farms, IL, USA; 1997–1998)

	Outcome variable						
	ABORT	INFERT	SOWDEATH	PWMORT	RESPNURS	RESPFIN	LONGTIME
Goodness-of-fit measures[a]							
G (P)	4.86 (0.088)	9.47 (0.024)	6.38 (0.041)	NA	18.16 (0.001)	NA	NA
HL (P)	0.36 (0.834)	0.03 (0.986)	5.67 (0.579)	NA	8.74 (0.365)	NA	NA
Predictor variable[b]							
Intercept (P)	0.04 (0.903)	0.77 (0.162)	−2.0 (0.001)	NA	2.77 (0.984)	NA	NA
HERDSIZE[c]	NR	NR	R, S	NR	R, S	NR	NR
β (P)	–	–	0.06 (0.042)	–	0.18 (0.011)	–	–
aOR (95% CI)	–	–	1.06 (0.98–1.14)	–	1.20 (1.00–1.45)	–	–
NURSAIAO	R, S	R, S	R, S	NR	NR	NR	NR
β (P)	−0.85 (0.032)	−1.05 (0.015)	−1.15 (0.012)	–	–	–	–
aOR (95% CI)	0.43 (0.24–0.76)	0.35 (0.25–0.49)	0.32 (0.10–1.03)	–	–	–	–
FINAIAO	R	R	NR	NR	R, S	NR	NR
β (P)	0.78 (0.067)	1.15 (0.010)	–	–	−1.33 (0.002)	–	–
aOR (95% CI)	2.18 (1.28–3.69)	3.15 (2.31–4.29)	–	–	0.26 (0.09–0.76)	–	–

[a] G = log likelihood ratio test for goodness of overall fit of the model; HL = Hosmer–Lemeshow residual test for goodness-of-fit of the logistic regression model.
[b] NR = not retained in the final model; R = retained in the final model; S = significant at the 0.05 level; NA = not applicable (no variables retained in the final model); β = parameter estimate for each variable; aOR = adjusted odds ratio for each variable. Predictors were considered significant only if they were in the predicted direction and if their associated one-tailed P values were <0.05. P values given for significant variables are one-tailed; all other P values are 2-tailed. Neither CONFINE nor VACCINE was retained in any model.
[c] Adjusted odds ratios for HERDSIZE are expressed as the risk associated with an increase in herd size of 1000 animals.

correlated with other clinical disease signs at all stages of production — but most strongly with respiratory disease in the finishing stages of production.

That pairs of farms experiencing deaths in their sow populations also tended to share genetically similar PRRSV isolates ($r = 0.69$; Table 2) supports the hypothesis that this aspect of PRRS might be under genetic influence. The fact that sow death was the only variable associated with genetic similarity of isolates is especially interesting to us. Sow death has been described as a hallmark of "atypical" PRRS (Mengeling et al., 1998). "Atypical" PRRS is generally considered the most-virulent type of PRRS, because it involves the deaths of immunologically mature animals. Our results suggest that a factor (or factors) responsible for PRRSV virulence may be genetically encoded, either within ORF5, or elsewhere in the viral genome, or both.

Our study also documents several associations between management practices and the probability that a PRRSV-infected herd will experience specific clinical-disease signs. Herd size was a significant risk factor for both sow death and for respiratory disease in nursery pigs. This observation is consistent with the assumption that large herd size is associated with greater opportunity for PRRSV transmission within herds, and thus with greater epidemic potential.

The protective effects of all-in–all-out management practices were predicted based on the assumption that such practices should tend to reduce transmission rates within herds. All-in–all-out management in the nursery was protective against signs associated with the aforementioned reproductive syndrome of abortion, infertility, sow death and preweaning mortality. This may reflect a direct effect of all-in–all-out management in reducing PRRSV transmission "backwards" in the production cycle. Such a phenomenon would also be required to explain the protective effect of all-in–all-out management in the finishing stages of production against respiratory disease in the nursery.

We failed to find any evidence that keeping pigs in total confinement increased the risk that a herd would experience any of the PRRS clinical disease signs surveyed. Unfortunately, the sample of farms in this study did not contain any in which pigs were kept on pasture. The study was not, therefore, able to examine the effects of confinement over the entire range of housing situations in which pigs are typically kept. This study also did not document any effect of vaccination on the probability that a herd would experience any of the PRRS clinical signs surveyed. Vaccination might be important for preventing the establishment of PRRS in a herd — but it does not appear to ameliorate clinical signs once a herd has become infected.

The trends discussed above should be interpreted with caution. The sample of farms in this study is necessarily biased (representing only those farms for which veterinarians had submitted tissue samples for virus isolation). Farms with mild or subclinical PRRS were therefore probably underrepresented. Also, many of the associations described above are weak. Given the number of separate statistical tests run, the possibility of type-I error should be considered (especially for marginally significant results). For example, the positive association between all-in–all-out management in the finishing stages of production and infertility (Table 3) was opposite to the predicted direction, and could be a statistical artifact.

Nevertheless, statistical associations between viral genetics and clinical disease in uncontrolled field settings are expected to be weak — as are associations between farm

characteristics and clinical disease signs. First, it is unlikely that the genetic locus examined (ORF5) is solely responsible for virulence in PRRSV. Virulence in PRRSV is probably under the influence of several interacting genetic loci. Second, the clinical-disease data indicated only whether a sign was present on a farm — but did not contain any quantitative estimates of associated morbidity and mortality rates. Such quantitative data were impossible to obtain due to lack of consistency in record keeping by submitting veterinarians (but might have increased the precision of the documented associations had they been available).

Most important, perhaps, was the lack of data pertaining to which other pathogens were present on the farms. Such data were impossible to collect (again, due to variation in the diagnostic and record keeping protocols of the submitting veterinarians). This was unfortunate, because such data (if collected objectively) would probably account for a large proportion of the clinical variability observed in field outbreaks of PRRS. In terms of the present study, the effect of such unmeasured variation would be to enhance type-II error, by obscuring weak epidemiologic associations. By the same reasoning, the strengths of the associations that were documented were probably underestimated.

Despite these shortcomings, our results suggest that the clinical manifestations of PRRS result from a combination of viral genetics and herd characteristics. Four of seven clinical disease signs (abortion, infertility, death in sows and respiratory disease in nursery pigs) were significantly associated with farm characteristics. Death in sows (among those examined, the clinical manifestation of PRRSV most indicative of high virulence) was the only clinical disease sign significantly associated with genetic similarity of viral isolates. Future studies incorporating larger sample sizes, quantitative data about morbidity and mortality rates, and data on other pathogens present should help clarify the precise nature of these associations. Also useful would be molecular genetic information about which loci are directly responsible for PRRS virulence. Only if such data were available could potential interaction effects between viral genetics and farm characteristics in predicting PRRS clinical severity be examined quantitatively.

Acknowledgements

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