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Effect of a major quantitative trait locus for porcine reproductive and respiratory syndrome (PRRS) resistance on response to coinfection with PRRS virus and porcine circovirus type 2b (PCV2b) in commercial pigs, with or without prior vaccination for PRRS[1]

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ABSTRACT: A major QTL for host response to porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) infection was identified in a previous study. Single nucleotide polymorphism WUR10000125 (WUR), which is in complete linkage disequilibrium with the putative causative mutation, can be used as a tag SNP for the QTL. However, the effect of WUR following PRRS vaccination and/or coinfection with other pathogens is not known. Therefore, objectives of this study were to estimate the effect of WUR on host response following PRRS vaccination and coinfection of PRRSV with porcine circovirus type 2b (PCV2b), to estimate genetic parameters for host response to vaccination and coinfection, and to estimate the effect of previously identified candidate SNP under PRRSV-only or PCV2b-only infection on host response to coinfection. Data from 2 trials, comprising a total of 396 commercial crossbred nursery pigs from a single genetic source, were used for all analyses. Pigs were preselected based on WUR genotype: approximately half AA and half AB, where B is the favorable and dominant allele. At weaning, pigs were shipped to Kansas State University, where half of the pigs were vaccinated with a PRRS modified live virus vaccine. Four weeks later, all pigs were coinfected with field strains of PRRSV and PCV2b and followed for 42 d. Body weight and serum viremia measurements were collected following vaccination and coinfection to calculate ADG and viral load (VL), respectively. Average heritability estimates for PRRS VL, PCV2b VL, and ADG were 0.29, 0.09, and 0.40, respectively. After vaccination, AB pigs had lower vaccination VL ($P = 0.03$) and faster gain ($P = 0.004$) than AA pigs, as expected. After coinfection, AB pigs had lower PRRSV VL ($P < 0.001$) but did not significantly differ from AA pigs in growth rate ($P = 0.86$). For PCV2b VL, suggestive evidence of an interaction between vaccination and WUR genotype ($P = 0.11$) was detected, where AB pigs had significantly lower PCV2b VL when vaccinated ($P = 0.007$) but not when they were not vaccinated ($P = 0.87$). In addition to WUR, several PRRS-associated SNP and a PCV2b-associated SNP had significant effects on host response to coinfection. In conclusion, marker-assisted selection based on WUR genotype alone, or along with other candidate SNP for PRRSV and PCV2b infection, is a promising strategy to select for improved host response to not just PRRS but also coinfection of PRRSV with PCV2b and perhaps other pathogens.

Key words: pigs, porcine circovirus type 2, porcine reproductive and respiratory syndrome, swine, WUR10000125

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INTRODUCTION

Porcine reproductive and respiratory syndrome (**PRRS**) is an economically devastating disease that has afflicted the U.S. pork industry since the late 1980s (Keffaber, 1989). Vaccine development has been underway nearly as long but with limited success. In recent years, the use of PRRS modified live virus (**MLV**) vaccines has risen in popularity but remain limited in their ability to protect against heterologous strains (Hu and Zhang, 2014). The PRRS virus (**PRRSV**) weakens the immune system, making pigs more susceptible to coinfection with other pathogens (Yin et al., 2013). Coinfection of PRRSV with porcine circovirus type 2b (**PCV2b**) is common worldwide and can increase morbidity and mortality compared with infection with PRRSV alone.

Previously, a major QTL on chromosome 4 was found to be associated with host response to PRRSV infection (Boddicker et al., 2012). The WUR10000125 (**WUR**) SNP was selected as a tag SNP for this region. However, the effect of this region on host response following PRRS vaccination or coinfection with other pathogens is not known and is important for predicting the effect of selecting on WUR genotype in the field. Therefore, the first objective of this study was to estimate the effect of WUR genotype following PRRS vaccination and coinfection with PRRSV and PCV2b. A second objective was to estimate genetic parameters for ADG and PRRS viral load (**VL**) following PRRS vaccination and for ADG, PRRS VL, and PCV2b VL following coinfection. The final objective was to estimate the effect of candidate SNP that were previously identified under PRRSV-only or PCV2b-only infection on ADG, PRRS VL, and PCV2b VL following vaccination and coinfection.

MATERIALS AND METHODS

This project was approved by the Kansas State University and Iowa State University Institutional Animal Care and Use Committees.

Animals

Data from 2 experimental coinfection trials of commercial Large White × Landrace crossbred pigs from the same genetic source (n = 199 barrows in trial 1 and n = 197 barrows in trial 2) were used for this study. Pigs were the same cross and also from the same genetic source as pigs from PRRS Host Genetics Consortium trials 1 through 3 and 11, used for analyses conducted by Boddicker et al. (2012, 2014) and Hess et al. (2016), although separated by several generations. This study adds to results presented by Niederwerder et al. (2015), which were based on the first of the 2 trials analyzed here by estimating genetic parameters, analyzing additional phenotypes, and conducting in-depth analyses of the effect of genotype at WUR and other candidate SNP.

Trial 1 pigs were from 12 sires and 48 litters and trial 2 pigs were from 10 sires and 79 litters, with sires and dams unique to each trial. Pigs originated from the same high-health multiplier farm, where sows were vaccinated for porcine circovirus type 2 (**PCV2**) but not for PRRS. Pigs were preselected based on WUR genotype at the source: approximately half for the AA genotype and half for the AB genotype. The B allele, corresponding to the "G" nucleotide, is favorable under PRRSV infection and has shown to be dominant to A, which corresponds to the "A" nucleotide but occurs at a low frequency in commercial populations (Boddicker et al., 2012). At weaning (between 18 and 28 d of age), pigs were shipped to a biosafety level 2 facility at Kansas State University (Manhattan, KS). Upon their arrival, pigs were randomly sorted into 1 of 2 rooms and placed into 10 pens per room, balanced by WUR genotype, with 11 to 12 pigs per pen. Pigs from trial 1 were allowed to acclimate to their new surroundings for 4 d and pigs from trial 2 for 3 d, after which all pigs in one of the rooms received a 2-mL dose of a commercial PRRS MLV vaccine (Ingelvac PRRS; Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO) according to the label instructions. Average age and weight at vaccination were 26.9 ± 4.0 d and 6.2 ± 1.4 kg, respectively. Four weeks after vaccination (**PostVx**), all pigs were coinfected with field strains of PRRSV and PCV2b that were isolated from a pig with postweaning multisystemic wasting syndrome, a porcine circovirus–associated disease. The inoculum was prepared according to Niederwerder et al. (2015). Forty-two days after coinfection (**PostCoX**), all surviving pigs were euthanized using pentobarbital sodium and tissue was collected for genotyping using the GeneSeek-Neogen PorcineSNP80 BeadChip (GeneSeek, Igenity, Lincoln, NE). Body weights were recorded weekly throughout the vaccination period (−28 to 0 d post-infection [**dpi**]) and throughout the coinfection period (0 to 42 dpi) on both vaccinated (**Vx**) and nonvaccinated (**NonVx**) pigs. Serum samples were collected on Vx pigs at −28, −24, −21, −17, −14, and −7 dpi and on all pigs at 0, 4, 7, 11, 14, 21, 28, 35, and 42 dpi during the coinfection period. Serum samples were used to quantify PRRS and PCV2b viremia using real-time PCR analysis according to Niederwerder et al. (2015).

Traits

Descriptive statistics for all traits are presented in Table 1. Pigs that died prior to coinfection (0 for trial 1

Table 1. Descriptive statistics by trial (1 and 2), vaccination status (vaccinated [Vx] and nonvaccinated [NonVx]), and genotype for the WUR10000125 SNP (AA and AB) for host response prior to (-28 to 0 d post-infection [dpi]) or after (0 to 21, 22 to 42, or 0 to 42 dpi) coinfection with porcine reproductive and respiratory syndrome (PRRS) and porcine circovirus type 2b (PCV2b)

	Count by trial[1]		Count NonVx[2]		Count Vx[3]		Trial 1		Trial 2	
Trait	1	2	AA	AB	AA	AB	Mean	SD	Mean	SD
PRRS VL[4]										
−28 to 0 dpi	98	88	–	–	87	99	55.0	26.2	88.6	12.1
0 to 21 dpi	193	175	85	96	86	101	77.6	15.5	63.1	17.6
ADG, kg/d										
−28 to 0 dpi	199	187	87	104	92	103	0.35	0.08	0.52	0.15
0 to 42 dpi	185	172	85	96	82	94	0.84	0.16	0.84	0.23
PCV2b VL										
0 to 42 dpi	173	163	82	91	79	84	160.1	41.5	94.4	52.5
Mortality[5]										
0 to 21 dpi	199	183	86	101	92	103	0.01	0.10	0.03	0.16
22 to 42 dpi	199	183	86	101	92	103	0.10	0.30	0.08	0.28
0 to 42 dpi	199	183	86	101	92	103	0.11	0.31	0.11	0.31
Blue ear[6]										
0 to 21 dpi	199	183	86	101	92	103	0.28	0.45	0.10	0.31
22 to 42 dpi	199	183	86	101	92	103	0.02	0.14	0.04	0.21
0 to 42 dpi	199	183	86	101	92	103	0.28	0.45	0.14	0.35
Treatment[7]										
0 to 21 dpi	199	183	86	101	92	103	0.08	0.27	0.15	0.36
22 to 42 dpi	199	183	86	101	92	103	0.21	0.41	0.18	0.38
0 to 42 dpi	199	183	86	101	92	103	0.24	0.43	0.24	0.43

[1]All analyses were conducted using 2 trials of 199 (trial 1) and 197 (trial 2) commercial crossbred nursery piglets.

[2]Pigs were not vaccinated against PRRS virus prior to coinfection with PRRS and PCV2b 28 d later.

[3]Pigs were vaccinated against PRRS virus prior to coinfection with PRRS and PCV2b 28 d later.

[4]VL = viral load; calculated as the area under the curve of log-transformed viremia between the indicated dpi.

[5]A pig died (= 1) or survived (= 0) during the coinfection period.

[6]A pig did (= 1) or did not (= 0) display clinical signs of blue ear for at least 1 d during the coinfection period.

[7]A pig did (= 1) or did not (= 0) receive veterinary treatment for at least 1 d during the coinfection period.

and 10 for trial 2) were excluded from all analyses because causes of death were unrelated to coinfection of PRRSV and PCV2b. Across the 2 trials, 89 NonVx AA, 106 NonVx AB, 95 Vx AA, and 106 Vx AB pigs were used for analyses. Average daily gain was calculated as the slope of BW regressed on dpi PostVx and PostCoX using all BW data collected from −28 to 0 and 0 to 42 dpi, respectively (Supplemental Fig. S1; see the online version of the article at http://journalofanimalscience.org). Body weight measurements at both −28 and 0 dpi were required to calculate ADG PostVx and at 0 and 35 dpi or 0 and 42 dpi to calculate ADG PostCoX. Body weight measurements at 35 dpi were used if weight at 42 dpi was not available and was justified by the high correlation (0.97) between ADG calculated using either 35 or 42 dpi (data not shown). Porcine reproductive and respiratory syndrome and PCV2b VL were calculated for each individual as the area under the curve of $\log_{10}$–transformed viremia values according to Boddicker et al. (2012). Viremia data from −28 to 0 dpi were used to calculate vaccination VL (Supplemental Fig. S2; see the online version of the article at http://journalofanimalscience.org) and data from 0 to 21 dpi were used to calculate PRRS VL PostCoX (Supplemental Fig. S2; see the online version of the article at http://journalofanimalscience.org), consistent with analyses of 3 PRRSV-only infection trials described by Boddicker et al. (2012). Porcine reproductive and respiratory syndrome viremia data after 21 dpi were not used because a portion of individuals enter a rebound phase after 21 dpi. Virus rebound was previously determined to be a property of the virus rather than host genetics (Islam et al., 2013). Because viremia data for PCV2b was much noisier and there was no clear evidence of rebound, all available data from 0 to 42 dpi were used to calculate PCV2b VL PostCoX (Supplemental Fig. S3; see the online version of the article at http://journalofanimalscience.org). For calculation of PRRS and PCV2b VL, at least 4 viremia measurements were required per pig to have sufficient data for estimating area under the curve, including measurements at 0 and 21 dpi for PRRS VL and at 0 and 42 dpi for PCV2b VL.

Table 2. Single nucleotide polymorphisms used for the candidate SNP analyses

SNP name	Minor allele[1]	Major allele[1]	MAF[2]	Position[3]	Prior association	Reference
MARC0056777	B/G	A/A	0.36	1, 294	Mortality after PRRSV[4] infection	Boddicker, 2013
DIAS0000349	A/A	B/G	0.06	7, 26.9	PRRSV N-protein specific IgG in serum at 42 dpi[5]	Hess, 2016
MARC0058875	B/G	A/A	0.37	7, 29.08		
ALGA0039771	B/G	A/C	0.34	7, 29.24		
ASGA0031860	A/A	B/G	0.25	7, 24	PRRS S:P ratio[6]	Serão et al., 2014
H3GA0020425	A/A	B/G	0.37	7, 27		
MARC0058875	B/G	A/A	0.37	7, 29.08		
ASGA0032151	A/A	B/G	0.47	7, 30.4		
MARC0037274	A/A	B/G	0.31	7, 128		
SNP1[7]	B/C	A/A	0.38	7, 28.8	PCV2b[8] viremia	Engle et al., 2014
SNP2[7]	A/A	B/G	0.30	12, 3.7		

[1]The left-most allele corresponds to the A/B designation and the right-most allele corresponds to the actual nucleotide.

[2]MAF = minor allele frequency.

[3]Chromosome, megabase.

[4]PRRSV = porcine reproductive and respiratory syndrome virus.

[5]dpi = days post-infection.

[6]PRRS = porcine reproductive and respiratory syndrome; S:P ratio = sample-to-positive ratio based on a PRRSV-specific ELISA.

[7]SNP1 and SNP2: candidate SNP.

[8]PCV2b = porcine circovirus type 2b.

Clinical signs of coinfection were also recorded from 0 to 42 dpi. Pigs were monitored daily by a veterinarian or trained personnel and clinical symptoms were recorded, including cyanotic or blue discoloration of the ears, requirement of veterinary treatment, and mortality. Antimicrobial and anti-inflammatory medications were administered under the direction of a veterinarian for moderate to severe clinical disease as previously described (Niederwerder et al., 2015). These clinical traits were analyzed as binary variables: whether a pig did or did not die, did or did not display clinical signs of blue ear for at least 1 d during the indicated period, and did or did not require veterinary treatment for at least 1 d during the indicated period.

Genotype Data

For all analyses presented, a genomic relationship matrix (**GRM**) was used to account for genetic relationships among the 396 individuals. Genotypes across 61,729 SNP (remaining after quality control) were available on 376 genotyped pigs. Sire-dam pedigree information was also available for these genotyped pigs, as well as 20 non-genotyped pigs, which was combined to construct a H matrix (Fernando et al., 2014) using the JWAS software (Cheng et al., 2016). Quality control of SNP genotypes was performed in 3 steps: 1) fixed SNP were removed, 2) genotypes with a gene call score lower than 0.7 were set to missing, and 3) SNP with at least 15% missing genotypes were removed. Missing genotypes were replaced with the average genotype (on a 0, 1, or 2 scale) by SNP within trial. Final genotyping rate was 90%.

Genotypes for 10 candidate SNP that were identified in previous studies to have associations with host response to PRRSV-only or PCV2b-only infection were extracted for further analyses. Candidate SNP names and references are presented in Table 2. For these analyses, SNP genotypes of the 10 candidate SNP were simultaneously fitted as fixed effects to estimate the effect of SNP genotype on PRRS VL, PCV2b VL, and ADG. Linkage disequilibrium, calculated as the squared correlation, was less than 0.3 for all pairs of candidate SNP. Multiple test correction was not performed because all SNP had previously identified associations with host response to infection.

Statistical Analyses

All analyses were performed using ASReml 4.0 (Gilmour et al., 2015).

Candidate SNP Analyses. The following univariate animal model was used to estimate the effect of genotype for each of the 10 candidate SNP (Table 2) previously identified under PRRSV-only or PCV2b-only infection on PRRS VL, PCV2b VL, and ADG separately by vaccination group (Vx or NonVx) and coinfection period (PostVx or PostCoX):

$$\begin{aligned} y_{ijklmno} &= \mathrm{Trial}_j + \mathrm{WUR}_k + \\ &\sum_{l=1}^{10} \mathrm{SNP}_{lm} + B_1 \times \mathrm{WtVx}_i + B_2 \\ &\times \mathrm{VxAge}_i + B_3 \times \mathrm{PCV2\_0}_i + \\ &\mathrm{Animal}_i + \mathrm{Litter}_n + \mathrm{Pen}_{o(j)} + e_{ijklmno} \end{aligned}, \quad [1]$$

in which $y_{ijklmno}$ is the observed phenotype, Trial_j is the fixed effect of the jth trial (trial 1 or 2), WUR_k is the fixed

effect of the WUR SNP genotype (AA or AB), SNP is the fixed effect of the *m*th genotype (AA, AB, or BB) of the *l*th candidate SNP (SNP 1 through 10), β_1 is the partial regression coefficient for the covariate weight at −28 dpi (WtVx), β_2 is the partial regression coefficient for the covariate age at −28 dpi (VxAge), β_3 is the partial regression coefficient for the covariate level of PCV2b viremia at 0 dpi, Animal is the random animal genetic effect with a variance–covariance structure proportional to the genomic relationship matrix based on SNP genotypes with the assumption ~N($\mathbf{0}$, $\mathbf{G}\sigma_g$), Litter is the random litter effect (127 levels), and Pen is the random effect of pen nested within trial (40 levels). For this model, interaction effects of trial with each fixed effect were also fitted and removed if not significant ($P > 0.10$). Animal, litter, and pen(trial) were fitted as random effects to account for genetic, common environmental, and random environmental effects, respectively.

The level of PCV2b viremia at 0 dpi was fitted as a covariate because 24 NonVx and 13 Vx pigs had nonzero PCV2b viremia values at 0 dpi. This suggests that some pigs were exposed to PCV2b prior to entry into the facility, likely from their mothers; although sows were vaccinated against PCV2, it is well known that vaccination reduces PCV2 virus replication but may not eliminate it (Gerber et al., 2011). To account for this, PCV2b viremia level on day of coinfection (**PCV2_0**) was fitted as an additional covariate and all phenotypes were adjusted to PCV2_0 = 0 for all pigs, rather than to the mean, as pigs should have been negative for PCV2b prior to coinfection. The genetic variance explained by significant SNP was computed as the difference between the sum of genetic and litter variance when fitting all 10 candidate SNP versus fitting all candidate SNP except for the SNP in question.

Although PCV2b VL, PRRS VL, and ADG were analyzed separately for Vx and NonVx pigs for the candidate SNP analyses, Vx and NonVx pigs were analyzed jointly for analyses of the binary traits (mortality, blue ear, and treatment) to aid with convergence for this more complex type of analysis. The model for these analyses was the same as model [1] but with the addition of (**VxStatus**; PRRS vaccination status, pigs were either Vx or NonVx against PRRSV) and WUR × VxStatus as additional fixed effects. A probit model, which assumes a residual variance of 1, was used for these analyses.

Multivariate Model. Multivariate animal models were used to analyze PCV2b VL, PRRS VL, and ADG by VxStatus and time period (PostVx or PostCoX), when applicable, to estimate the effect of WUR and the interaction of WUR × VxStatus by specifying contrasts and to estimate genetic parameters. Porcine circovirus type 2b VL of NonVx pigs and PCV2b VL of Vx pigs were analyzed as 2 separate traits based on the following 2-variate model:

$$\begin{bmatrix}\mathbf{y}_N\\ \mathbf{y}_V\end{bmatrix}=\begin{bmatrix}\mathbf{X}_N & 0\\ 0 & \mathbf{X}_V\end{bmatrix}\begin{bmatrix}\mathbf{b}_N\\ \mathbf{b}_V\end{bmatrix}+\begin{bmatrix}\mathbf{Z}_{\alpha_N} & 0\\ 0 & \mathbf{Z}_{\alpha_V}\end{bmatrix}\begin{bmatrix}\boldsymbol{\alpha}_N\\ \boldsymbol{\alpha}_V\end{bmatrix}+\begin{bmatrix}\mathbf{Z}_{l_N} & 0\\ 0 & \mathbf{Z}_{l_V}\end{bmatrix}\begin{bmatrix}\mathbf{l}_N\\ \mathbf{l}_V\end{bmatrix}+\begin{bmatrix}\mathbf{Z}_{p_N} & 0\\ 0 & \mathbf{Z}_{p_V}\end{bmatrix}\begin{bmatrix}\mathbf{p}_N\\ \mathbf{p}_V\end{bmatrix}+\begin{bmatrix}\mathbf{e}_N\\ \mathbf{e}_V\end{bmatrix}, \quad [2]$$

in which $\mathbf{y}_N$ ($\mathbf{y}_V$) is a vector of phenotypes for PCV2b VL for NonVx (Vx) pigs, $\mathbf{X}_N$ ($\mathbf{X}_V$) is the design matrix of fixed effects and $\mathbf{b}_N$ ($\mathbf{b}_V$) is the corresponding vector of solutions, $\mathbf{Z}_{\alpha_N}$ ($\mathbf{Z}_{\alpha_V}$) is the design matrix of random genetic effects and $\boldsymbol{\alpha}_N$ ($\boldsymbol{\alpha}_V$) is the corresponding vector of solutions, $\mathbf{Z}_{l_N}$ ($\mathbf{Z}_{l_V}$) is the design matrix of random litter effects and $\mathbf{l}_N$ ($\mathbf{l}_V$) is the corresponding vector of solutions, and $\mathbf{Z}_{p_N}$ ($\mathbf{Z}_{p_V}$) is the design matrix of random pen effects and $\mathbf{p}_N$ ($\mathbf{p}_V$) is the corresponding vector of solutions. Fixed effects were the same as those fitted for model [1], except that genotypes of the 10 candidate SNP were not included in the model. For the 3 PRRS VL traits (vaccination VL PostVx, PRRS VL of the NonVx group PostCoX, and PRRS VL of the Vx group PostCoX) and the 4 ADG traits (ADG of the NonVx group PostVx, ADG of the Vx group PostVx, ADG of the NonVx group PostCoX, and ADG of the Vx group PostCoX), similar 3-variate and 4-variate models were fitted, respectively.

The covariance matrix of random animal genetic, litter, pen, and residual effects for model [2] was specified as follows:

$$\begin{bmatrix}\boldsymbol{\alpha}_N\\ \boldsymbol{\alpha}_V\\ \mathbf{l}_N\\ \mathbf{l}_V\\ \mathbf{p}_N\\ \mathbf{p}_V\\ \mathbf{e}_N\\ \mathbf{e}_V\end{bmatrix}=\begin{bmatrix}\mathbf{G}_N\sigma^2_{\alpha N} & \mathbf{G}_{NV}\sigma_{\alpha N,V} & 0 & 0 & 0 & 0 & 0 & 0\\ \mathbf{G}_{NV}\sigma_{\alpha N,V} & \mathbf{G}_V\sigma^2_{\alpha V} & 0 & 0 & 0 & 0 & 0 & 0\\ 0 & 0 & \mathbf{I}\sigma^2_{l\,N} & \mathbf{I}\sigma_{lN,V} & 0 & 0 & 0 & 0\\ 0 & 0 & \mathbf{I}\sigma_{lN,V} & \mathbf{I}\sigma^2_{l\,V} & 0 & 0 & 0 & 0\\ 0 & 0 & 0 & 0 & \mathbf{I}\sigma^2_{p\,N} & 0 & 0 & 0\\ 0 & 0 & 0 & 0 & 0 & \mathbf{I}\sigma^2_{p\,V} & 0 & 0\\ 0 & 0 & 0 & 0 & 0 & 0 & \mathbf{I}\sigma^2_{e\,N} & 0\\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & \mathbf{I}\sigma^2_{e\,V}\end{bmatrix}$$

in which **G** represents the genomic relationship matrix for the NonVx (N) and Vx (V) individuals and **I** represents the identity matrix. For all analyses, covariances were allowed between traits for the animal genetic effect and for the litter effect. Covariances between traits were also fitted for pen effects and for residuals for traits that were defined for the same vaccination group but were constrained to 0 for traits that were defined for different vaccination groups. This is because Vx and NonVx pigs were allocated to different rooms. When the estimate of litter and/or pen(trial) variance

was 0 or near 0 for a trait, that effect was removed from the model for that trait to aid with convergence.

Initially, a 9-variate model was fitted to estimate genetic parameters across the 3 PRRS VL, 2 PCV2b VL, and 4 ADG traits, but this model did not converge, due to a limited amount of data. Therefore, genetic correlations between PRRS VL/PCV2b VL, PRRS VL/ADG, and PCV2b VL/ADG were obtained by fitting a series of 2-variate models for each pair of traits according to model [2].

An additional question that arose throughout the course of this study was whether the effect of WUR genotype on response to PRRSV challenge was different on initial versus secondary PRRSV exposure. To address this question, a contrast was fitted to test the average effect of WUR genotype across the 2 primary PRRSV exposure traits (Vx pigs PostVx and NonVx pigs PostCoX) versus the secondary exposure trait (Vx pigs PostVx). To obtain an accurate estimate for this contrast, a set of orthogonal contrasts that test these contrasts as well as other interesting effects of WUR genotype and PRRS VxStatus were fitted based on model [2] and are listed in Supplemental Table S1 (see the online version of the article at http://journalofanimalscience.org).

Genetic Parameters

Heritability estimates and genetic correlations among the 2 PCV2b VL, 3 PRRS VL, and 4 ADG traits were obtained using the corresponding 2-variate, 3-variate, and 4-variate models resembling model [2]. These same models were used to estimate genetic correlations among the 2 PCV2b VL, 3 PRRS VL, and 4 ADG traits, and genetic correlations between each pair of PRRS VL/PCV2b VL, PRRS VL/ADG, and PCV2b VL/ADG traits were estimated using bivariate models. Heritability estimates for the binary traits (mortality, blue ear, and treatment) were obtained based on model [1] but with the addition of VxStatus (Vx or NonVx for PRRS) and WUR × VxStatus as additional fixed effects in the model. All genetic parameters were calculated excluding the effect of WUR from the model to allow WUR genotype to contribute to the observed genetic variation.

RESULTS

Effect of WUR Genotype and Vaccination Status

Average Daily Gain. During the vaccination period, a significant interaction of VxStatus ×WUR genotype was detected ($P = 0.003$), where AB pigs grew numerically (2.7%) faster than AA pigs within the NonVx group ($P = 0.27$) and significantly (8.1%) faster than AA pigs within the Vx group ($P = 0.004$; Supplemental Fig. S1 [see the online version of the article at http://journalofanimalscience.org]).

During the coinfection period, no significant main effects or interaction effect of WUR genotype × VxStatus ($P = 0.75$) were detected, but Vx pigs grew numerically (2.7%) faster than NonVx pigs ($P = 0.52$) and AA pigs grew numerically (0.1%) faster than AB pigs ($P = 0.86$; Supplemental Fig. S1 [see the online version of the article at http://journalofanimalscience.org]). Pigs grew significantly faster PostCoX than PostVx ($P < 0.001$), which was expected because pigs were older PostCoX than PostVx (Fig. 1).

Porcine Reproductive and Respiratory Syndrome Viral Load. During the vaccination period, AB pigs had significantly (8.7%) lower vaccination VL than AA pigs, as expected ($P = 0.03$). The same relationship was observed PostCoX, for which AB pigs had (6.7%) lower PRRS VL than AA pigs ($P < 0.001$). In addition, Vx pigs had (28.5%) lower PRRS VL than NonVx pigs ($P < 0.001$), suggesting at least a partially protective effect of the vaccine (Supplemental Fig. S2; see the online version of the article at http://journalofanimalscience.org). No significant interaction of WUR genotype × VxStatus was detected ($P = 0.50$; Fig. 1).

Results of the PRRS VL analysis suggested that the magnitude of the WUR effect on PRRS VL was numerically greater following primary exposure, whether by vaccination (6.6 VL units) or coinfection (6.1 VL units), than following secondary exposure (3.7 VL units). To test this, additional contrasts were fitted, which indicated that, although not significant ($P = 0.39$), the average effect of WUR genotype for the 2 primary exposure traits was 72.0% greater than for the secondary exposure trait and that the effect of WUR genotype did not significantly differ between the 2 primary exposure traits ($P = 0.70$; Fig. 1; Supplemental Table S1 [see the online version of the article at http://journalofanimalscience.org]).

Additional contrasts showed that PRRS VL of Vx pigs during the vaccination period was significantly ($P < 0.001$) greater than PRRS VL of Vx pigs during the coinfection period and that AB pigs had significantly ($P < 0.001$) lower PRRS VL than AA pigs, when averaged over time period and VxStatus (Supplemental Table S1; see the online version of the article at http://journalofanimalscience.org).

Porcine Circovirus Type 2b Viral Load. Results indicated a tendency toward a significant ($P = 0.11$) interaction effect of WUR genotype × VxStatus during the coinfection period, for which AB pigs had significantly ($P = 0.007$; 10.6%) lower PCV2b VL than AA pigs within the Vx group but not within the NonVx group (0.8%; $P = 0.87$; Supplemental Fig. S3 [see the online version of the article at http://journalofanimalscience.org]). In contrast to results for PRRS VL, Vx pigs had significantly ($P = 0.001$; 10.9%) greater VL than NonVx pigs (Fig. 1).

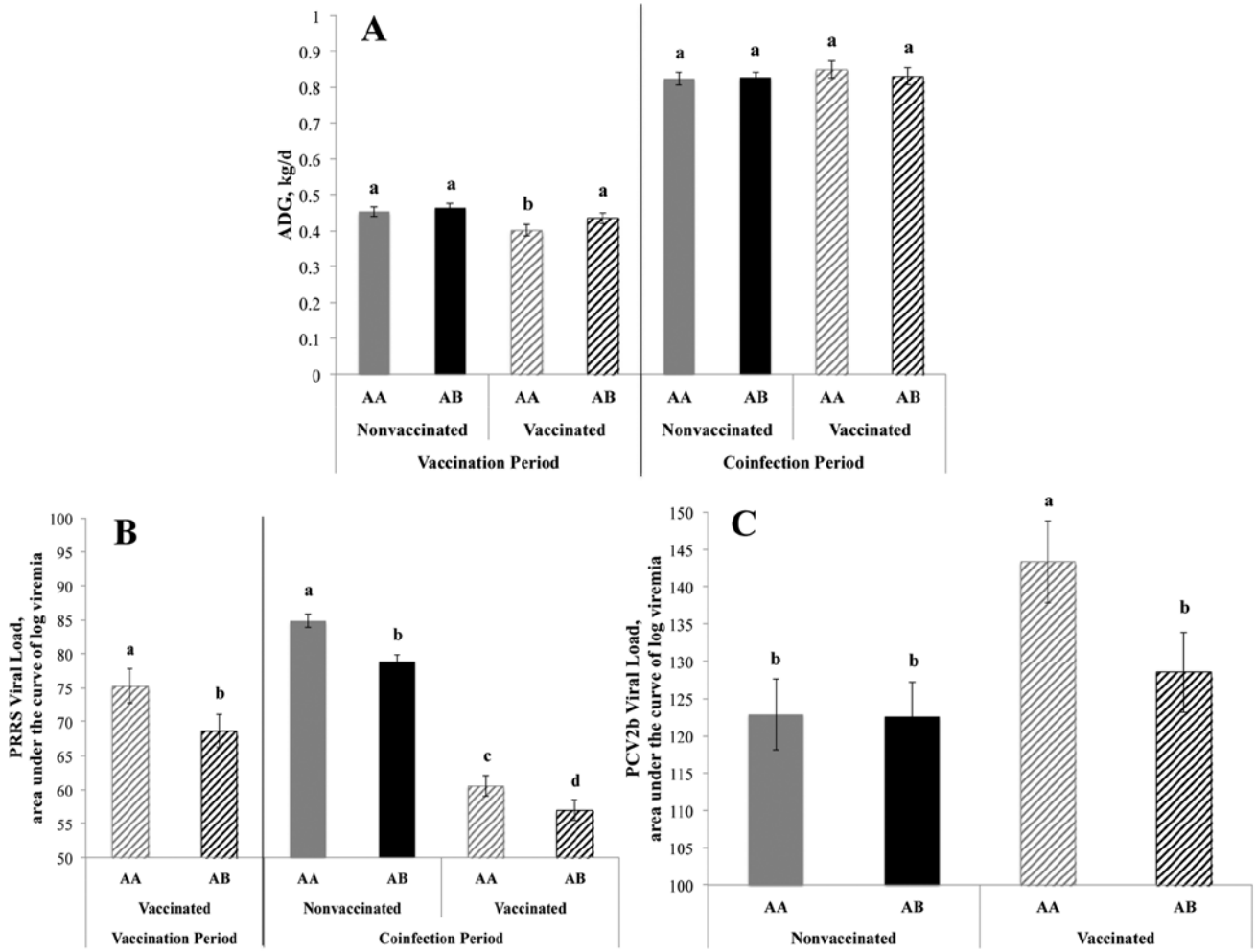

Figure 1. Least squares means of ADG (A), porcine reproductive and respiratory syndrome (PRRS) viral load (B), and porcine circovirus type 2b (PCV2b) viral load (C) following PRRS vaccination or PRRS virus and PCV2b coinfection by WUR10000125 SNP genotype (AA and AB). [a–d]Least squares means with different superscripts significantly differ ($P < 0.05$).

Blue Ear, Treatment, and Mortality. No evidence of a WUR genotype × VxStatus effect or WUR genotype effect was detected for analyses of mortality, blue ear, or treatment, but the effect of VxStatus was significant for every trait except for treatment from 0 to 21 dpi and from 0 to 42 dpi (Table 3). Analysis of mortality from 0 to 21 dpi and blue ear from 22 to 42 dpi did not converge.

For the significant VxStatus effects, a greater proportion of Vx pigs died from 22 to 42 dpi (10%; $P = 0.01$) and from 0 to 42 dpi (11%; $P = 0.04$) and a greater proportion of Vx pigs required treatment from 22 to 42 dpi (23%; $P = 0.01$) than NonVx pigs. However, a smaller proportion of Vx pigs displayed clinical signs of blue ear from 0 to 21 dpi (10%; $P = 0.001$) and from 0 to 42 dpi (13%; $P = 0.006$) than NonVx pigs.

Genetic Parameters

Within Traits. Heritability estimates (Table 4) and genetic correlations by time period and VxStatus are presented for ADG, PRRS VL, and PCV2b VL in Table 5. For ADG and PRRS VL, which were defined both PostVx and PostCoX, heritability estimates were higher for traits measured PostCoX than for those measured PostVx. Average daily gain PostCoX was moderately heritable at 0.41 ± 0.23 and 0.68 ± 0.22 for Vx and NonVx pigs, respectively. Porcine reproductive and respiratory syndrome VL PostCoX was also moderately heritable for NonVx pigs, at 0.61 ± 0.23, but less so for Vx pigs, at 0.13 ± 0.19. Porcine circovirus type 2b VL PostCoX was less heritable than either ADG or PRRS VL, at 0.09 ± 0.15 and 0.09 ± 0.19 for NonVx and Vx pigs, respectively. Heritability of ADG PostVx was lower for Vx pigs (0.18 ± 0.24) than NonVx pigs (0.33 ± 0.21).

Litter explained 13% or less of the total phenotypic variation for each PRRS VL trait and l9% or less for each ADG trait, except for ADG of Vx pigs PostVx, for which litter explained 40% of the phenotypic variation (Table 4). Substantial litter variances were observed for both PCV2b traits, where litter ex-

Table 3. Estimated proportion of affected individuals [95% confidence intervals] by porcine reproductive and respiratory syndrome (PRRS) vaccination status (VxStatus) and WUR10000125 (WUR) genotype (AA and AB)

	Nonvaccinated[1]		Vaccinated[2]		*P*-value		
Trait	AA	AB	AA	AB	VxStatus[3]	WUR[4]	VxStatus × WUR[5]
Mortality[6]							
0 to 21 dpi	–	–	–	–	NC[7]	NC	NC
22 to 42 dpi	0.02^{b} [0.00, 0.08]	0.04^{ab} [0.01, 0.10]	0.10^{ab} [0.05, 0.18]	0.11^{a} [0.06, 0.19]	0.01	0.55	0.69
0 to 42 dpi	0.03^{b} [0.01, 0.10]	0.07^{ab} [0.03, 0.14]	0.11^{a} [0.06, 0.20]	0.11^{ab} [0.06, 0.19]	0.04	0.55	0.36
Blue ear[8]							
0 to 21 dpi	0.24^{a} [0.15, 0.35]	0.22^{ab} [0.14, 0.33]	0.12^{bc} [0.06, 0.20]	0.09^{c} [0.04, 0.17]	0.001	0.94	0.80
22 to 42 dpi	–	–	–	–	NC	NC	NC
0 to 42 dpi	0.26^{a} [0.17, 0.37]	0.23^{a} [0.15, 0.34]	0.17^{ab} [0.10, 0.26]	0.09^{b} [0.04, 0.17]	0.006	0.50	0.36
Treatment[9]							
0 to 21 dpi	0.07^{a} [0.03, 0.14]	0.09^{a} [0.04, 0.16]	0.16^{a} [0.10, 0.25]	0.12^{a} [0.07, 0.20]	0.07	0.73	0.35
22 to 42 dpi	0.09^{b} [0.04, 0.17]	0.16^{ab} [0.09, 0.26]	0.24^{a} [0.16, 0.35]	0.22^{a} [0.14, 0.32]	0.01	0.52	0.18
0 to 42 dpi	0.14^{b} [0.07, 0.24]	0.24^{ab} [0.16, 0.35]	0.28^{a} [0.19, 0.39]	0.25^{ab} [0.17, 0.36]	0.11	0.44	0.13

$^{a–c}$Within each row, estimates with different superscripts significantly differ ($P < 0.05$).

[1]Pigs were not vaccinated against porcine reproductive and respiratory syndrome (PRRS) virus prior to coinfection with PRRS and porcine circovirus type 2b (PCV2b) 28 d later.

[2]Pigs were vaccinated against PRRS virus prior to coinfection with PRRS and PCV2b 28 d later.

[3]*P*-value for the effect of PRRS vaccination status.

[4]*P*-value for the effect of WUR SNP genotype.

[5]*P*-value for the effect of PRRS vaccination status by WUR SNP genotype.

[6]A pig died (= 1) or survived (= 0) during the indicated days post-infection (dpi).

[7]NC = no convergence: analysis did not converge.

[8]A pig did (= 1) or did not (= 0) display clinical signs of blue ear for at least 1 d during the indicated dpi.

[9]A pig did (= 1) or did not (= 0) receive veterinary treatment for at least 1 d during the indicated dpi.

plained 54 and 49% of the total phenotypic variation for PCV2b VL of Vx and NonVx pigs, respectively.

Estimates of genetic correlations are presented in Table 5. All estimates had large SE because of the small sample sizes, but these are the only estimates available for these hard-to-measure traits and they will, therefore, be presented with some detail. A strong, positive genetic correlation was detected between vaccination VL and PRRS VL of NonVx pigs PostCoX, at 0.94 ± 0.84, which are both related to primary PRRSV exposure; however, these primary exposure groups also had the weakest genetic correlation for ADG, at 0.10 ± 0.56. The phenotypic correlation for PRRS VL of Vx pigs PostVx and PostCoX was low, at −0.05 ± 0.12, but these 2 traits showed a moderate and positive genetic correlation (0.57 ± 1.12). A strong, positive genetic correlation was also detected between PCV2b VL of Vx and NonVx pigs at 0.99 ± 0.94.

For ADG, estimates of genetic and phenotypic correlations indicated a positive relationship between ADG traits for the NonVx group, whereas a negative genetic relationship but a positive phenotypic relationship was noted between ADG traits of the Vx group (Table 5). Genetic correlations were highest for ADG for the Vx and NonVx groups within a time period (i.e., among NonVx and Vx pigs PostVx and among NonVx and Vx pigs PostCoX) rather than among ADG across time periods for the same vaccination group. For example, the genetic correlation among ADG for Vx and NonVx pigs was 0.92 ± 0.92 PostVx and 0.75 ± 0.37 PostCoX. The lowest genetic correlation was detected among ADG for groups exposed to PRRSV for the first time, at 0.10 ± 0.56, as previously mentioned.

Heritability estimates for mortality, blue ear, and treatment are presented in Table 4. Results indicate that mortality and blue ear were low to moderately heritable for all phases of the coinfection period, with similar heritability estimates, ranging from 0.18 to 0.27. Litter did not explain any of the phenotypic variation for mortality or blue ear. Treatment was not heritable, although a sizeable litter component was detected for treatment from 22 to 42 and 0 to 42 dpi, suggesting that pigs from some litters received treatment longer than pigs from other litters during the second half of the co-infection period.

Across Traits. Phenotypic and genetic correlations between the 9 ADG, PRRS VL, and PCV2b VL traits analyzed by time period and VxStatus are presented in Table 5. Overall, phenotypic correlations were lower than genetic correlations and had lower SE. In general, phenotypic correlations of PRRS VL with ADG and PCV2b VL were weak, ranging from 0.04 ± 0.09 to 0.27 ± 0.08. Phenotypic correlations between PCV2b VL and ADG prior to coinfection were low, at 0.05 ±

Table 4. Estimates of heritability (SE) and litter components (SE) for traits after porcine reproductive and respiratory syndrome (PRRS) vaccination and after coinfection with PRRS and porcine circovirus type 2b (PCV2b)

Trait	Heritability	Litter component[1]
ADG		
NonVx,[2] −28 to 0 dpi[3]	0.33 (0.21)	0.13 (0.15)
Vx,[4] −28 to 0 dpi	0.18 (0.24)	0.40 (0.15)
NonVx, 0 to 42 dpi	0.68 (0.22)	0.13 (0.13)
Vx, 0 to 42 dpi	0.41 (0.23)	0.19 (0.16)
PRRS VL[5]		
Vx, −28 to 0 dpi	0.13 (0.22)	0.13 (0.13)
NonVx, 0 to 21 dpi	0.61 (0.23)	0.08 (0.14)
Vx, 0 to 21 dpi	0.13 (0.19)	0.12 (0.14)
PCV2b VL		
NonVx, 0 to 42 dpi	0.09 (0.15)	0.49 (0.11)
Vx, 0 to 42 dpi	0.09 (0.19)	0.54 (0.13)
Mortality[6]		
0 to 21 dpi	NC[7]	NC
22 to 42 dpi	0.27 (0.11)	0 (0)
0 to 42 dpi	0.24 (0.10)	0 (0)
Blue ear[8]		
0 to 21 dpi	0.18 (0.10)	0 (0)
22 to 42 dpi	NC	NC
0 to 42 dpi	0.20 (0.10)	0 (0)
Treatment[9]		
0 to 21 dpi	0.02 (0.15)	0 (0)
22 to 42 dpi	0 (0)	0.19 (0.08)
0 to 42 dpi	0.02 (0.15)	0.16 (0.12)

[1]Expressed as a proportion of the total phenotypic variance.

[2]NonVx = nonvaccinated: pigs were not vaccinated against PRRS virus prior to coinfection with PRRS and PCV2b 28 d later.

[3]dpi = days post-infection.

[4]Vx = vaccinated: pigs were vaccinated against PRRS virus prior to coinfection with PRRS and PCV2b 28 d later.

[5]VL = viral load; calculated as the area under the curve of log-transformed viremia between the indicated dpi.

[6]A pig died (= 1) or survived (= 0) during the indicated dpi.

[7]NC = no convergence: analysis did not converge.

[8]A pig did (= 1) or did not (= 0) display clinical signs of blue ear for at least 1 d during the indicated dpi.

[9]A pig did (= 1) or did not (= 0) receive veterinary treatment for at least 1 d during the indicated dpi.

0.09 for NonVx pigs and -0.18 ± 0.09 for Vx pigs. In contrast, PostCoX, phenotypic correlations between PCV2b VL and ADG were negative and moderately high, at -0.54 ± 0.07 for NonVx pigs and -0.58 ± 0.06 for Vx pigs.

For the correlations with PRRS VL, a negative phenotypic correlation was detected between ADG prior to co-infection with PRRS VL PostCoX for both NonVx (-0.09 ± 0.09) and Vx (-0.25 ± 0.08) pigs (Table 5), as expected. However, corresponding genetic correlations were in the opposite direction, at 0.63 ± 0.51 and 0.55 ± 1.18, respectively. The phenotypic correlation between PRRS VL and ADG of the Vx group PostCoX also indicated a negative relationship (-0.25 ± 0.08), as did the genetic correlation at -0.16 ± 0.79. Strong, positive genetic correlations were detected for PCV2b VL of Vx pigs with vaccination VL, PRRS VL of NonVx pigs PostCoX, and PRRS VL of Vx pigs PostCoX, at 0.92 ± 1.71, 0.97 ± 1.68, and 0.99 ± 1.83, respectively.

Similar to results for PRRS VL, ADG prior to coinfection had a negative phenotypic relationship (−0.18 ± 0.09) with PCV2b VL PostCoX for Vx pigs (Table 5) but the corresponding genetic correlation was in the opposite direction, at 0.55 ± 1.47. The strongest genetic correlations for PCV2b VL with ADG were observed for PCV2b VL and ADG PostCoX for NonVx pigs at -0.87 ± 0.40 and for PCV2b VL and ADG PostCoX of Vx pigs at -0.90 ± 0.67, with corresponding phenotypic correlations in the same direction.

Candidate SNP Analyses

When SNP genotypes of the 10 candidate SNP were simultaneously fitted in the model, several significant ($P < 0.05$) associations were detected (Table 6) for PRRS VL and ADG but not for PCV2b VL (Supplemental Table S2; see the online version of the article at http://journalofanimalscience.org). All but one of the significant associations were detected for traits analyzed PostCoX, with the greatest number of associations (3) for ADG of NonVx pigs (Table 6). Of candidate SNP with significant effects, SNP DIAS0000349 explained the greatest proportion of phenotypic variance for a trait (Table 6). Single nucleotide polymorphisms ALGA0039771 and ASGA0032151 were associated with both ADG and PRRS VL. For ALGA0039771, the B allele was associated with increased ADG for NonVx pigs PostVx ($P = 0.02$), representative of growth rate under normal, nonchallenged conditions, but with higher PRRS VL in Vx pigs PostCoX ($P = 0.02$). For SNP ASGA0032151, the BB genotype was associated with increased ADG ($P < 0.001$) and lower PRRS VL ($P = 0.02$) for Vx pigs PostCoX.

Single nucleotide polymorphisms DIAS0000349, H3GA0020425, MARC0058875, and SNP1 were all associated with a single trait (Table 6). For DIAS0000349 and H3GA0020425, the BB genotype was associated with increased ADG PostCoX for both NonVx ($P < 0.001$) and Vx pigs ($P = 0.01$). Pigs with the AA genotype for MARC0058875 ($P = 0.03$) and SNP1 ($P = 0.02$) had greater ADG during the coinfection period for NonVx pigs. Single nucleotide polymorphisms MARC0056777, ASGA0031860, MARC0037274, and SNP2 were not significantly ($P > 0.05$) associated with any trait.

Table 5. Estimates of phenotypic (SE; above the diagonal) and genetic correlations (SE; below the diagonal) for host response of pigs vaccinated (Vx) or nonvaccinated (NonVx) for porcine reproductive and respiratory syndrome (PRRS) prior to (−28 to 0 d post-infection [dpi]) or after (0 to 21 or 0 to 42 dpi) coinfection with PRRS and porcine circovirus type 2b (PCV2b)

Trait	Vaccination status and infection period	PRRS VL[1]			PCV2b VL		ADG			
		Vx (−28 to 0 dpi)	NonVx (0 to 21 dpi)	Vx (0 to 21 dpi)	NonVx (0 to 42 dpi)	Vx (0 to 42 dpi)	NonVx (0 to 42 dpi)	Vx (−28 to 0 dpi)	NonVx (0 to 42 dpi)	Vx (0 to 42 dpi)
PRRS VL	Vx (-28 to 0 dpi)	–	–	-0.05 (0.12)	–	0.14 (0.09)	–	-0.04 (0.08)	–	0.14 (0.08)
	NonVx (0 to 21 dpi)	0.94 (0.84)	–	–	0.04 (0.09)	–	−0.09 (0.09)	–	0.09 (0.09)	–
	Vx (0 to 21 dpi)	0.57 (1.12)	0.26 (0.57)	–	–	0.27 (0.08)	–	-0.25 (0.08)	–	-0.25 (0.08)
	NonVx (0 to 42 dpi)	-1.48 (2.15)	-0.13 (0.72)	0.30 (0.90)	–	–	0.05 (0.09)	–	-0.54 (0.07)	–
PCV2b VL	Vx (0 to 42 dpi)	0.92 (1.71)	0.97 (1.68)	0.99 (1.83)	0.99 (0.94)	–	–	-0.18 (0.09)	–	-0.58 (0.06)
	NonVx (-28 to 0 dpi)	0.61 (0.66)	0.63 (0.51)	0.47 (0.77)	-0.77 (1.92)	0.77 (1.10)	–	–	0.36 (0.09)	–
	Vx (-28 to 0 dpi)	0.44 (1.12)	-0.40 (0.63)	0.55 (1.18)	-0.80 (1.11)	0.55 (1.47)	0.92 (0.92)	–	–	0.14 (0.10)
ADG	NonVx (0 to 42 dpi)	0.67 (0.42)	0.70 (0.36)	-0.63 (0.40)	-0.87 (0.40)	-0.70 (0.58)	0.30 (0.28)	0.10 (0.56)	–	–
	Vx (0 to 42 dpi)	-0.37 (0.61)	-0.04 (0.47)	-0.16 (0.79)	-0.04 (1.16)	-0.90 (0.67)	-0.20 (0.48)	-0.48 (0.62)	0.75 (0.37)	–

[1]VL = viral load; calculated as the area under the curve of log-transformed viremia between the indicated dpi.

DISCUSSION

This is the first study to assess the effect of WUR genotype, previously associated with PRRSV-only infection (Boddicker et al., 2012, and Boddicker, 2013), on host response to PRRS vaccination and coinfection with PRRSV and PCV2b. Results from this study not only validate the effect of WUR genotype in a separate population of commercial crossbred pigs with another isolate of PRRSV but also indicate that the favorable (B) allele under PRRSV-only infection is also favorable following vaccination for PRRS with a commer-

Table 6. Least squares means (SE) and *P*-values for significant associations with host response for the candidate SNP analyses

Time period[1]	VxStatus[2]	Trait	SNP name	SNP genotype[3]			*P*-value	SNP variance[4]
				AA	AB	BB		
Post-vaccination	NonVx	ADG	ALGA0039771	0.44^{b} (0.02)	0.48^{a} (0.03)	0.44^{ab} (0.03)	0.02	0.03
Post-coinfection			DIAS0000349	–	0.61^{b} (0.06)	0.86^{a} (0.04)	<0.001	0.21
	NonVx	ADG	MARC0058875	0.85^{a} (0.06)	0.74^{b} (0.05)	0.62^{b} (0.08)	0.03	0.02
			SNP1[5]	0.83^{a} (0.05)	0.71^{b} (0.05)	0.65^{b} (0.08)	0.02	0.02
	Vx	ADG	H3GA0020425	0.63^{c} (0.09)	0.82^{b} (0.05)	0.98^{a} (0.09)	0.01	0.05
			ASGA0032151	0.57^{c} (0.09)	0.78^{b} (0.08)	1.09^{a} (0.09)	<0.001	0.05
		PRRS VL[6]	ASGA0032151	65.93^{ab} (5.15)	67.73^{a} (4.55)	51.29^{b} (5.59)	0.02	0.03
			ALGA0039771	56.08^{b} (3.69)	62.83^{a} (4.12)	66.02^{a} (4.93)	0.02	0.01

[a–c]Least squares means with different superscripts significantly differ ($P < 0.05$).

[1]After vaccination against porcine reproductive and respiratory syndrome (PRRS) or after coinfection with PRRS virus and porcine circovirus type 2b (PCV2b).

[2]VxStatus = PRRS vaccination status; pigs were either vaccinated (Vx) or nonvaccinated (NonVx) against PRRSV prior to coinfection with PRRS and PCV2b 28 d later.

[3]Least squares mean (SE) for each candidate SNP genotype.

[4]The proportion of phenotypic variance explained by the SNP.

[5]SNP1: candidate SNP (name not yet published) located on chromosome 7 at 28.8 Mb (Engle et al., 2014).

[6]PRRS = porcine reproductive and respiratory syndrome; VL = viral load: calculated as the area under the curve of log-transformed PRRS viremia between 0 and 21 d post-infection.

cial MLV vaccine and during coinfection with PCV2b. The AB genotype was associated with significantly reduced vaccination VL and significantly faster growth PostVx as well as significantly lower PRRS VL and PCV2b VL (within the Vx group) PostCoX. For practical reasons, only barrows were used in this study. Therefore, the effect of WUR on host response to vaccination and coinfection with PRRSV and PCV2b observed here must be validated for gilts in future studies.

Coinfection of PRRSV and PCV2b was identified as an ideal model to study the effect of WUR on coinfection, given the extensive literature documenting increased clinical signs in PRRSV/PCV2b coinfected pigs (Van Reeth et al., 1999; Shibata et al., 2000). The ubiquitous nature of PCV2b in swine populations was an additional motivating factor in selecting this virus to be used for the coinfection model. However, this very characteristic of PCV2b also added an extra layer of complexity to the study because complete elimination of exposure to the virus prior to coinfection proved difficult. Thirty-seven of the 396 pigs were found to have nonzero PCV2b titers at 0 dpi and were likely exposed to PCV2b from their mothers prior to arrival at Kansas State University. Although sows were vaccinated against PCV2, vaccination does not guarantee elimination of replicating PCV2. To account for the positive PCV2b titers of these 37 pigs prior to coinfection, the effect of PCV2b viremia at 0 dpi (PCV2_0) was fitted as a covariate for the analysis of each trait and all traits were adjusted to PCV2_0 = 0, to model the situation that all pigs were negative for PCV2b at the time of coinfection.

Effect of WUR Genotype on Host Response to PRRS Vaccination and Coinfection with PRRSV and PCV2b

It was of interest to estimate the effect of WUR genotype on host response following PRRS vaccination and coinfection with PCV2b for several reasons. First, PRRS vaccines are becoming more widely used and MLV vaccines are currently considered the most effective (Hu and Zhang, 2014). However, PRRS MLV vaccines, albeit less virulent than field strains, still result in PRRSV infection, and therefore, it was important to confirm that the favorable (AB) WUR genotype under infection with a field isolate of PRRS was also favorable following MLV vaccination. Furthermore, information regarding the effect of WUR genotype on host response following infection with diseases other than PRRS is limited. This is another important point to consider because we must establish that selecting for improved response to PRRS based on WUR genotype does not have a negative impact on response to other common diseases.

Previous studies have shown that AB pigs had significantly reduced PRRS VL following experimental infection with PRRSV (Boddicker et al., 2012; Hess et al., 2016), forming the basis for the hypothesis that AB pigs would have significantly lower PRRS VL than AA pigs following PRRS vaccination and coinfection with PRRSV and PCV2b. Our results supported this hypothesis. In a recent study conducted by Abella et al. (2016), no significant effect of WUR genotype on PRRS VL was detected. However, pigs from the Abella et al. (2016) study were older, only 80 pigs were used for analyses, and pigs were experimentally infected with a European PRRSV strain rather than a North American strain.

Our results for the analysis of PRRS VL also showed that PCV2_0 had a significant, positive effect on PRRS VL PostCoX of NonVx pigs. The trend was the same for Vx pigs PostCoX, although not significant. Although not a major point, the result is worth discussing because, to date, there is no evidence that PCV2b increases the replication of PRRSV, as this result suggests. However, there is evidence that PRRSV increases PCV2 replication (Allan et al., 2000; Harms et al., 2001). Although the exact mechanism is not clear, it is suspected that PRRSV increases PCV2 replication by stimulating immune cells, thereby increasing the number of cells that support PCV2b replication (Yin et al., 2013; Niederwerder et al., 2015).

For this reason, we hypothesized that AB pigs would not only have lower PRRS VL than AA pigs PostCoX but also lower PCV2b VL. Our results support this hypothesis because AB pigs had significantly lower PCV2b VL within the Vx group and numerically lower PCV2b VL within the NonVx group. Therefore, because AB pigs had significantly lower vaccination VL, they also had significantly lower PCV2b VL following coinfection. Little is known about the effect of WUR genotype on host response to PCV2b infection, except for a recent PCV2b experimental infection trial in which the effect of WUR genotype on PCV2b VL was not significant (D. Ciobanu, University of Nebraska – Lincoln, personal communication). However, pigs in the Ciobanu et al. study were neither vaccinated for nor coinfected with PRRSV.

The effect of WUR genotype on ADG following PRRSV infection has been investigated in previous studies, but its effect was not consistent. For example, AB pigs infected with the NVSL 97-7985 (**NVSL**) PRRSV isolate (GenBank accession number AY545985) had significantly greater ADG under infection (Boddicker et al., 2012). The same trend was observed for pigs infected with the KS2006-72109 (**KS06**) PRRSV isolate (Hess et al., 2016), but the effect was not significant. It was concluded that this observed inconsistency was a result of differences in virulence between the NVSL and KS06 isolates (Hess et al., 2016). Because MLV

vaccines are less virulent than field strains (Hu and Zhang, 2014), we hypothesized that AB pigs may not have significantly greater ADG than AA pigs PostVx but that the effect would be greater PostCoX. However, the opposite result was obtained. It may be that the vaccine virus, although modified, was still virulent enough to result in a significant WUR effect and that WUR genotype does not have a significant effect on ADG upon coinfection with PCV2b. However, the opposite would be expected, because WUR affected both PRRS VL and PCV2b VL PostCoX. Results from Abella et al. (2016), in which pigs were vaccinated with a PRRS MLV vaccine, support the finding that AB pigs had significantly greater ADG following PRRS vaccination.

Results indicate no evidence of a significant effect of WUR on blue ear, treatment, or mortality. Of these 3 traits, only mortality was analyzed for previous PRRSV-only infection trials, and, consistent with results from the current study, no significant effect of WUR on mortality was detected (Boddicker, 2013). There was, however, a significant trial × WUR effect for blue ear from 0 to 21 dpi and from 0 to 42 dpi, which was driven by the fact that more AB pigs had clinical signs of blue ear in trial 1 but more AA pigs had clinical signs of blue ear in trial 2. Each trait was analyzed separately for 0 to 21, 22 to 42, and 0 to 42 dpi, because a previous study of trial 1 showed evidence of more severe clinical signs during the latter half (22 to 42 dpi) of the coinfection period (Niederwerder et al., 2015).

In general, the findings that NonVx pigs were more likely to develop blue ear PostCoX, that a greater proportion of Vx pigs required treatment, and that a greater proportion of Vx pigs died during the coinfection period are consistent with results reported by Niederwerder et al. (2015) based on trial 1 of this study. Results from the current study show that increased clinical signs in Vx pigs are likely driven by significantly higher PCV2b VL for Vx AA pigs PostCoX. Therefore, our results support the conclusion presented by Niederwerder et al. (2015) that the early protective effect of the vaccine was outweighed by an increased incidence of clinical signs consistent with porcine circovirus–associated disease during the later phase of the coinfection period.

Effect of WUR Genotype, Depending on Previous Vaccination, or Not, for PRRS

The second objective of this study was to determine whether the effect of WUR genotype was consistent for PRRS VL, PCV2b VL, and ADG, regardless of whether or not pigs were previously vaccinated against PRRSV. Because Niederwerder et al. (2015) identified a protective effect of the vaccine on reexposure to PRRSV, we hypothesized that the level of PRRS viremia would be greater for NonVx than Vx pigs PostCoX, which could result in a greater magnitude of WUR effect for the NonVx than Vx pigs.

Although NonVx pigs did indeed have significantly greater PRRS VL than Vx pigs PostCoX, the effect of WUR genotype by × VxStatus PostCoX was not significant. However, based on results of fitting a contrast for the average effect of WUR genotype following primary versus secondary PRRSV exposure, the direction of the WUR × VxStatus effect PostCoX was in the expected direction. Results indicated that numerically, the effect of WUR was greater following primary PRRSV exposure, whether by vaccination or coinfection, than secondary exposure. Likewise, the magnitude of the WUR effect was greater within the NonVx group than the Vx group for the effect of WUR × VxStatus PostCoX.

These findings are consistent with the biological role of the putative causative gene for WUR, *guanylate binding protein 5* (*GBP5*), which has a known role in innate response to infection (Shenoy et al., 2012; Koltes et al., 2015). It is reasonable that an innate immune response gene has a larger effect on primary exposure than on secondary exposure because innate, rather than adaptive, immunity is predominantly responsible for controlling the initial response to infection.

The effect of WUR × VxStatus for PCV2b VL tended toward significance, with PCV2b VL significantly lower for AB pigs than AA pigs within the Vx group but not within the NonVx group. This result was likely because Vx pigs were positive for PRRSV at coinfection. Possibly, vaccination suppressed innate immune responses, allowing propagation of PCV2b, thereby increasing the magnitude of the WUR effect for this group. However, this difference between WUR genotypes for Vx pigs did not translate to differences in growth rate during the coinfection period. Results for ADG indicated that AB pigs grew numerically faster within the NonVx group and AA pigs grew numerically faster within the Vx group, but the effect of WUR genotype × VxStatus was not significant. Because AA pigs had significantly greater PRRS VL and PCV2b VL than AB pigs within the Vx group, we expected AA pigs to have lower ADG as a result.

Genetic Parameters Provide Novel Insight regarding Genetic Relationships among Host Response Traits

The third objective of this study was to estimate genetic parameters for host response following PRRS vaccination and coinfection. Except for days on treatment, low to moderate heritability estimates were obtained for all traits, indicating that selecting for improved response to vaccination and/or coinfec-

tion based on these traits is possible. Heritability estimates for vaccination VL, ADG of Vx pigs PostVx, and PRRS VL of Vx pigs PostCoX, were lower than reported by Boddicker et al. (2014), based on analyses of 8 trials in which pigs were experimentally infected with the NVSL PRRSV isolate, or by Hess et al. (2016), based on analyses of these same 8 trials plus 1 additional trial and 4 trials in which pigs were experimentally infected with the KS06 PRRSV isolate. There are many possible explanations for this, including differences in experimental design, such as challenge with the PRRS MLV vaccine versus the NVSL or KS06 PRRSV isolate. Pigs used in the current study had a shorter acclimation period compared with those in the trials described by Boddicker et al. (2012, 2014) and Hess et al. (2016), which may have contributed to the especially large phenotypic variance for vaccination VL (i.e. 474 VL units^2, versus 93 VL units^2 and 155 VL units^2 for PRRS VL of NonVx pigs and PRRS VL of Vx pigs PostCoX, respectively). The large phenotypic variance may also reflect the many stressors that pigs endured prior to vaccination, including postweaning stress, transportation stress, and the stress of being placed into new social groups with new pen assignments.

Several interesting genetic and phenotypic correlations were observed across and within traits. Standard errors were generally high, which was to be expected, given the limited number of animals for this type of genetic analysis, although 396 individuals is considered to be a large data set for an experimental challenge study of this nature. Because genetic parameters for host response traits following PRRS vaccination and PRRSV and PCV2b coinfection have not been previously reported, these estimates provide novel insight into the genetic relationships among these traits

Perhaps the most interesting genetic correlation identified was that between the 2 primary exposure traits: vaccination VL and PRRS VL of NonVx pigs PostCoX. The high, positive estimate for the genetic correlation (0.94), albeit with a large SE (0.84), suggests that the same genes that control response to vaccination also control response to primary infection with a field isolate. This suggests that response to vaccination could be used as an indicator trait for response to infection with a field isolate of PRRSV. The moderate, positive genetic correlation for PRRS VL between Vx pigs prior to and after coinfection (0.57 ± 1.12) suggests that some of the genes that control response to PRRS VL on primary PRRSV exposure also control response to secondary PRRSV exposure. However, the SE is large for both of these estimates, and therefore, additional research is needed before stronger conclusions can be drawn. For PCV2b VL, a high, positive genetic correlation (0.99 ± 0.94) was detected between the Vx and NonVx groups, suggesting that the same genes that control response to PCV2b infection in pigs previously vaccinated for PRRS also control host response in pigs not previously vaccinated for PRRS. Again, the SE of this estimate was large.

Results for ADG indicated a low genetic correlation between groups exposed to PRRS for the first time (0.10 ± 0.56), suggesting that ADG following vaccination is not a good indicator of ADG on primary exposure to PRRSV. Likewise, a moderate, negative genetic correlation (−0.48 ± 0.62) was detected between Vx pigs before and after coinfection, suggesting that different genes control growth upon vaccination versus reexposure. Among all ADG traits, the strongest genetic correlations were detected between NonVx and Vx groups prior to coinfection (0.92 ± 0.92) and between NonVx and Vx groups PostCoX (0.75 ± 0.37), suggesting that growth of Vx pigs PostVx is a better indicator of growth of NonVx pigs PostVx than of growth of Vx pigs PostCoX. The same applies to Vx and NonVx pigs PostCoX.

Between traits, the strong, positive genetic correlations of PCV2b VL of Vx pigs with vaccination VL, PRRS VL of NonVx pigs PostCoX, and PRRS VL of Vx pigs PostCoX suggest that the same genes that control response to vaccination VL and PRRS VL also control immune response to PCV2b VL for pigs previously exposed to PRRS. This is plausible, given what we know about the immunological interactions of these viruses, as previously mentioned (Allan et al., 2000). Although small, a negative phenotypic (−0.04 ± 0.08) correlation was observed between vaccination VL and ADG PostVx, which agrees with Boddicker et al. (2014), who reported negative genetic (−0.46 ± 0.35) and phenotypic (−0.25 ± 0.04) correlations between PRRS VL and weight gain upon PRRSV-only infection. The direction of these correlations is also consistent with genetic (−0.74 ± 0.10 and −0.52 ± 0.17) and phenotypic (−0.33 ± 0.03 and −0.23 ± 0.05) correlations reported by Hess et al. (2016) following experimental infection with the NVSL and KS06 PRRSV isolates, respectively. Genetic and phenotypic correlations between ADG and PRRS VL PostCoX were consistent with this observation for Vx pigs but not for NonVx pigs. However, SE were large. Negative genetic and phenotypic correlations were also observed between PCV2b VL and ADG PostCoX, which are consistent with results reported by Engle et al. (2014) following experimental infection with PCV2b.

Effect of Other Candidate SNP on Host Response to PRRS Vaccination and Coinfection with PRRSV and PCV2b

The final objective of this paper was to validate the effects of candidate SNP that were identified following PRRSV-only or PCV2b-only infection in pigs coinfected with PRRSV and PCV2b, with the hypothesis that the effect of SNP previously associated with either virus would also be significant for coinfected pigs. For these analyses, WUR genotype was fitted as a fixed effect because WUR genotype was part of the experimental design of the study and to allow the candidate SNP to explain variation due to regions other than the QTL on chromosome 4.

Results indicate that several SNP that were previously associated with host response to PRRSV-only infection also had an effect on host response to coinfection with PRRSV and PCV2b. However, for some SNP, the direction of the effect was not consistent with previously reported results. For SNP1, AA pigs within the NonVx group had numerically lower PCV2b VL and significantly greater ADG PostCoX than AB or BB pigs. The direction of the effect of SNP1 genotype on PCV2b VL is consistent with results reported by Engle et al. (2014) from a study in which pigs were experimentally infected with PCV2b. However, Engle et al. (2014) did not identify a significant effect of SNP1 on ADG.

Significant associations of candidate SNP genotype with PRRS VL and/or ADG were also detected for SNP ASGA0032151, H3GA0020425, ALGA0039771, MARC0058875, and DIAS0000349. It was not possible to compare the direction of these effects to those reported in the literature because previous associations were with PRRS antibody (Serão et al., 2014; Hess, 2016), which was not available for this study. However, results for SNP ASGA0032151, H3GA0020425, and ALGA0039771 were in the expected direction, based on the assumption that increased ADG and decreased VL are correlated with increased PRRS antibody production. Results for SNP MARC0058875 were also in the expected direction based on analyses of PRRSV-infected commercial nursery pigs (Hess, 2016) but not based on analyses of gestating multiplier females following a PRRS outbreak (Serão et al., 2014). Results for SNP DIAS0000349 were not in the expected direction.

For the current study, pigs with the BB genotype for SNP ASGA0032151 had significantly greater ADG and lower PRRS VL PostCoX. These findings agree with results reported by Serão et al. (2014), where the B allele was associated with increased PRRS antibody level. Similarly, BB pigs for SNP H3GA0020425 had significantly greater ADG PostCoX than AB or AA pigs, which is consistent with numerically greater PRRS antibody production reported by Serão et al. (2014). For SNP ALGA0039771, the AA genotype was associated with significantly lower PRRS VL PostCoX for Vx pigs. This finding is consistent with results from analyses of PRRSV-only-infected commercial nursery pigs, where AA pigs had significantly greater PRRS antibody production than AB or BB pigs (Hess, 2016). The opposite direction of effect was observed for NonVx pigs prior to coinfection, representative of growth under nonchallenged conditions, where AB pigs had significantly greater ADG than AA pigs.

For SNP MARC0058875, pigs with the AA genotype had significantly greater ADG PostCoX for NonVx pigs. This finding agrees with results presented by Hess (2016), where pigs with the AA genotype had significantly increased PRRS antibody production, but conflicts with results by Serão et al. (2014), where the B allele was associated with increased PRRS antibody level. For SNP DIAS0000349, pigs with the BB genotype had the highest ADG PostCoX. This conflicts with results reported by Hess (2016), where the AA genotype was associated with greater PRRS antibody production following PRRSV-only infection.

Conclusions

Results from this study not only validate the effect of WUR genotype, a major QTL for PRRS resistance, in a separate population of commercial crossbred pigs with another isolate of PRRSV but also indicate that the favorable allele following PRRSV-only infection is favorable following vaccination for PRRS with a commercial MLV vaccine and after coinfection with PRRSV and PCV2b. Results from this study provide novel insight regarding the role of this QTL on response to infection, including the finding that the WUR effect was numerically greater for PRRS VL following primary PRRSV exposure (whether by vaccination or coinfection) than secondary exposure and that the effect of WUR on PCV2b VL depends on whether or not pigs were previously vaccinated for PRRS. Our results also support the conclusion drawn by Niederwerder et al. (2015) that the early protective effect of the MLV vaccine was outweighed by increased mortality and days on treatment during the latter half of the coinfection period.

Heritability estimates indicate that ADG, PRRS VL, and PCV2b VL were lowly to moderately heritable, suggesting that genetic improvement of these traits is possible. Several interesting genetic correlations were detected, providing a first look at the genetic and phenotypic relationships among these traits. However, because the size of the data set was limited and, therefore, SE were large, care must be taken in drawing conclusions based on these estimates. Several candidate SNP associated with PRRSV-only or PCV2b-only infection from previous studies were also associated with host response to coinfection with PRRSV and PCV2b.

Taken together, these findings suggest that marker-assisted selection for WUR genotype alone, or alongside other candidate SNP for PRRSV or PCV2b-only infection, is a promising strategy to select for improved response to not just PRRSV infection but also coinfection of PRRSV and PCV2b and perhaps other pathogens. The genetic correlation between the 2 primary PRRSV exposure traits also suggests that response to vaccination can be used as an indicator for response to PRRSV infection with a field isolate, but because the SE of this correlation was large, further investigation is needed to solidify this conclusion.

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