Genetic Parameters and Effects for a Major QTL of Piglets Experimentally Infected with a Second Porcine Reproductive and Respiratory Syndrome Virus

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ABSTRACT: Blood samples were collected and weights recorded periodically for 42 days on commercial crossbred piglets for 4 trials, each of ~200 pigs, after being infected with Porcine Reproductive and Respiratory Syndrome Virus isolate KS-2006-72109. Blood samples were used to measure serum viremia, which was used to compute viral load (VL) from 0-21 days post infection. Heritability estimates of VL and weight gain from 0-42 days post infection (WG) were 0.65 and 0.44. Estimates of phenotypic and genetic correlations between VL and WG were -0.22 and -0.35. These estimates were similar to those previously reported for experimental infection with another PRRS virus. The effects of a SNP on SSC4 (*WUR10000125*) previously identified to be associated with VL and WG were significant but smaller than observed with the first virus, especially for WG, likely due to genetic and pathogenic differences between the two virus strains.
Keywords: Pigs; PRRSV; Susceptibility

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is found on nearly every continent of the world, affects all stages of pork production, and is a devastating disease to the pork industry, costing the U.S. pork industry alone $664 million per year (Holtkamp et al. 2013). To date, vaccines, biosecurity measures, and proposed methods for eradication have had limited success (Darwich et al. 2010). The aim of the PRRS Host Genetics Consortium (PHGC) is to identify genomic markers and pathways, associated with host response to PRRS, which could potentially be used for genetic selection of pigs for increased resistance or reduced susceptibility to PRRSV infection.

As part of the PHGC, Boddicker et al. (2012) identified a SNP on SSC4 (*WUR10000125*) for which the favorable allele (B) was associated with reduced viral load (VL) and increased weight gain (WG) after experimental infection with the NVSL-97-7895 PRRSV isolate. Virus genetic and pathogenic variability may result in differences in the effects of host response to infection. The objectives of this study were to compare 1) genetic parameters of host response and 2) the effects of the SSC4 SNP on animals infected with a more recent isolate (KS-2006-72109) that is genetically distinct from the previous isolate (89% amino acid sequence identity with NVSL-97-7895 in the GP5 viral gene). The aim of this study is to assess the usefulness of viral load and weight gain of piglets and the SSC4 marker, *WUR10000125,* for genetic selection of pigs with increased tolerance or reduced susceptibility to PRRS.

Materials and Methods

Experimental Design. This study followed the same experimental design as outlined by Boddicker et al. (2012). In short, this study involved 5 trials (trials 10-14) each of ~200 commercial crossbred piglets, which were transported to Kansas State University between 18-28 days of age. After a one week acclimation period, piglets were inoculated intramuscularly and intranasally with 10^5 $TCID_{50}$ of PRRSV isolate KS-2006-72109. Blood samples were collected at -6, 0, 4, 7, 11, 14, 21, 28, 35, and 42 days post infection (dpi). Weights were collected weekly. The piglets were euthanized at 42 dpi, and ear tissue was collected for DNA isolation for Illumina SNP 60k genotyping at the University of Alberta.

Phenotypes. From serum, viremia was measured at each time point using a semi-quantitative TaqMan PCR that is specific for PRRSV RNA. These results were reported as the log10 of PRRSV RNA per mL of serum, which is proportional to the number of replicating virion particles circulating in the host serum. These data were then used to calculate VL (area under the curve 0-21 dpi) using the algorithm developed by J.P. Steibel, as previously described by Boddicker et al. (2012). Weights collected were reported in kg and were used to calculate WG, which was defined as the total amount of weight gained from inoculation until the end of the trial (0-42 dpi).

Pedigree. For trials 10, 11 and 14, sire and dam information was provided for each piglet. Pooled semen was used for trials 12 and 13. Parent genotypes were available for trial 13. For this trial, parents were assigned to their respective offspring using a subset of ~1,250 of the highest quality SNPs in the parents, filtered by GC score (>0.8) and SNP call rate (>0.9). The parentage software Cervus 3.0 (Marshall et al. 1998) was used to assign a parent pair to each offspring. The output revealed that some piglets had parents that had not been genotyped. Individuals that could not be assigned to genotyped parents were assigned to sib groups using the genomic relationship matrix constructed from the Illumina 60k genotypes. Construction of the genomic relationship matrix also confirmed the assignments made by Cervus 3.0. Due to the absence of parent genotypes in trial 12, this trial was not used for variance component estimation but this trial was included in analyses to estimate the SNP effects of *WUR1000125*, with sire considered unknown.

Statistical Analysis. The presence of outliers were determined using the !OUTLIER statement in ASReml v 3.0 (Gilmour et al. (2009)). After removal of 10 outliers for WG and 28 outliers for VL (which were exclusively from trial 13), variance component analyses were carried out using ASReml v 3.0. For genetic parameter estimation, a univariate analysis was performed for VL and WG, with parity of sow nested within trial fitted as a fixed class effect, age and weight at infection (0 dpi) fitted as fixed covariates, and pen nested within trial, litter, and animal fitted as random effects. A bivariate analysis was also performed to estimate phenotypic and genetic correlations between VL and WG. To estimate the effects of the SSC4 SNP, the genotype was added as a fixed class effect in each univariate analysis. For these analyses, an additional 25 individuals for VL and 15 individuals for WG were excluded, either because they were missing the *WUR10000125* genotype, or they were determined to be outliers in the full data set, which included trial 12. The difference in least square means between the AA and AB genotypes was tested for significance. BB individuals were not considered in this test due to low numbers and because previous analysis had demonstrated that this SNP acts in a dominant manner (Boddicker et al. (2013)).

Results and Discussion

Genetic parameter estimates. Comparison of the summary statistics for the KS06 virus trials that were analyzed here and the NVSL virus trials analyzed by Boddicker et al. (2014) showed that the mean VL was lower and mean WG was higher for individuals infected with the KS06 isolate (Table 1). These data suggest that the KS06 virus may not be as virulent as its NVSL counterpart. However, more variation was observed for VL in individuals infected with KS06 than NVSL (Boddicker et al. (in press)). This is consistent with the observed viremia profiles for this virus, which also demonstrate that, while not having as high peak viremia, the KS06 isolate persists in the host for longer (data not shown). When comparing these two viruses, it is important to consider that these comparisons are confounded by time and genetics, although some of the trials analyzed here used the same genetic lines as some of the NVSL trials.

Heritability estimates for VL and WG (to a lesser extent) were both higher for the KS06 isolate than for the NVSL isolate, and the phenotypic and genetic correlations between VL and WG were also lower for the KS06 isolate (Table 2). This may be due to KS06 being less pathogenic; however, given the standard errors of these estimates, none of the estimates for the KS06 isolate are significantly different from those for the NVSL isolate.

Table 1. Summary of data used for viral load (VL) and weight gain (WG (kg)) following experimental infection with two PRRSV isolates.

Trait[§]	Virus Isolate	n	Mean	SD[¥]
VL	**KS06**	**684**	**92.8**	**7.95**
WG	**KS06**	**690**	**19.3**	**3.55**
VL	**NVSL**	**1416**	**106.9**	**7.34**
WG	**NVSL**	**1373**	**14.4**	**4.03**

[¥]Calculated as the square root of the sum of variance due to pen nested within trial, litter, animal, and residual variance, as estimated in ASReml.
[§]Values reported for the NVSL PRRSV isolate were reported by Boddicker et al. (2014).

Table 2. Estimates of genetic parameters for viral load (VL) and weight gain (WG (kg)) following experimental infection with two PRRSV isolates.

Trait[§]	Virus Isolate	$h^{2(\&)}$ (s.e.)	Litter[&] (s.e.)	r_p (s.e.)	r_g (s.e.)
VL	**KS06**	**0.62** (0.13)	**0.03** (0.04)	**-0.21** (0.04)	**-0.32** (0.21)
WG	**KS06**	**0.32** (0.12)	**0.02** (0.04)		
VL	**NVSL**	**0.44** (0.13)	**0.09** (0.05)	**-0.29** (0.03)	**-0.46** (0.20)
WG	**NVSL**	**0.29** (0.11)	**0.12** (0.05)		

[&]Reported as the proportion of phenotypic variance, which was computed as the sum of variance due to pen nested within trial, litter, animal, and residual, as estimated in ASReml.
[§]Values reported for the NVSL PRRSV isolate from Boddicker et al. (2014).

SSC4 SNP Effects on VL and WG. The SNP effects for *WUR10000125* for VL were in the same direction as those reported by Boddicker et al. (2014), although the effect of AA vs AB individuals was about 80% of the estimates obtained by Boddicker et al. (2014) for NVSL (Figure 1). The differences between these effects may be due to the fact that many individuals still have a substantial amount of virus circulating at 21 dpi for the KS06 virus, while individuals had nearly cleared the virus for the NVSL virus.

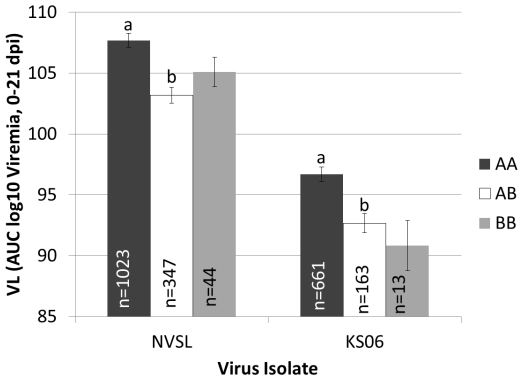

Figure 1. Estimates of the effects of SSC4 SNP (*WUR10000125*) on VL by PRRSV isolate[§*]

[*]Bars with different letters within the same virus isolate indicate a significant difference in the means ($p<0.05$). The mean BB genotype was not compared to other genotypes due to small numbers.

[§]Estimates of VL for the NVSL PRRSV isolate were as reported by Boddicker et al. (2014).

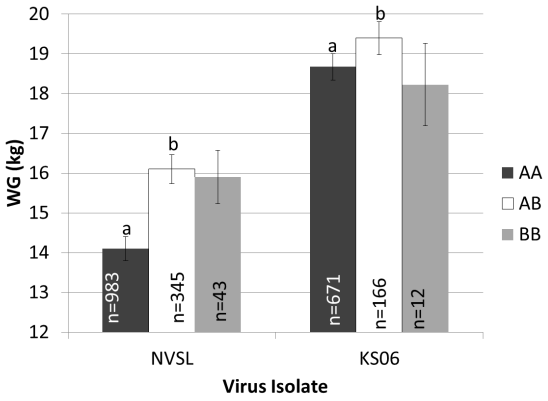

Figure 2. Estimates of the effects of SSC4 SNP (*WUR10000125*) on WG by PRRSV isolate[§*]

[*]Bars with different letters within the same virus isolate indicate a significant difference in the means ($p<0.05$). The mean BB genotype was not compared to other genotypes due to small numbers.

[§]Estimates of VL for the NVSL PRRSV isolate were as reported by Boddicker et al. (2014).

The SNP effects for *WUR10000125* for WG were also in the same direction as those reported by Boddicker et al. (2014), although the effect of AA vs AB individuals was about 36% of the estimates obtained by Boddicker et al. (in press) for NVSL (Figure 2). The much smaller effect of this SNP for WG may be due to lower pathogenicity of KS06 compared to NVSL, which could result in a smaller impact on weight gain, resulting in less of a difference between genotypes. As indicated previously, piglets in the KS06 trials grew faster than those in the NVSL trials; however, in order to make a fair comparison, trials with the same genetic backgrounds would need to be simultaneously infected with the different PRRSV isolates in the same location. This was not done, but it is possible to compare different trials with the same genetic background, while considering this confounding.

Conclusions

These results confirm that VL and WG have a highly heritable genetic component under experimental infection with PRRSV, and are antagonistically related traits, both phenotypically and genetically. The heritability and correlation estimates were numerically different from the estimates reported by Boddicker et al. (2014), for infections with the NVSL PRRSV isolate, suggesting that the genetic variance of VL and WG may depend on the virus isolate with which the pigs are infected. The SNP on SSC4 that was previously identified to be associated with WG and VL in piglets experimentally infected with the NVSL PRRSV isolate still had a significant effect on VL and WG with the KS06 isolate, although the size of the effects were smaller, especially for WG. Taken together, these results suggest that selection for increased resistance or tolerance to PRRS is feasible and that *WUR10000125* would be a useful marker for determining which individuals are expected to be less affected by PRRSV infection. The results of selection are expected to vary depending on the PRRS virus genetics, pathogenicity, or likely both. The results of selection are also likely to vary dependent on pig genetics. Genetic parameters of VL and WG and the effect of *WUR10000125* on these traits need to be confirmed in the more complex disease situations that animals are exposed to in the field.

Acknowledgements

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