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Regional Heritability Mapping of Production Traits in Epidemic Porcine Reproductive and Respiratory Syndrome

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ABSTRACT: Porcine Reproductive and Respiratory Syndrome (PRRS) is an important disease of pigs. Sow farrowing and service data were obtained from two commercial pig multiplication units which experienced several confirmed PRRS outbreaks. Genomic regions associated with reproductive failure during PRRS outbreaks were investigated using a regional heritability mapping (RHM) approach combining the two datasets. Covariates were explored both fitting and ignoring the shape of the epidemic. Heritability (h^2) of farrowing mortality (FMOR, proportion of dead piglets per litter) was 0.084 ignoring the epidemic shape and 0.059 fitting it. The additive genetic variance was non-estimable for the FMOR trait during non-epidemic phase. Two regions were significantly associated with FMOR at the genome-wide level, on Sus scrofa chromosomes (SSCs) 4 and 7, with several other regions approaching significance. A single SNP on SSC4 was significantly associated (P<0.001) with FMOR.

Keywords: Pig; PRRS; Regional Heritability Mapping

Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) is an infectious disease of pigs caused by the PRRS virus (PRRSV) with huge financial consequences to the pork industry. Reproductive failure, mummification of piglets and increased pre-weaning losses are direct outcomes of PRRSV infections. These losses appear alongside other clinical signs such as respiratory disease, reduced growth rates, increased incidence of co-infection and occasionally blue coloration on the ears, vulva or hind (Zimmerman et al. 2003). Compromised production plus increased monitoring and treatment costs contribute to losses estimated at $664 million p.a. to the US swine industry alone (Holtkamp, et al. 2012).

Control of PPRS continues to be problematic. The efficacy of current vaccines is limited by the rapid evolution of PRRSV and problems with escape mutants. An option for mitigating the effects of PRRS is the exploitation of host genetic variation in resistance or tolerance to infection; this would form part of a multifaceted solution, incorporating management strategies.

Heritable variation in response to the virus has been demonstrated both in-vivo and in-vitro (Lewis et al. (2007). Recent genome-wide association studies (GWAS) have shown genetic associations of resistance type traits and specific regions of the genome (Boddicker et al. 2012, Orrett et al. 2013, Serão et al. 2014). Boddicker et al. (2012) estimated the heritability (h^2) of variation of both weight gain and viral load at 0.3 in a challenge experiment

in growing pigs, indicating the potential for the genetic improvement of host resistance to PRRS.

This paper addresses the genetic control of the impacts of PRRS in reproductive sows. We combine reproductive performance data across two farms that experienced multiple PRRS outbreaks, one of which has been previously described by Lewis et al. (2009). The aim is to identify genomic regions common to the two datasets underlying reproductive failure in a PRRS outbreak.

Materials and Methods

Data. Data were available from two independent commercial pig multiplication units, collected from 2001 to 2007 in one farm and 2009 to 2012 in the other. These were combined to study reproductive traits during several confirmed PRRS outbreaks. The herds experienced repeated PRRS outbreaks over the period, confirmed by a commercial ELISA test (IDEXX, 2003, Maine, with sensitivity of 97.4% and specificity of 99.6%.).

These data described different reproductive outcomes, including numbers of live, stillborn and mummified piglets per litter. Also available were, additional information such as sow line, parity of sow, service date and farrowing date in addition to Illumina PorcineSNP60 BeadChip genotypes. We constructed a new trait, farrowing mortality (FMOR), defined as the number of dead piglets as a proportion of total piglets per litter.

Partitioning the data. Data were partitioned into two groups Epidemic (EPI) and Non-epidemic phase (NON). This was done according to the trend partitioning used by Lewis et al. (2009). The trend used was the rolling 30 day average dead piglets per litter, which showed discrete peaks identifying each of the ELISA confirmed outbreaks. Each farm was partitioned into the two phases separately. Within each farm a baseline period was identified when the herd was not exhibiting signs of PRRS. This period was used to calculate a 95th percentile of dead piglets per litter under non PRRS conditions. Confluent periods where the rolling 30 day average for the trait exceeded the 95th percentile, coinciding with the confirmed outbreaks, were defined as EPI phase data. One period on farm 2 showed a large peak over the threshold which did not coincide with a confirmed outbreak, and data from this period were treated as an unidentified epidemic and discarded. Two weeks either side of each identified epidemic were discarded as a buffer; the remaining data being treated as Non-epidemic phase data.

As only 31 animals had repeated farrowing events within the disease phase, there were insufficient repeated records to fit a permanent environmental effect and only the first farrowing event was used. Table 1 shows summary statistics for the FMOR in the EPI phase, detailing breakdown by farm.

Table 1. Descriptive statistics for EPI data subset

Farm	n	FMOR μ	FMOR sd
All	944	0.282	0.324
1	668	0.343	0.354
2	276	0.134	0.161

Statistical analyses. Regional heritability mapping (RHM) approach (Nagamine et al. 2012) is a variance components method of mapping quantitative trait loci (QTL). Firstly, a mixed model is fitted using Restricted Maximum Likelihood (REML), assuming no QTL (null model), as follows:

$$y_i = \mu + \sum_j \beta_j c_{ji} + G_i + \varepsilon_i$$

Where y_i is the phenotype of the ith individual; μ is the mean; β_j is the estimate of the jth fixed effect or covariate, c_{ji} is the value of the jth fixed effect or covariate for individual i and G_i is the random additive genetic effect for individual i. A genomic relationship matrix (GRM) describing the observed genomic relationships between animals is fitted to obtain the genomic estimated breeding values and estimate genome-wide additive genetic variation. Each chromosome is then divided into a series of overlapping windows with a given number of SNPs per window, and a region-specific GRM is then constructed for each window. Each window in turn is then fitted as an additional random effect to the null model, and the significance of each window tested using a likelihood ratio test (LRT). The derived statistic was taken to follow a distribution equal to ½ $\chi^2_{(0)}$ and ½ $\chi^2_{(1)}$. Significance thresholds were determined using a Bonferroni correction, scaling by half the number of windows fitted to correct for their overlap. The chosen window size was 50 SNPs with an offset of 25.

Table 2. Models fitted in Table 3

ID	Fixed Effects/ Covariates[§]	Random Effects
1	None	GRM
2	MumMean	GRM
3	MumMean	GRM + SSC4 Win 31
4	MumMean	GRM + SSC7 Win 50
5	ASGA0098976	GRM
6	ASGA0098976	GRM + SSC4 Win 31

[§]Additional to those included in all models Line + Parity + Epidemic + PC1 + PC2 + PC3

Once significant windows had been identified each of the SNPs in the window was fitted, in turn, as a fixed effect in a full mixed model to assess their significance, and their contribution to the region and genome-wide variance components. SNP genotype values (and their standard errors) were predicted, along with the SNP additive and dominance effects, and SNP-specific additive genetic variance.

Models fitted. Farm-specific epidemic was fitted as a fixed effect along with parity and line, to remove likely systematic effects in the data. In total, 11 lines were included, representing a range of breeds and F1/F2 crosses used on the two farms. Additionally, the 30 day mean mummified piglets per litter was fitted in some models (Table 3) as a covariate, to account for the environmental pressure of the epidemic.

Table 3. Heritability and variance component estimates for farrowing mortality (FMOR)

ID[§]	V_A	V_{Win}	V_E	V_P	$h^{2¥}$
1	0.00752	N/A	0.0820	0.0895	0.08
2	0.00433	N/A	0.0685	0.0728	0.06
3	0.272E-8	0.0231	0.0677	0.0908	0.00
4	0.00240	0.0171	0.0662	0.0858	0.03
5	0.00226	NA	0.0687	0.0710	0.03
6	0.407E-7	0.0015	0.0697	0.0712	0.00

[§]ID refers to the fitted model see Table 2 for key.
[¥]Heritability estimates are likely to underestimate the true value given the population diversity within the dataset and previously published results for one of the farms (Orrett et al. 2013.)

Given the range of breeding lines, hence the potential for population substructure, principal components (PCs) were also included in the model to account for these effects. The first ten PCs were investigated by plotting them against each other by line to understand the clustering. Although no PCs were significant under the Wald conditional F statistic, to further account for potential breed or population structure effects, the first three were added as these clustered the separate lines in keeping with known information about line origins.

Results and Discussion

Heritability estimates generated under the null model for EPI data are shown in Table 3 (ID 1 and 2) alongside variance component estimates. The standard errors of the heritabilities were ca. 0.04.

Figure 1 shows the likelihood ratio tests (LRTs) from the RHM analysis, for each window along the genome, along with the Bonferroni corrected genome-wide significance threshold. Two regions, on SSC4 and SSC7, reached genome-wide significance. These chromosomes are the same ones previously reported by Boddicker et al. (2012) for PRRS viremia in growing pigs and Serão et al. (2014) for antibody response in PRRS affected sows. The variances explained by the windows are shown in Table 2 (ID 3 and 4). Inclusion of the GRM of the SSC4 window #31 reduced the genomic heritability from 0.06 to 0.00 and gave a window heritability of 0.25. Inclusion of the GRM of the SSC7 window #50 reduced the genomic heritability to 0.03 from, 0.06, and gave a window heritability of 0.20. Neither QTL was seen in association analyses of the individual datasets, and the associations which were seen in

the individual datasets were much less significant (Orrett et al. (2013); unpublished results).

Figure 1. Regional Heritability Mapping showing LRT by region ordered by position on chromosome, identifying significant regions.
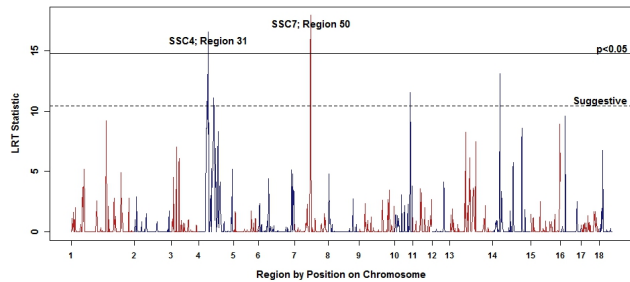
Farrowing Mortality ~ Line + Farm + Parity + EpidemicID + RollingTrend + PCs 1 to 3. Window = 50 SNPs Offset = 25

The two most significant windows were further explored by fitting the 50 SNPs within each window (SSC4 #31 and SSC7 #50) as fixed effects in a series of linear mixed models. For the SSC 7 QTL no single SNP had a large and significant effect, however a single SNP (ASGA0098976) appeared to explain the SSC 4 QTL effect. Fitting this SNP substantially reduced both the polygenic and regional heritabilities (ID 5 and 6, Table 3). If the SNP but no window was fitted, the polygenic heritability was 0.03, but if both were fitted the polygenic heritability was zero and the regional heritability was 0.02. The SNP effect was tested for sampling bias, no interactive effect was observed, with any of the other (co)variates.

The genotypic predictions for ASGA0098976 showed an associated additive effect (a=-0.063, P<0.001), with no dominance (d=-0.031, P=0.35). The additive variance ($2pqa^2$) was 0.002 representing 2.7% of total additive genetic variance. The AA genotype had a lower farrowing mortality than the heterozygote or rarer allele homozygote (GG). Linkage disequilibrium (r^2) of this SNP with adjoining SNPs were low, however the SNP lies within gene: Sus scrofa zinc finger protein, multitype 2 (ZFPM2), mRNA.

Since no specific single SNP association could be found within the SSC7 window, further work is needed to explore the haplotypes.

The precise regions and loci are as follows: SSC 4 Window #31 is a 1.8Mb region at position 33,962,423-32,121,356; SNP ASGA0098976 is on SSC4 at position 32,294,693 and SSC7 Window #50 is a 1.6Mb region at position 57,693,163-56,007,728. The loci identified by this study differ by 143 Mbp from the SSC 4 association reported by (Bodickker et al. 2012) and up to 27 Mbp from the SSC 7 association reported by (Serão et al. 2014).

Conclusion

RHM on the joint datasets has been very successful in identifying regions not identified on the individual datasets or by other methods. Associations also appear at a much higher level of significance. This method has not only identified regions of the genome in association with farrowing mortality, but helped isolate an individual SNP which shows no interactive effect with any other structure in the dataset.

There are different types of variation of response to infectious disease, e.g., resistance (or susceptibility), resilience or tolerance. Given the lack of infection status for individual animals, tolerance and resistance/ susceptibility are beyond the scope of this analysis. Resilience is modelled here in general terms as the ability to perform despite substantial infection pressure. The 30 day rolling mean was used as both a method of partitioning (as has been previously used) and as a covariate describing the mean effect of the epidemic, on contemporary farrowing animals. By accounting for this mean we model the environmental pressure on animals, as we are interested in how well animals perform in relation to their contemporaries at a given time-point. This signal would represent a resilience type trait. However, there is a risk that fitting the epidemic mean may also remove genetic information, hence we fitted with and without the rolling mean.

This study identified two regions of the porcine genome coinciding with previously reported chromosomes and, in one of these regions, a single SNP associated with farrowing mortality during PRRS outbreaks. Given the distance on these chromosomes from previously reported associations these are thought to represent new candidates for the genetic improvement of the host in terms of resilience.

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