RESEARCH ABSTRACT

SWINE HEALTH

Title: Impact of host immunity and genetics on persistence of PRRS virus in tonsils – **NPB #14-223**

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Scientific Abstract:

Despite extensive efforts to eliminate PRRS from US production facilities, it remains a key disease issue and poses a continued economic threat to the industry, particularly in pig dense areas. A major factor that complicates PRRS control is viral persistence. Viral survival is maintained because a proportion of the herd has persistent virus which is shed occasionally (due likely to other diseases or stress). This shed virus then infects the remaining herd pigs which are naïve and thus susceptible. This project proposed to identify pigs which have persistent PRRSV infections and to determine if there are immune or genetic correlates of PRRSV persistence.

Currently there is no good technology to accurately identify PRRSV carrier pigs. This proposal determined the frequency of pigs with persistent PRRSV using viral RNA levels in tonsil as a surrogate measure of persistence. To perform this, we took advantage of the repository of samples that were collected through the NPB funded PRRS Host Genetics Consortium (PHGC). Each PHGC pig, provided at weaning from current commercial breeding stocks, was infected with a type 2 PRRSV isolate and followed for 42 days post infection (dpi). Every pig that survived to 42 dpi had tonsil tissue archived. Moreover, the PHGC database (www.animalgenome.org/lunney/index.php) contains extensive data on each PHGC pig, including its pedigree, response to PRRSV infection (viral and antibody levels and weight gain data), and extensive genotypic information (60K SNP chip).

Our work with tonsils from pigs infected with virulent NVSL97 or moderately virulent KS-06 type 2 PRRSV isolates showed limited relationship between serum viral load (VL; 0-21 dpi) with tonsil viral RNA levels. This limited correlation is not unexpected since PRRSV is known to persist in tissues, particularly the tonsil, well past the time when serum viremia has cleared. When comparing the two viruses our data indicated that there is much lower cumulative virus with KS-06 as compared to NVSL97 infected pigs. This grant affirmed that pigs infected with a moderately virulent PRRSV (KS-06) exhibited similar tonsil persistence characteristics to the virulent NVSL infection.

Hess et al. (2018) verified that pigs identified as having persistent serum viremia levels for NVSL had significantly higher TV than pigs that had cleared serum viremia ($P<0.001$). There was a similar trend for KS-06 but differences were not as significant. Tonsil virus level was estimated to be lowly heritable for both type 2 PRRSV isolates (Hess et al. 2018). An earlier and faster virus clearance was associated with lower TV for both NVSL and KS-06 infected pigs. Analysis of weekly weight gain did not reveal any associations with TV. When anti-PRRS antibody responses were analyzed there were significant associations with TV in KS-06, but not

NVSL, infected pigs (Hess et al. 2018). Animals that had a higher antibody levels at the end of the trial had lower TV, while animals with higher neutralizing antibody titers had higher TV.

More detailed genome wide association studies (GWAS) revealed several genomic regions that explained a proportion of genetic variance and several strong candidate genes. Genes involved with the host's ability to control viral infiltration/replication and the ability to clear infected cells from tissue may be useful targets for gene expression analyses on tonsil tissue that are underway (Hess et al., 2018).

Finally, tonsil RNA was probed for host genes that might be involved in persistence. Overall, 12,597 genes were determined to be expressed in the tonsil with 1646 and 336 differentially expressed genes (DE genes, $q \leq 0.2$) identified between PRRSV isolate and TVclass, respectively (Dong et al. 2018 in preparation). Pathway analysis results showed that both KS-06 and high TV were associated with DE genes predicted to increase the quantity, proliferation, differentiation, cell movement and adhesion of immune cells, especially T cells, compared to NVSL and low TV. Preliminary results indicate that there were 4 DE genes that were significantly up-regulated in tonsils from pigs from high versus low tonsil viral level: CXCL10, TBX21, CCL5 and CCL19. Overall, nursery pigs infected with a less pathogenic PRRSV isolate, or that have higher tonsil viral level, have a stronger tonsil immune response.

Collectively, these DE gene results suggest that KS-06 infection may result in less tonsil tissue damage by regulating genes related to cell and tissue morphology. High tonsil virus levels may activate the expression of genes that trigger cellular immune responses to clear virus that persists in tonsils and inhibits virus replication. These findings contribute to our understanding the mechanisms involved in tonsil pathology induced by PRRSV infection in pigs. Based on this, efforts can be planned to selectively breed for pigs with lower tissue persistence or, alternately, to identify means of stimulating anti-viral responses in pigs with persistent PRRSV infections.