UNIVERSITY OF MINNESOTA
Swine Disease Eradication Center

November 5, 2012
Volume 1, Issue 10

www.cvm.umn.edu/sdec

SDEC Partners Research Update

Project Update: The effect of PRRSV infection on the expression of the antibody repertoire

Investigators: John C. Schwartz and Michael P. Murtaugh

Funded by the National Pork Board (10-139), Institute for Molecular Virology Training Program (NIH T32 AI83196), CVM Comparative Medicine Signature Program Grant

Background

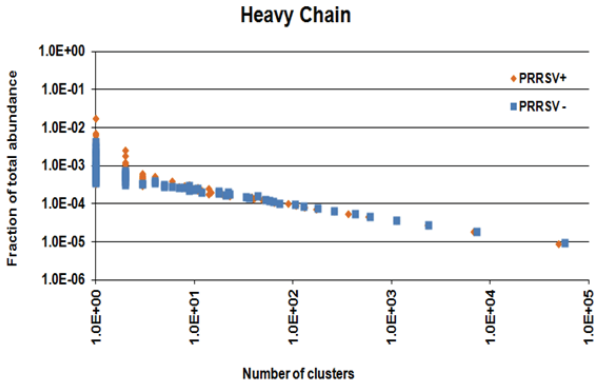
- PRRSV remains one of the most significant economically important diseases facing the swine industry. In PRRSV infections, the antibody response is variable in neutralization function, antigenic specificity, and duration.
- Whether the variation is due to the response capacity of individual swine or other factors is not known.

Objective

To compare the immunoglobulin mRNA diversity of PRRSV-infected and uninfected pigs to determine the effect of PRRSV infection on the expression of the antibody repertoire

Results

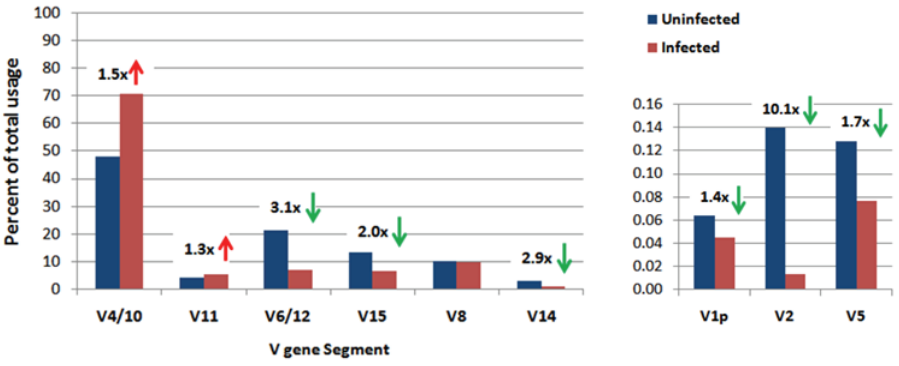
- The heavy chain library contained approximately 450,000 reads with an average length of 424 bases which allowed for the analysis of the entire variable region. The number of high-quality reads were similar for each group (220,857 reads for the infected group and 224,410 reads for the control group). Total repertoire richness was calculated using concatenated complementarity determining region (CDR) sequences and was calculated to be approximately $3x10^5$. Cluster analysis of all three concatenated CDRs revealed a power law distribution of heavy chain sequences where the vast majority are exceptionally rare and a small number are very common. (Figure 1A).



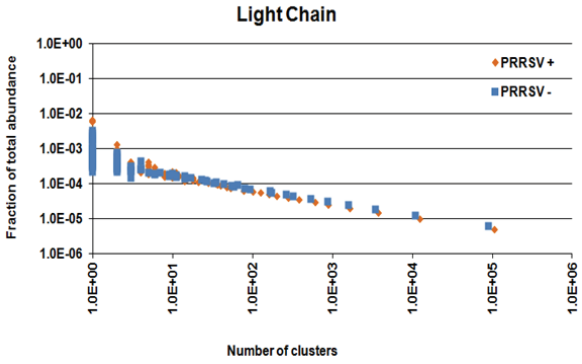
(Figure 1A).

Results

- There was a greater abundance for a small number of sequences in the infected group compared to the uninfected group, likely owing to antigenic stimulation and expansion of specific B-cell clones due to PRRSV infection. From these highly abundant sequences, we discerned the putative amino acid sequences for the full length transcripts. As these sequences were found to be exceptionally rare in the uninfected group, we postulate that these represent PRRSV-specific heavy chain sequences. It was found that many of these putative PRRSV-specific sequences derive from IGHV4. A chi-squared analysis of IGHV gene usage also found a significantly increased usage of IGHV4 in infected animals. In contrast, there was a significant compensatory decrease in usage for all other annotated IGHV genes in infected animals (Figure 2).



(Figure 2).



(Figure 1B).

- Our light chain library contained approximately 375,000 reads that were long enough to analyze the entire variable region and which contained no frameshifts or pre-mature stop codons. The number of reads per animal were similar (ranging from 19% to 23% of the total for 5 animals). The total richness of the light chain repertoire was slightly less than that of the heavy chain locus (~$2x10^5$), likely owing to the reduced activity of terminal deoxynucleotidyl transferase during light chain rearrangement. Cluster analysis of the light chain repertoire revealed essentially the same power law curve as seen for heavy chain (Figure 1B). Again, several highly abundant sequences were observed in the PRRSV-infected group. Two of the most abundant sequences differ by only two amino acids in CDR-L3 and result from the expression of the IGLV3-3 gene segment. Comparison of reads to the annotated light chain loci revealed a high level of expression of a previously undescribed IGLV3 family member in four of the five pigs investigated.

Conclusions

- Analysis revealed several heavy and light chain transcripts that were highly abundant in PRRSV infected pigs, yet rare in uninfected pigs.
- Genetic differences between pigs in the lambda locus resulted in significant alterations to the expressed antibody repertoire.

Implications

- This study is the first to investigate the immunoglobulin repertoire in pigs using deep sequencing and the first to compare the expression of the repertoire following infection.
- Knowledge at the molecular level of porcine antibody structures that respond to and recognize PRRSV antigens will fill a critical gap in our understanding of the pig's immune response to PRRSV that may reveal specific antibody molecules that mediate immune protection against PRRSV.