RESEARCH ABSTRACT

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SWINE HEALTH

Title: Development of a bELISA for serological diagnostics and surveillance of SVA infection -- **NPB #17-092**

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Scientific abstract:
Senecavirus A (SVA) is a small, non-enveloped, positive stranded RNA virus responsible for causing severe vesicular disease and neonatal mortality. The problem with this emerging virus is that it is clinically indistinguishable from foot and mouth disease virus (FMD). Currently, there are limited antibody reagents and diagnostic tests available for herd surveillance and confirmation of disease. To address these industry needs, we immunized mice to produce monoclonal antibody (mAb) reagents against capsid proteins VP1 and VP2 for immunohistochemistry (IHC), fluorescent antibody (FA) staining and virus isolation (VI) testing. In addition, we developed two separate serological assays: a blocking ELISA (bELISA), and a new mAb based, highly sensitive, serum virus neutralization (SVN) assay for immune surveillance of disease.

The first application of our resultant mAbs lead to the development of an immunohistochemical test (IHC) for routine *in-situ* detection of viral protein antigen within tissues. We are the first to report the development of an IHC test which should carry the same specificity as the bELISA and will provide a value-added tool for the differentiation of other diseases that cause vesicular lesions in swine. Second, early testing showed that SVA positive serum samples tested against the recombinant capsid proteins showed that VP2 had significantly greater immunoreactivity than VP1 and provided the rationale for the development of the VP2-bELISA. The VP2-bELISA was optimized by checkerboard titration using samples of known serostatus. Serum samples were obtained from uninfected pigs (n = 628) and SVA-infected animals (n=254). ROC analysis showed diagnostic sensitivity of 88.3% and a specificity of 93.6%. The bELISA demonstrated the ability to detect a broad serological response as soon as 5 DPV including the presumable appearance of IgM which is produced early during infection. Next, inter-rater (kappa) analysis was performed to assess testing agreement between the bELISA and SVN assays. The kappa value was calculated to be 0.806 demonstrating significant testing agreement. Although the sensitivity of the bELISA was lower than our previously developed indirect ELISA (iELISA), the specificity was significantly higher. We tested the ability of bELISA to resolve incidences of unexpected positive samples using 25 random “false-positive” samples generated by our previously developed iELISA. Notably, 23/25 samples (92%) were resolved as “true negative” samples while all 25 samples were resolved as true negatives via SVN testing. The antibody dynamics of the SVN test mirrored the performance of the bELISA in its ability to detect seroconversion at 5 DPV; however, it was able to detect a more robust antibody response over a longer period of time. Thus, the advantages of the bELISA and SVN are their ability to detect an early and broad range of antibodies regardless of isotype, along with improved specificity. Taken together, the antibody reagents and diagnostic tests will provide producers much needed, sensitive and specific assays to serologically monitor and differentiate incidences of vesicular disease outbreak.