RESEARCH ABSTRACT
SWINE HEALTH

Title: Development and evaluation of antibody detection methods in oral fluids for cost-effective and efficient surveillance and control of Senecavirus in swine population. **NPB#18-099**

Investigators: Pablo Pineyro, Luis G. Gimenez-Lirola

Institution: Iowa State University

Date Submitted: 2/29/2020

Scientific Abstract:

Senecavirus A (SVA) is an RNA virus in the family Picornaviridae. The virus has recently been detected in swine production systems, causing vesicular disease and neonate mortality. The viral capsid is composed of four structural proteins: VP1-VP4. Although the VP1 protein has been reported as the most immunogenic protein *in vivo*, no information on immunodominant regions of the SVA polyprotein is available. Besides the large amount of information generated regarding the humoral immune response and different tests reported to detect antibodies in serum, there is no information available regarding the antibody response in oral fluids.

The objectives of this study include **1)** identifying immunodominant regions of SVA polyprotein using epitope mapping, **2)** developing and validating SVA oral fluid ELISA in known status samples, **3)** determining the dynamic of immunoglobulins isotype in oral fluid during SVA active infection, and **4)** evaluating performance of assays.

The binding effect of a SVA polyclonal antibody (SVA-pAb), a set of SVA-VP1 monoclonal antibodies (mAb), and SVA positive sera from clinically affected animals was characterized using a set of SVA-VP1- and SVA-VP2-derived peptides by indirect and blocking ELISAs. Structural proteins' epitope array was based on 18 SVA-VP1 and 31 SVA-VP2 peptides, overlapping 20-mer and shifted by five amino acids. All VP1-derived peptides yielded significant signal against SVA-pAb and VP1-mAb by indirect ELISA. One peptide (aa 1-20) showed significantly higher OD on rVP1 and SVA whole virus indirect ELISAs. Peptides spanning aa 1-20, 60-80, 105-125 and 120-140 showed a significant half-maximal inhibitory concentration (IC50) on VP1-mAb against rVP1 by blocking ELISA. The VP2 epitope array was capable of detecting significantly higher signal from five overlapping amino acid residues (peptides 8, 9, 10, 11, and 12) spanning

amino acids 105-185 and two non-overlapping amino acid residues (peptides 27 and 29) spanning amino acids 390-410 and 420-440. Although several peptides showed variable binding inhibitory effects of SVA-pAb and SVA-positive clinical samples, none of the peptides reached a 50% inhibitory effect. These results suggest that the humoral immune response against SVA-VP1 and SVA-VP2 can be defined by a set of linear epitopes.

The presence of specific antibodies in oral fluids was evaluated in experimentally infected animals. Thirty pigs were randomly allocated to three different treatment groups and housed in pens, with two pigs per pen. Each pig received 5 mL 1 x 10^9 TCDI50/ml of historical SVV-1 strain (SVV group; n=10), SVA contemporary strain (SVA group; n=10) or RMPI media (Negative control group; n=10). Oral fluids and serum were collected at 0, 3, 7, 10, 14, 21, 28, 35, 42, 49, 54, 60 and 67 dpi. The presence of different antibody isotypes was evaluated in serum and oral fluids by a VP1 recombinant protein ELISA. Although animals inoculated with SVA showed a moderate IgG response in serum that lasted four weeks, no significant increment on IgG levels on oral fluids was observed. The presence of IgA was observed by the SVA-VP1 IgA ELISA in both inoculated groups after 7 dpi. The presence of specific Senecavirus antibodies was detected and sustained throughout the duration of the study. Although there were significant differences in S/P detected between both strains used for this study on specific time points (42 dpi), the test was able to discriminate positive groups. ROC analysis was performed for both Ig isotypes among animals infected with both Senecavirus strains. For IgG, an S/P value of 0.17 showed a sensitivity of 39% and specificity of 93%, while for IgA, an S/P value of 0.25 showed a sensitivity of 77% and specificity of 93%.

Based on these findings, it can be concluded that SVA immunological response could be driven by a small set of linear epitopes in the VP1 and P2 structural proteins. These immunodominant epitopes could be used as a target for immunological diagnostic assays. The immune response against Senecavirus infection can be detected in oral fluids and is largely IgA isotype based. This result supports the idea that oral fluid has the potential to replace individual pig serum samples for both SVA routine and emergency disease surveillance.