RESEARCH ABSTRACT

SWINE HEALTH

Title: Development and validation of Singleplex and Multiplex Luminex Assays for Detection of antibodies to foot-and-mouth disease (FMD), swine vesicular disease (SVD), classical swine fever (CSF) and African swine fever (ASF) viruses in porcine oral fluids – NPB# 15-177

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Scientific Abstract:
Swine oral fluids (OF) are increasingly being used for the diagnosis of diseases in pigs. We have previously shown (NPB# 14-286) that FMDV, SVDV, CSFV and ASFV nucleic acids and antibodies can be measured in OF. Antibodies were detected by enzyme-linked immunosorbent assay (ELISA), which might be of less sensitivitiviy for OF than for serum. Luminex assays are potentially more sensitivity and have multiplexing capability. The objective of this project was to develop and validate singleplex and multiplex Luminex assays for detecting antibodies to FMDV, CSFV, ASFV and SVDV in OF. Specific objectives were to generate swine OF for test method development and validation through experimental inoculations of pigs with FMDV, SVDV, CSFV and ASFV; obtain samples from other members of the FAD Oral Fluid Consortium and laboratories in endemic countries; develop individual Luminex assays for detection of antibodies to FMDV, SVDV, CSFV and ASFV in swine OF; validate and compare methods for antibody detection for the 4 viruses in OF and serum by singleplex and multiplex Luminex assays.

Groups of pigs were either directly inoculated intradermally in the heel bulb of one hind limb with cell culture supernatants containing FMDV or were inoculated by contact with the directly inoculated pigs. For SVDV, each pig was inoculated intradermally in the heel bulb of one hind limb and oronasally with cell culture supernatants containing SVDV (4 groups of 4pigs /group). CSFV and ASFV inoculations were performed by administering virus oronasally to each animal. Oral fluids were collected from each group of pigs using cotton ropes. Serum and swabs of the mouth and nares were also collected from individual animals.

Luminex assays were developed using recombinant 3ABC (FMDV), 3D (SVDV), E^{rns} (CSFV) and p54 (ASFV) antigens produced in the baculovirus expression system. Optimal amounts of antigen were coupled to fluorescent beads. The antigen-coupled beads were mixed with equal volume of OF in predetermined wells. Wells of negative and positive control sera were included on each plate. Plates were incubated in the dark at room temperature with shaking for 2 hours, washed 3 times with PBS (no tween)

and a cocktail of biotinylated goat anti-swine IgA, IgM and IgG at 1/400 in StabilGuard® Immunoassay Stabilizer (BSA-Free**,** Surmodics IVD, Inc., USA**)** was added to each well at 50 uL/well. Plates were further incubated for 30 min, washed, streptavidin- phycoerythrin diluted 1/100 in StabilGuard was added to all wells at 50 uL/well and plate incubated for 15 minutes followed by a final wash. Beads were resuspended in 150 uL of PBS and analyzed on the MAGPIX® instrument. Median fluorescence intensity (MFI) for each sample was recorded and the data expressed as a percentage of the ratio of the MFI for the test sample (S) to the MFI of the positive (serum) control (P) ie %S/P = MFI S/MFI P X 100.

For multiplexing, the haemorrhagic diseases (ASF and CSF) and the vesicular diseases (FMD and SVD) were combined into duplex assays. At least 391 samples from naïve animals as well as oral fluids from experimentally infected animals were tested to establish the specificity and sensitivity respectively for each assay. Oral fluids were also tested by ELISAs optimized for OF (Senthilkumaran et al, 2016, 2017; NPB #14-286 report) to confirm the presence of relevant antibodies.

Singleplex Luminex assays for the detection of antibodies to FMDV, SVDV, ASFV and CSFV in OF were successfully developed and partially validated. Positive antibody detection for FMDV 3ABC started as early as 4 DPI. Positive antibody responses to SVDV 3D started at 14 DPI. Similarly positive antibody detection for ASFV and CSFV started at 10 and 14 DPI respectively. Comparable results were obtained in the duplex and singleplex assays. These results also mirrored those for corresponding sera from groups of pigs. The data demonstrates that Luminex assays can be used for the detection of antibodies to FMDV, SVDV, CSFV or ASFV in OF. There is therefore a high potential for the use of OF for FMDV, SVDV, CSFV or ASFV surveillance using both Luminex assays and ELISAs.