RESEARCH ABSTRACT
SWINE HEALTH

Title: Use of oral fluid (OF) samples to monitor virus shedding and antibody responses in pigs experimentally infected with high consequence swine viruses (foot and mouth disease, African swine fever, swine vesicular disease and classical swine fever viruses); **NPB #14-286.**

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Date Submitted: August 2, 2017

Scientific Abstract:

The use of swine oral fluids (OF) for the detection of virus nucleic acids and antibody response to pathogens is becoming common practice. Consequently, assays have been developed for this purpose for endemic and foreign animal diseases of swine. Detection of foot-and-mouth disease virus (FMDV) genome in swine OF has previously been demonstrated. Confirmatory assays for FMDV diagnosis such as virus isolation and viral antigen detection have not been evaluated using swine OF. We have now validated molecular detection of FMDV in OF, evaluated antigen detection and FMDV isolation from swine OF and developed an IgA isotype ELISA for anti-FMDV antibody detection in OF. FMDV genome was detected in OF from experimentally infected pigs by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) from 1day post-infection (DPI) to 21 DPI. Live virus was isolated from OF at 1-5 DPI. Additionally, FMDV antigen was detected in OF from 1-6 DPI by a lateral flow immuno-chromatographic strip test and from 2-3 DPI using a double antibody sandwich ELISA. In addition, FMDV-specific IgA was detected in OF using an isotype-specific indirect ELISA starting at DPI 14.
These results further demonstrate the potential use of OF for FMDV genome, live virus, and viral antigen detection, as well as quantification of mucosal (IgA) antibody response.

For SVDV, viral RNA was detected in OF by qRT-PCR from 1 DPI to 21 DPI; and live virus isolated at 1-5 DPI. A competitive ELISA based on the monoclonal antibodies 5B7 was modified to detect antibodies to SVDV in OF starting at 6 DPI. Using isotype-specific indirect ELISAs, SVDV-specific IgM response was detected in OF starting at 6 DPI, peaking at 7 or 14 DPI and declining sharply at DPI 21 while IgA response started at DPI 7, peaked at DPI 14 and remained high until the end of the experiment.

In the first experiment, CSFV genome (at Ct considered positive) was detected in OF starting at 10 DPI and levels increased until the end of the experiment at 18 DPI. In experiment 2, CSFV genome was detected in OF starting at 14 DPI, coinciding with detection in oral swabs. However, CSFV detection in serum was earlier and stronger compared to OF and swabs. Using the commercially available IDEXX ELISA kit for anti-CSFV antibodies in swine OF, positive antibody respo

nse was detected at 9 and18 DPI in experiment 1 (n = 1 group) and at 14 to 21 DPI in experiment 2 (in 2 of 4 groups).

ASFV Malta genome was detected in OF starting at 6 DPI to 14 or 21 DPI. Detection of virus in oral and nasal swabs mirrored detection in OF. However, whole blood was the best sample type for ASFV detection, becoming positive at 4 DPI and containing higher levels of virus genome in most pigs.

A fully integrated and automated assay for simultaneous detection and differentiation of FMDV, SVDV, CSFV and ASFV was developed. The fully integrated/automated assay was optimized, validated and used to successfully process and detect cell culture amplified viruses, as well as FMDV, SVDV, CSFV and ASFV in OF.

Our data demonstrates that OF can be used for the detection of genome and live virus of FMDV, SVDV, CSFV and ASFV. Additionally, FMDV antigen can be detected in OF. Furthermore, antibodies to these viruses can be detected in OF by a variety of serological assays, including competitive and isotype-specific (IgA and IgM) ELISAs. Furthermore, detection of IgA in OF has the potential use for detecting antibody response following vaccination. All these results point to the high potential for the use of OF for FMDV, SVDV, CSFV or ASFV surveillance employing both established and partially validated assays. However, there is the possibility that virus detection in OF may vary for different virus species, between serotypes of the same virus, under different experimental settings, and/or sample handling and testing conditions.