RESEARCH ABSTRACT

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SWINE HEALTH

Title: Evaluation of African swine fever virus antibody detection and viral nucleic acid detection dynamics in swine in various sample types – NPB #20-176

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Scientific Abstract:
This study evaluated the detection of African swine fever virus (ASFV) nucleic acid and antibodies against ASFV in pigs inoculated with an attenuated strain (MK200) of the Mozambique 1978 virulent variant. There were two groups of 8 inoculated pigs. The first group was inoculated with MK200 and the second was vaccinated with a porcine reproductive and respiratory syndrome (PRRS) modified live vaccine of the European type 10 days prior to inoculation with MK200 ASFV. There were two control groups of three pigs each, one was inoculated with PRRS MLV and one was not. The latter group was cross contaminated with a virulent form of ASFV at day 20 and were euthanized. These results were discarded.

Buccal swabs, tonsillar scrapings, oral fluids (individual), serum and blood were collected every 3 to 7 days over a 93-day period. Ear notches were also taken during the first 11 days and last 10 days. All sample types were extracted using two commercial nucleic acid extraction kits and the extracted material was tested on two commercially available real-time ASF PCR kits. The serum and oral fluids were also tested on five commercial enzyme linked immunosorbent assay (ELISA) kits from three companies with one being the Ingenasa ASF ELISA. All procedures and validation confirmation were done following the manufacturers' directions.

The positivity rate was calculated stratified by group, period, sample type and diagnostic kits. For purposes of this study, we assumed all animals were positive for the duration of the 93 days for nucleic acid testing and evaluated serum and oral fluids after day 7, 12 and 16/17 dpi. We confirmed that the assays properly classified known negative samples as well. These included all day 0 samples for Group 1 and 2, day 10 from Group 2 prior to ASFV inoculation and all control group samples were used. The 95% confidence intervals for proportions were calculated using the Wilson interval for sample sizes <50 and the Agresti-Coull interval for samples ≥ 50. Agreement between kit results was evaluated using the Brennan & Prediger method to control for prevalence and bias. All data analysis was performed in STATA IC 16.1 (STATA Corp, College Station, Texas). Data was stored in Microsoft Excel files (Microsoft, Redmond, Washington).

This study showed that ASFV nucleic acid was detectable from day 1 to 93 in all sample types and antibodies were detectable from day 6 to 93 in all sample types. The number of animals that had viral nucleic acid detected decreased over time, but this differed with sample type. Oral fluids had the best period of detection from day 1 to 7 in Group 1 with a percent positivity of 83.3% and in Group 2 of 36.8% (MagMax Core extraction and IDEXX REALPCR™ ASFV DNA TEST). Blood samples had a high positivity rate of 85.2% (IDEXX RealPCR™ DNA/RNA Magnetic Bead extraction

and IDVet PCR) from day 8-21 in Group 1 and of 60.7 (IDEXX RealPCR™ DNA/RNA Magnetic Bead extraction and IDEXX REALPCR™ ASFV DNA TEST) in Group 2. There was no combination of extraction and PCR kits that returned a significantly higher positivity rate over time and sample type but using the measure of highest positivity rate alone, the MagMax Core extraction with the IDEXX REALPCR™ ASFV DNA TEST gave the highest values. Antibodies were detectable from day 6 to 93 with high rates of sensitivity in blood for Group 1 after 14 days post infection (>90%) and 80% for Group 2. The level of detection was lower in oral fluids and the Ingenasa ELISA only classified one oral fluid sample as positive. There was no statistical difference in the kits rates of positive detection, but the highest positivity rate was consistently returned by the Innovative Diagnostics IDScreen ELISA assays.

There was a consistent trend between groups 1 and 2 where the positivity rate dropped in the PRRS vaccinated group among all sample types with the PCR and in ELISA when using blood. Confidence intervals did overlap, which may suggest there is no real difference, or that we were constrained by the sample sizes and are unable to tell if this trend is real or not.

Attenuated ASFV variants are common today due to vaccine development and field trials. It is clear that we can detect viral remnants for up to 93 days in a variety of tissues, although oral fluids does do best in the long term and blood in the short term. Co-infections with other viruses, mimicked here by a PRRS MLV, showed a consistent trend of a lesser ability to detect, but did have overlapping confidence intervals, meaning the trend may be an artifact between groups. A future study should evaluate co-infection with a pathogen and not a vaccine strain to elucidate a difference.

Early detection of attenuated viruses will require consistent ongoing testing within a system and a positive result should classify the herd as positive and not just a singular animal since animal level testing is inconsistent. Antibody testing of animals that have been in a location for greater than 14 days when coupled with robust biosecurity protocols may be the best combination of assets to ensure a herd is not infected with an attenuated virus.