RESEARCH ABSTRACT

SWINE HEALTH

Title: Development of classical swine fever virus diagnostic assays for porcine oral fluid samples - **NPB #11-001**

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Scientific Abstract:

The primary objective of this project is to optimize and validate technology capable of rapidly identifying premises infected with classical swine fever virus (CSFV) following its introduction into North America or other CSFV-free areas using oral fluid samples. The *in vivo* pilot experiment was previously conducted and found that a high virulence CSFV strain (Bangkok-1950) at the titer of 10^3TCID$_{50}$/pig given intramuscularly killed the piglets within 5 days and infected pigs were unable to chew the rope while a low virulence CSFV strain (ALD) showing mild clinical signs did not kill the inoculated animals. The protocol, therefore, was modified to answer the field questions on vaccinated animals or animals previously exposed to the low virulence CSFV strain being re-challenged with the high virulence CSFV strain acting as CSFV reservoir. Producing subclinically CSFV-infected pigs allowed us to successfully collect the oral fluid samples mimicking the situation of using modified live CSFV vaccines in CSFV-endemic countries. In the present study, 20 3-week-old pigs were obtained from a free-CSFV farm with sow vaccination and divided into 3 groups. Pigs in group A were intramuscularly inoculated with a low virulence CSFV strain (10^5TCID$_{50}$/pig) on day 0 and re-challenged with the virulence strain (10^3TCID$_{50}$/pig) intramuscular at 14 days post inoculation (DPI). Pigs in group B was intramuscularly vaccinated with a commercial modified live CSFV (LOM) vaccine on day 0 and challenged with the virulence strain similar to pigs in group A at 14 DPI. Group C was a negative control group. It should be noted that all vaccinated- and low virulence CSFV infected-pigs had no obvious clinical sighs showing a few hemorrhagic lesions when necropsied. All samples including serum and oral fluid samples were tested negative using a routine RT-PCR method (sensitivity at 10^3 TCID$_{50}$/ml). Based on the modified real-time RT-PCR results (sensitivity at $10^{1.5}$ TCID$_{50}$/ml), a few vaccinated pigs showed viremia for at least 2 weeks after vaccination. When re-challenged with a high virulence strain, a few vaccinated- and low virulence CSFV-infected pigs were sporadically tested positive and 1 of 8 vaccinated-pigs was tested positive at the end of the experiment (16 days post re-challenged). However, none of the oral fluid samples from all groups were tested positive using either the real-time RT-PCR or the routine RT-PCR. It could be that the sensitivity of the tests was not sensitive enough to detect the low CSFV levels in the oral fluid samples or it could be due to the difficulty of the genetic material extraction method hampered by mucous component of the saliva.

A potential use of a commercial blocking ELISA of classical swine fever antibody detection was also evaluated comparing to a neutralizing peroxidase-linked assay (NPLA) using oral fluid samples from a CSFV negative herd mixed with serially diluted known CSFV antibody tiers. Interestingly, the low levels of NPLA antibody titers ($\log_2$ 1- $\log_2$ 3) could evidently be detected in the oral fluid samples (lower than average serum NPLA titers of pigs in the same group, $\log_2$ 3- $\log_2$ 6) but none of the oral fluid samples from this present study were tested positive by a commercial ELISA (sensitivity at least $\log_2$ 4). It should be noted that NPLA titers detected in the pooled oral fluid samples in each group were equal to or less than $\log_2$ 3, resulting in all negative detection when tested by the ELISA test kit. However, the present study proved that CSFV antibody could be detected in the oral fluid samples using NPLA and ELISA methods. Validation of the commercial blocking ELISA is undergoing to improve the sensitivity and specificity of the detection when using oral fluid samples. This evaluation contributes not only in the facilitation of CSFV antibody detection method, but nevertheless helps in the rapid diagnosis and controlling aspects of CSFV, particularly in the CSFV-free countries.

In conclusion, the modified real time RT-PCR yielded satisfactory sensitivity than the routine RT-PCR, but the sensitivity was not good enough to detect low CSFV levels, particularly in the oral fluid samples. Effectiveness of a genetic material extraction method from oral fluid samples might be hampered by the mucous component in the saliva or other organic matters. Achievement of this objective could be done by developing a better genetic material extraction method from oral fluid samples to enhance the sensitivity of the molecular diagnosis. However, detecting CSFV antibody in the oral fluid samples is another alternative method for improved surveillance in CSFV-free areas, thereby enhancing elimination and control efforts much faster than using molecular diagnostic methods.