RESEARCH ABSTRACT

SWINE HEALTH

pork checkoff

Title: Development and validation of real-time RT-PCR assays for the detection of swine acute diarrhea syndrome coronavirus (SADS-CoV) and investigation of its presence in US swine. **NPB: #18-142 IPPA**

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Scientific Abstract:

Swine enteric coronaviruses, such as porcine epidemic diarrhea virus (PEDV), porcine deltacoronavirus (PDCoV), transmissible gastroenteritis virus (TGEV), and swine acute diarrhea syndrome coronavirus (SADS-CoV), are important enteric pathogens and cause indistinguishable clinical signs, making differential diagnosis crucial. SADS-CoV had only been reported in China; its presence in the U.S. remained unknown. We aimed to develop and validate a PEDV/PDCoV/TGEV/SADS-CoV/XIPC 5-plex reverse transcription real-time PCR (RT-rtPCR) for simultaneous detection and differentiation of the four viruses (XIPC serves as an internal positive control for the PCR) and investigate the detection frequency of these viruses in U.S. swine.

Four singleplex SADS-CoV RT-rtPCR assays were evaluated for analytical specificity, analytical sensitivity, and diagnostic performance by testing 140 clinical samples in China. One SADS-CoV PCR with best performance was chosen for the 5-plex assay development. One PDCoV PCR that did not cross-react with sparrow deltacoronaviruses was developed and included for the 5-plex PCR development. The 5-plex PCR including an exogenous internal positive control (XIPC) was optimized for the concentrations of primers and probes for each target. The 5-plex PCR had excellent analytical specificity by testing against 32 different swine viral and bacterial pathogens. Based on testing serial dilutions of IVT RNAs, the limits of detection of the 5-plex PCR was 8 genomic copies/reaction for PEDV, 4 genomic copies/reaction for PDCoV, 16 genomic copies/reaction for TGEV, and 6.8 genomic copies/reaction for SADS-CoV when Ct cut-off value was set at 37 for each virus target. Based on testing 219 clinical samples to evaluate the diagnostic performance, the 5-plex PCR had diagnostic sensitivity, specificity, and agreement of 98.96%, 95.12% and 96.80% for PEDV, 100%, 97.81% and 98.63% for PDCoV, and 100%, 100% and 100% for TGEV when compared to a commercial PEDV/TGEV/PDCoV PCR.

Then the 5-plex PCR was used to determine if SADS-CoV was present in US swine. First, 288 clinical samples archived during 2019-2020 from diarrheic pigs negative for PEDV, PDCoV, TGEV, rotavirus A, B, C were tested and all 288 samples were negative for SADS-CoV. Next, the feces, fecal swabs, and oral fluid samples submitted to the ISU VDL during Mar-Oct 2019, Nov-Dec 2020, and Feb-Apr 2021 were randomly selected for testing. All samples were negative for SADS-CoV. Among the 1,028 samples positive for at least one of PEDV, PDCoV, and TGEV, the following data were found: (1) 71.2% PEDV positive only, (2) 18.1% PDCoV positive only, (3) 0% TGEV positive only, (4) 10.1% PEDV&PDCoV positive, (5) 0.19% PEDV&TGEV positive, (6) 0% PDCoV&TGEV positive, and (7) 0.39% PEDV&PDCoV&TGEV positive.

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

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In summary, the novel PEDV/PDCoV/TGEV/SADS-CoV/XIPC 5-plex RT-rtPCR developed and validated in this study is sensitive and specific and provides a convenient tool for detection and differentiation of these important swine enteric coronaviruses. Although there is no evidence of SADS-CoV presence in the U.S. now, the availability of the PEDV/PDCoV/TGEV/SADS-CoV/XIPC 5-plex PCR will enable us to conduct ongoing surveillance and thereby we are better prepared to respond to introduction.