RESEARCH ABSTRACT

SWINE HEALTH

Title: Feasibility of viability PCR and ex-vivo bioassay to detect viable PED virus in feed. Identification - **NPB# 14-153**

Investigator: Dr Montserrat Torremorell

Institution: University of Minnesota

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Scientific Abstract:
Porcine epidemic diarrhea virus (PEDV) was first detected in the United States in April 2013. During the first year, PEDV spread rapidly, affecting most of the swine producing states and causing significant economic losses. In this study, a viability PCR (v-PCR) was developed to differentiate viable and non-viable PEDV to assist in the study of risk factors associated with PEDV transmission. Propidium monoazide (PMA) is a dye capable of penetrating cell membranes of non-viable organisms. PMA intercalates into the DNA/RNA through penetration of structurally damaged or compromised capsids of inactivated viruses limiting the replication of the viral DNA/RNA during the PCR reaction. In this study, we report various v-PCR experiments to test the effects of PMA concentration, incubation temperature and time, and sample pre-treatment conditions in infectious (viable) and heat inactivated (non-viable) samples. We also evaluated the performance of the v-PCR in a solution containing infectious and heat treated PEDV, and determined the impact and limit of detection of infectious and heat-treated PEDV concentration using this method. Lastly, the feasibility of this technique was evaluated in fecal samples from experimentally infected animals, feed samples spiked with PEDV, and slurry and feed-samples of known PEDV status based on pig bioassay data.
Infectious virus samples treated with PMA had a change in Ct value (ΔCt) of ~<3. In contrast, heat inactivated samples treated with PMA had a ΔCt ~>5. In the suspension of infectious and heat inactivated PEDV, differentiation was detected in the ratio of alive:dead PEDV equal or greater than 50:50. Differences in Ct values for infectious PEDV samples treated with PMA increased as the concentration of PEDV decreased, with ranges of ΔCt of 2.4 to 3.89 for 10^0 to 10^{-5} PEDV dilutions. The change in Ct values for heat- inactivated samples ranged from 5.86 to 12.19 for10^0 to 10^{-4} PEDV dilutions. ΔCt >~5 of heat inactivated samples was shown in 4 out of6 fecal samples collected from experimentally infected animals (ΔCt value could not be estimated in two samples because starting Ct was >35), compared to ΔCt < 5 for 3 out of 6 samples in non-heat treated samples. A ΔCt of <3.70 was shown in infectious PEDV spiked feed when incubated in solution but not as a solid paste. In contrast, a ΔCt of 5.98-8.90 was shown in heat inactivated PEDV spiked feed samples incubated in solution but not as a solid paste. While samples from the bioassay studies and vPCR were in agreement 8 out 9 in slurry samples, agreement was poor or unobtainable in feed samples and plasma protein samples that had high Ct values in the starting material. Overall, this study differentiates between infectious and heat inactivated PEDV by combining RT-PCR with PMA treatment. However, further research is needed to evaluate the feasibility of this technique in field samples.