RESEARCH ABSTRACT

SWINE HEALTH

Title: Real-time PCR detection of PRRSv and rapid identification of vaccine in serum and semen **NPB #03-052**

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Abstract: The challenge studies completed in this study highlights the need for improving our Porcine Reproductive and Respiratory Syndrome virus (PRRSv) diagnostic capabilities. Correlating the current PRRSv infectious status of a boar using only one diagnostic approach from only one type of sample can result in misdiagnoses. The intent of this study was to evaluate the efficiency of a SYBR green "real-time" PCR for detecting PRRSv in boar semen and serum. When SYBR green PCR was compared to other PCR detection methods the SYBR green PCR was unable to detect the presence of PRRSv RNA in all serum and semen samples evaluated. While traditional electrophoretic gels stained with ethidium bromide (EtBr) did result in positive results and were superior to the SYBR green PCR results, Southern blot hybridizations with an internal DNA probe in hybridizations with the EtBr-stained PCR amplicons we were able to detect PRRSv RNA in samples that were otherwise undetectable by SYBR green or EtBr fluorescence detection. This highlights the need for a DNA sequence detection step in any PCR assay for detecting PRRSv RNA in semen based on these results and the potential for false-negative misdiagnoses. To overcome this, we recommend the development and evaluation of PCR approaches that incorporate automated DNA hybridization steps into the PCR analysis. Examples would be TaqMan or Molecular Beacon detection systems. These fluorgenic-based PCR assays would allow for improvement in "real-time" detection sensitivity and specificity that is lacking with the SYBR green detection system.

Also recognized in this study was the need for effective RNA recovery procedures from semen samples and their importance in the development of a sensitive PRRSv PCR detection procedure. Additional research will be required to identify and optimize the most efficient PRRSv RNA recovery processes from semen that ultimately meets the needs and requirements of the pork producer. The goal of the process should be the development of a straight-forward, user-friendly RNA recovery process that can be totally automated and be a component in a self-contained PRRSv-RNA detection system for on-farm applications.