RESEARCHREPORT
SWINE HEALTH

Title: Novel DNA-vaccine for PRRSV with improved heterologous protection. **NPB project: #11-089**

Investigator: Alexander N. Zakhartchouk, Ph.D. and DVM

Institution: University of Saskatchewan

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Scientific Abstract:

Though a modified live attenuated vaccine is available against porcine reproductive and respiratory syndrome virus (PRRSV), its limitations in protection efficacy, safety and few others warrant the development of newer vaccines.

In this study, we have constructed a novel DNA vaccine that consists of two plasmids designed to express modified synthetic PRRSV genes for GP5 and M. To achieve the highest possible levels of gene expression in porcine cells, the gene sequences were codon-optimized using the GeneScript's OptimumGene™ computer program. In addition to codon-optimization, the GP5 gene has the following modifications. First, the decoy epitope (aa 27-33) in the GP5 sequence was mutated: the sequence VLANASN was changed to AVAGASA. Second, to eliminate glycan shielding, the aspargine triplet (AAC) in the GP5 gene sequence at positions N33 and N51 was replaced with the alanine triplet (GCC). Third, to facilitate the protein detection, the sequence encoding 15 amino acids HA tag was introduced at the 3'-end of the gene. The sequence of M gene was also codon-optimized, and the sequence encoding 8 amino acids FLAG tag was added to the 3'-end of the gene. Next, for gene expression, both GP5 and M genes were cloned under the control of the cytomegalovirus (CMV) promoter, intron and polyA signal. Furthermore, internal ribosome entry sequence (IRES) was introduced between the genes. Introduction of IRES allows expression of both genes from one mRNA, and this facilitates formation of GP5-M heterodimers.

Another construct was designed to increase M-specific cell-mediated immune responses. To stimulate aggregation of the M protein in cells, the M gene sequences were fused with polyglutamine (polyQ) domains, which are expected to enhance its antigenic potential. To this end, CAG/CAA repeats coding for 103 glutamine residues was added to the 3'-end of the M gene. To facilitate the M protein detection, the sequence encoding 8 amino acids FLAG tag was added to the 5'-end of the gene.

Two eukaryotic vectors were explored in order to express PRRSV antigens in pig cells: pMASIA and pCI-neo. All plasmids were sequenced and showed correct PRRSV gene sequences. However, several attempts to detect GP5 and M proteins in the transfected with the plasmids swine (ST) cells were unsuccessful despite using three different immunological methods: indirect immunofluorescence, Western blotting and immunoprecipitation. More work is needed to achieve abundant PRRSV antigens expression in swine cells.

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.

For more information contact:
National Pork Board • PO Box 9114 • Des Moines, IA 50306 USA • 800-456-7675 • Fax: 515-223-2646 • pork.org