RESEARCH ABSTRACT

pork checkoff

SWINE HEALTH

Title: Development of a modified live vaccine against PRRSV with optimal DIVA marker potential" project - **NPB#08-248**

Investigator: Fernando A. Osorio, DVM, PhD

Institution: University of Nebraska-Lincoln

Co-Investigator: Asit Pattnaik, PhD

Date Submitted: August 2, 2010

Keywords: PRRSV, DIVA Vaccine, Live Vaccines, Epitopes, Marker Vaccine

Scientific Abstract

This proposal has been aimed at developing an optimal marker differential vaccine system for the new generation of vaccines currently under development. The central notion is that the optimal *new generation* PRRSV vaccine will be of the live attenuated type. Previous research in our laboratory has indicated that one specific epitope located on ORF6 gene (epitope contained in peptide 201) would be highly conserved in the vast majority of PRRSV type II strains and would then constitute an ideal candidate as a marker for serologic differentiation of infected and vaccinated animals (DIVA principle). Our previous results had indicated that the totality of infected pigs (15/15) mounted antibody response to an epitope in peptide 201 suggesting that this epitope is highly immunogenic. Monoclonal antibody against the epitope in peptide 201 (MAB 201) was then developed. Genomic alignment indicated great level of conservation amongst PRRSV type II strains. Our initial attempts (using reverse genetics) at deleting such epitope (pep 201) from the PPRSV genome ended up in failure. We were able to rescue only one viable deletion mutant which still maintained the immunogenicity corresponding to epitope peptide 201. The specific objectives of this proposal #08-248 have then been : 1) to develop a live amino-acid substitution mutant (rather than a deletion mutant) deprived of the 201 ORF6 epitope reactivity, an epitope which we consider the ideal marker, based on its level of conservancy among strains and its immunodominance, 2) Testing of this epitope 201-deprived mutant in vivo , using an standard experimental design for animal inoculation which has been previously tested and described 3) Field testing of the companion peptide-ELISA specific for the marker epitope, validating its specificity and sensitivity based on the analysis a large number of field serum samples.
Overall, we indentified a highly conserved and immunogenic epitope (designated as epitope 201) in the M protein of type-II PRRSV which can be used as marker for vaccine. We confirmed such conserved character by immunofluorescent analysis of a large number of field isolates, assessed for reactivity with MAB 201 A large majority of field isolates (91 %) was positive for MAB 201 reactivity. Using aa substitution we were able to generate 2 different mutant viruses which harbour a disrupted epitope 201. However, both of these mutants

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.

(table 1) were not stable in infected pigs, reverting to wt type after infection of pigs, as early as 7 days pi, as detected in viremic samples collected post-inoculation. These experiments have helped to pinpoint in a more exact manner, the aa residues that are involved in the MAB 201 reactivity. Likewise, the frank back mutations of these constructs obviously indicate a strong selection pressure for wt. As a continuation of these experiments, but beyond the termination of NPB #08-248, we are pursuing an alternative approach. We are now working in constructing mutants that mimic the exact epitopic 201 structure in one of those isolates (~ 9 % of total isolates analyzed) that are naturally occurring with a negative phenotype for epitope peptide 201.We hypothesize that by mimicking the amino acid sequence of a 201–negative field isolate, we can generate a virus that is stable in infected pigs, thus inducing a permanent marker negative serologic profile.