RESEARCH ABSTRACT

SWINE HEALTH

Title: Development of protein based ELISA for the rapid strain specific identification of PRRSV and responding to novel emerging variants. **NPB #06-132**

Investigator: Dr. Julian A. Hiscox

Institution: Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, UK

Date Submitted: 30th May 2008.

Scientific Abstract:
The major aim of this project was to develop and evaluate diagnostic reagents, tools and methodologies to identify both generic and specific strains of PRRSV as well as future proofing this technology for the rapid identification of new and emerging strains. The project focused on evaluating and characterising various virus proteins and protein expression systems for use in ELISA. Two proteins were immediately selected as being of interest. The viral RNA binding protein, nucleocapsid (N) protein and one of the major virus surface glycoproteins, GP5 protein. These undergo post-translational modification in the host cell. Many protein based ELISAs are based on E.coli expressed protein and do not take this into account. Using gene sequence provided by our collaborator at the University of Nebraska-Lincoln, the respective genes were cloned into a variety of different expression vectors for evaluation of different ways of making modified and unmodified protein. In the first methodology, proteins were expressed using two different vector systems utilising a histidine tag (for nickel affinity purification) in E.coli, which is commonly used for expression strategies. In the second methodology these proteins were expressed in insect (Sf9) cells and mammalian cells. This had the added advantage over E.coli based systems that the proteins would be modified post-translation and thus would mimic those found in virus infected cells and perhaps would have greater immunogenic coverage. In the third methodology proteins were expressed with fluorescent labelled tags for identification and isolation in mammalian cell culture and subsequent enrichment using fluorescent activated cell sorting (FACS) to select positive expressing cells. In the fourth methodology proteins were expressed using rapid large scale *in vitro* translation systems which have recently been developed. Although unmodified proteins are produced, these systems offered the added advantage of rapid protein expression and purification as many of the gene cloning steps could be eliminated as well as some of the purification steps. In addition, recombinant protein expression in bacteria/cell based systems often fails or is less than efficient due to toxicity; the *in vitro* translation systems eliminated this concern. A large number of reagents in terms of gene expression constructs for the N and GP5 proteins were generated in this project including several different truncation variants of N protein. All of the resulting proteins had different tags to aid purification, for example from histidine tags to fluorescent labelled proteins for enriching positive expressing cells and immuno-affinity purification. Good protein yields were generated from histidine tagged proteins expressed in E.coli and insect cells, and from mammalian cells. Although proteins were obtained from enriched cells expressing fluorescent labelled tags, yields were low.

Poor production of proteins in mammalian cells was probably a result of protein toxicity rather than an expression/purification problem *per se*. Overall, high level expression and purification of both histidine tagged N protein and GP5 protein was obtained. Evaluation as reagents in ELISA using sera from PRRSV infected swine indicated that although the modified proteins expressed in mammalian cells were the better immunogenic reagent the rapid and efficient production of proteins using the *in vitro* translation system may outweigh this, as highlighted in our publication describing these methodologies. Finally, live cell imaging data utilising the fluorescent labelled N proteins defined trafficking of the protein in the cell in real-time - the first description of this. As highlighted in our publication of this work, this information can be rapidly exploited for the development of growth attenuated recombinant vaccines.