2A-103: *Comparing the Mucosal and Systemic Immune Response after APP Vaccination with Natural Challenge*.

Executive Summary

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APP-Alive is a live intranasal vaccine to prevent *Actinobacillus pleuropneumoniae* (APP) in pigs. When vaccine failures became apparent, we developed IgA based ELISA tests to investigate the immune response at the mucosa of the respiratory tract. The antibody responses found in blood and lungs of a serovar 5 vaccinated herd were compared to those of an apparently APP-free herd and two herds with endemic serovar 7 infection.

During test development, the vaccine at the collaborating farm was changed from APP-Alive to intramuscular immunisation (IM), with a killed whole cell (bacterin) vaccine which was unlikely to stimulate the same level of mucosal IgA response. However, we continued with the project to develop the tests as potential diagnostic tools and to examine the mucosal and serum antibody profiles in the different herds.

To develop APP serovar specific IgA based tests, seven different APP antigens were isolated. We found that lipopolysaccharides (LPS) were the most specific antigens for the serovars of interest when tested against anti-sera to Serovars 1, 7 and 15.

On-farm results from these two new ELISA tests poorly supported the APP classification status originally given to each farm. Therefore, test results from the EMAI ELISA's were compared to results from two other IgG ELISA tests, Swinecheck® APP (5a-5b) and ID Screen ® APP 4-7 Indirect using the same samples. The EMAI ELISA's for both serum IgG and IgA were rated as good for serovar 5 and excellent for serovar 7 at detecting allegedly truly positive animals using Swinecheck® and ID Screen® APP results as the "gold standards". As these gold standards were not developed for use with lung washings a similar assessment of the EMAI ELISA tests using lung mucosal samples was not possible. We could only conclude, on the basis of the serum sample correlations and a single vaccinated pig with a high level of IgG present in Bronchoalveolar Lavage(BAL), that if elevated levels of antibodies to either serovar *were* present in BAL they would be detected by the EMAI IgA ELISAs.

The EMAI ELISAs, Swinecheck® and ID Screen® test results all supported the assertion that there were Serovar 7 negative pigs in the Serovar 7 herd; Serovar 7 positive pigs in the serovar 5 vaccinated herd; one Serovar 7 positive sample in the APP free herd and serovar 5 negative pigs in the serovar 5 vaccinated herd. The results of all tests were compared with lung damage scores obtained at abattoir sampling. There was no correlation between EMAI ELISA antibody levels in individual pigs and their pneumonia score at slaughter.

Neither vaccination nor infection appeared to produce a consistent detectable rise in mucosal IgA in the herds. However, the triple IM vaccination protocol, followed by an 8 week interval prior to sampling, was too long for the detection of the IgA mucosal response which recent work has shown occurs in the initial stages of vaccination/infection. However, the sera IgA response, which occurs in the later stages, was evident and more reliable than the induced IgG response for detecting antibody response.

The EMAI ELISA tests will be suitable for future live vaccination efficacy studies but more frequent collection of blood and /or mucosal fluid samples, pre and post immunisation will be necessary to better define and quantify the specifics of the immune response to vaccination.