RESEARCH ABSTRACT

SWINE HEALTH

Title: Development and Validation of Novel Diagnostic Assays and Investigation of Disease Pathogenesis for Infectious Arthritis in Growing Pigs **- NPB #16-107**

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Scientific Abstract:

Background:

M. hyorhinis and *M. hyosynoviae* are common causes of polyarthritis in swine. The overall goal of this study was to develop reliable antibody ELISA assays for the detection of *M. hyorhinis* and *M. hyosynoviae*. These assays will provide antemortem diagnostics, surveillance methods, and pertinent information to practitioners concerning vaccination or antibiotic intervention timing resulting in better management strategies for swine production systems that will enhance production efficiency, animal well-being, and improve animal health.

Methods:

To generate a panel of specimens (serum and oral fluid) and specific polyclonal antibodies against *M. hyorhinis*, *M. flocculare*, *M. hyopneumoniae*, and *M. hyosynoviae*, development of oral fluid and serum antibody assays specific against *M. hyorhinis* and *M. hyosynoviae*, and
establish an association between detection of either agent in oral fluids by PCR and clinical disease, 50 cesarean derived colostrum deprived (CDCD), crossbred, mixed-sex, 8-week-old pigs were inoculated with Friis media, *M. hyorhinis*, *M. flocculare*, *M. hyopneumoniae*, or *M. hyosynoviae* (n=10/group). Serum samples were collected on day post-inoculation (DPI) 0, 3, 7, 10, 14, 17, 21, 24, 28, 35, 42, 49, and 56. Pen-based oral fluids (5 pens, 2 pigs per pen) were collected daily throughout the study. To investigate the immunopathogenesis and bacterial dissemination pattern of *M. hyorhinis* in a single and multiple inoculation model, CDCD pigs were inoculated once or four times with *M. hyorhinis* or sham-inoculated. We then designed and produced a recombinant polyprotein (rVlpA-G) consisting of the chimeric variable lipoproteins of *M. hyorhinis* cloned in frame in a prokaryotic vector system (pET32a). For *M. hyosynoviae,* we obtained a bacterial protein extract from a pure culture of *M. hyosynoviae* treated with Tween 20 detergent. Both antigens were used to develop indirect ELISAs for antibody (IgG and IgA) detection in serum and/or oral fluids.

Results:

M. hyosynoviae was detected by PCR in oral fluids daily for 11 days starting at DPI 3. Seven out of ten animals developed clinical signs consistent with *M. hyosynoviae*. *M. hyorhinis* was detected intermittently

in oral fluids by PCR throughout the study period starting at DPI 2. Clinical signs consistent with *M. hyorhinis*-associated disease started at DPI 11 and eight out of the ten inoculated animals developed clinical signs that lasted through DPI 56. Lesions consistent with *M. hyorhinis* were observed at necropsy in nine out of ten *M. hyorhinis*-inoculated animals. The diagnostic performance, i.e., diagnostic sensitivity and specificity, and analytical specificity (cross-reactivity) of the *M. hyorhinis* rVlpA-G and *M. hyosynoviae* T20 indirect ELISAs was evaluated by testing a panel of samples of precisely known immune status (*M. hyorhinis*, *M. hyosynoviae*, *M. hyopneumoniae*, and *M. flocculare*). *M. hyorhinis* or *M. hyosynoviae* inoculated animals showed specific seroconversion between DPI 14 (IgA) and 28 (IgG). No cross-reactivity (100% analytical specificity) was observed under experimental conditions.

Discussion:

The newly developed antibody ELISA assays for the detection of *M. hyorhinis* or *M. hyosynoviae* are currently available at the ISU-VDL and will assist in the diagnosis and surveillance of *M. hyorhinis*-associated disease and *M. hyosynoviae*-arthritis.