

SWINE HEALTH

Title: Early detection of *Mycoplasma hyopneumoniae* infections in live pigs: Comparison of current methods and development of new assays – NPB #12-047

Investigator: Dr. Maria Pieters

Co-Investigator: Dr. Albert Rovira

Institution: University of Minnesota

Date Submitted: Nov 1st 2013.

Scientific abstract:

In vivo detection of *M. hyopneumoniae* infected animals constitutes a difficult diagnostic task. The location of the pathogen in the lower sections of the respiratory tract makes it inaccessible for direct pathogen detection, and the shedding in the upper sections of the respiratory tract seem to be late and non-continuous. Moreover, specific antibody detection is of slow onset and sometimes unspecific, as represented for the detection of false positive animals and presumably *M. hyopneumoniae* free herds. Various sample types and testing methods have been developed over time in an effort to achieve early diagnosis of *M. hyopneumoniae* infections. However, comparisons have been performed under different conditions which make it difficult to establish the most accurate diagnostic tool. Therefore, the objectives of this study were to identify the most accurate current diagnostic tools for early detection of *M. hyopneumoniae*, through a side-by-side comparison. And to develop alternatives to current diagnostic procedures for early detection of *M. hyopneumoniae* in live pigs. Twenty three 8-week old pigs obtained from a source negative to *M. hyopneumoniae* and other major pathogens were employed in this investigation. Pigs were randomly allocated to 2 experimental groups. One group of 2 non- infected pigs considered as a negative control and a group of 21 experimentally infected pigs. Pigs in the control group were intra-tracheally inoculated with 10mL of sterile Friis medium, while pigs in experimentally infected group were inoculated with 10mL of Friis medium containing 1×10^5 CCU/mL of *M. hyopneumoniae* strain 232 from lung homogenate. All pigs were sampled at 0, 2, 5, 9, 14, 21, and 28 days post inoculation (dpi) by means of nasal swabs, laryngeal swabs, trachea-bronchial lavages, oral fluids and blood samples. Bronchial swabs and lung lavages were collected after euthanasia on day 28 dpi. Gross and microscopic lesions were blindly scored in all animals after euthanasia. Negative control pigs were negative to *M. hyopneumoniae* by all tests and at all sampling dates. All pigs in the experimentally infected group were detected positive to *M. hyopneumoniae* by various sample types and at various dpi. *M. hyopneumoniae* was not detected in the experimentally inoculated pigs at 0 and 2 dpi. PCR detection of *M. hyopneumoniae* was achieved with greater sensitivity by means of laryngeal swabs, followed by trachea-bronchial lavages, nasal swabs and oral fluids. Antibody detection using commercial ELISA kits was observed at 21 and 28 dpi, with no statistical significance among the tests used. *M. hyopneumoniae* specific IgM was detected as early as 9 dpi in a small

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

percentage of experimentally infected pigs, increasing at 14 dpi and decaying at 21 and 28 dpi. IgA antibodies were not detected in oral fluids at any point after infection, but were detected in trachea-bronchial lavages at 28 dpi. C reactive protein detection was similar between pigs of the infected and non-infected groups. Specific protein detection by Western blot showed an undifferentiated pattern in most pigs, regardless of their experimental group, with a small proportion of pigs showing specific bands attributable to a *M. hyopneumoniae* specific response. Under the conditions of this study, detection of *M. hyopneumoniae* after experimental infection was achieved earlier and with greater sensitivity by using laryngeal swabs in combination with real-time PCR testing, compared to the other samples and assays used in this investigation.