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Development of an ELISA for the Detection of Antibodies to *Mycoplasma hyopneumoniae* in Swine Sera

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Enzootic pneumoniae or *Mycoplasma Pneumoniae* Swine (MPS), a chronic disease with a high morbidity and a low mortality is caused by *Mycoplasma hyopneumoniae*. An ELISA has been developed to detect antibody to *Mycoplasma hyopneumoniae* (Mhyo) present in swine serum. This assay has been designed in the microtiter format, in which purified Mhyo antigen has been coated onto the solid phase and an anti-swine IgG horseradish peroxidase conjugate is used for detection. This Mhyo ELISA was designed as a rapid, standardized, herd screening method, to be used as an indicator of exposure to the agent. It has been shown to be sensitive, specific, and correlates well with the Tween 20 ELISA.

Sensitivity was assessed through the testing of temporal bleeds from pigs inoculated with an Mhyo bacterin vaccine. Three pigs of known serologic status for Mhyo were inoculated according to the vaccine manufacturer's recommendation. Serum was collected from each animal at 0, 7, 14, 21 and 28 days post inoculation. Samples were tested by Tween 20 ELISA at the Iowa State Veterinary Diagnostic Laboratory and tested on the experimental Mhyo ELISA at IDEXX Laboratories. The Mhyo ELISA detected seroconversion in 1 pig by day 14, and 2 pigs by day 21, while the Tween 20 ELISA detected seroconversion in 3 pigs by day 21.

Additional sensitivity data, demonstrating correlation of the Mhyo ELISA to Tween 20 ELISA was obtained through the testing of field samples from Mhyo-infected herds.

Samples were obtained from two sites with accompanying Tween 20 ELISA data. From 540 samples tested, 292 samples were considered positive or suspect by Mhyo ELISA and 255 samples were considered positive or suspect by Tween 20 ELISA. Of these 255 samples, the Mhyo ELISA detected 228 positive or suspect samples. This results in 89.4% sensitivity for the Mhyo ELISA as compared to the Tween 20 ELISA for this population. The overall correlation between the Mhyo ELISA and the Tween 20 ELISA was 77.2%. When all suspect samples for either test were considered positive, the correlation between the two methods was 83.1% and when they were considered negative, the correlation was 82.2%.

A set of 190 field samples from Mhyo negative herds, obtained from two sites in the United States, were used to evaluate specificity. Serum samples were assayed on the Mhyo ELISA and results were compared to the Tween 20 ELISA. All of the samples tested were negative (S/P less than 0.30) on the Mhyo ELISA, resulting in 100% specificity for this population.

Specific swine serum samples against *Mycoplasma flocculare*, *Mycoplasma hyorhinis*, *Mycoplasma hyosynoviae*, Encephalomyocarditis virus, Hemagglutinating Encephalomyelitis virus, Porcine Adenovirus, Porcine Parvovirus, Porcine Reovirus, Porcine Rotavirus, Swine Influenza virus, and Transmissible Gastroenteritis virus were assayed on Mhyo ELISA and all of them were negative.

The IDEXX *Mycoplasma hyopneumoniae* Antibody test described above provides good correlation to other serological method in the detection of seropositive pigs, while maintaining excellent specificity.