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Evaluation of *Actinobacillus pleuropneumoniae* diagnostic tests using samples from experimentally infected pigs

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Introduction: *Actinobacillus pleuropneumoniae* (APP) is the causative agent of porcine pleuropneumonia, a highly contagious disease that causes important economic losses worldwide. Although APP prevalence is low among US swine herds, testing to establish herd status is routinely performed to avoid the introduction of carrier pigs into negative populations. Recently, new serological tests have been introduced for APP diagnosis. No information is currently available regarding how these tests compare for detection of subclinically infected pigs. The objective of this study was to compare the performance of all diagnostic tests available for detection of pigs subclinically infected with APP using samples from pigs experimentally infected with clinically relevant serotypes.

Materials and Methods: This study was approved by the University of Minnesota IACUC committee. Eighty pigs from an APP negative source were divided into 8 groups. Each group was housed in a separate room at the University of Minnesota isolation facility. Groups of pigs were experimentally infected with 7 different APP serotypes: 1, 3, 5, 7, 10, 12 and 15, with group 8 remaining as the negative control. Pigs were inoculated intranasally with 1mL per nostril using a dose of 10^6 CFU/mL. Inoculated pigs were observed for three consecutive days post-inoculation for the presence of clinical signs and weekly thereafter. Blood samples and tonsil swabs were collected prior to infection and for 7 consecutive weeks thereafter. Serum samples were tested weekly using 2 commercially available kits: the Swinecheck® APP (Biovet) and Multi-APP ELISA (University of Montreal). Swabs were cultured for bacterial isolation and tested by PCR. All pigs were euthanized at 49 days post-inoculation. At necropsy, tonsil swabs, whole tonsils, and lung tissues were collected and subsequently tested by PCR and cultured for isolation.

Relevant results: Carrier status was successfully reproduced in pigs inoculated with serotypes 1, 3, 7, 10, and 15. Pigs inoculated with serotypes 5 and 12 did not become infected. Pigs infected with serotypes 10 and 15 showed respiratory clinical signs and were treated with antibiotics. The Multi-APP ELISA detected seroconversion one week earlier than the Swinecheck® APP ELISA in groups infected with serotypes 1, 3, and 10. The earliest seroconversion detected was at 1 week post-infection (serotype 10) and the latest was at 3 weeks post-infection (serotype 1). Seroconversion at day 49 post-infection was serotype serovar-dependent, with a a minimum of 4 (44%) positives detected in the serotype 10 group and a maximum of 9 (100%) in the serotype 15 group. Thirty-one pigs were serologically positive for APP at 49 days post-infection and only 15 still carried APP on the tonsils based on PCR results. No cross-reactions were observed when serum samples were tested using all Swinecheck® APP ELISA tests. APP was not isolated from tonsillar swabs collected throughout the experiment due to heavy contamination by the resident flora.

Recommendations: The Multi-APP ELISA test should be used for serological screening of pigs prior to introduction into naïve populations. The serotype-specific ELISA tests can be used to identify the serotype circulating in the herd following the detection of positives by the Multi-App test. Positive serum samples based on the Multi-APP ELISA results may test negative with the serotype-specific ELISA. Re-test of these samples 1-2 weeks later with the serotype-specific ELISA compensates for the difference in sensitivity among these tests. PCR can be used to troubleshoot serological results in the earlier stages of infection.

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