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Research article

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

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ABSTRACT

New serological tests have recently been introduced for *Actinobacillus pleuropneumoniae* diagnosis. No information is currently available on how these tests compare regarding the detection of antibodies from subclinically infected pigs. To answer this question, 80 pigs were randomly assigned to experimental groups infected with *A. pleuropneumoniae* serovars 1, 3, 5, 7, 10, 12, 15 and a non-inoculated control group. Blood samples and oropharyngeal swabs were collected prior to infection and for 7 consecutive weeks thereafter. Serum samples were tested using the Swinecheck® APP ELISA and the Multi-APP ELISA (University of Montreal). All pigs were euthanized at 49 days post-inoculation. Tonsil and lung samples were cultured for isolation and tested by PCR. The Multi-APP ELISA detected seroconversion 1 week earlier than the Swinecheck® APP ELISA with the earliest seroconversion detected at 1 week post-infection (serovar 10) and the latest at 3 weeks post-infection (serovar 1). Seroconversion at day 49 was serovar-dependent and varied from 4 (44%) positives detected in the serovar 10 group to 9 positives (100%) detected in the serovar 15 group. Thirty-one pigs were serologically positive for *A. pleuropneumoniae* at 49 days post-infection and only 15 still carried *A. pleuropneumoniae* on their tonsils based on PCR results. No cross-reactions were observed when serum samples were cross-tested using the Swinecheck® APP ELISA. *A. pleuropneumoniae* was successfully isolated from the lung of 2 pigs that developed pleuropneumonia, but was not isolated from tonsils due to heavy contamination by the resident flora. This study offers a comprehensive evaluation of the diagnostic tools currently available for detection of *A. pleuropneumoniae* subclinical infection.

1. Introduction

Actinobacillus pleuropneumoniae is the causative agent of swine pleuropneumonia, a highly contagious disease responsible for significant economic losses worldwide (Jessing et al., 2003; Gottschalk et al., 2003; Dreyfus et al., 2004). This pathogen is absent from most U.S. swine herds. Consequently, monitoring for negative status is very important to avoid the introduction of positive animals into naïve populations (Gottschalk et al., 2003).

Currently, there are at least three different ELISA tests commercially available to diagnose *A. pleuropneumoniae* infections, namely the serovar-specific Swinecheck® APP ELISA offered by Biovet, the Multi-APP ELISA offered at the University of Montreal and the Chekit APP-ApxIV® ELISA offered by IDEXX. The serovar-specific Swinecheck® APP and the Multi-APP ELISA tests target similar antigens, specifically the long chain lipopolysaccarides (LC-LPS) of *A. pleuropneumoniae*. The Chekit APP-ApxIV® ELISA targets a species-specific toxin produced by *A. pleuropneumoniae*

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during infection (Schaller et al., 1999). In our experience, results obtained using LC-LPS and ApxIV-based tests frequently disagree, likely due to differences in the target antigens, test sensitivity and specificity among these tests. Unexpected or conflicting serological results are usually sorted out based on clinical history and detection of carriers by bacterial isolation and PCR testing (Schaller et al., 2001; Fittipaldi et al., 2003).

At this time, there is no information available on how the variety of diagnostic tests available for *A. pleuropneumoniae* diagnostics compare regarding detection of subclinical infection or which combination of tests provides an accurate definition of true infection. Although many studies have evaluated some of the tests mentioned separately, none have compared all of them in a single experiment. The objective of this study was to evaluate the capacity of different diagnostics tests to detect *A. pleuropneumoniae* subclinical infection using samples derived from pigs experimentally infected with 7 clinically relevant *A. pleuropneumoniae* serovars.

2. Materials and methods

2.1. Animals

This experiment was approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC) (protocol # 0806A37381). Eighty six-week old pigs obtained from a source free of *A. pleuropneumoniae* based on clinical history, tonsil PCR and serological testing (Chekit APP-ApxIV® ELISA) were transferred to the isolation unit at University of Minnesota. Pigs were randomly divided into 8 different rooms with 10 pigs per room. Each group was inoculated with a different *A. pleuropneumoniae* serovar and one group remained as the negative control (Table 1). Pigs were acclimatized for 1-week pre-infection and housed for 7 weeks post-inoculation. All pigs were euthanized at 49 days post-infection when post-mortem examinations were conducted to evaluate the presence and severity of *A. pleuropneumoniae* lesions.

2.2. Bacterial strains

The reference strains used to experimentally infect naïve pigs with *A. pleuropneumoniae* serovars 1 (4074), 3 (1421), 5 (K17) and 7 (WF83) were obtained at the American Type Culture Collection (ATCC). Reference strains for serovars 10 (22009), 12 (1096) and 15 (HS143) were kindly provided by Dr. Pat Blackall (Blackall et al., 2002) (Table 1). The serovars of all reference strains were confirmed by toxin profiling (Rayamajhi et al., 2005) and indirect hemagglutination (Mittal et al., 1983) prior to inoculation. These serovars were chosen based on their prevalence in U.S. swine herds as identified by querying the Minnesota Veterinary Diagnostic Laboratory (MVDL) database between 2002 and 2008. Although serovar 10 was not isolated from any clinical cases at the MVDL in the past 6 years, it was included in this study due to the detection of antibodies by the serovar-specific Swine-check® APP ELISA test in pigs from herds with no clinical history of *A. pleuropneumoniae* infection.

Table 1
Strain identification and group assignment for *Actinobacillus pleuropneumoniae* serovars used for experimental infection.

Group	*A. pleuropneumoniae* serovar	Strain
1	1	4074
2	3	1421
3	5	K17
4	7	WF83
5	10	22009
6	12	1096
7	15	HS143
8	Negative control	–

2.3. Inoculum and challenge

The growth curve for each challenge strain was determined prior the preparation of the inoculum. *A. pleuropneumoniae* strains were plated on chocolate agar and incubated at 37 °C in 5% CO_2 for 24 h. Two full loops of each plate were transferred to 50 ml of PPLO broth with added NAD and this suspension was incubated for 18 h. Following incubation, *A. pleuropneumoniae* concentrations were determined using a spectrophotometer (optical density at 280 nm) and bacterial counts were confirmed by plate dilutions. One milliliter of each tube was transferred to new tubes with additional 50 ml of PPLO. The bacterial load for each suspension was determined at 2, 4, 6 and 8 h of incubation using a spectrophotometer and confirmed by plate dilutions. Colony counting for each serovar was performed at 24 and 48 h. The growth curve was determined for each serovar and the exponential growth phase identified. Challenge inoculum cultures were harvested at 6 h of growth in PPLO and the concentration was estimated using a spectrophotometer and adjusted to 10^6 CFU/ml using PBS. All inoculums were plated for colony counting prior to inoculation. All pigs were inoculated intranasally with 1 ml of the respective inoculum in each nostril within 2 h of its preparation.

2.4. Clinical signs

Animals were observed for three consecutive days post-inoculation for the development of clinical signs of *A. pleuropneumoniae* infection and weekly thereafter. Rectal temperatures were collected for three consecutive days after inoculation to monitor the course of infection. Pigs that were found to be lethargic with evident respiratory signs and rectal temperature above 41 °C were treated with ceftiofur sodium at a dosage of 3 mg/kg body weight administered intramuscularly for two consecutive days.

2.5. Blood samples

Blood samples were collected prior to the inoculation (day 0) and weekly thereafter for seven consecutive weeks. Five milliliters of blood were collected from each pig by venipuncture of the jugular vein. Samples were left in the refrigerator (4 °C) overnight and centrifuged the next day to separate the serum. All serum samples were divided into