Title: Evaluation of *Actinobacillus pleuropneumoniae* diagnostic tests using samples from naturally and experimentally infected pigs – NPB #08-095

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Date Submitted: 07/02/098

Industry Summary:

The objective of this study was to compare the performance of different diagnostic tests for the detection of pigs subclinically infected with *Actinobacillus pleuropneumoniae*. To address this question, we infected pigs with APP serotypes 1, 3, 5, 7, 10, 12, and 15 and collected weekly tonsil swabs for isolation and PCR and serum samples for serological testing. Our results showed that the Multi-APP ELISA test tended to detect infection one week earlier than the serotype-specific ELISA tests. The Multi-APP ELISA was also more consistent in identifying positives throughout the experiment. This is the first study to compare different diagnostic tests for the detection of subclinical infected pigs with APP serotypes clinically relevant to the U.S. swine industry. We recommend the use of the Multi-APP ELISA test for serological screening followed by testing of positive serum samples with the serotype-specific ELISA test offered by BIOVET.

Scientific Abstract:

New serological tests have recently been introduced for *Actinobacillus pleuropneumoniae* (APP) diagnosis. No information is currently available on how these tests compare regarding the detection of subclinically infected pigs. To answer this question, 80 pigs were experimentally infected with APP serotypes 1, 3, 5, 7, 10, 12 and 15, with group 8 remaining as the negative control. Blood samples and tonsil swabs were collected prior to infection and for 7 consecutive weeks thereafter. Serum samples were tested using 2 commercially available kits: the Swinecheck® APP (Biovet) and Multi-APP ELISA (University of Montreal). Pigs were euthanized at 49 days post-inoculation. Tonsil swabs, whole tonsils, and lung tissues were collected and tested by PCR and cultured for isolation. The Multi-APP ELISA tended to detect seroconversion one week earlier than the Swinecheck® APP ELISA. 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 serovar-
dependent, with 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 cross-tested using the Swinecheck® APP ELISA. APP 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 available for detection of APP subclinical infection.

**Introduction:**

*Actinobacillus pleuropneumoniae* (APP) is the causative agent of the swine pleuropneumonia, a highly contagious disease responsible for significant economic losses worldwide (Stine et al, 2003; Dreyfus et al, 2004; Gottschalk et al, 2003). This pathogen is absent in most U.S. swine herds. Consequently, monitoring negative status is very important to avoid the introduction of positive animals into naïve populations (Gottschalk et al, 2003). The early identification of subclinically infected animals is very important to prevent the spread of this pathogen (Rosendal et al, 1982; Gottschalk, 1999-AASV; Taylor et al, 1999). A wide range of diagnostic tools can be used to diagnose APP infection and most are successful in confirming infection when lesions are present. The detection of carriers, on the other hand, is usually not straightforward due to limitations of the current tests regarding sensitivity.

Bacterial isolation and serotype-specific ELISA tests were for many years the techniques of choice to diagnose and monitor APP infections in naïve and positive swine herds. Given that isolating APP from subclinically infected pigs is frequently unsuccessful, serological testing has become the cornerstone of identifying carriers (Montaraz et al, 1996). A number of different serological tests have been used to detect of APP antibodies, including the hemolysin neutralization test (HNT), enzyme labeled immunosorbent assays (ELISA), and the complement fixation (CF) test. ELISA tests reportedly have the best sensitivity and specificity among these tests, with those based on long-chain LPS antigens being the most used (Klein, et al 2003). The complement fixation test (CF), which was once considered the reference serological test for APP, is now rarely used as it lacks sensitivity and is relatively complex to perform (Gottschalk et al, 2006). In addition, serum titers in naturally infected animals are highly variable; some infected animals never manifest significant titers and other animals move from positive to negative status in a short period of time (Klausen et al, 2002).

Currently, there are at least three different ELISA tests commercially available to diagnose APP infections, namely the serotype-specific ELISA offered by Biovet, the Multi-APP ELISA offered at the University of Montreal, and the Apx IV-based ELISA offered by IDEXX (temporarily out of the market due to QAQC issues). These tests are used to monitor exposure to APP and to identify the serotype circulating in the herd. The serotype-specific and the Multi-APP ELISA tests target similar antigens, specifically the long chain lipopolysaccharide (LC-LPS) of APP. The Apx IV ELISA targets a species-specific toxin produced by APP during infection. Apx IV is a recently described toxin and it has only been found in APP (Schaller, 1999). Results from LC-LPS based ELISA tests and APX IV based tests frequently disagree due to differences in target antigens, sensitivity, and specificity. When serological tests fail to define herd status, clinical history, bacterial isolation, and PCR testing are used to confirm or rule out actual infection.

Considering the limitations of isolating APP from tonsils due to heavy contamination with the resident flora, detection by PCR has become the technique of choice to troubleshoot serological results. Several PCR
techniques have been described. These tests target different genes, including omIA, dsbE-like, aroA, cps-cpx, and Apx IV. A comparison of these tests using samples from naturally and experimentally infected animals demonstrated that the Apx IV-based PCR was highly specific and sensitive, detecting a minimum of $10^2 \text{CFU/g}$ of tonsil (Fittipaldi, 2003).

At this time, there is no information available on how the diagnostic tests described above compare regarding detection of APP subclinical infection or which combination of tests provides an accurate definition of the true infection status. Although many studies have evaluated some of the tests mentioned separately, none have compared all of them in one single experiment. These studies also vary regarding the experimental design, APP serotypes evaluated, type of infection (clinical or subclinical), and duration. These variables are important confounders during the development of a standard protocol for APP diagnostics.

**Objective:**

Evaluate the performance of tests used to detect antibodies and antigen using samples from pigs experimentally infected with *A. pleuropneumoniae*.

**VI. Materials & Methods:**

**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 APP based on clinical history, tonsil PCR, and serological testing (Apx IV ELISA) were transferred to the isolation unit at University of Minnesota. Pigs were randomly divided into 8 different rooms, with 10 pigs in each room. Each group was inoculated with a different APP serotype and one group remained as the negative control (Table 1). Pigs were acclimatized for one week pre-infection and housed for additional seven weeks post-inoculation. All pigs were euthanized at 49 days post infection when post-mortem examination was completed to evaluate the presence of APP lesions.

**Bacterial strains**

The references strains for APP serotypes 1 (4074), 3 (1421), 5 (K17), 7 (WF83), 10 (22009), 12 (1096) and 15 (HS143) used to experimentally infect naïve pigs in this experiment were kindly provided by Dr. Pat Blackall (Blackall et al, 2002) and are listed in Table 1. These serotypes 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 serotype 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 frequent detection of reactive antibodies in pigs from herds that never experience any clinical signs of APP infection.
Inoculum and challenge

The growth curve for each challenge strain was determined prior the preparation of the inoculum. APP strains were plated on chocolate agar and incubated at 37ºC in 5% CO2 for 24 hours. Two full loops of each plate were transferred to 50mL of PPLO broth with added NAD and this suspension was incubated for 18 hours, when APP concentrations were determined using a spectrophotometer (optical density at 280nm) and bacterial counts confirmed by plate dilutions. After 18 hours of incubation, 1mL of each tube was transferred to new tubes with additional 50 mL of PPLO. The bacterial counting for each suspension was determined at 2, 4, 6 and 8 hours of incubation. At each point, APP concentrations were again determined using a spectrophotometer and confirmed by plate dilutions. Colony counting for each serotype was performed at 24 and 48 hours. The growth curve was determined for each serotype and the exponential phase identified. Challenge inoculum cultures were harvested at 6 hours 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 hours of its preparation.

Clinical signs

Animals were observed for three consecutive days post inoculation for the development of clinical signs of APP infection and weekly thereafter. Rectal temperatures were collected daily for three days after inoculation to monitor the course of infection. Pigs that were found to be prostrated, with evident respiratory signs, and rectal temperature above 105°F were treated with ceftiofur.

Blood samples

Blood samples were collected prior to the inoculation (day 0) and weekly thereafter for seven consecutive weeks. Five ml of blood were collected from each pig by venipuncture of the jugular vein using vacutainer tubes. Samples were left in the refrigerator (4 ºC) overnight and centrifuged in the next day to separate the serum. All serum samples were divided into 1.5 mL aliquots, placed into identified boxes, and frozen at -80ºC until assayed. Serum samples were tested weekly using the BIOVET serotype-specific ELISA tests at the MVDL and with the Multi-APP ELISA test at the University of Montreal. The Apx IV ELISA offered by IDEXX was not available for purchase when this experiment was performed. Cross-reactivity among the serotypes that successfully infected the pigs was evaluated by testing serum samples from day 49 post-infection using all serotype-specific BIOVET ELISA tests.

Tonsillar swabs

Tonsillar swab samples were taken prior inoculation (day 0) and weekly thereafter for seven consecutive weeks. Two swabs were taken every week from each pig, one for bacterial isolation and one for DNA extraction. The tonsillar swabs collected for bacterial isolation were inoculated in 5% sheep blood agar plates with a nurse Staphylococcus epidermidis streak. All plates were incubated at 37ºC in a 5% CO2 atmosphere for 48 hours. Plates were checked for the presence of APP suspect colonies at 24 and 48 hours. Suspect colonies (hemolytic
with satellitism to the *S. epidermidis* nurse streak) were re-isolated onto blood agar and incubated in similar conditions for 24 additional hours. DNA from pure cultures was extracted and tested by PCR described by Schaller et al. (1999) to confirm the identity of the isolate. Tonsillar swabs collected for DNA extraction were placed into identified centrifuge tubes (1.5mL), suspended in 300µL of PBS (phosphate buffer saline) and vortexed for 1 minute. Swabs tips were removed from the tubes and the remaining suspensions were centrifuged at 13,000 rpm during 3 min. The supernatant was discarded and the remaining pellet was re-suspended in 200 µL of Prepman Ultra (Applied Biosystems, Foster City, CA.). The re-suspended content was vortexed for an additional 15 seconds and then boiled for 20 min using a heat block. After boiling, the suspension was again centrifuged at 13,000 rpm during 3 min to separate the DNA from cell debris. A total of 50 µL of DNA from the supernatant was mixed with 50 µL of DNA-Rnase-free water and this was used as template for the PCR reactions.

**Necropsy**

At the necropsy, all pigs were examined for the presence of lesions and tonsillar swabs, whole tonsils, and lung swabs were collected for APP isolation and PCR. Whole tonsils were removed using sterile scissors and forceps for each pig. Fragments of 1 cm² were obtained from each tonsil and utilized for DNA extraction. Each fragment was placed on a sterile plastic bag with 300 ul of PBS, homogenized using a ‘stomacher’ and 100 ul of the tissue homogenate was transferred to a 1.5 ml tube for DNA extraction. DNA extraction from tissue homogenate was done using the Qiagen DNeasy Blood & Tissue Kit following the manufacture’s instructions. DNA extraction from swabs and bacterial isolations were performed as described previously (tonsillar swabs section).

**Results:**

**Inoculum**

The serotype of all reference strain was confirmed by toxin profiling (Rayamajhi et al. 2005) and indirect hemagglutination (Mittal et al. 1992) prior to inoculation. The expected inoculum dose of 10⁶ CFU/mL was also confirmed for all serotypes by plate counting. Successful infection was confirmed in groups inoculated with serotypes 1, 3, 7, 10, and 15 based on serology and tonsil PCR results at day 49 post-infection. Pigs in groups inoculated with serotypes 5 and 12 did not become infected and remained serologically and PCR negative until the end of the study.

**Clinical response to infection and necropsy findings**

All pigs were clinically examined during three consecutive days post-inoculation and weekly after that. Only pigs from groups infected with serotypes 10 and 15 showed clinical signs characterized by respiratory distress and lethargy within 24 hours post-inoculation. Elevated rectal temperatures (average greater than 105°F) were observed among pigs infected with serotype 10 (Table 2). One pig from this group died after being treated for two consecutive days with ceftiofur and APP serotype 10 was isolated from the lungs. One pig from the group infected with serotype 15 was euthanized at 3 weeks post-infection due to a leg injury unrelated with the
experimental infection. At necropsy, lesions characteristic of APP infection (necrosis and hemorrhage in the diaphragmatic lobes of the lungs) were observed and APP serotype 15 was isolated from the lungs. At 49 days post-infection, mild focal fibrous adherences between the surface of the lungs and the parietal pleura were observed in pigs infected with serotypes 5, 7, 10, and 15. No lesions were observed in pigs inoculated with serotypes 1, 3, and 12 (Table 2).

**Bacterial isolation**

APP was not isolated from tonsillar swabs collected weekly throughout the experiment or from clinical samples collected at necropsy on day 49 post infection. APP was only isolated from pleuropneumonia lesions observed in one pig inoculated with serotype 10 and one pig inoculated with serotype 15 euthanized at 3 and 21 days post infection, respectively.

**PCR results**

The swabs utilized in this study to collect weekly tonsil samples contained a gel-based media in the bottom which interfered with the efficiency of the PCR. Results were inconsistent and not reproducible due to this interference. Reliable PCR results were only obtained at 49 days post-infection, when tonsil fragments were collected and tested. Table 3 shows the number of positive animals per serotype group obtained by PCR on tonsil’s fragments collected at 49 days post-infection. Infection was confirmed in groups infected with serotypes 1, 3, 7, 10, and 15. All pigs inoculated with serotypes 5, 12 or from the non-inoculated control groups were negative for the presence of APP in the tonsils by PCR.

**Serological findings**

Table 3 summarizes serological and PCR results obtained throughout the experiment. No seroconversion was observed in pigs infected with serotype 5 and 12 or in the non-infected control groups at any time point by either ELISA test. Pigs from these groups were also PCR negative at day 49 post infection. The Multi-APP ELISA detected seroconversion one week earlier than the serotype-specific ELISA tests in pigs infected with serotypes 1, 3, and 10. Both ELISA tests detected seroconversion 2 weeks post infection in pigs infected with serotypes 7 and 15. At day 49 post-infection, the Multi-APP ELISA detected a higher number of positives in groups infected with serotypes 1, 3, 10, and 15 compared with the serotype-specific ELISA tests. Both ELISA tests detected 8/9 positive pigs infected with serotype 7 at this time point. Seroconversion varied greatly depending on the serotype utilized for infection. Lower seroconversion rate was observed in groups inoculated with serotypes 1, 3 and 10, whereas 89-100% seroconversion was detected in groups inoculated with serotypes 7 and 15, respectively. The number of pigs with positive tonsil PCR at day 49 post-infection was consistently lower than the number of pigs with positive antibody titers detected by Multi-APP ELISA tests in all groups (Table 3). There was no direct association between positive tonsil PCR results and seroconversion at day 49 post infection.
Discussion:

*Actinobacillus pleuropneumoniae* serological monitoring is commonly used in the U.S. to avoid the introduction of subclinically infected pigs into naïve populations. *A. pleuropneumoniae* has been successfully eliminated from most U.S. swine herds and the prevalence of infection is now relatively low. The serotype-specific ELISA tests offered by BIOVET were for many years utilized as the gold standard for serological screening. Although these tests generate reliable and specific data regarding APP infection, the costs associated with screening for several APP serotypes are prohibitive for many swine herds. In 2005, two new APP screening tests were introduced into the U.S. – the Apx IV ELISA offered by IDEXX and the Multi-APP ELISA offered by the Veterinary Diagnostic Laboratory at the University of Montreal. The Apx IV ELISA targets antibodies against the Apx IV toxin produced by APP during infection, whereas the Multi-APP ELISA and the serotype-specific ELISA tests target antibodies against the LC-LPS from APP. With many tests available for APP serological screening, discordant results were a frequent finding among field veterinarians. The objective of this study was to compare the performance of all available serological tests to detect APP subclinical infection under identical experimental conditions.

Subclinically infected pigs are the main source of APP introduction into naïve populations. In this study we aimed to reproduce subclinical infection by exposing pigs to a sub-lethal dose of APP (10⁶ CFU/ml). Subclinical infection was accomplished in groups infected with serotypes 1, 3, 7, and 15. Pigs infected with serotype 5 and 12 did not become infected, whereas pigs infected with serotype 10 showed evident clinical signs 24-48 hours post infection. All pigs were infected with the same dose, indicating that infectivity varies depending on the serotype used.

The unsuccessful infection of pigs with serotypes 5 and 12 was unexpected, since these serotypes are commonly associated with disease in the field. Although there is no data in the literature supporting the successful experimental infection of pigs with serotype 12, typical pleuropneumonia lesions have been reproduced following experimental infection with 10⁸-10⁹ CFU/ml of serotype 5 (Ueda et al, 1995). The dose required to reproduce a subclinical infection for serotypes 5 and 12 may be higher than that used in this study. The development of severe clinical signs following infection with serotype 10 was also unexpected. A query of APP isolation from clinical cases at the Minnesota Veterinary Diagnostic Laboratory from 2002 to 2008 revealed that serotype 10 was not isolated from clinical cases in the U.S. during this period. The few isolates that were classified as serotype 10 by IHA were not confirmed as APP based on toxin profile, genotyping, and sequencing of the 16 rRNA gene (Oliveira et al, 2008). Although serotype 10 is not commonly isolated from field cases in the U.S., we decided to include it in the study due to the fact that a few swine herds reported seroconversion to this serotype in the absence of clinical infection. The serotype 10 strain utilized to infect pigs in this study was obtained from Dr. Blackall’s collection, and it may differ from strains that are currently circulating in the U.S. There is no information in the literature regarding the experimental infection of pigs with this serotype.

Our initial design included weekly testing of serum samples post-infection using the serotype-specific ELISA tests, the Multi-APP ELISA tests, and the Apx IV ELISA offered by IDEXX; however, IDEXX interrupted commercialization of the Apx IV ELISA test prior to the beginning of the experiment and we were not able to utilize this kit. Consequently, we were only able to compare the performance of the BIOVET serotype-specific ELISA tests and the Multi-APP ELISA test. Although both of these tests detect antibodies to the LC-LPS of...
APP, there were important differences in the consistency of results obtained weekly for each infected group, and more importantly, important differences in sensitivity to detect early seroconversion. The Multi-APP ELISA tended to detect seroconversion one week earlier than the serotype-specific ELISA tests. It also tended to be more consistent in maintaining positive and negative results for individual pigs throughout the experiment. The serotype-specific ELISA tests first detected true positives (as defined by Multi-APP ELISA) as suspects, followed by weak positive results in the subsequent week. Inconsistencies in suspects, positives, and negative results were mainly observed with the serotype-specific ELISA tests detecting antibodies against the serogroup 1-9-11 and serotype 10. The Multi-APP ELISA contains a mix of LC-LPS antigens obtained from all APP serotypes and may represent a more complete antigen pool for detection of seroconversion compared with the serotype-specific ELISA tests, which contains a single antigen for each serotype.

Our results demonstrated that the serotype-specific ELISA tests formulated to detect antibodies against the serogroup 3-6-8 can also detect antibodies against the serotype 15. S/P ratios were in fact higher for pigs infected with serotype 15 compared with those infected with serotype 3 (data not shown). This data raised the question whether other cross-reactions could occur using the serotype-specific ELISA tests. To address this questions, we cross-tested all serum samples obtained at day 49 post-infection from pigs infected with serotypes 1, 3, 7, 10, and 15 with the serotype-specific ELISA tests 1-9-11, 2, 3-6-8, 4-7, 10, and 12. No cross-reactions were observed, confirming that these tests specifically detect the serogroups indicated on each kit.

Although tonsil swabs were collected weekly for isolation and PCR, both tests failed to confirm infection throughout the experiment. Tonsil swabs utilized for isolation also carried the normal flora, which made identification of APP colonies a difficult task. Although APP has been successfully isolated from the tonsils of infected pigs (Ueda et al, 1995), this was not reproduced in this study. Techniques such as immunomagnetic isolation are known to improve the chances of isolating APP from tonsils (Gagne et al, 1998). However, our laboratory was not set up to run this technique. APP was successfully isolated form the lungs of pigs that developed pleuropneumonia, which indicates that isolation still has value to confirm APP infection. PCR failed mostly due to the use of incorrect swabs for sample collection. The gel media in the bottom of the swab interfered with the PCR reaction and severely affected the sensitivity of the technique. PCR of tonsil fragments collected during necropsy at 49 days post-infection did yield positive results. These results, in addition to the serological results, were useful to confirm infection of pigs inoculated with APP serotypes 1, 3, 7, 10, and 15 and to confirm that pigs inoculated with serotypes 5 and 12 did not become infected. The number of pigs per group that were positive by tonsil PCR was lower than the number of pigs that were seropositive at 49 days post infection. There was no direct correlation between a positive PCR results and a positive serological result. These differences may be associated with the fact that pigs with positive serological results may have cleared APP from the tonsils, whereas pigs with negative serological results but positive tonsil PCR results could be recently infected through nose-to-nose contact within a group. Although we failed to detect APP in tonsil swabs by PCR, we did detected APP DNA in the saliva obtained from ropes hanged in the rooms with pigs infected with serotypes 7 and 10 at 1 week post infection (data not shown). These results suggest that shedding was likely more active during the acute phase of infection, and that tonsil PCR would still have been a good alternative diagnostic method to troubleshoot unexpected serological results during this phase provided proper swabs are used for sample collection.

Based on the results obtained in this study, we recommend that the Multi-APP ELISA test be used for serological screening of pigs prior to introduction into naive 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.

ACKNOWLEDGEMENTS

This work was supported by the grant 08-095 of NPB (National Pork Board) and in part by the Swine Disease Eradication Center at the University of Minnesota.

REFERENCES


**TABLES**

Table 1 – Strain identification and room assignment for *Actinobacillus pleuropneumoniae* serotypes used for experimental infection.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>APP SEROTYPE</th>
<th>STRAIN*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>4074</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>1421</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>K17</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>WF83</td>
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<td>5</td>
<td>10</td>
<td>22009</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>1096</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>HS143</td>
</tr>
<tr>
<td>8</td>
<td>Negative control</td>
<td>-</td>
</tr>
</tbody>
</table>

*Blackall et al (2002)

Table 2 – Average rectal temperature and number of pigs with *post mortem* lesions per group.

<table>
<thead>
<tr>
<th>SEROTYPE</th>
<th>TEMP. ºC</th>
<th># LESIONS*</th>
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<tr>
<td>1</td>
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</tr>
<tr>
<td>3</td>
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<td>5</td>
<td>104.07</td>
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<tr>
<td>7</td>
<td>104.03</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td><strong>106.31</strong></td>
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</tr>
<tr>
<td>12</td>
<td>103.82</td>
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<tr>
<td>15</td>
<td>103.96</td>
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</tr>
<tr>
<td>Control group</td>
<td>104.23</td>
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*Focal fibrous adherences in the lungs*
Table 3 – Summary of serological and PCR results for all experimental groups

<table>
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<tr>
<th>Serotype</th>
<th>n</th>
<th>Earliest + detection (wk)</th>
<th>+/suspect/- at 49 d.p.i</th>
<th>Earliest + detection (wk)</th>
<th>+/- at 49 d.p.i</th>
<th>(+/-)</th>
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<td>10</td>
<td>5 **</td>
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<td>3</td>
<td>5/5</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
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<td>3</td>
<td>1/4/5</td>
<td>2</td>
<td>5/5</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>-</td>
<td>0/0/10</td>
<td>-</td>
<td>0/0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>9*</td>
<td>2</td>
<td>8/0/1</td>
<td>2</td>
<td>8/1</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>9*</td>
<td>2</td>
<td>1/3/5</td>
<td>1</td>
<td>4/5</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>-</td>
<td>0/0/10</td>
<td>-</td>
<td>0/0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>9*</td>
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<td>5/2/2</td>
<td>2</td>
<td>9/0</td>
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<tr>
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<td>-</td>
<td>0/0/9</td>
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<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

*All groups started with 10 pigs. This represents the number of pigs at 49 days post infection.

**data for 4 weeks was not available for serotypes 1, 5 and 7.