Marsteller TA, Fenwick B. *Actinobacillus pleuropneumoniae* disease and serology. *Swine Health Prod.* 1999;7(4):161–165.

Actinobacillus pleuropneumoniae disease and serology

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Summary

Actinobacillus pleuropneumoniae is a primary bacterial pathogen causing swine respiratory disease and has been described in most major swine-producing regions of the world. Preliminary diagnosis of *A. pleuropneumoniae* is based on clinical signs and gross pneumonic lesions. Isolating and identifying the causative bacteria confirms the diagnosis. Twelve serotypes of *A. pleuropneumoniae* and a number of serotype variants have been identified. Serum diagnostic tests have been developed for some of these serovars. The serological test to be implemented in a herd diagnostic effort should be determined by the herd veterinarian in consultation with the laboratory. The herd veterinarian should evaluate the advantages and disadvantages of each serological test prior to determining which test to apply in a specific herd situation. Several tests may need to be employed, as in some cases multiple serotypes are present in the same herd. Each laboratory assigns its own sensitivity and specificity to the tests they provide. Diagnostic cutoff points are set for herd evaluations and thus, serological evaluations should only be completed on a herd basis as they are not valid when applied to the individual animal. It is important to ensure that an adequate sample size of pigs is evaluated to determine the correct serological status of a swine population.

Keywords: swine, *Actinobacillus pleuropneumoniae*, pneumonia, respiratory disease

Received: December 21, 1998
Accepted: April 19, 1999

Actinobacillus pleuropneumoniae disease is a common primary bacterial pneumonia in swine populations worldwide.[1] Because 12 serotypes of *A. pleuropneumonie* plus serotype variants exist worldwide, identifying serotypes and determining what serological methods to employ in herd situations has been confusing.[2] The use of polymerase chain reaction (PCR) has been helpful in solving serotype and bacterial strain issues. New serology methods have been developed that improve the sensitivity and specificity of *A. pleuropneumonie* serum tests.[3–7] Application of the new serum tests within herd situations has improved the ability of the herd veterinarian to monitor populations of pigs for *A. pleuropneumoniae*. This paper reviews *A. pleuropneumonie* disease, the bacterial characteristics, pathology, serological methods, and application of serology for herd situations.

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This article is available online at http://www.aasp.org/shap.html.

The disease

The diagnosis of *A. pleuropneumoniae* disease in the United States was first reported in 1963.[8] Since then, there have been reports of the clinical signs, pneumonic lesions, bacterial isolation, and identification from herds throughout North America.[9] When taken together, these remain the "gold standard" for diagnosing *A. pleuropneumoniae* disease.

During herd outbreaks of *A. pleuropneumoniae* disease, the herd may simultaneously contain individual pigs that demonstrate all the clinical signs, including peracute, acute, subacute, and chronic pneumonia. All ages of pigs may be affected, but pigs 12–16 weeks old are most frequently diagnosed with the disease. The serological status of the breeding and growing herd may help explain the timing of outbreaks (Cruijen A, et al; *Proc IPVS Cong,* 1992: 227).[10]

Sow herds with *A. pleuropneumoniae* are typically seropositive and the sows pass high concentrations of maternal antibodies via colostrum to their offspring. Pigs lose their maternal antibody protection by approximately 4 weeks of age, and evidence of antibody titer has disappeared by 9 weeks of age.

Actinobacillus pleuropneumoniae may colonize the neonatal pig's respiratory tract while the pig is nursing the infected sow. *Actinobacillus pleuropneumoniae* has been described as a late colonizer compared to other bacteria. Therefore, weaning and segregating the piglets prior to colonization can prevent vertical *A. pleuropneumoniae* transmission (Wiseman BS; *Proc Minn Swine Conf*, 1992:223–231. Harris DL; *Hog Farm Mgmt*, 1989:50–55). However, in some herds using age-segregated weaning with a relatively early weaning age, vertical transmission may still occur. Antibiotics may be given to sows and/or their offspring to prevent the transmission of *A. pleuropneumoniae* bacteria from the sow to the pig when weaning age adjustment and segregation are ineffective (Desrosiers R; *Proc Minn Swine Conf*, 1988:244–250. Geiger JO, O'Hare WJ; *Proc AASP Ann Meet*, 1995:447–451. Schultz RA; *Proc AASP Ann Meet*, 1997:407–409). Each herd's production site, pig management, health, and immune status may be different. The herd veterinarian must be familiar with the herd and understand the interactive nature of factors associated with *A. pleuropneumoniae* transmission to produce a positive result with intervention strategies.

It has been shown that *A. pleuropneumoniae* disease expression is dose dependent.[11] Lower exposure results in seroconversion without clinical disease, while a slightly higher level of exposure results in fatal infections. In theory, the infectious dose of *A. pleuropneumoniae* increases logarithmically with pig age in the environment of *A. pleuropneumoniae*-positive herds. In an endemically infected herd, this

theoretical model would suggest that growing animals are exposed to increasing doses of *A. pleuropneumoniae* bacteria over time, which may help to explain why outbreaks occur in 12- to 16-week-old pigs.

The economic losses associated with *A. pleuropneumoniae* disease are greatest in pigs with gross lung lesions.[12] In contrast, it has not been possible to measure the economic impact of *A. pleuropneumoniae* colonization in pigs without overt clinical disease.[13] Pigs with clinically apparent pleuropneumonia do not grow at their genetic potential due to chronic respiratory disease, and consequently, their average daily gain (ADG) is reduced and feed conversion ratios are increased.[14,15] Other economic losses due to *A. pleuropneumoniae* disease include mortality, intervention costs, and slaughter trim losses (Rodibaugh M; *Swine Pract*, Feb 1993:20–22. Tubbs R, Deen J; *Proc AASP Ann Meet*, 1997:361–366). Feed, facility, and labor inputs increase with pig age and weight. Therefore, economic losses due to *A. pleuropneumoniae*-related mortality increase with pig age.

Increased feed cost is the major factor in calculating loss associated with clinical *A. pleuropneumoniae* disease in market weight swine. Lost opportunity from market sales may also be a significant economic loss. Intervention costs such as injected, water, and feed medications are greater in herds with *A. pleuropneumoniae* disease. Vaccine costs may also add to the intervention costs of *A. pleuropneumoniae* disease. Slaughter trim losses due to injection site trims or pleural adhesion trims are important when determining the full impact of losses due to *A. pleuropneumoniae* disease.[16] Assessing all these factors when determining the economic impact of *A. pleuropneumoniae* in a herd, while important, is difficult at best due to ever-changing input and output variables.

Treating animals with clinical pneumonia during *A. pleuropneumoniae* outbreaks can be successful (Derosiers R, *Proc AASP Ann Meet*, 1997:333–344).[17] Treatment early in the course of the disease is of paramount importance. Group therapy intended to control the spread of *A. pleuropneumoniae* disease in the subclinically infected portion of the group is also important. Animal groups must be observed daily and dead pigs necropsied to make a preliminary diagnosis of an *A. pleuropneumoniae* outbreak. Mortality or treatment records (recorded on a daily timeline) are the best tool for farm managers to use when instituting a control program. Scanning records can allow one to predict when a pleuropneumonia outbreak is likely to occur in an endemic herd so that appropriate control measures can be strategically applied.

The bacteria

In a recent diagnostic laboratory survey, *A. pleuropneumoniae* was observed to be the most common primary bacterial pneumonia infection (20% of cases).[18] Thirty percent of the cases of *A. pleuropneumoniae* were uncomplicated by other pathogens. The most common bacterial combination with *A. pleuropneumoniae* was *Pasteurella multocida*.

Actinobacillus pleuropneumoniae was first classified as *Haemophilus pleuropneumoniae*.[8] In 1983, the bacteria was reclassified and given the name *Actinobacillus pleuropneumoniae*, after DNA homology studies demonstrated the close relationship of *H. pleuropneumoniae* to *Actinobacillus lignieresii*.[19]

Bacteria with *Pasteurella haemolytica*-like growth characteristics have been isolated from swine with pleuropneumonia-like lesions.[19] Differentiation of these bacteria and further classification within the species was accomplished by comparing bacterial DNA. Further DNA differentiation work was needed to classify the bacterial isolate as *A. pleuropneumoniae*.

Actinobacillus pleuropneumoniae is a gram-negative, nonmotile, nonspore-forming, small coccoid- or rod-shaped bacteria.[1] The bacteria grow readily from the lung lesions of untreated pigs on chocolate or blood agar (supplemented with NADA found in lysed blood or "V strips") in a CO_2 chamber. *Actinobacillus pleuropneumoniae* colonies grown on blood agar plates will demonstrate a zone of β-hemolysis. *Staphylococcus aureus* streaked across a blood plate will allow the colonies to demonstrate the CAMP reaction. The bacteria are facultatively anaerobic and may require CO_2 for primary growth.

Care should be exercised when evaluating only the hemolytic characteristics of *A. pleuropneumoniae* isolates (X and V factors). Frank described an *A. pleuropneumoniae* biotype 2 isolate from swine that did not require V factor on blood agar media.[20] Further biochemical testing and polymerase chain reaction (PCR) aided in differentiating the *A. pleuropneumoniae* biotype 2 isolate from *A. suis*.

Blanchard described a urease-negative variant of *A. pleuropneumoniae* serotype 1.[21] Therefore, if a question arises over the classification of the bacteria that cause swine respiratory disease due to biochemical reactions or growth characteristics, a strain-specific PCR may be required for final bacteria identification.[22]

Pathology

Preliminary diagnosis of porcine pleuropneumonia should be based on clinical signs and gross pneumonic lesions. Isolating and identifying the causative bacteria confirms the diagnosis (Sanford SE, *Proc AASP*, 1998:357–360).

Gross pathological lesions of *A. pleuropneumoniae* are well described.[9] The classic presentation of *A. pleuropneumoniae* disease is characterized by demarcated lesions in the middle, cranial, and the caudal lobes of the lung. The pneumonic areas of lung are dark and consolidated. Fibrinous pleurisy is obvious, especially in these areas of pneumonic lung. Chronically infected pigs typically have pleural adhesions, and abscesses may be found in the lung and other tissues. In contrast, the lung lesions in uncomplicated infections may resolve completely in only a few weeks, diminishing to only pleural adhesions at slaughter.

A number of other organisms can cause clinical signs and lung lesions that are difficult to distinguish from *A. pleuropneumoniae*. This difficulty is exacerbated by the sporadic nature of *A. pleuropneumoniae* disease outbreaks, even in infected herds. The causative agent cannot be determined from the appearance of gross lesions of

Erratum

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Several errors were published in Table 1 (page 163). Biochemical and bacterial growth characteristics are important in the classification of bacteria. However, the enzymes used in biochemical reactions can be up-regulated or down-regulated by the bacteria and may cause confusion with bacterial classification. Polymerase chain reaction, which uses the DNA of bacteria, may be important in classifying any bacteria when confusion exists concerning bacterial biochemical reactions. *Actinobacillus pleuropneumoniae* is especially noted for its variable biochemical reactions.[21] The corrected Table 1 appears here.

Table 1

Biochemical and bacterial growth characteristics

	Actinobacillus pleuropneumoniae	*Actinobacillus suis*	*Haemophilus parasuis*
Urease	±	+	–
Porphyrin	+	+	+
X- and V-factor dependent	variable	independent	V-factor dependent

Table adapted from Kilian M, Frederiksen W, Biberstein EL. *Haemophilus, Pasteurella,* and *Actinobacillus*. London: Academic Press Inc, Ltd. 1981; 283–288.

Table 1

Biochemical and bacterial growth characteristics[19]

	A. pleuropneumoniae	*A. suis*	*H. parasuis*
Urease	Positive	Negative	Negative
Porphyrin	Positive	Negative	Positive
X or V factor dependent	V factor	X and V factor	V factor

pneumonia alone,[23] because many septicemic diseases may potentially cause lung lesions. Bacterial culture with proper enrichment media may be needed to confirm the diagnosis.

Actinobacillus suis, an emerging disease, may be confused with *A. pleuropneumoniae* (Sanford SE; *Proc AASP Ann Meet*, 1998:357–360). However, *Actinobacillus suis* is a septicemia and clinical signs and gross pathological lesions may be somewhat different than those produced by *A. pleuropneumoniae* (Yaeger M; *Proc AASP Ann Meet*, 1997:475–477) (Table 1).[19] *Actinobacillus pleuropneumoniae* typically demonstrates as a focal pneumonic lesion while *A. suis* causes a multifocal pneumonia. *Actinobacillus pleuropneumoniae* also produces a focal fibrinous pleuritis and/or pericarditis while *A. suis* tends to be a diffuse serofibrinous pleuritis, pericarditis, and peritonitis. *Actinobacillus pleuropneumoniae* lesions are typically found only in the caudal lobe of the lung whereas *A. suis* will typically produce a diffuse pneumonia.

Haemophilus parasuis, another septicemic pathogen, may also be confused with *A. pleuropneumoniae*. *Haemophilus parasuis* lesions include meningitis, pleuritis, pericarditis, peritonitis, and arthritis, which occur in various combinations or singly (Table 1).[19] The most predominant lesion is meningitis.

The histopathology of *A. pleuropneumoniae* pneumonia cases is characterized by lung necrosis, hemorrhage, neutrophil infiltration, macrophage and platelet activation, vascular thrombosis, edema, and a fibrinous exudate.[9] Histopathology may not provide confirmatory evidence that the etiologic pathogen is *A. pleuropneumoniae*. Bacteriology is necessary to form a definitive diagnosis.

Serotypes and serology

Twelve serotypes of *A. pleuropneumoniae* and a number of serotype variants have been reported in the literature.[2] Serotypes 1, 5, and 7 are most prevalent in the United States swine population.[24,25] In our experience, the occurrence of serotype 3 is increasing. Serotypes 2 and 9 are more prevalent in the European swine population (Kobisch M. *Proc AASP Ann Meet*; 1996, 445).

The severity of clinical signs within a population of pigs may be dependent on the serotype involved. Serotype 1 has been noted as a highly virulent pathogen in most swine herds (Sanford SE; *Proc AASP Ann Meet*, 1998:357–360).[17] Serotypes 2, 5, 9, 10, and 11 are described as moderately virulent. Serotypes 3, 6, 7, and 12 are typically least virulent.[10] However, even within a specific serotype, certain strains may be more virulent than others. Regardless of the *A. pleuropneumoniae* serotype involved, disease severity depends on the exposure dose and the susceptibility of the pig. Multiple serotypes of *A. pleuropneumoniae* may exist within the same herd,[3,6] making diagnosis and serological testing a challenge. In certain cases, multiple serological tests may be required in herds with multiple *A. pleuropneumoniae* serotype isolates.

Differentiating *A. pleuropneumoniae* serotypes can be quite puzzling. Cross-reaction of *A. pleuropneumoniae* isolates with the tube agglutination test makes this an inexact science. Cross-reaction between serovars 1, 9, and 11; serovars 3, 6, and 8; and serovars 4 and 7 have been reported. Using PCR to identify the serotype-related genetic characteristics of the bacterial isolate has been useful in unraveling the serotype issues.[20,22,26,27] PCR-based DNA fingerprinting may also prove helpful in tracking a particular *A. pleuropneumoniae* strain through pig populations and among herds.[26,28]

Actinobacillus pleuropneumoniae capsular polysaccharides are antigens used in some complement fixation (CF) and enzyme linked (ELISA) serologic tests.[4,29,30] These same antigens are present in many vaccines (Thacker B, et al; *Proc IPVS Cong*, 1996:197). Lipopolysaccharides of *A. pleuropneumoniae* also vary among the *A. pleuropneumoniae* serotypes, and cross-reacting antigens are present. A number of cytotoxins have been described with *A. pleuropneumoniae* (Sanford SE; *Proc AASP Ann Meet*, 1998:357–360). These bacterial cytotoxins interact with the immune system response in the animal and the hemolysin neutralization test (HNT). Cytotoxins, which induce a neutralizing immune response, are not present in the *A. pleuropneumoniae* vaccines currently on the market. Therefore, the HNT test will not detect immunological responses with current vaccine use.

Many diagnostic serology laboratories offer *A. pleuropneumoniae* ELISA tests. The ELISA test has shown promise of improved sensitivity and specificity for field application over the CF test (Morrison R, et al; *Proc IPVS Cong*, 1984:102).[5,7,31–33] *Actinobacillus pleuropneumoniae* serotype-specific ELISAs have been developed for serotypes 1, 4, 5, and 7.[33–35] Recently, an inhibition enzyme immunoassay (inhibition EIA) technique has been developed for serotype 5.[36] When using the inhibition EIA technique, no cross-reactivity was observed with serum from pigs infected with other *A. pleuropneumoniae* serotypes, *H. parasuis,* or *A. suis*. An *A. pleuropneumoniae* strain-specific ELISA could potentially be developed for any *A. pleuropneumoniae* isolate. However, this task can not be completed without an investment in laboratory materials and expertise.

Cross reactions to the Apx toxins of *A. suis* may cause serological false positives with the CF and HNT test (MacInnes JI, Frey J; *Proc AASP Ann Meet*, 1997:471–474).[37] Cross reaction of serum from *A. suis*-exposed pigs to an *A. pleuropneumoniae* ELISA has not been demonstrated.

The herd veterinarian, in collaboration with the supporting diagnostic laboratory, should determine the best serologic test to use in each herd

situation. There is no one best *A. pleuropneumoniae* serological test for all herds. To confirm *A. pleuropneumoniae* infection:

1. Isolate and confirm *A. pleuropneumoniae* bacteria from pleuropneumonic lung.
2. Serotype the *A. pleuropneumoniae* isolate(s) from the lung cultures.
3. Confer with the serology laboratory concerning which test to use for each serotype isolated from each herd. You may need two or more serologic tests when two or more *A. pleuropneumoniae* serotypes are present in one herd.
4. The sample size depends upon herd size and suspected prevalence (Gardner I. *Proc Leman Swine Conf.* 1994; 1–5). In most situations, 30 animals per cohort group will be sufficient.

Many endemically infected sow herds are 100% serologically positive.[38] The number of 8- to 10-week-old pigs with antibody titers should be relatively low when the seroconversion rate is increasing as pigs are exposed to the organism.[10] More frequent serological evaluation will help to determine when the organism exposure is highest. If seroconversion is not noted in the seroprevalence survey, then you need to reevaluate whether you're using the proper serological test for a particular herd. After the initial serological survey, you can then determine whether *A. pleuropneumoniae* control strategies change the clinical picture or serological prevalence between groups of pigs.

Table 2

Herd *A. pleuropneumoniae*-status categories[38]

	Clinically positive- C	Clinically negative- c
Serologically positive - S	C S - category 3	c S category 1
Serologically negative - s	C s - undefined category	c s category 2

Applied serology

The *A. pleuropneumoniae* status of herds will fall into one of four categories with respect to the presence of clinical signs and serological status (Table 2).[38] From a serological perspective, clinically positive and serologically negative *A. pleuropneumoniae* herds do not exist. Herds with clinical signs of *A. pleuropneumoniae* should also contain serologically positive pigs. When herds are clinically positive but serologically negative, it means that the serology test chosen was not sensitive or specific enough to detect serum antibodies for that particular serotype of *A. pleuropneumoniae*. This might happen, for example, if an ELISA test that was developed for serotypes 1, 5, and 7 is used on a herd clinically positive for serotype 3, or if an HNT serology test that detects serotypes 1, 5, 9, 10, or 11 is used for a herd clinically positive to either serotype 3 or 7. Given the potential for false negatives, it is important to complete steps 2 and 3 (above) before selecting a herd serology test. Early in the course of an acute *A. pleuropneumoniae* outbreak in a naïve herd, you may find the animals to be clinically positive but serologically negative. This phenomenon will last 3–4 weeks when antibodies develop from *A. pleuropneumoniae* exposure.

In our experience, the most common category of herds is the Category 1 herd (Table 2). Approximately 80% of herds fit this category. Category 2 and 3 herds (Table 2) make up the remaining 20% of herds in the industry. The concern with Category 1 herds is that an environmental or management practice challenge will occur that causes an outbreak of disease.[39] This would cause the Category 1 herd to achieve Category 3 status without any recent herd-to-herd spread of *A. pleuropneumoniae* bacteria.

Even within these three *A. pleuropneumoniae* herd categories, there are frequently herds in which the breeding and growing herd are in different categories. In herds that appropriately deploy SEW and AIAO production methods, it is possible for the breeding herd to be a Category 1 herd and the growing herd to be a Category 2 herd (Wiseman BS; *Proc Minn Swine Conf*, 1992:223–231. Harris DL; *Hog Farm Mgmt*, 1989:50–55. Geiger JO, O'Hare WJ; *Proc AASP Ann Meet*, 1995:447–451). As long as management of the growing pig is maintained and biosecurity is adequate, clinically negative *A. pleuropneumoniae* pigs can be produced from an endemically infected *A. pleuropneumoniae* sow herd.

Tonsil swabbing is another screening tool that may be used alone or together with serology. Tonsil swabbing may be most important for animal additions to a clinically and serologically negative (Category 2) herd, especially when a thorough veterinarian-to-veterinarian conference does not take place during animal purchases. The sensitivity for detecting *A. pleuropneumoniae* carriers using tonsil swabbing via direct culture is low; however, newly developed techniques such as PCR, monoclonal antibody, and immunomagnetic separation (IMS) tests have improved the sensitivity of the tonsil swab screen (Gottschalk M, Bilodeau R; *Proc Leman Conf*, 1997:82–88).[40] IMS has demonstrated 1000-fold higher sensitivity than a direct tonsil swab for detecting *A. pleuropneumoniae* carriers.

You should determine which serological test to implement in a herd diagnostic effort in consultation with the laboratory.[3,4] You'll need to evaluate the advantages and disadvantages of each serological test before deciding which test to apply in a specific herd situation.[5] You may need to use several tests, if multiple serotypes are present in the same herd.[6] Be aware that each laboratory assigns its own sensitivity and specificity to the tests it provides.[7] Diagnostic cutoff points are set for herd evaluations and thus, serological evaluations should only be completed on a herd basis —they are not valid when applied to the individual animal.[3,7] Assuring that an adequate sample size within the population of pigs being evaluated is an important factor in determining the correct serological status of a swine population (Gardner I; *Proc Leman Swine Conf*, 1994:1–5).[6]

Implications

- Bacterial isolation is still the "gold standard" for *A. pleuropneumoniae* diagnosis.
- Final identification and serotype classification of an *A. pleuropneumoniae* isolate may require PCR. PCR also may be useful when tracking *A. pleuropneumoniae* isolates through pig populations.

- Serotype each *A. pleuropneumoniae* isolate from a herd and consult a diagnostic laboratory prior to applying serology on a herd basis. Serology is a herd test, not an individual animal test.
- Each herd diagnostic process may be unique because different serotype or serotypes may be involved.

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