Relationships among seroconversion to *Mycoplasma hyopneumoniae*, lung lesions, and production parameters in pigs

Maria Sitjar, DVM; Elizabeth P. Noyes, DVM, PhD; Xavier Simon, DVM; Carlos Pijoan, DVM, PhD

Summary

Objective—*To assess the value of observing lung lesions at slaughter as a measure of lifetime pneumonia, to investigate the role of* Mycoplasma hyopneumoniae *in lifetime pneumonia, and to investigate the relationship between lifetime pneumonia and growth performance.*

Materials and methods—*Forty-eight pigs from a 150-sow farrow-to-finish farm with a history of respiratory problems were weighed and bled every 2 weeks until slaughter. Serum samples were processed for serology to measure antibodies against* M. hyopneumoniae, Actinobacillus pleuropneumoniae, *and pseudorabies virus (Aujeszky's disease). In addition, the thorax of each pig was radiographed every 2 weeks until slaughter. Lung parenchymal densities were determined from the radiographs and an average lifetime pneumonia (ALP) score was calculated. For each pig, radiographs were evaluated over time and used to determine when the pneumonia involved the highest percentage of the lung (pneumonic peak). Lungs were collected at slaughter and superficial lung lesions were measured. At the same time, pneumonic tissue was dissected and its relative volume was determined for each lung. For each weekly group of pigs, average daily gain (ADG) was calculated and correlated with pneumonic lesion measures (average lifetime pneumonia, volumetric pneumonia, and superficial pneumonia) for each pig as well as for each group. Onset of seroconversion to* M. hyopneumoniae *and* A. pleuropneumoniae *was tested for correlation with onset, extent, and severity of radiographic pneumonic lesions (ALP) and lung lesions seen at slaughter.*

Results—*A significant correlation between peak average pneumonia and seroconversion to* M. hyopneumoniae *was found. Groups showing early onset of infection had higher ALP and higher pneumonia peaks. Lesions seen at slaughter were a poor indicator of both average lifetime pneumonia and peak pneumonia.*

Implications—M. hyopneumoniae *appears to play an important role in the pneumonic peak of pigs. The growth of pigs infected later in the finishing phase with* M. hyopneumoniae *is less compromised than that of pigs infected earlier in the finishing phase.*

Keywords: *swine, pneumonia,* Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae, *radiography*

MS, XS: Upjohn Farmoquímica SA, Avda. Prat de la Riba 171, 08780 Palleja, Barcelona, Spain; EPN, CP: Department of Clinical and Population Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota. Reprint requests to: Dr. Carlos Pijoan, 385 Animal Science/Veterinary Medicine Building, 1988 Fitch Ave, St. Paul, Minnesota 55108

Received: *May 27, 1996*
Accepted: *September 26, 1996*

Porcine lower respiratory tract disease (pneumonia), is routinely evaluated at slaughter by observing gross lesions in lungs.[1–3] The effects of pneumonia on production have been studied by comparing average daily gain (ADG) and feed:gain ratio (F:G) between pigs that had pneumonia at slaughter to those values of pigs that did not.[4] Much debate has occurred regarding whether lung lesions found at slaughter are associated with production parameters.[5–7] Noyes, et al., found that pneumonia observed at slaughter is not a good estimator of lifetime pneumonia, while pneumonia observed during the life of the pig has a pronounced effect on production.[8] They used radiography to determine lifetime pneumonia in pigs, which cannot be determined by conventional slaughter methods. However, their study was not able to determine the microbiological cause of the pneumonia because appropriate serological techniques for *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae* were not available at that time.

Recently, workers in Sweden[9] reported a relationship between *M. hyopneumoniae* seroconversion and pneumonia. They found that animals that seroconverted earlier in life tended to have less pneumonia or regressing lesions, whereas late seroconverters had extensive pneumonic lesions. This study was conducted in an off-site, all-in–all-out (AIAO) finisher. These units commonly show delayed seroconversion as compared to conventional farrow-to-finish units, which seroconvert soon after weaning. Because of this, estimations of pneumonia in farrow-to-finish farms by slaughter examination tend to underestimate the extent of the problem and may explain the lack of correlation between pneumonia and growth.

This study aimed to determine whether there was a correlation between onset of pneumonia, extent and duration of lesions with seroconversion to *M. hyopneumoniae*, ADG, and weight at 180 days. An attempt was also made to assess the efficacy of several methods for determining lung lesions at slaughter by comparing them to radiologic measurements.

Materials and methods

Farm

A 150-sow, farrow-to-finish conventional herd with a history of cough and high prevalence of pneumonia at slaughter was selected. This farm

was located in northeast Spain, where pseudorabies virus (Aujeszky's disease) is endemic.

The starter feed for the piglets was medicated with oxytetracycline and neomycin. Other than the respiratory problems described above, no other chronic diseases had been reported during the 2 years previous to the start of the trial. All animals including the experimental pigs were maintained under identical management protocols.

Animals

Forty eight Landrace × Large White pigs from different weekly weaning groups were selected. At 3 weeks of age, pigs were weighed, sexed, tagged, and then systematically assigned to one of four weekly groups of 12 animals each. From 4 weeks of age until slaughter, pigs were weighed, bled, and radiographed every 2 weeks. All pigs that died during the study were examined by necropsy within 12 hours of death. At 180 days of age, regardless of their body weight, all pigs were slaughtered and their lungs examined.

To ensure that manipulation and radiography of the animals did not affect growth parameters, another 48 pigs from the same farrowing groups were used as a control group. These animals were weighed and tagged at 3 weeks of age and then weighed again when sent to slaughter. These animals received no other manipulation beyond the normal management practices of the farm.

Radiographic technique

Every 2 weeks, beginning at 4 weeks of age, pigs were weighed and then anesthetized by IM administration of 2 mg of azaperone (Stresnil®, Pitman-Moore Co., 36201, Vigo. Spain) per kg bodyweight followed by anesthetic induction with isofluorane (Abbott Co., 08037, Barcelona, Spain)[8,10,11] given by mask. When muscular relaxation was achieved, anesthesia was discontinued. A blood sample was taken and ventrodorsal and lateral radiographic views of the thorax were obtained using a portable x-ray machine (Min-X-Ray®, X803G 30–20 mA 50–80 Kv). All radiographs were taken during expiration. Each pig was returned to the appropriate pen after recovery from anesthesia, usually within 10–15 minutes after anesthesia was induced.

At the end of the study, radiographs were blind-read with assessment by a radiologist[12] (D.A. Feeney, University of Minnesota). Lung parenchymal densities were classified as interstitial or alveolar on the basis of apparent opacity, relative coalescence, and presence of air bronchograms.[13,14] All densities were considered to be evidence of pneumonia. Percentage of the lung with lesions was determined by the method described by Noyes, et al.[8] Radiographically defined lung lesion percentages observed over the life of each pig were used to determine ALP by adding the individual radiographic pneumonia scores and dividing by the number of observations.

Lung analysis

Lungs from experimental pigs were collected at slaughter and the pneumonic lesion was evaluated for each individual pig. Slaughter superficial pneumonia (SSP) lesion was measured with the corrected method described by Morrison, et al.[15] Briefly, each lung lobe was assigned a percentage based on the lung area and the percentage of pneumonia was calculated on the basis of the amount of total lung affected, as determined by observation and palpation of lung tissue. Slaughter volumetric pneumonia (SVP) was determined from the amount of water displacement by the affected area as described by Hill, et al.[16]

Serological analysis

Blood was collected every 2 weeks with 5-mL vacutainer tubes from either the vena cava or jugular veins of the animals from 4 weeks of age until the end of the experiment. Samples were marked with the animal ID and allowed to settle until a clot was formed. Blood samples were kept refrigerated in a polyurethane box with ice packs and on the day of collection were delivered to the Diagnostic Laboratory of the University of Veterinary Medicine of Barcelona for processing. Samples were then centrifuged to separate the serum and frozen at –20°C until processed for serology at the end of the experiment.

A monoclonal-antibody-blocking ELISA (Mycoplasma ELISA kit, DAKO A/S, DK-2600, Glostrup, Denmark)[4,17,18] was used to measure antibodies against *M. hyopneumoniae*. To measure antibodies against *A. pleuropneumoniae*, a blood-agar hemolysin assay developed by Utrera and Pijoan[19] was used. Briefly, this test is based on detecting *A. pleuropneumoniae* hemolysin I (APX I), which appears to be important in the pathogenesis of the disease and in inducing protective immunity against infection. The antigen was prepared from an overnight culture of the reference strain of *A. pleuropneumoniae* serotype 1. Bacterial cells were then harvested, washed with PBS, and resuspended in 0.15% NaCl solution to an A650 of 0.2. For the test, 50 µL of the bacterial suspension were mixed 1:1 with 50 µL of the serum to be tested and incubated at room temperature for 30 minutes. After incubation, 20 µL of the mixture was placed in triplicate onto the surface of 1% sheep blood agar plates and incubated overnight at 37°C. A positive reaction was characterized by a complete lack of hemolysis in the area of bacterial growth. A negative result was characterized by a complete halo of hemolysis, similar to that observed with the negative control sample.

For pseudorabies virus, a commercial kit (Ingelvac Aujeszky Diagnostic Kit®, Boehringer-Ingelheim, 08017, Barcelona, Spain) to detect GI and GII antibodies was used.

Statistical analysis

Average lifetime pneumonia (ALP), SSP, and SVP were calculated as described above for each animal. Correlation analysis (r values) was performed:

- comparing ALP, SSP, and SVP;
- between these values and ADG, and
- between these values and weight at slaughter.

Linear regression between ALP or slaughter pneumonia and weight was performed and compared to previous results.[8] Animals were also analyzed when divided into initial lightweight pigs or initial heavyweights, depending on whether their weight was above or below

average. Seroconversion to *M. hyopneumoniae* and *A. pleuropneumoniae* was established for each animal. Time of seroconversion was correlated against pneumonia peak and ALP.

Results

In general, the lungs of the study pigs had a very low percentage of lesions, with ALP < 10%. Pneumonic scores at slaughter varied widely, but were usually lower than industry standards which commonly average approximately >15% lung lesion.

Animals assigned to treatment groups and handled every 15 days had significantly ($P = .0001$) lower weights (mean of 7 kg lower) than nonhandled controls.

Effect of ALP on growth

Average lifetime pneumoina was negatively correlated with ADG ($r = .39$), ($P = .080$) in the lightweight animals, which had more lesions than the heavier weight pigs. ALP was not significantly correlated (although there was a positive trend) to ADG in initially heavyweight animals.

Effect of slaughter pneumonia on gain

Both superficial (SSP) and volumetric (SVP) slaughter pneumonia showed a marginally significant positive correlation ($r = .26$, $P < .1$) with weights at 180 days, but not ADG. This was probably due to variability in the initial weights of the pigs. Animals with higher percentage of lung slaughter lesions had heavier weights compared to animals without lesions.

Relationship between pneumonia measurements

Slaughter volumetric pneumonia and SSP showed a strong significant correlation ($r = .83$, $P < .01$), indicating that they are essentially the same measurements. On the other hand, ALP was not significantly correlated to SSP ($r = .23$, $P < .2$) or SVP ($r = .17$, $P < .1$).

Slaughter lesions were better correlated to age of onset of pneumonia than to ALP, being more extensive in animals that were infected late in life. These animals also tended to have higher 180-day weights.

Seroconversion to different microorganisms versus ALP

There were small differences in ALP among the four weekly groups of pigs (Figure 1). For example, group 1 pigs showed seroconversion to *A. pleuropneumoniae* during the finishing period, while animals in group 2 did not.

All groups showed variable pneumonia at the beginning, steadily increasing to a distinct pneumonic peak between 12–14 weeks of age, then tending to decrease. However, pneumonia lesions were minimal, never exceeding an average of 10% of the lung. All groups showed some seropositive animals to PRV at the beginning of the study, but most of these became seronegative by 12 weeks, suggesting the presence of maternal antibodies. Maternal antibodies were also detected against *A. pleuropneumoniae* for up to 8–10 weeks of age, but not to *M. hyopneumoniae*, even as early as 3–4 weeks of age. All groups showed seroconversion to *M. hyopneumoniae* between 18–20 weeks of age (about 6–8 weeks after onset of peak pneumonia). A trend to earlier seroconversion when the pneumonic peak was higher was also observed, although it was not significant ($P = .05$). *A. pleuropneumoniae* seroconversion followed *M. hyopneumoniae* seroconversion closely in group 1 pigs, but was absent in pigs in the other two groups. Pigs that seroconverted to *A. pleuropneumoniae* had higher ALP scores ($r = .077$, $P < .01$).

Discussion

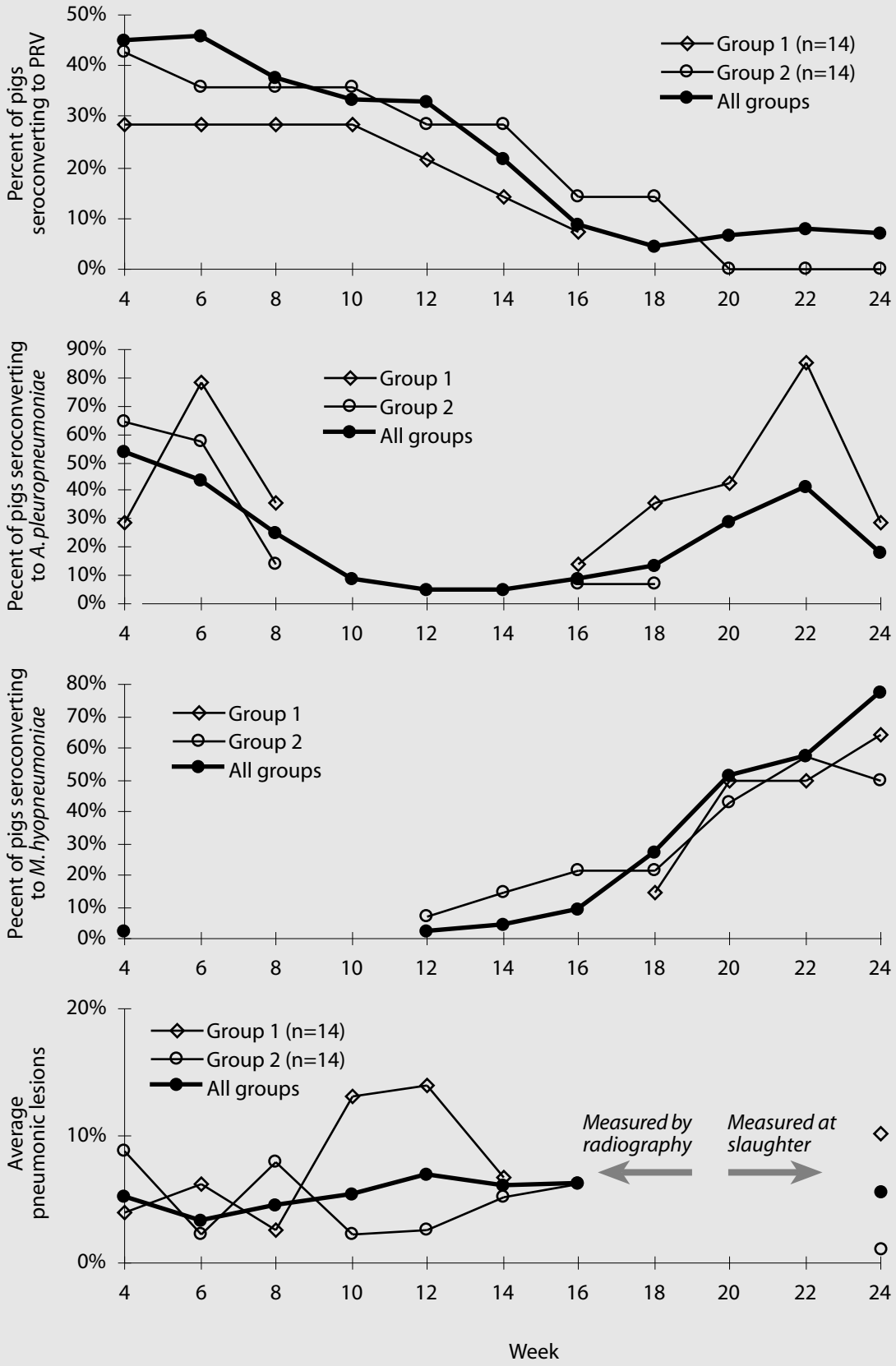
An association between radiographically measured lifetime pneumonia (ALP) and slaughter pneumonia (SSP and SVP) was not found. This result confirms earlier observations that slaughter pneumonia is a poor indicator of lifetime pneumonia.[8]

Hill, et al.,[16] suggested that some of the inconsistency reported between slaughter pneumonia and production could be due to inaccuracies in measuring superficial pneumonia lesions. They suggested that a volumetric measurement might be more precise, although they did not compare the two techniques directly. We found these two measurement techniques (SSP and SVP) to be essentially identical, as long as the proper percentage of SSP was given to each lobe as described by Morrison, et al.[15] However, commonly used simplified techniques that assign arbitrary 10% values to anteroventral lobes probably overestimate pneumonia lesions.

Slaughter lesions were poorly correlated with gain. Generally, animals with the largest percentage of affected lung tissue had a tendency to have the heaviest weights. This can be explained by the fact that slaughter lesions were found to be highly correlated with the time in which pigs developed pneumonia. Apparently, pigs developing pneumonia late in life reached market weights with large unresolved lesions in the lungs. These animals tended to grow better, probably because they spent most of their life without pneumonia (Figure 1). Conversely, animals that became affected at an early age may have been capable of resolving the lesions before they reached market age, but suffered growth retardation through most of their growth period, and may have tended to be more severely affected with pneumonia.

The pneumonia dynamics on the farm were very striking, with a clear peak at 12–14 weeks of age. This coincided with pigs being moved from the nursery to the grow-finisher at about 10 weeks of age. There was also a clear (albeit delayed) association between *M. hyopneumoniae* seroconversion with this pneumonic peak. The delay of approximately 8 weeks has been reported previously[4,18,20,21] and is probably due to the fact that the serological test used measures IgG. This IgG response is apparently a delayed response in *M. hyopneumoniae* infections, because the organism is poorly invasive. This delay in IgG response should be taken into account when seroprofiling herds for *M. hyopneumoniae* for treatment and control strategies. Time to seroconversion to *A. pleuropneumoniae* on this farm was more variable, but appeared to coincide with *M. hyopneumoniae*

Figure 1



Antibody response to specific disease agents and presence of pneumonic lesions

seroconversion. This variability may again be related to the serological test used — measuring Apx I antibody — only found in pigs after infection with *A. pleuropneumoniae* serotypes 1, 5, 9, and 10. These are not the most prevalent serotypes in Spain.

Correlation between ALP and ADG was weaker than found previously.[8] This discrepancy might be due to the fact that the farm studied had a less severe respiratory problem than the one studied previously. Even so, there was a strong correlation between ALP and decreased ADG in lightweight pigs, which generally had more pneumonia than heavier weight animals.

Implications

- The use of slaughter lung lesions to assess the health status of the population or its performance is limited, and should be accompanied by seroprofiling of the herd.
- To accurately detect *M. hyopneumoniae* infection, seroprofiling of the herd should be performed when pigs reach 12–14 weeks of age.
- Seroconversion to *M. hyopneumoniae* was strongly associated in this study with the onset and peak of pulmonary lesions.

References

1. Pijoan C. Respiratory System. In: Leman A, Straw B, Mengeling W, D'Allaire S, Taylor D, eds. *Diseases of Swine*, 6th ed. Ames, Iowa: Iowa State University Press; 1986:152–162.

2. Huhn RG. Swine enzootic pneumonia: incidence and effect on rate of body weight gain. *Am J Vet Res.* 1970;31(6):1097–1108.

3. Straw B, Burgi EJ, et al. Pneumonia and atrophic rhinitis in pigs from a test station. *JAVMA*. 1983;182(6):607–611.

4. Sorensen V, Barford K, Feld NC, Vraa-Andersen L. Aplication of enzyme-linked immunosorbent assay for the surveillance of *Mycoplasma hyopneumoniae* infection in pigs. *Rev Sci Tech Off Int Epiz*. 1993;12(2)593–604.

5. Morrison RB, Pijoan C, Leman AD. Association between enzootic pneumonia and performance. *Pig News Info*. 1986;7(1):23–31.

6. Aalund O, Willeberg P, Mandrup M, et al. Lung lesions at slaughter: associated factors in the pig herd. *Nord Vet Med.* 1976;28:487–495.

7. Brogdon BG, Kelsey CA, Moseley RD. Factors affecting perception of pulmonary lesions. *Radiol Clin North Am*. 1983;21:633–654.

8. Noyes EP, Feeney DA, Pijoan C. Comparison of the effect of pneumonia detected during lifetime with pneumonia detected at slaughter on growing swine. *JAVMA*. 1990;197(8):1025–1029.

9. Fellstrom C, Wallgren P. The relationship between seroconversion to *Mycoplasma hyopneumoniae* and lung findings at slaughter. *IPVS Proc*. 1993:308.

10. Claxton-Gill MS, Cornick-Seahorn JL, Gamboa JC, Boatright BS. Suspected malignant hyperthermia syndrome in a miniature pot-bellied pig anesthetized with isofluorane. *JAVMA*. 1993;203(10):1434–1436.

11. Lucke JN, Hall GM, Lister D. Anaesthesia of pigs sensitive to malignant hyperthermia. *Vet Rec.* 1977;100:45–48.

12. Feeney DA, Gordon BJ, Johnston GR, et al. A 200-centimeter focal spot-film distance (FFD) technique for equine thoracic radiography. *Vet Rad*. 1982;23(1):13–19.

13. Heitzman ER. Pneumonia and lung abscess. In: Harshberger SE. ed. *The lung: radiologic pathologic correlation*. St Louis: CV Mosby, 1984:194–352.

14. Wood AKW, Lloyd LC. A radiological investigation of enzootic pneumonia in the pig. *Res Vet Sci.* 1980;29:8–20.

15. Morrison RB, Hilley HD, Leman AD. Comparison of methods for assessing the prevalence and extent of pneumonia in market weight swine. *Can J Vet Res*. 1985;26:381–384.

16. Hill MA, Scheidt AB, Teclaw RF, Clark LK, Knox DE, Jordan M. Association between growth indicators and volume of lesions in lungs from pigs at slaughter. *Am J Vet Res*. 1992;53(12):2221–2223.

17. Feld NC, Qvist P, Ahrens P, Friis NF, Meyling A. A monoclonal blocking ELISA detecting serum antibodies to *Mycoplasma hyopneumoniae*. *Vet Microbiol*. 1992;30:35–46.

18. Sorensen V, Barford K, Feld NC. Evaluation of a monoclonal blocking ELISA and IHA for antibodies to *Mycoplasma hyopneumoniae* in SPF-pig herds. *Vet Rec*. 1992;130:488–490.

19. Utrera V, Pijoan C. Agar Hemolisis Inhibition Assay for detection of antibodies to *Actinobacillus pleuropneumoniae* in pig serum. *IPVS Proc*. 1993:218.

20. Suter M, Kobisch M, Nicolet J. Stimulation of Immunoglobulin-containing cells and Isotype specific antibody response in experimental *Mycoplasma hyopneumoniae* infection in SPF pigs. *Infec and Immun*. 1985;49(3)615–620.

21. Young T, Ross RF. Assessment of antibody response of swine infected with *Mycoplasma hyopneumoniae* by inmunoblotting. *Am J Vet Res*. 1987;48(4)651–656.

Practice tips

Necropsy equipment

Plastic fishing tackle boxes are excellent containers for necropsy kits. These can be easily cleaned and cold sterilized with disinfectant. They also contain numerous compartments that can be adapted to carry exam gloves, culturettes, swabs, knife, hatchet, etc.

For central nervous system signs and dissecting out the brain and backbone, a small Barns dehorner with metal handles is an excellent tool. With a little practice, a brain could be removed from a pig in 1–2 minutes without damaging or contaminating the brain excessively. The metal-handled Barns dehorner can also be sterilized and kept in a separate sterile pack or with the necropsy knives.

—Butch Baker, DVM