Diagnosis of viral respiratory disease in swine

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Viruses reported to cause respiratory disease in swine include swine influenza virus (SIV), porcine reproductive and respiratory syndrome virus (PRRSV), pseudorabies virus (PRV), porcine respiratory coronavirus (PRCV), porcine cytomegalovirus (PCMV), porcine paramyxovirus (PPMV), hemagglutinating encephalomyelitis virus (HEV), encephalomyocarditis virus (EMC), porcine parvovirus (PPV), porcine adenovirus, and porcine enterovirus.[1-11] The first three viruses (SIV, PRRSV, PRV) are the viruses most likely to be involved in respiratory problems in field situations, and initial diagnostic efforts should be directed toward detecting these agents. Past studies in Europe and the United States and more recent reports from Canada suggest PRCV may be an emerging pathogen that should also be considered.[12,13] Additional efforts to identify the other viruses are probably not warranted until the more common pathogens have been ruled out. In severe respiratory disease problems, multiple viruses may be involved. In our experience, the two viruses most frequently found concurrently in severe endemic nursery and grower pig pneumonia problems are SIV and PRRSV. European studies have identified concurrent PRCV, PRRSV, and SIV infections.[14,15]

Differential diagnosis in the field

Clinical signs

Determining whether or not a virus may be involved during on-site investigation of a respiratory problem can be difficult. In general, viral epidemics tend to present as outbreaks of high morbidity, sudden in onset and of short duration if uncomplicated. Bacterial infections, whether primary or secondary, tend to move through a group of pigs more slowly. However, in many situations, the clinical effect of the virus is overshadowed by the effect of concurrent or secondary bacterial infections. Likewise, differentiating one virus infection from another by clinical signs is rarely reliable. Differences may be subtle, inconsistently present, or less prominent than the effect of secondary bacterial infections. Clinical signs indicative of a particular virus are most likely to be observed in acute uncomplicated infections. Depression, anorexia, fever, slight nasal and ocular discharge, occasional soft cough, and slight hyperpnea may be observed with any of the viruses, depending on the severity of the infection. A severe cough is most likely to occur with SIV infection because of the damage the virus causes to the epithelium lining the airways. PRV infection also can result in a mild cough in some pigs. Coughing is rarely observed in uncomplicated PRRSV infection because the most significant damage induced by the virus is thickening of alveolar walls. This thickening apparently interferes with oxygen transfer, resulting in prominent dyspnea and hyperpnea (thumping) in severe infections. Both PRRSV and PRV infections can induce significant rhinitis, which may result in sneezing in the early stages of infection. Central nervous system signs in a few pigs is suggestive of possible PRV infection and occasionally PRRSV infection.

Age of affected pigs also can be a clue to the virus that is most likely to be involved. When PRRSV infection initially occurs in pregnant animals, resulting in reproductive problems, respiratory problems are often seen in surviving neonatal pigs from affected litters. In endemic herds, nursery pigs are commonly affected, as maternal antibody wanes relatively quickly and pigs become susceptible to infection by 3–4 weeks.[16] PRCV also typically infects pigs during the first 1–2 weeks after weaning.[13,17] In contrast, maternal antibody against SIV persists for 2–4 months, and infection with this virus is more likely to occur in older 60–200 pound pigs.[1] Maternal antibody against PRV provides protection for 10–12 weeks, thus respiratory disease due to PRV is unlikely in pigs less than 2 months old in vaccinated herds.[3] Introduction of any of these viruses into naive herds may result in infection and clinical disease in pigs of any (and sometimes all) ages.

Gross lesions

Both experimentally and in the field, SIV infection most frequently results in cranioventral consolidation of the lung, not unlike the appearance of *Mycoplasma hyopneumoniae*-infected lungs.[1] Severe infections may exhibit a diffuse increase in lung firmness. A diffusely consolidated, meaty-appearing lung is most characteristic of severe PRRSV infection, but such a lesion can also be seen with severe SIV infection, concurrent SIV-PRRSV infections, or *Salmonella choleraesuis* septicemia. PRRSV infection may also present as a cranioventral or patchy to coalescing lesion. In mild infections, or possibly with less virulent strains, the interstitial pneumonia induced by PRRSV may be subtle and not readily apparent on cursory examination. Enlarged tan lymph nodes are a consistent finding.[18] Pseudorabies virus typically does not induce grossly identifiable lesions, but occasional pigs may exhibit inflamed turbinates or trachea, or necrosis in tonsil or trachea.[3] Porcine respiratory coronavirus usually does not induce distinctive gross lesions.[18] As expected, in many pigs the lesions induced

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by a virus may be overwhelmed by the consolidation resulting from concurrent or secondary bacterial infections.

Laboratory aid in diagnosis

Pig selection

In new epidemics, well-defined in time, place, and pigs affected, the choice of sample is relatively easy. There is no better sample than several live acutely-affected pigs delivered to the laboratory (Table 1). However, samples taken carefully from such pigs, preserved well, and delivered promptly, can provide similar test results. Chronic ongoing respiratory problems can be more difficult to sort out. Consider the stages of production at which the problems appear to be most severe. Viruses are likely to be early initiators, and samples to detect them will need to be taken before pigs develop the secondary problems that result in poor end-stage pigs. Different viruses may be infecting pigs at different stages. In these situations, separate samples should be taken from each stage. Sampling acute, subacute, and chronic pigs may allow one to determine the sequence of multiple viral and bacterial agents that are contributing to the overall problem.

Histopathology

All submissions that include tissue samples should also include formalin-fixed tissues (Table 2). Microscopic examination allows the diagnostician to evaluate whether the organisms recovered from the tissues are significant contributors to the lesion and also whether organisms not detected may also be involved. Necrotizing bronchiolitis (with interstitial pneumonia) is the hallmark of SIV infection. Porcine respiratory coronavirus also induces mild interstitial pneumonia with necrosis of bronchiolar epithelium, but damage tends to be limited to small and terminal bronchioles. Variable thickening of alveolar walls by macrophage infiltration and pneumocyte swelling is characteristic of PRRSV infection. In severe infections, aggregates of necrotic inflammatory cells in alveolar lumens is suggestive of PRRSV. Pseudorabies virus usually does not induce recognizable microscopic lesions in the lung of older pigs, but in a few, usually younger, pigs focal necrosis of the parenchyma may be present. Vasculitis, focal gliosis, and nonsuppurative meningitis in the brain are more consistent lesions. Both PRRSV and PRV may induce prominent rhinitis. Porcine cytomegalovirus may induce intranuclear inclusion bodies in glandular epithelial cells in the nasal mucosa (and occasionally in other organs) with or without significant inflammation or necrosis.

Fluorescent antibody test

The fluorescent antibody (FA) test is the quickest test available. Portions of fresh lung are frozen and thin sections cut and placed on glass slides, and fluorescein-labelled antisera against specific viruses are applied. The slides are then examined with a special fluorescent microscope. Two to 3 hours are required to prepare and stain the samples and read the test. In

Table 1

Specimens to submit to detect respiratory viruses

Pig

Several live, acutely affected pigs are always the sample of choice.

Lung

Fresh

- One entire lung (small pig).
- Most of the cranial and middle lobes and the cranial portion of the caudal lobe, including the hilar area (larger pig). Portions with larger airways are best for FA tests.
- Chill before shipment but do not freeze.
- Do not submit in glycerin.
- Do not submit in the same bag as liver or intestine.
- Fresh tissue can be used for FA test for SIV, PRCV (PRRSV in some labs), and for VI for PRV, PRRSV, SIV, PRCV.

Formalin-fixed

- Remove 1 or 2 thin (6mm, ¼") slices through consolidated and adjacent unaffected lung and fix in buffered formalin. Cross-sections of entire lobes near the junction with the hilar area often contain the best areas for histopathologic examination.
- Formalin-fixed tissue can be used for histopathologic examination and for IHC test for PRRSV (some labs).

Nasal swabs

- Must be from acutely affected pigs early in the course of disease (clear nasal discharge).
- Swabs must be kept moist (saline or cell culture media) and chilled.
- Preferably delivered to the laboratory within 24 hours.
- Nasal swabs can be used for VI for SIV, PRCV, PRV.

Serum

Serum can be used for VI for PRRSV only (keep chilled).

Serum can be used for serologic studies.

- Acute & convalescent are preferable to diagnose specific disease outbreak.
- Herd samples can be used to detect the presence of a specific virus (Table 3).

Tonsil

Fresh

- FA test for PRV

Formalin-fixed

- IHC test for PRRSV (some labs)

Brain

Fresh

- FA and VI for PRV

Formalin-fixed

- Histopathologic examination for PRV, PRRSV

Nasal turbinate

Formalin-fixed

- Remove most of the double scroll from one side at the junction with the midline septum with a scissors after cross-sectioning the snout.
- Histopathologic examination for PRRSV, PRV, PCMV.

laboratories with heavy case loads, it may take most of the day to prepare the numerous samples received each day and slides won't be ready to read until the end of that day. Samples must be taken early in the course of disease. Fluorescent antibody tests are used routinely to detect SIV (lung) and PRV (tonsil, brain stem). The FA test does not efficiently identify PRV or PRCV in lung tissue. Some laboratories also use the FA test for PRRSV (lung). The FA test can be difficult to interpret on lung tissue with extensive damage due to bacterial infection.

Immunohistochemistry

The immunoperoxidase (IP) test is an immunohistochemical (IHC) test that is available in a few laboratories to detect PRRSV antigen. The IP test is similar in principle to the FA test but is applied to formalin-fixed tissue. Tissue sections are prepared in a manner similar to that used for histopathology but are then stained with antiserum against a specific virus. This is followed by an anti-antibody linked to an enzyme that will cause precipitation of colored reagent in the tissue section when the final substrate is applied. An additional biotin-streptavidin linkage step is included in most current IHC tests. Formalin-fixed tissues received at the laboratory can be processed into histopathologic sections the following day. Immunohistochemical staining techniques work best with overnight incubation periods. Thus, IHC test results can be available on the second day. Because of the expense and labor involved in the test, diagnosticians prefer to examine histopathologic sections first, requesting the IHC test only on likely tissues rather than using this technique indiscriminately. The advantages of the test include better control of tissue preservation by fixation and direct visualization of the location of viral antigen by light microscopy. In theory, the test should be more sensitive than the FA test because the steps have been amplified. Virus isolation should be more sensitive. Buffered formalin is preferable for fixation to reduce precipitation of acid hematin in tissues, especially those with considerable congestion or hemorrhage. This brown precipitate artifact resembles the stain produced in the IP test, making interpretation difficult. Lung is the tissue of choice, but the virus can also be detected in tonsil, lymph node, and spleen. An IHC test for SIV antigen also is being developed.

Virus isolation

Virus isolation (VI) is the most definitive method of diagnosis. This test also would be the most sensitive if all viruses could be cultured efficiently in vitro. Unfortunately, PRV is the only virus that grows readily in routinely used cell lines. Lung can be used to isolate PRV but brain is much preferred. Porcine reproductive and respiratory virus can be isolated in a few continuous cell lines, but alveolar macrophages apparently are a more sensitive medium.[2] Preparation of alveolar macrophages is time and labor intensive and may not be available in all laboratories. Lung and serum (lymph node, spleen) are the tissues of choice. Egg inoculation is the best method to isolate SIV, because continuous cell lines do not effectively support growth of this virus. Lung or nasal swabs are the samples of choice. Eggs are time-consuming to prepare for inoculation and susceptible to bacterial contamination. This method is not available in all laboratories. PRCV will grow on swine testis (ST) cell lines, which are available in most laboratories, but may not be used routinely on swine respiratory cases. Nasal swabs and lung are the samples of choice.Virus isolation cultures are usually followed for 2 weeks before being terminated.

Table 2

Preferred samples for specific respiratory viruses

SIV
- Fresh lung for FA.
- Nasal swabs and fresh lung for VI (some labs).
- Formalin-fixed lung for histopathologic examination.

PRRSV
- Fresh lung and serum (lymph node, spleen) for VI.
- Fresh lung for FA (some labs).
- Formalin-fixed lung (tonsil, lymph node) for IHC (some labs) and for histopathologic examination.

PRV
- Fresh tonsil for FA.
- Fresh brain stem (spleen, lung) for FA and VI.
- Formalin-fixed brain (lung) for histopathologic examination.

PRCV
- Nasal swabs for VI.
- Fresh lung for FA and VI.
- Serum for serology (TGE).
- Formalin-fixed lung for histopathologic examination.

Other viruses

Check with laboratory for test availability.

Serology

Serologic studies are most useful in determining whether or not pigs in a herd have been exposed to a particular virus. Statistically, a sample size of five will allow 95% confidence of detecting infection in a herd of any number of animals over 40 if the seroprevalence is at least 50%. If the seroprevalence is only 25%, 10 samples would be needed to detect infection in a herd of 40–100 pigs. Eleven samples would achieve 95% confidence in any number of pigs over 120 at this infection rate. If the seroprevalence is only 10%, 25 samples would be necessary to be 95% sure of detecting infection in a herd of 100 pigs, 28 samples in a herd of 500 pigs, 29 samples in 1000 or more pigs. The number of animals that must be tested can be predicted by estimating the clinical morbidity rate and considering the number of pigs in the group (Table 3). Periodic surveillance of herd titers may provide clues about virus activity within a herd over time.

Serologic diagnosis becomes more difficult when you wish to identify the cause of a specific disease episode, especially if the pre-disease serologic status is unknown, or the pigs have been vaccinated for the virus(es) in question. Acute and convalescent sera may be needed to achieve interpretable results in situations involving an epizootic or a predictable recurring problem that affects successive groups of pigs.

It is easiest to evaluate serologic results with PRV because of the amount of research that has been conducted in the past with regard to infection, vaccination, and differential tests. Maternal antibody may persist for 10–12 weeks.[3] Some researchers believe that serologic studies may be unreliable for the diagnosis of SIV infection because of reports describing pigs that did not develop titers detectable by routine hemagglutination inhibition (HI) tests after confirmed infection.[16,19] Whether or not this is due to antigenic variation in the virus (atypical SIV) is unknown. Implication of PRCV is provided by detecting serologic titers to TGEV in herds in which evidence for enteric disease due to TGEV infection is not present. An ELISA test that will differentiate PRCV and TGEV antibody is being developed.[13]

PRRS antibody, as detected by the commonly used IFA (indirect-fluorescent antibody) test, appears during the second week after infection and peaks ($\geq$ 1:640) by 4–6 weeks.[2,16,20,21] Antibody may be undetectable again by 10–12 weeks, but more routinely persists for 4–5 months. Maternal antibody persists for 3–5 weeks. A commercial enzyme-linked immunosorbent assay (HerdChek®: PRRS, IDEXX Labs, Inc., Westbrook, Maine) is available in some labs. The rate of rise and decay of antibody, as measured by this test, closely parallels the IFA test.[20] Peak titers are represented by 2.0–3.0 S:P ratios. A value of <0.4 is negative.

Help in interpreting of titers is best obtained from the laboratory that performed the tests, because results may vary between laboratories because there are slight differences in techniques used to perform the tests.

Sample submission—Problems to avoid

Experience has shown that certain types of samples rarely yield positive results. Avoid, if possible, sending in lung tissue in which the cranioventral half of the lung is so chronically consolidated that abscessation has developed. The most significant initiating pathogens are likely to no longer be present or be undetectable amidst the necrotic and inflammatory debris. The lower tips or edges of such consolidated lobes should be especially avoided.

Table 3

Abbreviated table for predicting sample size (*n*) needed to detect infection at the 95% confidence level in a herd of population size (*N*) with estimated morbidity rate (%).

N	50%	25%	10%
10	4	7	10
50	5	10	22
100	5	10	25
250	5	11	27
500	5	11	28
1000	5	11	29

The table gives the sample size (*n*) required to be 95% certain of including at least one positive if the disease is present at the specified level (%) in the population (*N*). Excerpted from Thrushfield MV. *Veterinary epidemiology*. London: Butterworth & Co Ltd; 1986: 158.

Likewise, submitting only the relatively unaffected caudal lobe from such lungs may lead to misleading negative results as this portion seldom holds valuable clues. If such a lung must be used, the junction or transition area between consolidated and normal tissue should be sampled. In particular, formalin-fixed samples for histopathologic examination should include transition areas and the middle of the lesion rather than lobe tips and edges. Portions of lung containing larger airways are best for FA tests. Be aware of chronic and more acute lesions that may be present in the same lung, which may represent the activity of different pathogens. Each should be sampled. Finally, do not freeze fresh tissue or submit in glycerin. Glycerin has not proven to significantly improve bacterial culture results and does interfere with freezing of sections for FA tests. Do not package lung tissue for VI with liver or intestine. Autolyzed tissue is not suitable for any histopathologic, bacteriologic, or virologic tests.

Other viruses

As mentioned previously, SIV, PRRSV, and PRV are the most commonly detected respiratory viruses in swine. Porcine respiratory coronavirus is less commonly found, but tests for detecting this virus are available in most laboratories. If other viruses are suspected, special efforts will be needed to identify them as tests to detect these viruses are not routinely applied to samples submitted to diagnostic laboratories. Porcine cytomegalovirus, encephalomyocarditis virus (EMC), and hemagglutinating encephalomyelitis virus (HEV) primarily affect other organ systems but may concurrently infect the lung. Porcine cytomegalovirus can be detected only by identifying the characteristic intranuclear inclusion bodies in the nasal turbinates (or in lungs and other tissues in generalized systemic infections) by histopathologic examination. Fluorescein-conjugated antiserum (FA test) specific for EMC and HEV and permissive cell lines in which these viruses can be cultivated are available by special request in some laboratories. Several isolated reports of porcine paramyxovirus (PPMV) infection in swine in association with respiratory disease have been documented. Porcine parvovirus, adenovirus, and enterovirus also have been reported to cause respiratory diseases. These viruses will not be detected by routine examination, but additional tests may be conducted by some laboratories by special request or by forwarding tissues to reference laboratories.

Comment

The various tests available for respiratory diagnostics have been described above. In a typical diagnostic case submission, not all of the above tests are applied to every case and the type of test applied may vary somewhat between laboratories. The disease prevalence in the area, specimen availability, history, gross lesions, and histopathological findings are all important considerations when the diagnostician considers which tests to order. If you are concerned about testing for a particular virus, be sure to convey that desire to the diagnostician.

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Windows Tip

Does Windows look different every time you start it up? Would you like it to always look the same, with a particular group (or groups) open? It's simple to set up. Here's how:

- In the Program Manager, click on the "Options" menu.
- If there is a check mark in front of the item "Save Settings on Exit" click on that line to UN-check it. (If there is NO check mark, click anywhere else to dismiss the menu without making any changes.)
- Now arrange your desktop the way you want it to appear. Close your seldom-used groups. Open the groups you use most often, size them to your liking and position them where you want them. Size them by dragging the edges to make them the size you want. You can tell when to drag because the cursor changes to a double headed arrow when it is on the edge. If you change the size very much, you may have to rearrange the icons to fit better. To do this, click on the "Window" menu and choose "Arrange Icons." To move a window drag its title bar.
- Now to fix that arrangement, **pretend** that you are going to exit Windows. Click on "File" but when you click on "Exit Windows," hold down the <Shift> key. You should see the hard disk drive light flash briefly as the setup is saved. Now Windows will always look the same at startup.