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Pathology in Practice

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In cooperation with

ACVP

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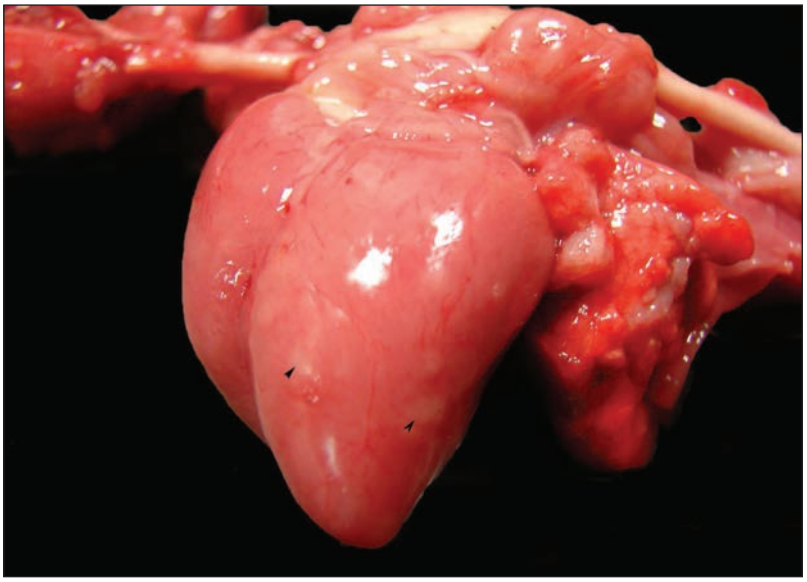

Figure 1—Photograph of the heart of a stillborn piglet (approx gestational age, 110 days). The piglet was 1 of 7 variably mummified near-term fetuses and stillborn piglets aborted by an apparently healthy sow. Notice the slightly raised, pale, white-tan foci (arrowheads).

History

A 1.5-year-old second-parity Large Black X Tamworth cross sow from a well-managed 20-sow, unvaccinated, pasture-raised herd of pigs in upstate New York aborted a litter of 7 variably mummified near-term fetuses and stillborn piglets. This sow had no signs of ill health other than abortion; it was housed in a group with 4 other sows and a 2-year-old Gloucestershire Old Spot boar. One of the stillborn piglets from this litter was submitted to the New York State Animal Health Diagnostic Center for necropsy; no placental tissue was submitted with the piglet. A second sow from this group had aborted a litter at the same late stage of gestation within the preceding 2 weeks; however, none of the fetuses were submitted for diagnostic investigation. No signs of ill health were reported for the rest of the herd. The referring veterinarian indicated that these pigs had access to corn standing in the field and that, because of recent wet weather, there was concern that mycotoxin contamination might be the cause of the abortions.

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Gross Findings

The submitted fetus was in thin body condition for a near-term fetus and had mild autolysis. The crown to rump length was 22 cm, which corresponds to an approximate gestational age of 110 days (a gestation period of 114 days [ie, 3 months, 3 weeks, and 3 days] is considered normal). Multifocal areas of cutaneous erythema were present. The most striking gross change was the presence of approximately a dozen slightly raised (0.1 × 0.1 × 0.1 cm to 0.1 × 0.2 × 0.1 cm) white to tan foci on the epicardial surface of the heart (**Figure 1**). On section, these foci extended into the myocardium and were also evident along the endocardial surface. Approximately 9 mL of serosanguineous fluid was present in the thoracic cavity. Widespread petechial hemorrhages were present on the cortical surfaces of the kidneys and along the intercostal muscles.

Formulate differential diagnoses from the history, clinical findings, and Figure 1—then turn the page →

Gross and Histopathologic Findings

Specimens of brain, heart, lung, liver, kidney, spleen, and gastrointestinal tract tissues were collected, fixed in neutral-buffered 10% formalin, and routinely processed. Changes were evident in the heart, spleen, and kidney tissues. The myocardium had moderate numbers of perivascular lymphocytes and histiocytes that extended into the adjacent interstitium (**Figure 2**). In areas of inflammation, the myocardiocytes occasionally had mild degenerative changes consisting of myocardiocyte thinning (atrophy) and cytoplasmic vacuolation. There was marked depletion of the splenic lymphoid tissue with replacement by loose histiocytic aggregates that frequently contained Langhans-type multinucleated giant cells. The renal petechiae observed grossly corresponded to areas of moderate glomerular congestion and mild interstitial hemorrhage; however, there was no evidence of inflammation in the kidneys or in the remaining tissues.

Microbiological Findings

Samples of stomach content and lung tissue were submitted for bacterial culture and kidney, heart, lung, and spleen tissues were submitted for fluorescent antibody testing for *Leptospira* spp, porcine parvovirus (PPV), and porcine reproductive and respiratory syndrome virus (PRRSV); a PCR assay[a] was used for detection of porcine circovirus (PCV)-2 DNA. For all evaluated samples, fluorescent antibody test results for *Leptospira* spp, PPV, and PRRSV were negative, whereas results of PCV-2 PCR analysis were positive. Sections of heart, spleen, lungs, and liver underwent immunohistochemical analysis for PCV-2 (by use of polyclonal rabbit anti–PCV-2 antibody[b]) and PRRSV. There was moderate to strong intracytoplasmic staining for PCV-2 within histiocytes infiltrating the myocardium (**Figure 3**) and spleen and within Kupffer cells in the liver. Immunohistochemical analysis of the selected tissue samples for PRRSV antigen yielded negative results. Bacterial cultures of fetal lungs and stomach content yielded moderate growth of coagulase-negative *Staphylococcus* spp. Gram staining of sections of fetal myocardium and spleen did not reveal the presence of bacteria.

Morphologic Diagnosis and Case Summary

Morphologic diagnosis: moderate, multifocal, chronic, lymphohistiocytic myocarditis and severe, multifo-

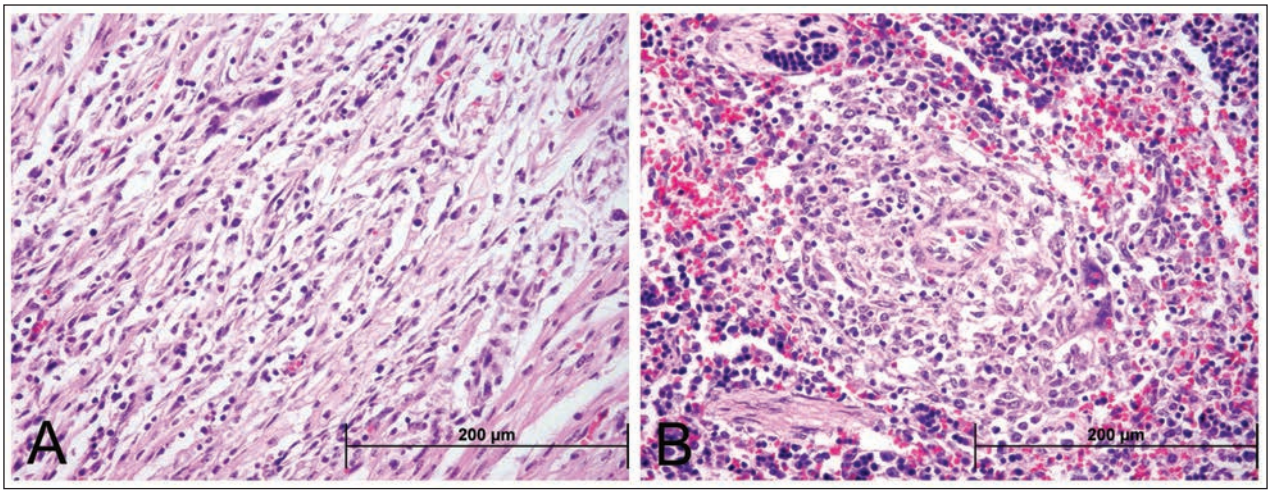

Figure 2—Photomicrographs of sections of myocardium (A) and splenic lymphoid tissue (B) from the piglet in Figure 1. There is evidence of lymphohistiocytic myocarditis and severe lymphoid depletion and multifocal granulomatous splenitis with Langhans-type multinucleated giant cells. H&E stain; in each panel, bar = 200 µm.

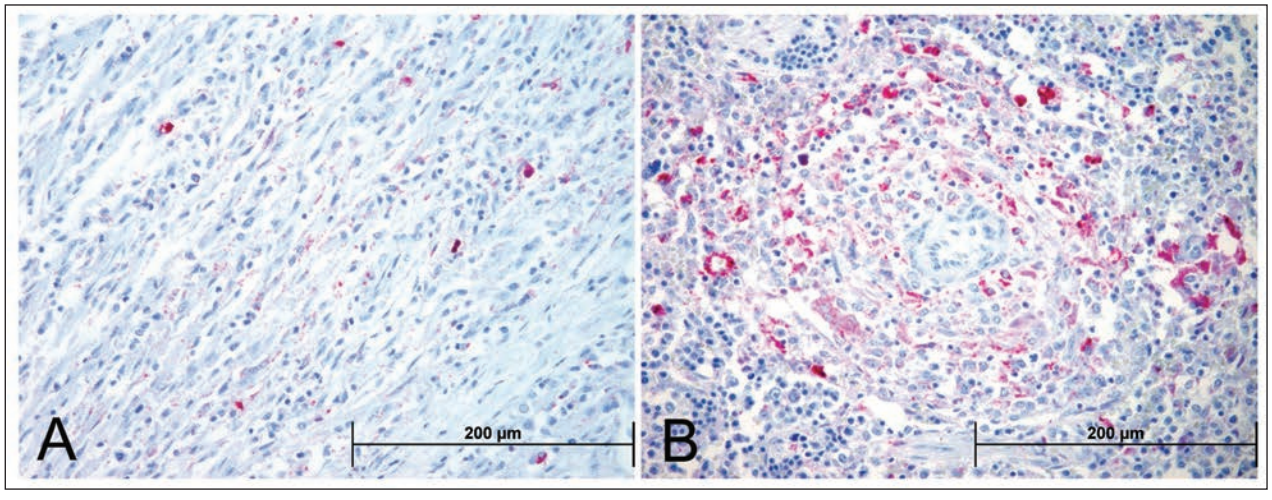

Figure 3—Photomicrographs of sections of myocardium (A) and splenic lymphoid tissue (B) from the piglet in Figure 1 following immunohistochemical staining for PCV-2 antigen. Notice that the histiocytes in the myocardium and the histiocytes and multinucleated giant cells in areas of granulomatous inflammation in the spleen are positive (red staining) for PCV-2 antigen. 3-amino-9-ethylcarbazole chromogen with hematoxylin counterstain; in each panel, bar = 200 µm.

cal to coalescing, chronic, granulomatous splenitis with marked lymphoid depletion.

Case summary: myocarditis attributable to PCV-2 infection in a pig fetus.

Comments

In pigs, the presence of multiple fetuses in various stages of mummification is strongly suggestive of a viral infection that has progressed from fetus to fetus, resulting in death at various stages of gestation. Historically, this finding was considered pathognomonic for PPV infection in pigs, but making a diagnosis on the basis of these gross changes has become more challenging given the more recent emergence of PCV-2, PRRSV, and encephalomyocarditis virus (EMCV), 3 viruses that can result in similar clinical and gross changes in infected pigs. As such, testing for multiple viral agents must be considered when evaluating abortions in pigs with these clinical signs. To increase the likelihood of identifying viral agents and detecting diagnostic histologic changes, samples of lymphoid tissues (notably enlarged lymph nodes and spleen) and nonlymphoid tissues (including heart, liver, and lungs) from affected fetuses should be submitted for complete diagnostic evaluation.[1,2] The histologic presence of foci of granulomatous inflammation within the splenic parenchyma and lymphohistiocytic myocarditis is highly suggestive of PCV-2 infection. In the case described in the present report, the presence of PCV-2 in the fetal tissues was confirmed via PCR assay and immunohistochemical analysis. However, intracytoplasmic basophilic botryoid viral inclusion bodies, which are associated with PCV-2 infection, were not identified in the examined tissues of this aborted piglet. Although inclusion bodies can be present,[3] they are not always identified[4]; thus, one should not rely on their detection to make a diagnosis of PCV-2 infection. Negative results of fluorescent antibody testing for PPV and PRRSV and negative results of immunohistochemical analysis for PRRSV confirmed that the abortion of the piglet of the present report was not attributable to a mixed viral infection involving PCV-2 and these other viral agents. Because of financial constraints and detection of PCV-2 viral antigen and nucleic acid in samples of tissues with lesions, testing of the piglet for EMCV infection was declined. Given that coagulase-negative *Staphylococcus* spp were isolated from the samples of stomach contents and lungs obtained from this piglet, Gram staining of sections of the myocardium and spleen was performed to determine whether bacteria were associated with the development of the lesions; however, no bacteria were detected. Therefore, it is unlikely that these bacteria contributed directly to the myocarditis and splenitis.

The hallmark histologic change in early fetal infection with PCV-2 is nonsuppurative myocarditis that can include myocardial fibrosis or necrosis,[5] which correlates to the findings in the case described in the present report. This finding has been verified through experimental inoculations, which revealed that early in utero infection (at approx day 52) is associated with myocardial viral tropism and that later infections (after day 92) are associated with lymphoid viral tropism.[2,6] Because the fetus of this report had both myocardial and lymphoid tissue lesions, it is likely the sow's infection developed early during pregnancy and persisted until the time of abortion, allowing for infection of both fetal tissue systems.

Although infection with PCV-2 by itself can cause disease in pigs, it is more commonly identified in cases of so-called porcine circoviral–associated disease (PCVAD). Porcine circoviral–associated disease results from coinfection of PCV-2 with PPV, PRRSV, EMCV, swine influenza virus type A, or *Mycoplasma hyopneumonia* or a combination of these agents or from PCV-2 infection in association with recent vaccination with a vaccine containing a potent immune-stimulating adjuvant.[1,3,4,7–9] It has been postulated that the lymphoid proliferation that occurs as a result of coinfections or adjuvant-induced immune stimulation elicits a greater pool of proliferating lymphoid cells for PCV-2 infection and therefore clinical disease expression.[5] With the reproductive form of PCVAD, PPV, and PRRSV appear to be the most common concurrent infections. Based on the paucity of reported cases, EMCV appears to be a rare coinfecting agent, but considering that a diagnostic assay specific for EMCV was not performed in the case described in the present report, coinfection cannot be completely ruled out.

Other outcomes of PCVAD that may be detected concurrently on a farm where abortions occur, but which were not recorded in the present case, include postweaning multisystemic wasting syndrome, porcine dermatopathy and nephropathy syndrome, and pulmonary disease.[1,5] Although the nonreproductive effects of PCVAD are more frequently associated with postnatal infections, they can develop in piglets that acquire subclinical PCV-2 infections in utero and, after birth, are infected with PPV or immune stimulated with a potent vaccine adjuvant.[4] Currently, there is no effective vaccine available in the United States to directly control the spread of infections with PCV-2.[1,5] Management factors such as maintaining strict biosecurity, quickly removing infected animals from the herd, and managing the presence of other pathogens that potentiate PCVAD are recommended in the control of PCV-2 infection.[1,5]

a. Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa.
b. Provided by Dr. Tanja Opriessnig, Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa.

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