History and Clinical Signs

Tissues from 3 juvenile mixed-breed dog littermates that each died naturally were examined over a 3-week period. These puppies were from a litter of 13. Two littermates had died in the immediate neonatal period, and no diagnostic investigation had been performed; a third littermate had died at 2.5 weeks of age with a clinical diagnosis of coccidiosis. The first examined tissues were from a puppy (dog 1) that died at 5 weeks of age and also had a clinical diagnosis of coccidiosis. Within days after receipt of the tissues from the fourth dog, a fifth puppy (dog 2) died at 6 weeks of age and was submitted for full necropsy. A sixth puppy (dog 3) died and was submitted for full necropsy 1 week later.

Prior to death, dog 2 had no premonitory signs and dogs 1 and 3 had suddenly gasped for breath, collapsed, and died shortly thereafter. All 13 puppies in the litter had received their initial routine vaccinations. Although all puppies were reported to have received colostrum from their dam, they were weaned at 4.5 weeks of age because the bitch was cachectic (it had been stray while gravid). The bitch recovered following weaning of the puppies and was healthy at the time that the tissues from the fourth puppy were submitted. The vaccination history, age, and previous reproductive history of the bitch were unknown.

Gross Findings

In all 3 puppies, the lungs failed to collapse on opening the thorax or thereafter and were slightly firm and heavy on palpation; the lungs of dogs 2 and 3 had dark red discoloration. In all 3 dogs, the heart was subjectively enlarged, the cardiac apex was slightly wide, and the cardiac muscle was mottled pale tan on epicardial and cut surfaces (Figure 1). In addition, dog 2 had 9, 3, and 4 mL of serosanguineous thoracic, pericardial, and peritoneal effusion, respectively, as well as miliary, white-tan foci in the splenic parenchyma.

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

Various tissues, including specimens of the heart, lungs, and liver, from the 3 dogs underwent histologic examination. Multifocal to locally extensive cardiomyocyte degeneration and necrosis were found in all cases. The degeneration and necrosis were characterized by variable fiber size and staining affinity, fragmentation, and loss of cross striations. Within the myocardium, occasional discrete, homogeneous, basophilic, intranuclear inclusion bodies that peripheralized the chromatin were evident (Figure 2). There was lymphoplasmacytic infiltrate throughout the myocardium, accompanied by interstitial fibrosis. The degree of myocardial degeneration and necrosis as well as the degree of the inflammatory infiltrate ranged from moderate in dogs 1 and 2 to marked in dog 3. The degree of fibrosis was mild in dog 1, moderate in dog 2, and marked in dog 3.

In the lungs of all 3 dogs, there was mild hypercellularity of the alveolar septa and moderate, diffuse pulmonary edema, which was characterized by light pink, finely granular fluid within the alveoli and small airways and expanding the interstitium. In sections of the liver of dog 3, mild periportal lymphoplasmacytic infiltrate with diffuse, severe hepatic congestion and edema were observed. The sinusoids and the perivascular interstitium were expanded by a light pink, finely granular, protein-rich fluid.

Immunohistochemical analysis of cardiac tissue samples from the 3 dogs was performed with a custom monoclonal antibody purified from tissue culture supernatant specific for canine parvovirus (CPV) and feline panleukopenia virus (FPV). Immunohistochemical labeling for CPV antigen was found within the nuclei and occasionally the cytoplasm of cardiomyocytes of all 3 dogs (Figure 3). Results of a PCR assay to detect CPV were positive for cardiac tissue from dogs 1 and 2 and for samples of small intestine from dog 2; the PCR assay was not performed on tissues from the third dog.

Morphologic Diagnosis and Case Summary

Morphologic diagnosis: mild to severe, multifocal, lymphoplasmacytic myocarditis with degeneration and necrosis, fibrosis, and intranuclear inclusion bodies; moderate, diffuse, pulmonary edema, and passive congestion; and passive congestion of the liver (in dog 3 [the dog that died at 7 weeks of age]).

Case summary: canine parvoviral myocarditis causing sudden death in 3 juvenile mixed-breed dog littersmates born to a bitch with unknown vaccination history.

Comments

For the 3 dogs of the present report, results of immunohistochemical analysis of cardiac tissue confirmed canine parvoviral myocarditis, and results of PCR assays corroborated this diagnosis for the 5- and 6-week-old dogs. Canine parvovirus is a nonenveloped, single-stranded negative-sense DNA virus, with a 25-nm capsid and a tropism for rapidly dividing cells. Canine parvovirus, FPV, and mink enteritis virus are closely related, host-specific members of the parvovirus genus. Canine parvovirus is well-known as the most pathogenic viral agent of enteritis in dogs. Specifically, the etiologic agent of parvoviral enteritis is CPV-2, of which there are 3 currently identified strains, CPV-2a, CPV-2b, and CPV-2c. Canine parvovirus-2c has emerged within the past 12 years and is reported to have increased pathogenicity relative to the other 2 strains. canine parvovirus is thought to have evolved from FPV or a related virus in the 1970s. The genetic distinction that dictates species specificity of CPV and FPV is an alteration in the code for <10 amino acids in the viral capsid. The clinicopathologic hallmark of CPV infection is leukopenia. Transient lymphopenia is most common because lymphoid tissues are rapidly dividing and often the first infected tissues. The resultant lymphocytolysis is most dramatic in the thymus.
throughout life. Similarly, feline panleukopenia is the result of FPV infection, a virus that preferentially infects progenitor cells in the bone marrow. The biochemical rationale for this tropism lies in the incomplete DNA replication machinery of members of the genus.

Neonatal CPV infection can result in severe, commonly acutely fatal parvoviral myocarditis. Clinical signs develop from birth to 8 weeks. The cardiac form often develops without enteric involvement. Whether infection of the bitch during gestation leads to infection of the fetus is under debate. Some authors contend this type of exposure would not lead to vertical transmission, but rather, it would confer immunity. Others contend that natural exposure of a pregnant bitch can lead to neonatal parvoviral cardiomyositis.

Clinical signs of CPV cardiomyositis include vocalization, retching, dyspnea, and sudden death. Viral tropism for neonatal cardiomycocytes is not surprising because these cells are mitotically active until 15 days after birth. Infection with CPV leads to variable degrees of cardiomyocyte degeneration and necrosis and interstitial lymphoplasmacytic inflammation, edema, and fibrosis with amphophilic to basophilic intranuclear inclusion bodies in cardiomycocytes. There can be a secondary neutrophilic and histiocytic infiltrate associated with necrotic cardiomycocytes, although it is not consistently observed. Experimental CPV infection of 9 puppies followed by necropsy at 4, 8, and 16 weeks revealed cardiomyocyte degeneration and necrosis that decreased in severity with increased age, lymphoplasmacytic infiltrate that peaked at 8 weeks of age, and interstitial fibrosis that increased in density, thickness of the bundles, and overall expanse with increased age. Similar temporal changes in necrosis, inflammation, and fibrosis were observed in the cases described in this report. In addition, the pulmonary edema and passive hepatic congestion in the dog that died at 7 weeks of age indicated a degree of heart failure, likely secondary to marked fibrosis and cardiomyocyte loss. There were no lesions of parvoviral enteritis in any of the experimentally infected puppies or in the dogs of the present report.

Canine parvovirus travels to the heart via leukocyte trafficking or freely in blood and lymph. The major mechanism of cell death in parvovirus infection is apoptosis. In addition to apoptosis, parvovirus infection leads to cell cycle arrest (as a result of DNA damage induced by the virus) and necrosis (by variable mechanisms depending on host species). One study investigating the pathogenesis of CPV revealed increased global oxidative stress in dogs with enteritis involving CPV infection, compared with dogs with enteritis without CPV involvement. Whether this outlines a mechanism of CPV pathogenicity or indicates that CPV-associated diarrhea is more severe and leads to increased oxidative stress as a result is unclear.

Whether due to apoptosis or oxidative stress, the acute myocarditis induced by CPV infection can lead to ectopic irritable foci, dysrhythmia, and sudden death. If an infected puppy survives, there is a likelihood of development of fibrous scar tissue, which predisposes to future dysrhythmia or contractile dysfunction. Exposure to the virus or vaccination imparts lifelong immunity; as such, puppies represent the CPV-susceptible population. Marked viremia develops 1 to 5 days after infection. Recently, CPV-2b was reported as the cause of erythema multiforme in multiple puppies born to a 5-year-old English Setter bitch residing on a horse farm in southern Georgia. The puppies developed clinical signs at 2 weeks of age; signs progressed to severe, painful, mucocutaneous crusting, scabbing, and blistering. Canine parvovirus-2b infection of the skin, tongue, lymph node, and intestines was confirmed via fluorescent antibody testing, PCR assay, immunohistochemical analysis, and transmission electron microscopy. However, in that report, no mention was made of lesions in the myocardium or testing of myocardial tissue for CPV-2b. There is an additional report of erythema multiforme due to CPV-2b infection in four 2-month-old English Setters. A parvovirus has been reported to cause myocarditis in neonatal cats. In a report published in 1985, two 13-day-old kittens were described to have degeneration, inflammation, and inclusion bodies within their intestines, liver, and myocardium. The inclusions consisted of parvovirus particles, although it was unclear whether this represented sequelae of FPV infection or aberrant infection with CPV.

Vaccination and improved husbandry have made parvoviral cardiomyopathy an infrequent problem among puppies. It should, however, remain a differential diagnosis for collapse, sudden onset dyspnea, or sudden death in puppies, especially littermates or those born to an unvaccinated bitch. There exists a dilemma as to whether it is ethical to offer puppies from an affected litter for adoption, given that they may have increased risk of sudden death from dysrhythmia or heart failure secondary to myocardial fibrosis.

References