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Pathology in Practice

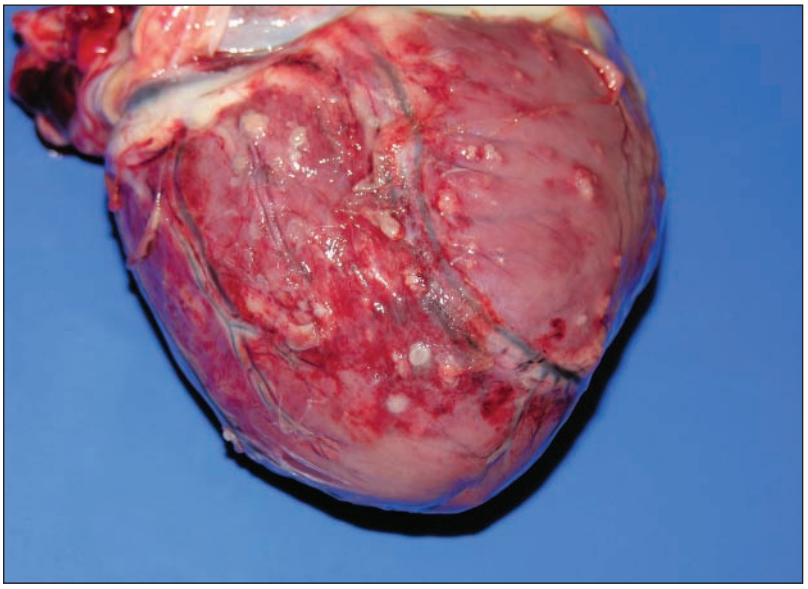

Figure 1—Photograph of the heart of a sexually intact female German Shepherd Dog that was evaluated by a veterinarian because of lethargy, anorexia, progressive lameness, and projectile vomiting. Multifocal to coalescing, white, firm, slightly raised nodules (0.2 to 0.5 cm in diameter) are present on the epicardium. Multiple strands of fibrin are also visible on the epicardial surface.

History

A 3-year-old 22.4-kg (49.3-lb) sexually intact female German Shepherd Dog was evaluated by a veterinarian because of lethargy, decreased appetite, progressive lameness, and projectile vomiting.

Clinical and Gross Findings

Physical examination by the veterinarian revealed poor body condition (body condition score, 2/5) and noticeable lameness. A CBC revealed moderate leukocytosis (30.2×10^3 WBCs/µL; reference interval, 4.0×10^3 to 15.5×10^3 WBCs/µL) that was characterized by neutrophilia and monocytosis. Döhle bodies, which were consistent with toxic change, were detected within the cytoplasm of many neutrophils. Serum biochemical analyses revealed mild to moderate azotemia (BUN concentration, 62 mg/dL [reference interval, 6 to 25 mg/dL]; creatinine concentration, 2.6 mg/dL [reference interval, 0.5 to 1.6 mg/dL]) and high phosphorus concentration (6.5 mg/dL; reference interval, 2.5 to 6.0 mg/dL). The dog was mildly to moderately hyperglobulinemic (4.7 mg/dL; reference interval, 1.6 to 3.6 mg/dL) and mildly hypoalbuminemic (2.4 g/dL; reference interval, 2.7 to 4.4 g/dL). Although aggressive medical treatment was attempted, the dog was euthanatized 2 days later and submitted for necropsy.

At necropsy, the dog had severe muscle atrophy. Approximately 20 mL of fibrinous, serosanguineous fluid was present within the pericardial sac. Multiple, white, firm, slightly raised nodules (0.2 to 0.5 cm in diameter) were disseminated throughout the epicardium, myocardium, and endocardium (**Figure 1**) as well as kidneys, liver, spleen, pancreas, and diaphragm. Bilaterally, the parietal cortices of the brain had multiple depressed red areas. On cross section, these foci were red with a liquefied necrotic center. Lymph nodes were diffusely large and firm; on cut surfaces, no distinction between cortex and medulla was seen. The hip joint capsules were thick, and the joints were filled with yellow turbid fluid.

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

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Cytologic and Histopathologic Findings

Microscopic examination of impression smears of the myocardium revealed abundant macrophages, fewer degenerate neutrophils, and plump fibroblasts admixed with numerous, variably sized mats of fungal hyphae. The hyphae were septate with parallel walls and both right-angled and acute-angled dichotomous branching. Occasional conidiophores that formed subspherical vesicles were evident, along with abundant conidia (**Figure 2**).

Histologically, multiple organs, including the heart, kidneys, spleen, brain, liver, pancreas, diaphragm, synovium of the hip joints, and lymph nodes, had signs of severe multinodular pyogranulomatous inflammation. Larger foci were associated with extensive areas of parenchymal necrosis and were surrounded by numerous epithelioid macrophages admixed with fewer degenerate neutrophils, occasional Langhans-type multinucleated giant cells, moderate numbers of lymphocytes, and fewer plasma cells. Numerous unstained longitudinal and transverse cross sections of translucent, refractile hyphae were embedded within necrotic areas or within the cytoplasm of multinucleated giant cells (**Figure 3**). Fungal hyphae were 6 to 8 µm in diameter and septate, with relatively parallel walls. There was irregular (frequently right-angled) branching of hyphae. Many hyphae had a spherical terminal bulb (conidiophores); some hyphae had occasional laterally branching spherical vesicles (aleuriospores or aleuroconidia) that were characteristic of *Aspergillus terreus* (**Figure 4**). *Aspergillus* organisms were isolated by means of mycological culture of brain tissue but not identified further.

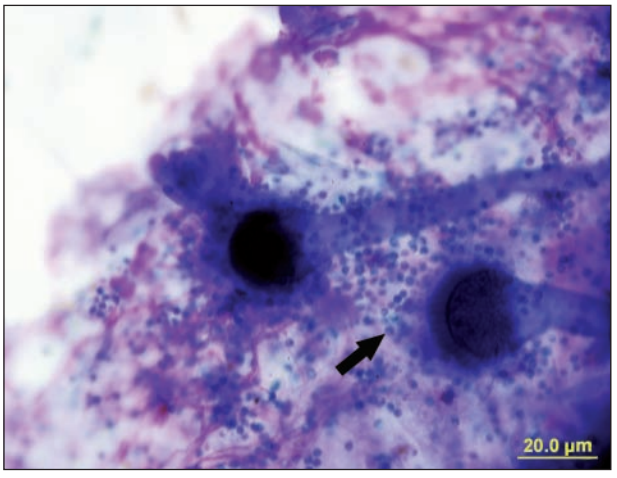

Figure 2—Photomicrograph of an impression smear of the myocardium of the dog in Figure 1. Notice the 2 fungal conidiophores that are forming numerous 1.5- to 2-µm-diameter conidia (arrow). Wright stain; bar = 20 µm.

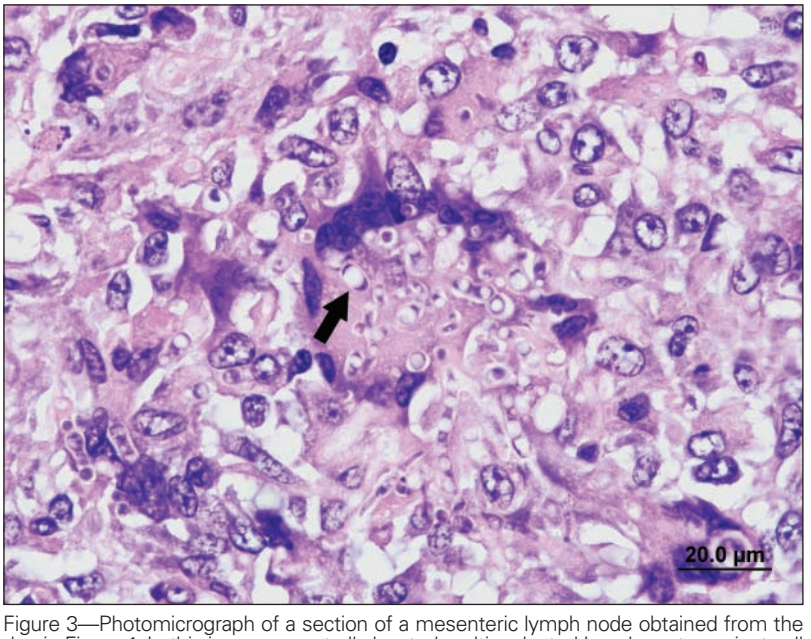

Figure 3—Photomicrograph of a section of a mesenteric lymph node obtained from the dog in Figure 1. In this image, a centrally located multinucleated Langhans-type giant cell is surrounded by numerous epithelioid macrophages. The cytoplasm of the Langhans cell is filled with multiple cross sections of fungal hyphae (arrow) that measure 6 to 8 µm in diameter. H&E stain; bar = 20 µm.

Morphologic Diagnosis

Severe, chronic, multinodular, necrotizing, and pyogranulomatous pancarditis; lymphadenitis; splenitis; pancreatitis; nephritis; encephalitis; synovitis; and myositis with intralesional fungal hyphae consistent with *A terreus*.

Comments

The dog of this report had disseminated aspergillosis, most likely attributable to infection with *A terreus*. Disseminated aspergillosis is a rare condition, and most commonly affects young female German Shepherd Dogs.[1–5] Because of this strong breed predilection, it has been proposed that German Shepherd Dogs have a selective immunodeficiency that predisposes them to develop disseminated aspergillosis.[2,6] Despite low serum concentrations of IgA and high serum concentrations of IgG in German Shepherd Dogs with disseminated aspergillosis, major deficiencies in either humoral or cell-mediated immunity that could explain the prevalence of the disease in this breed have not been identified.[7,8]

Common clinical signs associated with systemic aspergillosis are often nonspecific and include weight loss, anorexia, muscle wasting, weakness, vomiting, fever, and lameness.[6] In dogs with aspergillosis, the musculoskeletal system is most consistently affected and the site of the lameness typically correlates with the location of granulomas.[3] The lameness in the dog of this report was likely a result of fungus-associated arthritis of both hip joints. Radiographically, evidence of disease is often detected at multiple locations, including the scapulae, femurs, tarsi, and sternebrae. Some affected dogs develop acute renal failure in the latter stages of the disease.[6] Uveitis or endophthalmitis may precede more generalized signs of illness by several months.[2,3]

In the dog of this report, leukocytosis (characterized by neutrophilia and monocytosis) was detected. This, along with the presence of toxic changes in the

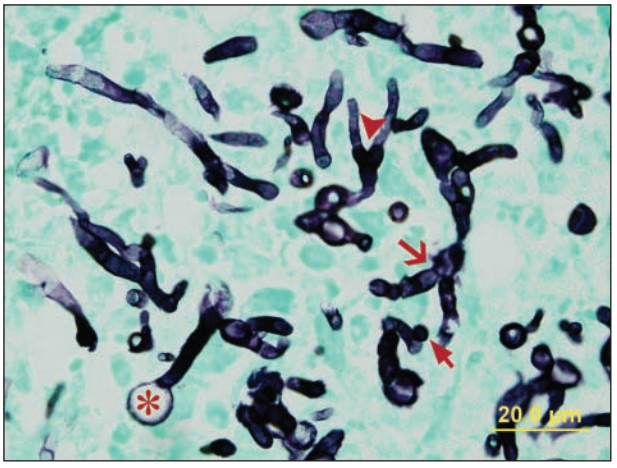

Figure 4—Photomicrograph of a section of a kidney obtained from the dog in Figure 1. In the tissue granulomas, there are multiple longitudinal and transverse cross sections of fungal hyphae. The hyphae are approximately 6 to 8 µm in diameter and regularly septate (arrow with open head) with dichotomous branching (arrowhead), terminal bulbous conidiophores (15 µm in diameter [asterisk]), and laterally branching aleuriospores (arrow with closed head). Gomori methenamine silver stain; bar = 20 µm.

neutrophils, was consistent with a systemic inflammatory response. Azotemia and hyperphosphatemia often develop in patients with disseminated aspergillosis, likely because of the presence of fungal hyphae in the renal parenchyma and subsequent infection-associated inflammation. The dog's urine specific gravity was not measured, but dogs with systemic aspergillosis are commonly isosthenuric with complete loss of renal concentrating ability.[2] Hyperglobulinemia in the dog of this report was likely due to antibody production secondary to systemic infection. An acute-phase response may have been concurrent with ongoing inflammation, contributing to both the high serum concentration of globulins and the mildly low concentration of albumin. Many animals with this condition and other granulomatous diseases are hypercalcemic because of excessive production of 1,25-dihydroxycholecalciferol by macrophages.[9] Unfortunately, accurate assessment of circulating calcium concentration in the dog of this report was not possible because the blood sample collected by the veterinarian prior to euthanasia of the dog was submitted to the analyzing laboratory in a tube containing Na-K EDTA anticoagulant. Because it is a powerful chelator, EDTA binds calcium and artifactually lowers both the total and ionized calcium concentrations in blood samples.

Grossly, disseminated aspergillosis is characterized by multiple, pale, discrete, variably sized, and sometimes friable nodules.[1,4,6] The kidneys, spleen, lymph nodes, and skeleton are most commonly affected.[2–4,6] It is suggested that the location of lesions in these organs is a result of vascular stasis in terminal capillary loops that allows the organisms to easily invade blood vessels, thereby causing thrombosis and infarction followed by infiltration of neutrophils and macrophages.[1,4,6] Other commonly affected sites include the pancreas, CNS, liver, heart, and eyes.[3,5] However, any organ can be affected. Disseminated aspergillosis with uterine involvement and transuterine transmission in a German Shepherd Dog with pyometra was recently described.[10]

Aspergillus terreus is most frequently associated with systemic aspergillosis, but *Aspergillus deflectus* and *Aspergillus niger* can also cause disease.[3,4] Cytologic or histologic evidence of *Aspergillus* organisms is often sufficient to confirm a diagnosis of systemic fungal infection. However, results of mycologic culture are needed to definitively diagnose aspergillosis. *Aspergillus terreus* can be differentiated from other *Aspergillus* spp by the presence of laterally branching conidia—called aleuriospores or aleuroconidia[4]—that are readily seen in tissue sections stained with Gomori methenamine silver stain. These structures are the agent of hematogenous spread in experimentally induced *A terreus* infection in mice.[1,4] Oral and respiratory routes have been implicated as the portal of entry for this organism.[1–4] In the dog of this report, the fungus had morphological characteristics of *A terreus*.

The prognosis for animals affected with disseminated aspergillosis is grave. Despite aggressive antifungal treatment (typically administration of amphotericin B and azoles), clinical signs often progressively worsen until the affected animal is euthanized, especially when signs of neurologic dysfunction, severe pain, or respiratory distress develop. However, treatment with antifungal drugs is recommended in cases with mild clinical signs because long-term resolution of infection is possible.[3]

References

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