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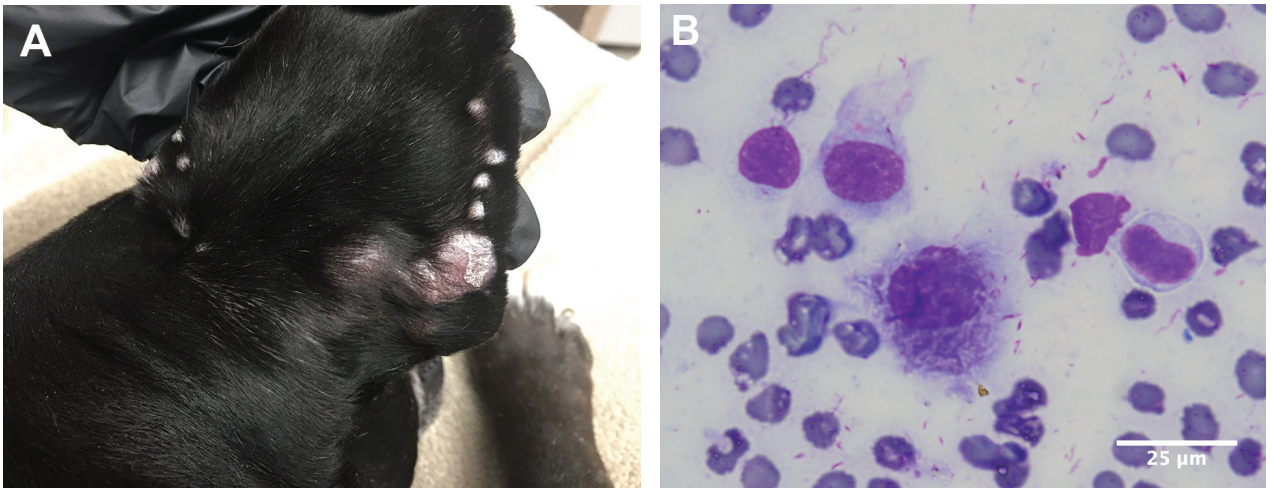

Figure 1—Photograph of dermal nodules on the right pinna (A) of a 5-year-old Boxer cross and photomicrograph of a direct smear of a fine-needle aspirate specimen collected from one of the nodules (B). Grossly, multiple multifocal to coalescing, firm, alopecic dermal nodules ranging from approximately 0.5 to 1.5 cm in diameter are visible at the caudal base and margins of the pinna. In the direct smear of the fine-needle aspirate specimen, notice the pale background with many erythrocytes and a macrophage filled with numerous negatively staining, 2- to 5-µm-long bacilli. Modified Wright stain; bar = 25 µm.

History

A 5-year-old 22.7-kg (49.9-lb) spayed female Boxer cross was presented to a primary care veterinarian for evaluation of multiple dermal nodules on both pinnae **(Figure 1)**. Approximately 2 weeks earlier, the dog had been examined because of a suspected interdigital foreign body on the left forelimb. Sterile exploration of the lesion revealed no foreign material. Cephalexin (22 mg/kg [10 mg/lb], PO, q 12 h for 10 days) was prescribed. The dermal nodules began emerging several days after initiation of cephalexin administration. The dog was also maintained on diphenhydramine (2.2 mg/kg [1 mg/lb], PO, q 8 to 12 h) for seasonal allergies.

Clinical and Cytologic Findings

On physical examination, the dog was bright, alert, and responsive. Pulse and heart rate were reported as within reference limits. Core body temperature was not recorded. Multiple multifocal to coalescing, firm, alopecic dermal nodules ranging from approximately 0.5 to 1.5 cm in diameter were noted on both pinnae (Figure 1). A CBC revealed mild erythrocytosis (Hct, 55.9%; reference interval, 37.0% to 55.0%) and mildly high hemoglobin concentration (19.5 g/dL; reference interval, 12.0 to 18.0 g/dL). Mild hyperglycemia (142 mg/dL; reference interval, 75 to 125 mg/dL) was the only detected serum biochemical abnormality.

A fine-needle aspirate specimen of the largest dermal nodule was obtained for cytologic examination. A modified Wright–stained direct smear preparation of the aspirate specimen had a mixed inflammatory cell population predominated by macrophages within a light blue, mildly hemodiluted background (Figure 1). Fewer plasma cells, small lymphocytes, eosinophils, and rare mast cells were also present. Occasional nonstaining, 2- to 5-µm-long bacilli were observed both within macrophages and freely throughout the background of the preparation.

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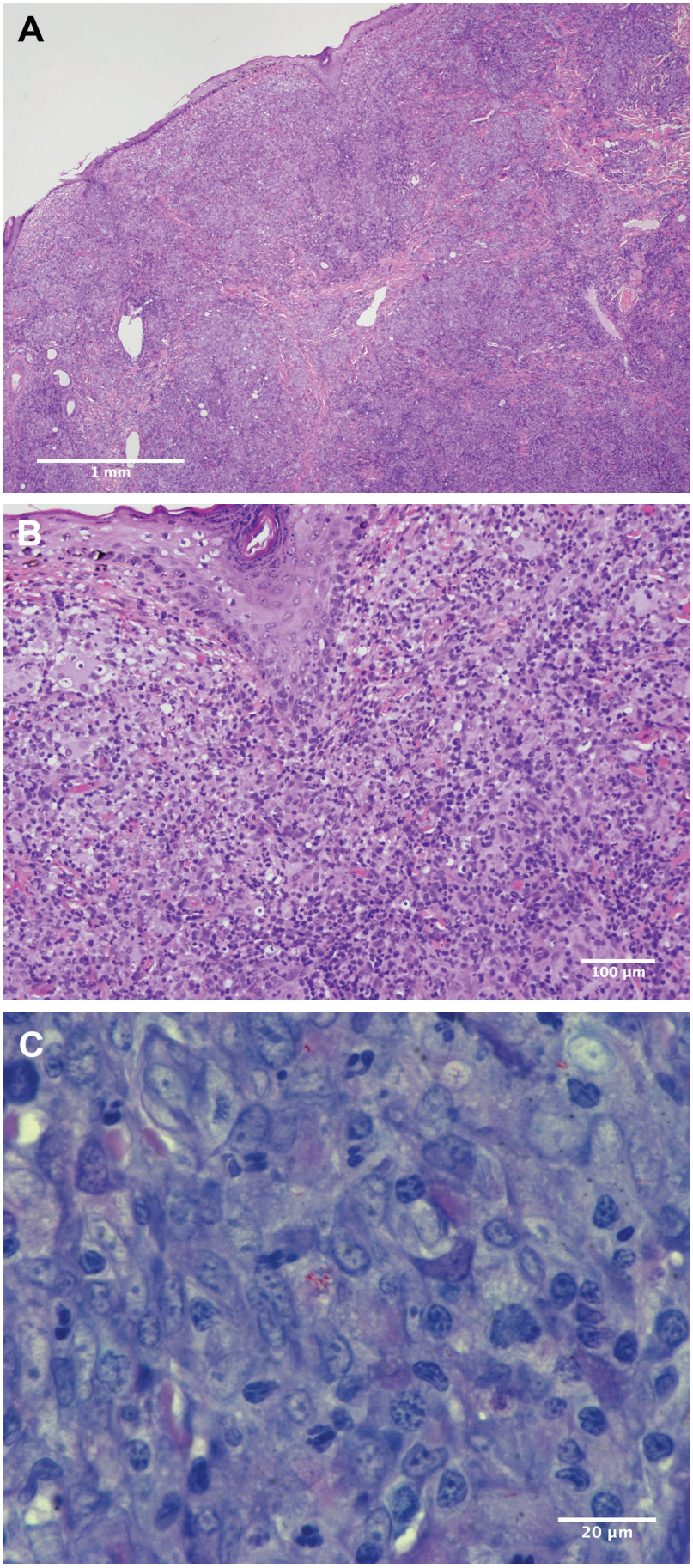
Formulate differential diagnoses from the history, clinical findings, and Figure 1—then turn the page→



Additional Diagnostic Test Results

Both fresh and formalin-fixed biopsy specimens of the dermal lesions were collected for examination. Formalin-fixed skin specimens were paraffin embedded, and several sections were stained with H&E stain **(Figure 2)**. Expanding the superficial and deep dermis and replacing the adnexal structures were numerous macrophages admixed with myriad multinucleated giant cells (3 to 7 nuclei/cell), degenerated and nondegenerated neutrophils, few lymphocytes, occasional plasma cells, and rare mast cells. Staining of sections with a Fite acid-fast method revealed scattered macrophages containing several acid-fast, short (2- to 4-µm-long) rod bacteria. A nested PCR assay[a] with previously published primers[1,2] that targeted the 16s DNA sequence of *Mycobacterium* sp Murphy was performed on the fresh tissue specimens. Sequencing of the amplified PCR products revealed 100% homology with *Mycobacterium* sp Murphy. This nucleotide sequence has also been referred to as *Mycobacterium* sp CLGS.[2]

Morphologic Diagnosis and Case Summary

Morphologic diagnosis and case summary: canine leproid granulomas (CLGs) associated with *Mycobacterium* sp Murphy in a dog.

Comments

The genus *Mycobacterium* contains > 150 known species of aerobic,

Figure 2—Photomicrographs of sections of formalin-fixed paraffin-embedded tissue from a punch biopsy of a dermal nodule on the pinna of the dog in Figure 1. A—The superficial and deep dermis are diffusely expanded by myriad macrophages, multinucleated giant cells (3 to 7 nuclei/cell), and degenerated and nondegenerated neutrophils admixed with fewer lymphocytes, occasional plasma cells, and rare mast cells. Normal adnexal structures have been replaced by these loosely organized granulomas with indistinct and converging borders. H&E stain; bar = 1 mm. B—Higher-magnification view of the loose aggregates of inflammatory cells. H&E stain; bar = 100 µm. C—In this section, notice the macrophage with intracellular acid-fast positive, 2- to 4-µm-long bacilli consistent with mycobacteria. Fite acid-fast stain method; bar = 20 µm.

non–spore-forming, gram-positive bacilli.[3] Mycobacterial infections consistently cause granulomatous or pyogranulomatous lesions (or both). However, the severity, extent, and chronicity of the lesions depend on the infecting mycobacterial species. On the basis of characteristics of the infection-associated pathological lesions and the organism's in vitro growth rate, mycobacteria are classified into 3 groups: slow-growing, rapidly growing, or leproid granuloma-inducing mycobacteria.[4] Slow-growing mycobacteria are further subdivided into tubercle-inducing and non–tubercle-inducing species. Slow-growing tuberculous bacteria include the well-known obligate intracellular pathogens *Mycobacterium tuberculosis* and *Mycobacterium bovis*. In contrast, nontuberculous slow-growing mycobacteria are opportunistic pathogens, such as those species within the *Mycobacterium avium* complex. Rapidly growing mycobacteria are often nontuberculous and easily cultured and identified in vitro. A notable example is the *Mycobacterium fortuitum* group of organisms, which has been implicated in mycobacterial panniculitis and pneumonia in both dogs and cats. Finally, leproid granuloma-inducing mycobacteria are nontuberculous mycobacterial species that are notoriously challenging to successfully cultivate in vitro. The mycobacterium that causes CLGs falls within this category.

Until the application of PCR assay–based diagnostic testing, knowledge about the mycobacteria associated with CLG lesions was scarce. In 2000, however, Hughes et al[1] reported the identification of a novel mycobacterial 16s rRNA gene sequence in 100% of fresh tissue sections from 9 dogs with CLGs. This novel sequence was referred to as *Mycobacterium* sp Murphy in the GenBank database. Identical sequences in samples from CLG lesions in dogs from Australia, New Zealand, Brazil, and Italy have since been reported.[2,5–7] The exact phylogeny of the *Mycobacterium* sp Murphy nucleotide sequence is incompletely understood, yet it appears most closely related to mycobacterial organisms within the *Mycobacterium simiae* clade.[1,2]

Canine leproid granuloma, also known as CLG syndrome, is a form of canine leprosy that has a worldwide distribution. Canine leproid granulomas were first described for 2 dogs from Zimbabwe in 1973.[7] Today, most reported CLG cases originate from Australia with fewer documented cases from North America, South America, Africa, and Europe.[5–6,8–10] Affected dogs develop 1 or more, well-circumscribed, multifocal to coalescing, dermal or subcutaneous nodules. These firm, nonpainful nodules are typically confined to the head, pinnae, or limbs. Although ulceration and secondary superficial bacterial infections may develop in affected dogs, neither systemic illness nor lesion dissemination has been reported.[6] In general, CLG cases involve apparently immunocompetent, short-coated, large-breed dogs. Boxers and Boxer-cross dogs are particularly overrepresented and comprised 21 of 45 affected animals in 1 report.[8] Inoculation of mycobacterial organisms via wounds or biting arthropods is the most widely accepted route of transmission proposed to date; however, the exact mechanism of transmission is unknown.[6] The development of CLG lesions in sparsely haired anatomic regions of short-coated dogs seems to support this hypothesized transmission route.

Traditionally, diagnosis of CLGs has relied on visualization of intracellular, nonstaining bacilli on examination of either cytologic or histologic specimens in conjunction with the clinical signs.[6] However, CLG lesions may contain sparse numbers of organisms, which can interfere with rapid and accurate diagnosis.[5,10] In 1 report,[10] a lengthy histologic search of sections of 60% of 37 CLG lesions was required to identify mycobacterial organisms even when aided by Ziehl-Neelsen acid-fast staining of mycobacterial cell wall constituents. The tendency for CLG lesions to spontaneously regress may contribute to the low number of easily detectable organisms within lesions. For the dog of the present report, organisms were easily identified in histologic sections following application of the Fite acid-fast stain method.

For many mycobacterial species, microbial culture can be used for identification of the disease-causing organism. However, for decades, the mycobacteria in CLG lesions have not been successfully grown in in vitro culture despite observation of the organisms in biopsy samples of CLG lesions.[10] Mycobacterial culture samples collected from these lesions may be overgrown with members of the rapidly growing mycobacterium subgroup, such as *M fortuitum*.[8] Cleaning the skin surface with 70% isopropyl alcohol prior to tissue collection for mycobacterial culture is now recommended to avoid *M fortuitum* contamination.[6]

Molecular techniques to identify *Mycobacterium* sp Murphy nucleotide sequences in granulomatous lesions may be particularly useful when organisms are not easily identified in cytologic or histologic specimens. Similar gross and histologic lesions may develop with other disease processes, such as idiopathic canine sterile granuloma-pyogranuloma syndrome, cutaneous leishmaniasis, and cutaneous fungal infections, among many others.[11] Treatment protocols and prognoses for each of these differential diagnoses can be highly variable. For instance, misidentification of CLG as canine sterile granuloma-pyogranuloma syndrome could result in administration of immunosuppressive doses of corticosteroids to an affected animal, which is contraindicated in the treatment of CLGs.[6,9,11] Detection of *Mycobacterium* sp Murphy nucleic acid sequences may help ensure appropriate treatment is administered.

Interestingly, up to 86% of CLG lesions resolve spontaneously within 1 to 3 months without medical or surgical intervention.[9] For the dog of the present report, the dog's lesions resolved within 8 weeks without specific treatment. Intermittent refractory cases have also been reported. Surgical excision alone is often curative.[9] Rarely, refractory lesions may persist, causing severe scarring and disfigurement of affected areas. Medical treatment for refractory cases involving various combinations of clarithromycin, rifampin, doxycycline, and clofazamine

has been reported with variable outcomes.[4,9] Regardless of the selected antimicrobial protocol, drug administration for 4 to 6 weeks or until complete resolution of lesions has been achieved is recommended.[4] Treatment of secondary superficial bacterial infections in ulcerated lesions with appropriate antimicrobials is also warranted.

To our knowledge, this is the first report of *Mycobacterium* sp Murphy nucleotide sequences associated with a CLG lesion in a dog from the central United States. In the case described in the present report, an interdigital lesion was observed initially, and the dog was administered cephalexin; the lesion subsequently resolved, and the cause remained unknown. Furthermore, the temporal association between antimicrobial administration and the onset of CLG lesions was considered coincidental because a link between antimicrobial treatment and development of CLG lesions has not been reported. The present report has highlighted the importance of cytologic evaluation and adjunct molecular techniques in the diagnostic workup of canine cutaneous mass lesions. In this case, mycobacterial organisms were identified in both cytologic and histologic specimens; however, the absence of observable mycobacterial organisms in such specimens should not preclude a diagnosis of CLG. Molecular testing of samples of lesions for the presence of *Mycobacterium* sp Murphy should be considered to ensure an accurate diagnosis is made when infective organisms are not detected.

Acknowledgments

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Footnotes

a. Developed by the Kansas State Veterinary Diagnostic Laboratory's Molecular Diagnostics Service, Manhattan, Kan.

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