tivity over a range of preclinical and clinical disease events, as well as optimization for different humidity, collection times, collection temperatures, and proximity placement under different management scenarios. Further development efforts are warranted by the preliminary findings, which are also encouraging for routine surveillance or targeted detection of other viral pathogens in environments where animal density is high, such as sale yards, confinement barns, or enclosed shipping containers.

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Sources and manufacturers
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Fatal mycobacteriosis with hepatosplenomegaly in a young dog due to
*Mycobacterium avium*

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Abstract. Cases of disseminated *Mycobacterium avium* infections in dogs are rare because it appears that the species is innately resistant to infection. A 2-year-old, castrated, 5 kg Shih Tzu-Poodle-cross developed anemia, abdominal pain, lethargy, and splenomegaly. Histological examination of surgically removed spleen indicated marked granulomatous splenitis with myriad intracytoplasmic acid-fast bacterial rods. Ultrastructural examination revealed the presence of 3–4-μm-long mycobacteria in phagolysosomes of epithelioid macrophages. Tissue extract of lightly fixed spleen was positive for *M. avium* 16S ribosomal RNA and negative for *M. tuberculosis* complex IS6110 DNA by polymerase chain reaction testing. Anemia was associated with the presence of mycobacteria-infected macrophages in bone marrow. The animal’s condition deteriorated, and euthanasia was performed after a clinical course of 2 months. The principal morphological findings at necropsy were severe diffuse granulomatous hepatitis, enteric lymphadenomegaly, and segmental granulomatous enteritis with intralesional mycobacteria present. *Mycobacterium avium* was cultured from enteric lymph nodes sampled at necropsy. The source of infection was not established but was presumed to be environmental with an enteric portal of entry.

Key words: Anemia; chronic disease; dogs; hepatitis; *Mycobacterium avium*; pathology; polymerase chain reaction.

Mycobacterial infections with *Mycobacterium avium* complex (MAC), which includes *M. avium* and *M. intracellulare*, as well as *M. tuberculosis* complex (which includes *M. tuberculosis* and *M. bovis*) are rare in dogs.6,7 Dogs are relatively less susceptible to infections with MAC organisms compared with *M. tuberculosis* complex.15 In a recent report, which summarized the clinical, microbiological, and morpholog-
ical features of disseminated mycobacteriosis in 18 dogs, certain breeds, such as Miniature Schnauzers and Basset Hounds, appeared to be predisposed to mycobacterial infection, although the basis for this was unclear. The present report involves generalized granulomatous disease in a young dog infected with *M. avium*.

A 2-year-old, castrated, 5 kg Shih Tzu-poodle crossbred dog in northern Wyoming was presented for lethargy to a veterinary practitioner. The dog was afebrile and anemic. It was treated with amoxyccinilin and prednisolone for 5 days. Three weeks later, the dog was observed to exhibit pain when handled. At this time, the dog was anemic, an enlarged spleen was palpated, and a tentative diagnosis of autoimmune hemolytic anemia was made. Prednisolone was dispensed. On re-examination 9 days later, the owner reported that the dog had had an episode of diarrhea 1 day earlier. The dog was given a blood transfusion, and a splenectomy was performed under general anesthesia. Fixed samples of the enlarged spleen were submitted for histological examination. The dog was prescribed dexamethasone and enrofloxacin and discharged. Five days later, the dog was reexamined, and the owner reported that it exhibited pain on rising. It was examined at a veterinary teaching hospital, and a bone marrow aspirate was performed for cytology. Despite continued treatment with enrofloxacin, the dog’s condition deteriorated, and it was euthanized after a total clinical course of 2 months. The carcass was submitted for necropsy to the WSVL.

The samples of surgically removed spleen exhibited the characteristics of severe, diffuse granulomatous splenitis on light microscopic examination (Fig. 1). The red pulp was heavily infiltrated by a monomorphic population of epithelioid macrophages, without evidence of necrosis, calciumization, or fibrosis (Fig. 2). The cytoplasm of macrophages was abundant, lightly basophilic, and finely stippled. Special stains revealed innumerable, lightly periodic acid–Schiff (PAS)-positive, diastase-resistant, lightly argyrophilic, intensely acid-fast, auramine red–positive bacterial rods. Ultrastructural examination revealed numerous 3–4-μm-long bacterial rods, some degenerate, almost all of which were in phagolysosomes (Figs. 3, 4). Organisms had the characteristic mycobacterial envelope, composed of cytoplasmic membrane, cell wall skeleton, and capsule (marked 2, 4, and 5, respectively, in Fig. 5).13 Polymerase chain reaction (PCR) was performed on sections of formalin-fixed, paraffin-embedded spleen, as described previously, except that amplification conditions were 94°C for 10 minutes, 50 cycles of 94°C for 45 seconds followed by 72°C at 135 seconds, and a single cycle of 72°C for 10 minutes. The test was positive with primers for 16S ribosomal RNA (rRNA), which identify *M. avium* species, and negative with primers for IS6100, which identify *M. tuberculosis* complex species. The procedure follows previously described methods.10

Antemortem hematological examination revealed the presence of a regenerative anemia (red blood cell count of 2.2 × 10^6/μl [reference interval 5.5–8.5 × 10^6/μl]; an absolute reticulocyte count of 110,160/μl; hemoglobin 5.2 g/dl [reference interval 13.0–20.0 g/dl]; and a packed cell volume of 19.0% [reference interval: 40.0–55.0%]). The automated nucleated cell count was elevated (21.8 × 10^3/μl [reference interval 4.5–15 × 10^3/μl]), and a differential count revealed that most nucleated cells (59%) were nucleated erythrocytes. Numerous metarubricytes, occasional rubricytes, and scant pronubricytes were also noted. Examination of a bone marrow aspirate showed numerous macrophages (5% of total nucleated cells) containing abundant intracytoplasmic, negatively stained filamentous bacterial rods. The myeloid-to-erythroid ratio was 1.5:1.0 (reference interval: 0.75–2.5). The normal myeloid-to-erythroid ratio was interpreted to be the result of concurrent erythroid and myeloid cell line hyperplasia.

Necropsy examination was performed 45 days after splenectomy. The 4.6-kg carcass was in adequate nutritional condition. There was a transmural, 30×15×15mm firm, white nodule in the duodenum situated about 22 cm distal to the pyloric sphincter. On cut section, the nodule corresponded to marked segmental thickening with white discoloration of mucosa and submucosa. There was moderate diffuse intraabdominal lymphadenomegaly that was most evident in jejunal nodes (11 g; 60×15×15 mm). Histologically, there was marked granulomatous lymphadenitis affecting all abdominal lymph nodes. The duodenal mass consisted of a similar granulomatous infiltrate. There was marked granulomatous portal hepatitis in which approximately 50% of liver was composed of granulomatous infiltrate. Other changes noted included mild disseminated granulomatous pneumonia, moderate diffuse granulomatous thymitis, moderate segmental granulomatous mucosal-submucosal duodenitis, and locally extensive granulomatous pachymeningitis of lumbar sacral spinal cord. In all affected tissues, epithelioid macrophages were distended with acid-fast bacilli. *Mycobacterium avium* was isolated in pure culture from jejunal lymph nodes, and its identity was confirmed using a commercial kit. The *M. avium* complex has traditionally included 28 serotypes of 2 distinct species, *M. avium* and *M. intracellulare*. They may be distinguishable by application of genetic methods such as DNA probes, 16S rRNA sequencing, and PCR–restriction fragment length polymorphism typing of the gene encoding the
Figure 1. Dog; spleen. Red pulp is replaced by a monomorphic population of epithelioid macrophages. Periarteriolar sheath is depleted of lymphocytes. Hematoxylin and eosin (HE).

Figure 2. Dog; spleen. Epithelioid macrophages have finely vacuolated, stippled cytoplasm. Necrosis, fibrosis, and mineralization are absent. HE.

Figure 3. Dog; spleen. Electron micrograph of 3 macrophages containing intracytoplasmic bacterial rods. Bacteria are membrane bound with distinct electron-lucent halo corresponding to Mycobacterium avium capsule (arrowhead). Uranyl acetate/lead citrate (UA/LC).

Figure 4. Dog; spleen. Higher magnification of an intracytoplasmic vesicle interpreted as phagolysosome containing intact bacilli (white arrowhead). Note one bacterium is damaged with clumped electron-dense internal structure (black arrowhead). L: lipid droplet. UA/LC.

Figure 5. Dog; spleen. Electron micrograph of M. avium in cross section. The complex mycobacterial cell envelope has 5 components (1–5)—1: electron-lucent separation between cytoplasm and cytoplasmic membrane; 2: cytoplasmic membrane; 3: electron-lucent space between cytoplasmic membrane and cell wall skeleton; 4: electron-dense cell wall skeleton; 5: capsule (electron-transparent zone). Terminology after Rastogi et al.13 UA/LC.
65-kD heat shock protein. Members of the MAC group are opportunistic, saprophytic, or facultative, intracellular, slow-growing organisms. *Mycobacterium avium* complex organisms may survive for long periods in the environment, particularly in acid soils with high organic content or in bodies of water. Contamination of the environment with *M. avium* presumably originates from feces or carcasses of birds infected with *M. avium*. Other sources of infection for mammals are birds, water, wood shavings, and contaminated litter such as sawdust and litter, feed, and compost. The source of infection in this case was not established. The presence of lesions in duodenum and abdominal lymph nodes, combined with inconsequential lesions in lungs, suggests an enteric portal of entry. Ingestion was speculated to be the basis for systemic mycobacteriosis in 7 Basset Hounds.

Anemia is a common finding in veterinary and human patients with mycobacterial infections and may be explained by the occurrence of secondary, immune-mediated hemolytic anemia, hemophagocytic syndrome, anemia of inflammatory disease, gastrointestinal blood loss, or a combination of these. The finding of rubricytosis on hematology and erythroid precursors extending as far back as prorubricytes prompted the performance of bone marrow aspirates. Although rubricytosis can be an appropriate response to anemia, the number of nucleated red cells observed in this case was higher than that could be explained by regeneration alone. The combination of splenectomy and granulomatous inflammation in bone marrow resulted in rubricytosis due to the persistence of nucleated erythrocytes, which are normally removed by the spleen. Granulomatous inflammation probably contributed to the anemia because of the bystander damage of sinusoidal endothelium and premature release of erythroid precursors.

Systemic mycobacteriosis due to MAC organisms in dogs is rare, despite their ubiquity in soil and water, because the species is usually refractory to infection (reviewed in reference 8). In a previous study in which healthy dogs were inoculated with *M. avium* by intravenous, intrabronchial, intraperitoneal, and oral routes, progressive infection could be established only by intracerebral inoculation. Of 18 reported spontaneous cases of generalized MAC infection in dogs, 8 were in Basset Hounds, 5 in Miniature Schnauzers, and the remainder were in mixed-breed dogs and various pure-breeds. Defective cell-mediated response involving either T cells or macrophages may be involved in Miniature Schnauzers and Basset Hounds, but this hypothesis has not been rigorously tested. A similar mechanism may account for sporadic cases in other breeds. No antemortem testing of the competence of the immune system was attempted in the current case. Most reports of illness in dogs with disseminated mycobacteriosis noted features similar to those observed-in this case. Disease tends to involve young dogs, which present with lethargy, weight loss, pain, and gastrointestinal symptoms. In most cases, extensive involvement of bowel, spleen, liver, and mesenteric lymph nodes are found at necropsy. Large numbers of mycobacteria are present in macrophages. In addition, caseation, fibrosis, and mineralization tend to be minimal.

The character of histological lesions due to MAC can be used to differentiate it from disease due to other mycobacteria, particularly *M. tuberculosis* and *M. bovis*. Most cases of canine MAC infection, including this one, have lesions characterized by a florid, monomorphic, epithelioid macrophage response, with numerous intracytoplasmic mycobacteria. In contrast, lesions associated with tuberculosis due to *M. tuberculosis* or *M. bovis* are generally caseous with a more pleocellular granulomatous response, including the presence of giant cells. *Mycobacterium avium* complex infection can produce either tuberculoid lesions with well-organized granulomas and scant bacteria (e.g., in cattle) or lepromatous lesions composed of sheets of epithelioid macrophages and numerous bacteria (e.g., in dogs, swine, and cervid species). To achieve definitive identification, bacterial cultures on fresh tissue samples followed by the use of molecular genetic techniques on the culture growth or PCR on formalin-fixed tissue are required.

The zoonotic potential of systemically MAC-infected mammals, including dogs, is considered to be low. *Mycobacterium avium* complex organisms are ubiquitous in soil and water, and most healthy people are exposed yet do not acquire infection; immunocompromised individuals are at greater risk. The owner of the dog and her immediate family were tested using the Mantoux tuberculin skin test intradermally by personnel from the Wyoming Department of Health for evidence of tuberculosis, after the initial diagnosis of unidentified mycobacterial infection in her dog. All the skin tests for in-contact family members were negative.

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Sources and manufacturers

a. Accuprobe®, Gen Probe Inc., San Diego, CA.

References
