

Case Report

A Case of Disseminated *Mycobacterium avium* Infection in a Dog in Greece

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A Basset Hound dog was presented with anorexia, fever, diarrhea, significant level of splenomegaly, and enlargement of mesenteric and superficial lymph nodes. Cytology of fine-needle-aspiration material, obtained from popliteal lymph node, revealed macrophages with intracytoplasmic, nonstaining, slender, rod-like structures, indicative of mycobacteria. Bacterial culture of lymph node aspirated material produced a colony which by means of molecular techniques (PCR amplification and hybridization of PCR products) was subsequently identified as *Mycobacterium avium*. This is the first report of disseminated *M. avium* infection in a dog in Greece.

1. Introduction

The genus *Mycobacterium* contains various obligate or opportunistic microorganisms. The latter are mostly members of the *Mycobacterium avium* complex, acquired mainly from environmental sources, for example, soil, water, dust, and feed. *M. avium* complex strains can cause disease in humans and in a wide range of animals, including dogs and cats, mainly in immunocompromised individuals [1]. In general, infections with *M. avium* complex strains are rare in dogs [2], as these animals are considered less susceptible to infections with these organisms in comparison to those with *M. tuberculosis* [3]. Objective of this paper is the description of a case of disseminated *M. avium* infection in a dog in Greece. To the best of the authors' knowledge, this is the first report of such infection in dogs in Greece.

2. Case Description

A 3.5-year-old, intact male, Basset Hound-breed dog was presented with a 10-day-long history of anorexia, fever, and diarrhea. The administration of broad-spectrum antibiotics and

antidiarrheals resulted in transient improvement of appetite and clinical signs. Nevertheless, the general health status of the animal deteriorated and was presented for reexamination two weeks later. During general clinical examination, weight loss, fever (39.8°C), pink mucous membranes, significant level of splenomegaly, and enlargement of mesenteric and superficial lymph nodes were recorded.

Hematological examination revealed increased leucocyte count (27.000 μL^{-1}), predominantly neutrophils and monocytes (4.000 μL^{-1}). Serum biochemistry revealed increased activity of alanine transferase (254 IU L⁻¹) and aspartate aminotransferase (173 IU L⁻¹). Cytology of fine-needle-aspiration (FNA) material from popliteal lymph node revealed increased number of inflammatory cells (neutrophils, macrophages) but no *Leishmania* spp. amastigotes or malignant lymphocytes were observed. Many macrophages contained intracytoplasmic, nonstaining, slender, rod-like structures, indicative of mycobacteria (Figure 1). Serology (indirect fluorescent antibody test) was negative for antibodies against *Leishmania* spp. and *Ehrlichia* spp. Based on these results leishmaniosis, lymphoma and ehrlichiosis

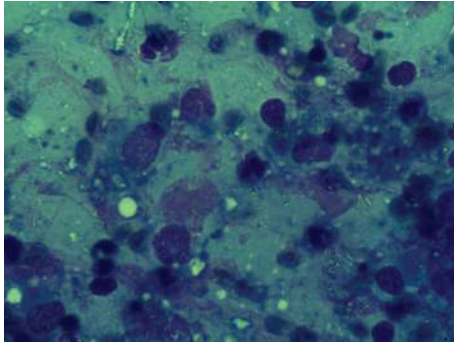


FIGURE 1: Results of cytological examination of smears of material from popliteal lymph node, collected with fine-needle-aspiration: presence of macrophages with intracytoplasmic, nonstaining, slender, rod-like structures, indicative of mycobacteria (Diff Quick stain, $\times 400$).

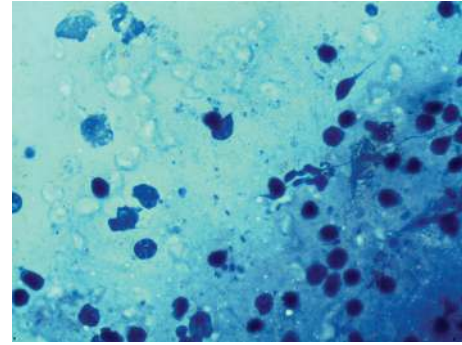


FIGURE 3: Results of cytological examination of smears of material from popliteal lymph node, collected with fine-needle-aspiration: presence of acid-fast intracytoplasmic bacilli (Ziehl-Neelsen stain, $\times 400$).

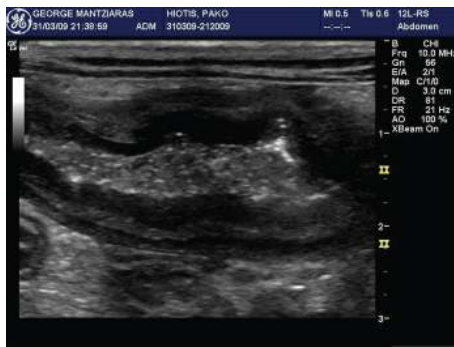


FIGURE 2: Results of ultrasound examination of the abdomen: thickening of intestinal loops and enlargement of mesenteric lymph nodes.

were excluded as differentials. Lymphadenitis secondary to tuberculosis was suspected.

Ultrasonographic examination of the abdomen revealed mild hepatomegaly, severe splenomegaly (approximately 5-fold the normal size of the spleen), significant thickness of intestinal loops, and enlargement of mesenteric lymph nodes (Figure 2). Ultrasonography of the thorax revealed no abnormality.

Subsequently, blood samples and FNA material from lymph nodes were submitted for additional laboratory tests (direct smears stained with Ziehl-Neelsen stain, bacterial culture and PCR from FNA material from popliteal lymph node). On cytological examination, acid-fast intracytoplasmic bacilli presented as slender, purple-color rods were observed (Figure 3). Bacteriological examination was carried out at the Greek National Reference Laboratory for Mycobacteria. Bacterial culture was performed on Lowenstein-Jensen medium enriched with glycerol, at 37°C in 5% carbon dioxide environment, for 4–6 weeks. Microorganisms were recorded in two samples. A loopful of the colonies was suspended in 1 mL of distilled water and DNA was extracted by sonication for 15 min, followed by heating to 100°C for 15 min. DNA was partially purified by centrifugation for 2 min. Subsequently, $5\ \mu\text{L}$ from the supernatant was used for molecular

identification by means of the GenoType Mycobacterium CM/AS assay (Hain Lifescience, Nehren, Germany), which was performed according to the manufacturer's instructions. The process involved PCR amplification, hybridization of PCR products to probes bound to test strips, and detection and interpretation of the results [4]. Finally, the isolates were identified as *Mycobacterium avium* spp. However, the subspecies of isolated *Mycobacterium avium* could not be identified.

Based on the above, a diagnosis of disseminated infection by *M. avium* was made. After final diagnosis, the owner elected euthanasia of the dog and refused necropsy and additional laboratory tests.

3. Discussion

M. avium bacilli are ubiquitous and may remain viable in the environment for over two years [5]. In dogs, infections caused by *M. avium* have been reported sporadically [6]. In Europe, previous cases of this disease have been reported in Germany [4], Italy [2], and United Kingdom [7]. To the best of the authors' knowledge, this is the first report of disseminated *M. avium* infection in dogs in Greece.

Certain breeds of dogs, for example, Basset Hounds and Miniature Schnauzers, seem to be at higher risk of infection [8]. In general, it is thought that immunocompromised animals are at higher risk of the disease.

Mycobacteria invade hosts through the respiratory or the digestive system. The source of infection is possibly the animal's environment, perhaps as the result of contamination from infected birds [3]. Another possible source of infection is contaminated chicken or porcine livers ingested by affected animals [9, 10].

Clinical signs of canine *M. avium* complex infections are nonspecific and, often, are associated with the disseminated form of the disease with multiple organs involvement [7, 11]. In the present case, the prominent abdominal findings probably suggest infection by the oral route. Initial diagnosis of the disease was based on cytological examination of lymph node aspirated material. The accuracy of this diagnostic approach can be increased, if Ziehl-Neelsen stain is used

[12]. Nevertheless, definitive diagnosis could only be reached with bacteriological culture of such material and subsequent bacterial identification by molecular techniques [11].

M. avium is a confirmed zoonotic pathogen, especially in immunocompromised persons. Therefore, there is always a risk for infection for the owners of affected animals and prescription of treatment protocols should consider the zoonotic risks before their instigation. Intestinal involvement, as in the present case, can increase potential zoonotic risk due to faecal excretion of the organism. The latter may lead the owner to decide euthanasia for his animal.

4. Concluding Remarks

M. avium infection in the dog may be presented as disseminated form with nonspecific clinical signs and involvement of intestinal tract, liver, spleen, and lymph nodes. Since specific laboratory tests are needed for isolation and identification of the bacillus, the clinician should come in contact with an appropriate laboratory for this purpose, if this disease is suspected.

Conflict of Interests

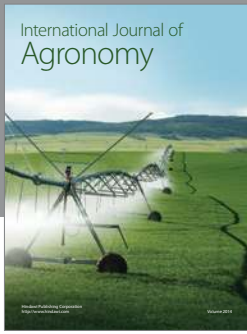
None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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References

- [1] D. A. Ashford, E. Whitney, P. Raghunathan, and O. Cosivi, "Epidemiology of selected mycobacteria humans and other animals," *OIE Revue Scientifique et Technique*, vol. 20, no. 1, pp. 325–337, 2001.
- [2] L. Campora, M. Corazza, C. Zullino, V. V. Ebani, and F. Abramo, "*Mycobacterium avium* subspecies *hominissuis* disseminated infection in a Basset Hound dog," *Journal of Veterinary Diagnostic Investigation*, vol. 23, no. 5, pp. 1083–1087, 2011.
- [3] M. F. Thorel, H. F. Huchzermeyer, and A. L. Michel, "*Mycobacterium avium* and *Mycobacterium intracellulare* infection in mammals," *Revue Scientifique et Technique*, vol. 20, no. 1, pp. 204–218, 2001.
- [4] V. Haist, F. Seehusen, I. Moser et al., "*Mycobacterium avium* subsp. *hominissuis* infection in 2 pet dogs, Germany," *Emerging Infectious Diseases*, vol. 14, no. 6, pp. 988–990, 2008.
- [5] F. Biet, M. L. Boschiroli, M. F. Thorel, and L. A. Guilloteau, "Zoonotic aspects of *Mycobacterium bovis* and *Mycobacterium avium*-intracellulare complex (MAC)," *Veterinary Research*, vol. 36, no. 3, pp. 411–436, 2005.
- [6] A. G. Gow and D. J. Gow, "Disseminated *Mycobacterium avium* complex infection in a dog," *Veterinary Record*, vol. 162, no. 18, pp. 594–595, 2008.
- [7] K. Gerber, J. Hargreaves, A. Iveson, and D. Worth, "Disseminated *Mycobacterium avium* in a young Basset Hound located in a suburban area in the United Kingdom," *European Journal of Companion Animal Practice*, vol. 19, no. 1, pp. 31–36, 2009.
- [8] B. Horn, D. Forshaw, D. Cousins, and P. J. Irwin, "Disseminated *Mycobacterium avium* infection in a dog with chronic diarrhoea," *Australian Veterinary Journal*, vol. 78, no. 5, pp. 320–325, 2000.
- [9] J. L. Carpenter, A. M. Myers, M. W. Conner, S. H. Schelling, F. A. Kennedy, and K. A. Reimann, "Tuberculosis in five Basset hounds," *Journal of the American Veterinary Medical Association*, vol. 192, no. 11, pp. 1563–1568, 1988.
- [10] M. A. Miller, C. E. Greene, and A. E. Brix, "Disseminated *Mycobacterium avium*—intracellulare complex infection in a miniature schnauzer," *Journal of the American Animal Hospital Association*, vol. 31, no. 3, pp. 213–216, 1995.
- [11] J. F. Naughton, K. L. Mealey, K. J. Wardrop, J. L. Oaks, and D. S. Bradway, "Systemic *Mycobacterium avium* infection in a dog diagnosed by polymerase chain reaction analysis of buffy coat," *Journal of the American Animal Hospital Association*, vol. 41, no. 2, pp. 128–132, 2005.
- [12] M. Bezabih, D. W. Mariam, and S. G. Selassie, "Fine needle aspiration cytology of suspected tuberculous lymphadenitis," *Cytopathology*, vol. 13, no. 5, pp. 284–290, 2002.



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