

Intra-abdominal *Mycobacterium tuberculosis* Infection in a Dog

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Key words: Intra-abdominal; Tuberculosis; Computed tomography; Canine.

A 4-year-old, 30.6-kg castrated male mixed-breed dog was referred for evaluation of acute diarrhea, inappetence, weight loss, and lethargy. The dog had otherwise been healthy since being adopted from a Greek animal shelter 18 months before presentation. The only other household pet was its clinically healthy littermate. On examination, the dog was lethargic, hyperthermic (102.4°F), had a body condition score of 3/9, and was moderately painful upon palpation of the cranial abdomen.

Abnormal hematologic findings included a moderate leukocytosis ($19.3 \times 10^9/L$; reference range [RR], $5.5\text{--}13.7 \times 10^9/L$) with neutrophilia ($15.6 \times 10^9/L$; RR, $2.8\text{--}8.7 \times 10^9/L$) and a mild microcytic nonregenerative anemia (PCV, 35%; RR, 39–56%). Serum biochemistry profile results disclosed moderate hyperglobulinemia (56.4 g/L; RR, 22.9–35.6 g/L), hypoalbuminemia (19.9 g/L; RR, 29.6–37.0 g/L), and moderately increased alkaline phosphatase activity (169 U/L; RR, 0–130 U/L). Urinalysis was unremarkable.

Results of serologic assays for anti-*Leishmania infantum* antibody and *Dirofilaria immitis* antigen were negative. Fecal flotation was negative. Thoracic radiographs were within normal limits, but abdominal radiographs showed marked loss of detail in the cranial abdomen caudal to the stomach with a mass effect causing caudal intestinal displacement.

Ultrasonographic examination of the abdomen (Fig 1) identified an elongated, well-defined, multilobular, mixed echoic mass 5 cm in length within the cranial to midabdomen caudal to the pyloric antrum and porta hepatis, surrounded by hyperechoic mesentery and including the portal vein as well as parts of the mesenteric vein. Multifocal hypo- to anechoic areas and hyperechoic foci with acoustic shadowing were noted. The surrounding mesenteric fat had mildly increased echogenicity. The stomach and pancreas were normal. Multiple hypoechoic nodules of varying

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Abbreviations:

CT computed tomography

size were visible throughout the parenchyma of all liver lobes. Based on the ultrasonographic findings, the most likely origin of the abdominal mass was thought to be mesenteric and hepatic lymph nodes. A multicentric neoplastic process or granulomatous disease of the mesenteric and hepatic lymph nodes and liver was suspected. Ultrasound-guided fine needle aspirates of the abdominal mass were stained using May-Grünwald-Giemsa stain and cytologic evaluation indicated mild cellular necrosis and moderate pyogranulomatous inflammation consisting of nondegenerate neutrophils, small mature lymphocytes, and activated macrophages containing nonstaining rods suspicious for acid-fast bacteria. Because these bacteria were positive on Ziehl-Neelsen staining, infection with *Mycobacterium* spp. was suspected. After consulting with the National Reference Center for *Mycobacteria* and Supranational Reference Laboratory of the World Health Organization (WHO, Borstel, Germany), 2 weeks after the 1st presentation a laparotomy was performed to obtain multiple diagnostic biopsy specimen samples. To determine the organ of origin, the extent of the disease throughout the abdomen, and possible involvement of the lungs and hilar lymph nodes, which would have led to euthanasia because of public health safety issues, a computed tomographic (CT) examination was performed before laparotomy.

Computed tomography examination (Fig 2) was performed using a 16-slice helical scanner^a with a slice

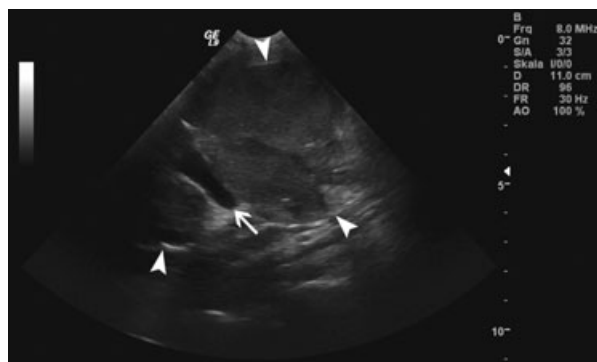


Fig 1. Longitudinal gray-scale ultrasonographic image: An ovoid slightly heterogeneous lobulated mass of low echogenicity (white arrowheads) containing a large vessel (white arrow) is visible. The cranial and caudal extent of the lesion is not included in the images.

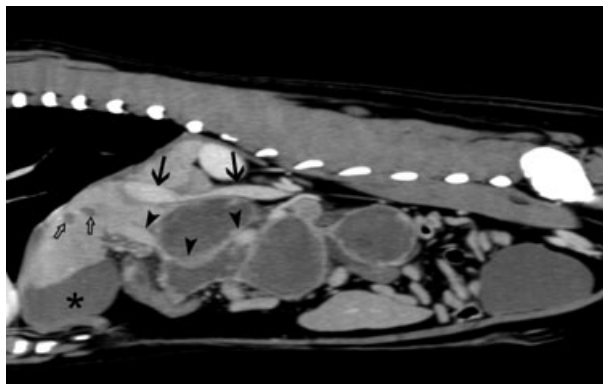


Fig 2. Postcontrast sagittal computed tomographic images of the abdominal cavity, soft-tissue algorithm. The sagittally reformatted image is slightly to the right of the midline: Note the ring enhancement of the lesion while the center noncontrast enhancing and hypoattenuating (Mean 20 HU) indicating central necrosis or exudate. The caudal vena cava (arrows) is narrowed at the level of the cranial aspect of the space-occupying lesion. Ventral displacement of the portal vein (arrowheads) caudal to the liver hilum is present. Several ovoid hypoattenuating noncontrast enhancing areas are visible within the liver parenchyma (open arrows). The asterisk (*) indicates the gallbladder.

thickness of 3 mm. Pre- and postcontrast abdominal CT images after manual IV injection of nonionic iodinated contrast medium^b at a dosage of 600 mg/kg were acquired. An elongated, well-defined, multilobulated, cavitary space-occupying lesion 19 cm in length was noted, adjacent to the portal vein and caudodorsal to the gastric body and pylorus, extending caudally to the level of the caudal pole of the left kidney. Cranioventral displacement of the gastric body, pylorus, and left limb of the pancreas was present and the right limb of the pancreas and the duodenum were displaced to the right.

Narrowing of the caudal vena cava was present at the level of the mass. The interior was heterogeneous with attenuation values ranging from 15 to 45 Hounsfield Units (HU). Overall, the peripheral margins of the mass were slightly hyperattenuating, and the central aspects exhibited lower attenuation values but were interspersed by amorphous hyperattenuating foci (up to 82 HU). There was subtle hazy increased attenuation of the fat in the mesentery and liver hilum surrounding the lesion. On postcontrast CT images ring enhancement in the periphery of the subdivisions of the space-occupying mass along with largely nonenhancing lesion centers resulted in a heterogeneous appearance. Part of the portal vein including the junction with the splenic and gastroduodenal veins was localized centrally within the cranial aspect of the mass and appeared to be severely compressed and distorted in shape. The gastric and pancreaticoduodenal lymph nodes had mild spherical enlargement with a short-to-long axis ratio above the reported reference range of 0.5.¹

Multiple round, well-defined, noncontrast-enhancing nodules 5–25 mm in diameter and with HU of 30 were

present throughout the liver parenchyma. A CT diagnosis of necrotic mesenteric and hepatic lymphadenopathy with early calcification and hepatic nodules was made. The CT investigation of the thorax was unremarkable.

Laparotomy was performed to obtain biopsy samples and identified multifocal nodular lesions affecting the serous membranes of the diaphragm, stomach, intestines, and liver as well as liver parenchyma and multiple severely enlarged mesenteric lymph nodes. Biopsy specimens of abdominal lymph nodes were submitted to the National Reference Center for *Mycobacteria* in Borstel, Germany and processed according to WHO guidelines. The specimens were inoculated on 2 solid^c and 1 liquid^d media. There was only 1 strain grown, isolated from 2 different specimens. The strain was grown on all solid media with the typical morphology for *M. tuberculosis*. Differentiation was performed with a DNA strip assay, a molecular based technique (GenoType MTBC^e) and *Mycobacterium tuberculosis* was identified. Drug susceptibility testing was carried out for first-line drugs and performed using the Mycobacteria Growth Indicator Tube (MGIT) system.^d

The owners were educated about the potential health risk and were asked to contact their physician. Furthermore, they were advised to avoid contact between their dog and young, old, or immunosuppressed people. The case was reported to the public health officer. Monthly evaluation of fecal smears from both the dog and its littermate using Ziehl-Neelsen staining failed to detect any *Mycobacteria* spp.

In accordance with statutory provisions and the owners' wishes, treatment was initiated whereas susceptibility testing was pending with 3 antibiotics described for their use in *M. tuberculosis* infections²: rifampicin^f 10 mg/kg administered PO q24h, clarithromycin^g 12 mg/kg administered PO q12h, and enrofloxacin^h 5 mg/kg administered PO q12h for the 1st 3 months. Results obtained 2 months after diagnosis indicated *M. tuberculosis* to be susceptible to rifampicin, isoniazid, ethambutol, and pyrazinamid, but resistant to streptomycin. Subsequently, rifampicin and clarithromycin were continued indefinitely.

The dog was reevaluated every 4 weeks for the next 6 months, and from then on monthly including physical examination, hematology, serum biochemistry, fecal smear examination using Ziehl-Neelsen staining, thoracic and abdominal radiographs, and abdominal ultrasound and CT examinations at 7, 12, and 20 months postdiagnosis. Appetite was restored and diarrhea resolved 4 weeks after treatment was initiated, and all blood parameters except albumin (25.6 g/L) and alkaline phosphatase activity (143 U/L) were within reference intervals 7 months after diagnosis. Thoracic and abdominal radiographs were unremarkable.

CT examinations at 7, 12, and 20 months postdiagnosis (Fig 3A,B) identified progressive multifocal calcification and decreased size of the mesenteric and hepatic lymph nodes. At the time of writing

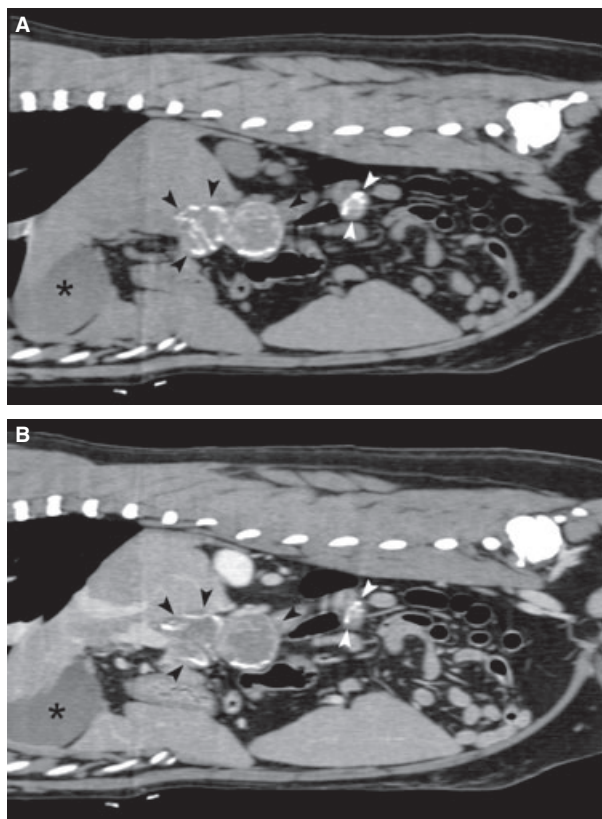


Fig 3. (A, B) Follow-up CT 20 months after diagnosis: Pre-contrast (A) and corresponding postcontrast (B) sagittal computed tomographic images of the abdominal cavity, soft-tissue algorithm, slightly to the right of the midline. A progressive decrease in size and increase in mineral content predominantly in the lesions peripheries is evident. Mineralization of the mass wall is visible (black arrows). The gallbladder is indicated by the asterisk (*). Because of reduction in size, the lesions comprise 2 separate entities (black and white arrowheads).

(31 months postdiagnosis), the dog is clinically healthy.

Canine *M. tuberculosis* infection is rarely reported, and so far an intra-abdominal localization and successful treatment in a dog has not been documented. *M. tuberculosis* is a nonmotile, nonspore-forming, obligate intracellular, gram-positive aerobic bacillus that is characteristically acid-alcohol fast. Culture is difficult and takes several weeks because the organism divides only once every 15–20 hours and needs special culture conditions such as Lowenstein-Jensen Medium. Its unique cell wall composition (hydrophobic waxy mycolate layer and peptidoglycan layer held together by arabinogalactan) makes *Mycobacterium* spp. highly resistant to heat, pH changes, routine disinfection, and antibiotics.²

Humans are reservoir hosts for the organism and dogs have not been reported to spread *M. tuberculosis* infection to people.² Thus, infections in dogs are considered an anthrozoosis, with dogs becoming infected after prolonged exposition to human respiratory secretions. Transmission of *M. tuberculosis* to

dogs almost always remains unclear. A case report proved positive by genotyping for anthrozoosis from a woman to her dog.³ Furthermore in 2011, 3 veterinary pathologists with low risk before necropsy of a dog with disseminated clinical *M. tuberculosis* infection tested serologically positive a few weeks later.⁴

Considering that the owners of the present dog never showed any signs of tuberculosis, the Mediterranean background of the dog, and the long incubation period, it is likely that this dog became infected in Greece, possibly by ingestion of garbage contaminated by infected human sputum, before being obtained by the current owners.

Few cases of infection of dogs with *Mycobacteria* spp. are reported throughout the world. *M. tuberculosis* usually infects the respiratory system of mammals because it needs high oxygen tension for survival and replication. Thus, aerogen disease transmission by inhaled infectious sputum is the most common route of infection. Until now, all reported cases of infections with *M. tuberculosis* in dogs were localized in the pulmonary system.^{5–9} In a review published in 1962 of 48 dogs and cats with known exposure to human tuberculosis, 14.6% were culture positive for *M. tuberculosis*.¹⁰ Recent studies in a high-risk setting in South Africa reported a prevalence of 1% of nonclinical tuberculosis in dogs which is similar to the prevalence in Europe in the early part of the 20th century when 0.1–6.7% of dogs were *M. tuberculosis* positive.^{11,12} Rare cases of *Mycobacterium* spp. infection of the digestive tract in the dog described so far are caused by *M. bovis* (member of the *Mycobacterium tuberculosis* complex) or members of the *M. avium* complex.^{13–18}

Computed tomography is a useful tool for diagnosing *M. tuberculosis* infections in human patients.¹⁹ It helps in differentiating 3 types of *M. tuberculosis* infection: the “wet ascitic” type, the “dry-plastic” type, and “fibrotic-fixed” lesions.²⁰ The most frequently encountered form is the “wet ascitic type”, which is characterized by a large amount of high-density ascitic fluid because of its exudative content. The “dry-plastic” type is difficult to diagnose on CT scans because of the bulky mesenteric lymph nodes, which can result in a misdiagnosis of abdominal lymphoma. The “fibrotic-fixed” lesion is defined by distinctive abdominal or omental masses.^{20,21} In the present case, CT findings were consistent with the “dry-plastic” type (ie, enlarged mesenteric lymph nodes, well-circumscribed areas with central low density, and peripheral enhancement with low-attenuation centers).^{19–21} This enhancement is highly suggestive, but not pathognomonic of *M. tuberculosis* infection.^{19,21} The central low density areas indicate central caseation necrosis.^{19–21} Advanced lesions such as peripheral calcification, seen in the CT examination performed after 7 months, have only been described in *M. tuberculosis* infection in the liver or spleen.¹⁹

The hepatic hypoattenuating foci seen in this patient are similar to lesions described as a micronodular form of hepatosplenic tuberculosis (diameter of 1–3 cm) in

human patients. This is caused by a secondary hematogenous dissemination of the primary form of the disease. Although the micronodular form usually is accompanied by miliary pulmonary tuberculosis, the CT findings of the thorax in the present dog were inconspicuous.^{19,20}

Treatment options in human and small animal patients offer few choices. Standard treatment of uncomplicated *M. tuberculosis* infections in human patients consists a minimum of 4 antibiotics (ie, isoniazid, rifampicin, ethambutol, and pyrazinamid for the initial treatment over a period of 2 months) followed by a maintenance treatment with 2 antibiotics (ie, isoniazid and rifampicin for several months).²² Streptomycin is an antibiotic of last resort and should only be administered after susceptibility testing.²² Options for treatment of multi-drug-resistant *M. tuberculosis* infection include fluoroquinolones, aminoglycosides, and thiamides over a period of at least 21 months starting with 5 antibiotics.²² Current recommendations for treatment of *M. tuberculosis* infections in small animals start with a minimum of 2 antibiotics (some combination of isoniazid, rifampicin, ethambutol, dihydrostreptomycin, and pyrazinamid).² While susceptibility testing was not available, a combination of rifampicin, clarithromycin, and enrofloxacin was chosen for several reasons. Firstly, streptomycin should be kept as a last-resort antibiotic for human patients and should not be used in dogs. Secondly, isoniazid can cause severe adverse neurological effects and has led to euthanasia in a case of pulmonary *M. tuberculosis* infection in a dog.⁶ Thirdly, pyrazinamid is useful in *M. tuberculosis* infections, but is ineffective in *M. bovis* infection,²³ which initially was the presumed organism in this case because of its localization.¹⁵ Rifampicin, which later turned out to be effective according to susceptibility testing, has no antagonistic effect with other antibiotics but is potentially hepatotoxic. Therefore, liver enzyme activity was monitored monthly and showed no change over a 1-year period. Enrofloxacin is an established and well-tolerated antibiotic in small animal medicine. It was chosen as the 3rd antibiotic in case of multi-drug-resistant *M. tuberculosis* infection. Enrofloxacin was discontinued after 3 months and maintenance treatment was continued with rifampicin and clarithromycin.

Although low serum vitamin D concentrations have been described in cats with active *Mycobacteria* infections,²⁴ the addition of vitamin D₃ in *M. tuberculosis*-infected patients had no clinically relevant effect on antigen-induced lymphoproliferative response.²⁵ Therefore, vitamin D₃ concentrations were neither measured nor were vitamin D supplemented.

Treatment of *M. tuberculosis* infections in dogs is controversial, mainly because of the human health risk. Nevertheless, there are no documented cases of *M. tuberculosis* infection spreading from dogs to humans, thus this disease is known to be an anthroponosis. With that in mind, we tried to limit the potential risk by taking several measures: detailed

owner education, isolation of the dog from immunosuppressed persons as well as young and elderly people, serial fecal examination for acid-fast bacteria to detect if the dog was shedding organisms, and serial physical examinations and diagnostic imaging to document potential progression to the pulmonary system. In treating the *M. tuberculosis* infection in this dog, we walked a fine line between public health protection and saving a beloved companion animal.

Footnotes

- ^a Philips Brilliance, Philips Deutschland GmbH, Hamburg, Germany
^b Xenetix 300, Guerbet, Sulzbach, Germany
^c Löwenstein Jensen and Stonebrink, Becton Dickinson, Cockeysville, MD
^d MGIT 960 Becton Dickinson
^e Hain Lifescience, Nehren, Germany
^f Eremfat 300 mg, Riemser Arzneimittel AG, Greifswald, Germany
^g Clarithromycin HEXAL 500 mg, Holzkirchen, Germany
^h Baytril 150 mg, Bayer HealthCare, Leverkusen, Germany
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Conflict of Interest: Authors disclose no conflict of interest.

References

- De Swarte M, Alexander K, Rannou B, et al. Comparison of sonographic features of benign and neoplastic deep lymph nodes in dogs. *Vet Radiol Ultrasound* 2011;52:451–456.
- Greene CE, Gunn-Moore DA. Infections caused by slow-growing mycobacteria. In: Greene CE, ed. *Infectious Diseases of the Dog and Cat*, 4th ed. St. Louis, MO: Saunders, Elsevier; 2006:495–510.
- Erwin PC, Bemis DA, Mawby DI, et al. *Mycobacterium tuberculosis* transmission from human to canine. *Emerg Infect Dis* 2004;10:2258–2260.
- Posthaus HT, Bodmer T, Alves L, et al. Accidental infection of veterinary personnel with *Mycobacterium tuberculosis* at necropsy: A case study. *Vet Microbiol* 2011;149:374–380.
- Parsons SDC, Gous TA, Warren RM, van Heiden PD. Pulmonary *Mycobacterium tuberculosis* (Beijing strain) infection in a stray dog. *J S Afr Vet Assoc* 2008;79:95–98.
- Sykes JE, Cannon AB, Norris AJ, et al. *Mycobacterium tuberculosis* complex infection in a dog. *J Vet Intern Med* 2007;21:1108–1112.
- Deppenmeier SA, Schiesler A, Nolte I, et al. Pulmonary tuberculosis with evidence of *Mycobacterium tuberculosis* in a Golden Retriever. *Tierärztl Prax K H* 2007;35:111–115.
- Hackendahl NC, Mawby DI, Bemis DA, Beazley SL. Putative transmission of *Mycobacterium tuberculosis* infection from a human to a dog. *J Am Vet Med Assoc* 2004;225:1573–1577.
- Turinelli V, Ledieu D, Guilbaud L, et al. *Mycobacterium tuberculosis* infection in a dog from Africa. *Vet Clin Path* 2004;33:177–181.
- Hawthorne VM, Lauder IM. Tuberculosis in man, dog, and cat. *Am Rev Respir Dis* 1962;85:858–869.

11. Parsons SDC, Warren RM, Ottenhoff THM, et al. Detection of *Mycobacterium tuberculosis* infection in dogs in a high-risk setting. *Res Vet Sci* 2012;92:414–419.
12. Snider WR. Tuberculosis in canine and feline populations – Review of literature. *Am Rev Respir Dis* 1971;104:877–887.
13. van der Burgt GM, Crawshaw T, Foster AP, et al. *Mycobacterium bovis* infection in dogs. *Vet Rec* 2009;165:634.
14. Haist V, Seehusen F, Moser I, et al. *Mycobacterium avium* subsp *hominissuis* infection in 2 pet dogs. Germany. *Emerg Infect Dis* 2008;14:988–990.
15. Ellis MD, Davies S, McCandlish IAP, et al. *Mycobacterium bovis* infection in a dog. *Vet Rec* 2006;159:46–48.
16. Naughton JF, Mealey KL, Wardrop KJ, et al. Systemic *Mycobacterium avium* infection in a dog diagnosed by polymerase chain reaction analysis of buffy coat. *J Am Anim Hosp Assoc* 2005;41:128–132.
17. O'Toole D, Tharp S, Thomsen BV, et al. Fatal mycobacteriosis with hepatosplenomegaly in a young dog due to *Mycobacterium avium*. *J Vet Diagn Invest* 2005;17:200–204.
18. Bauer N, Burkhardt S, Kirsch A, et al. Lymphadenopathy and diarrhea in a Miniature Schnauzer. *Vet Clin Path* 2002;31:61–64.
19. Harisinghani MG, McCloud TC, Shepard JAO, et al. Tuberculosis from head to toe. *Radiographics* 2000;20:449–470.
20. Hanson RD, Hunter TB. Tuberculous peritonitis – CT-appearance. *Am J Roentgenol* 1985;144:931–932.
21. Engin G, Acunas B, Acunas G, Tunaci M. Imaging of extrapulmonary tuberculosis. *Radiographics* 2000;20:471–488.
22. Schaberg T, Bauer T, Castell S, et al. Recommendations for therapy, chemoprevention and chemoprophylaxis of tuberculosis in adults and children. German Central Committee against Tuberculosis (DZK), German Respiratory Society (DGP). *Pneumologie (Stuttgart, Germany)* 2012;66:133–171.
23. de Jong BC, Onipede A, Pym AS, et al. Does resistance to pyrazinamide accurately indicate the presence of *Mycobacterium bovis*? *J Clin Microbiol* 2005;43:3530–3532.
24. Lalor SM, Mellanby RJ, Friend EJ, et al. Domesticated cats with active Mycobacteria infections have low serum vitamin D (25(OH)D) concentrations. *Transbound Emerg Dis* 2012;59:279–281.
25. Chandra G, Selvaraj P, Jawahar MS, et al. Effect of vitamin D-3 on phagocytic potential of macrophages with live *Mycobacterium tuberculosis* and lymphoproliferative response in pulmonary tuberculosis. *J Clin Immunol* 2004;24:249–257.