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Infection by *Mycobacterium bovis* in a dog from Brazil

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ABSTRACT

Tuberculosis (TB) is a chronic disease caused by bacteria belonging to the *Mycobacterium tuberculosis* complex (MtbC). This disease rarely affects dogs. Canine infections are usually caused by *M. tuberculosis*. *Mycobacterium bovis* infections are rare in dogs and associated with consumption of raw milk or contaminated products. Here, we report a Boxer dog who had a *M. bovis* infection and was admitted to a Brazilian veterinary hospital with a presumptive diagnosis of chronic ehrlichiosis. Despite receiving treatment for chronic ehrlichiosis, it progressed to death. TB was diagnosed during post-mortem examinations using histopathological analysis. Ziehl-Neelsen staining revealed acid-fast bacilli in the kidneys, liver, mesentery, and a mass adhered to the liver. Further, PCR-restriction analysis was performed to identify mycobacteria in the samples. A restriction profile compatible with MtbC was found in the lungs. In addition, PCR-based MtbC typing deletions at different loci of chromosome 9 enabled the identification of *M. bovis* in the lungs. Therefore, it is very essential to perform differential diagnosis of TB in dogs with non-specific clinical signs and who do not respond to treatment, particularly those who had been in contact with TB-infected cattle or owners. Further, we highlight the use of molecular methods for the identification of bacilli, improving the diagnosis and aiding epidemiological studies.

Introduction

Tuberculosis (TB) is a chronic infectious disease caused by bacteria belonging to the *Mycobacterium tuberculosis* complex (MtbC).[1] Although the disease is generally observed in humans and animals, infections in dogs are rare. To the best of our

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knowledge, only a few studies, thus far, have reported the incidence of TB among dogs.[2–5] Canine infections are usually caused by *M. tuberculosis* due to a close relationship with the infected owners.[6] *Mycobacterium bovis* is pathogenic to numerous species; however, infections sporadically occur in both urban and rural dogs. Such infections are generally associated with a close proximity to TB-infected herds or consumption of contaminated animal by-products.[4]

Dogs with TB present various clinical signs, such as pulmonary, gastrointestinal, cutaneous, or disseminated manifestations. Amongst all types of TB, pulmonary TB is the most common among dogs.[7] Ante mortem diagnosis of TB is very difficult in dogs because the initial stages may be asymptomatic. Even animals presenting extensive lesions may remain asymptomatic for a long period of time.[8] The clinical signs are not pathognomonic for the infection and can be easily confused with those of other diseases.[3] In addition, the tuberculin test is not routinely performed in dogs.[9] The presence of acid-fast bacilli (AFB) or the isolation of the agent in exudates and tissue biopsies are considered definitive for the diagnosis. However, these techniques have low sensitivity and are laborious, time-consuming, and unviable, when the sample is preserved in formalin. Thus, the amplification of specific DNA sequences by PCR is a useful tool for the diagnosis of TB, aiding epidemiological studies.[10]

Material and methods

A five-year-old male Boxer dog was admitted at the Veterinary Hospital of Faculty of Veterinary Medicine (FMVZ)-Universidade Estadual Paulista (UNESP) in Botucatu, São Paulo, Brazil, with the chief complains of dark feces, progressive weight loss over a period of eight months despite regular feeding, and restricted mobility due to cachexia.

In the anamnesis, it was found that the dog was born on a farm, where it was in contact with other animals including cattle. The dog had been previously treated with antibiotics and antiparasitic drugs; however, their prescriptions were not available with the owners. In addition, vitamin supplements had been prescribed to treat the weight loss. However, the outcomes of these previous treatments were unsatisfactory.

During the clinical examination, the animal presented prostration, discrete tachycardia, severe dehydration, pale mucous membranes, ulcers in the oral cavity, and sensitivity to abdominal and renal palpation. Therefore, a presumptive diagnosis of chronic ehrlichiosis associated with chronic renal failure was made. This was supported by the previous history tick exposure, endemic situation of the region to ehrlichiosis, and the apparent renal failure, a common complication in chronic ehrlichiosis.

Results

Hematological and biochemical examinations revealed severe pancytopenia and elevated levels of urea, creatinine, and alkaline phosphatase, which are observed in cases of ehrlichiosis. Based on the presumptive diagnosis, the dog was treated with chloramphenicol (50 mg/kg/BID), dexamethasone (0.30 mg/kg/SID), and fluids. However, the treatment was ineffective and the dog progressed to death.

Post-mortem examinations revealed a high concentration of nodular lesions in the abdominal cavity. The involvement of the thoracic cavity was smaller with the presence of whitish nodules in the pulmonary parenchyma. In addition, unilateral pneumonia, pulmonary edema, and emphysema were observed in lungs. The kidneys presented an irregular surface, pelvis dilation, medullar cysts with whitish pinhead calcified lesions on the surface, similar to those found in the lungs. These calcified lesions were widespread in the mesentery and liver. Furthermore, a whitish mass, with irregular dimensions and approximately two-inch diameter, was found attached to the liver. The mass presented calcification points with similar characteristics to other organs.

Samples were collected from these organs and the mass to perform histopathological examination using hematoxylin & eosin (HE) and Ziehl-Neelsen staining. The results revealed pulmonary emphysema, interstitial nephritis, and presence of AFB in the kidneys, liver, mesentery, and mass attached to the liver.

In addition, these samples were sent to the Laboratory of Bacterial Zoonoses, FMVZ-USP in São Paulo, SP, Brazil, for identification of mycobacteria by PCR. After extracting DNA from the lungs, mesentery, liver, and kidneys using the ChargeSwitch gDNA Micro Tissue Kit (Life Technologies®), following the manufacturer's instructions, PCR-restriction analysis (PRA) was performed, according to the protocol reported by Telenti et al.[11] PCR-based MtbC typing of the chromosomal region-of-difference deletion loci (RD) was also conducted to differentiate the *Mycobacterium* sp., as previously described by Warren et al.[12]

Furthermore, the samples were tested at the Laboratory of Parasitic Diseases, FMVZ-USP in São Paulo, Brazil, using quantitative real-time PCR (qPCR) for the detection of *Ehrlichia canis*, according to the protocol reported by Doyle et al.[13] According to the PRA, Mycobacterium-compatible amplification was only observed in the lungs. The restriction pattern of BstEII and HaeIII observed in lungs showed the presence of MtbC bacteria. In the PCR-based MtbC typing of chromosomal RD, a sample from the lungs showed amplification of a 108-bp in the analysis of RD9 locus that is consistent with the absence of RD9 region, characteristic of *M. bovis*. In addition, qPCR analysis did not detect *E. canis* in the lungs, mesentery, liver, and kidneys.

Discussion and conclusion

Ante mortem TB diagnosis is difficult[8] because the clinical signs of the infection are unspecific and easily mistaken for other diseases.[3] In this case report, the non-specific clinical signs associated with the epidemiological history of parasitism by ticks and the severe changes in both hematological and biochemical examinations, similar to those observed in chronic ehrlichiosis, led to the misdiagnosis. Unfortunately, before differential diagnosis for other conditions could be performed, the dog died.

During the autopsy, nodular and pinpoint lesions were observed in several organs and the mass attached to the

liver. Therefore, initially, neoplasia with metastasis to multiple organs was suspected, which also could explain the clinical signs as well as biochemical and histopathological changes observed during the examinations. Samples from the lesions were collected and preserved in formalin for histopathological analysis. Ziehl-Neelsen staining showed the presence of AFB, confirming the diagnosis of TB.

In our case, the presence of AFB in the kidneys, liver, mesentery, and mass attached to the liver was definitive for the diagnosis. However, the samples were fixed in formalin, hindering the isolation and identification of bacteria during culture.

PCR analysis was particularly useful for detecting the presence of mycobacteria in the lung sample that was negative for the presence of AFB by the Ziehl-Neelsen stain. However, the amplifications were not obtained in samples from other tissues that were positive. PCR is more sensitive than other techniques used for the detection of AFB in tissues; therefore, it could possibly detect the bacteria in the lungs. In general, formalin degradation can hinder molecular studies of certain tissues.[14] Therefore, we hypothesized that formalin degradation hampered specific DNA amplification from the kidneys, liver, and mesentery damaging the detection of mycobacteria during the execution of the PRA.

The PRA enabled DNA amplification as well as the differentiation of MtbC bacteria from the lungs. The RD9 locus analysis identified *M. bovis* as the cause of the infection. The dog's epidemiological data such as birth on a farm and having been fed raw milk and animal products ad libitum validated the *M. bovis* infection. In addition, the distribution of lesions suggested that the infection occurred through an oral route as the maximum occurrence of the lesions was found in the abdominal cavity. Snider[8] and Pesciarelli et al.[15] stated that when dogs live in close contact with heavily diseased cattle, common infections are transmitted orally via the consumption of infected raw milk or via aerosols.

Many cases of mycobacterial infection in dogs have been diagnosed by molecular tests,[4,5,16,17] showing the feasibility and superiority of this diagnostic procedure. Considering the worldwide prevalence of TB and the zoonotic importance of *M. bovis*, we highlight the importance of using molecular techniques to not only identify but also characterize the bacilli species, which are very useful for further epidemiological studies. Molecular typing can help explain the possible routes of infection and establish effective control strategies for both human and animal TB.[18] Therefore, PCR assays have been developed for the rapid diagnosis of TB and MtbC typing[11,12] as they are more sensitive and specific compared to culture or histopathological methods.[1] However, these techniques not only require sophisticated equipment and skilled personnel but also are cost intensive; therefore, their clinical implementation on a routine basis is limited, particularly in the developing countries.[19]

In our case, the dog was probably an isolated victim of *M. bovis* infection; however, we did not have any information about animal or human contacts that dog had on the farm. Recently, two cases of cat-to-human *M. bovis* transmission were reported in the Great Britain, corroborating the risk to public health.[20] In addition, *M. tuberculosis* was isolated from a dog with generalized infection in nasal fluids, urine, and feces,[17] suggesting that dogs can transfer the mycobacteria into the environment, potentially acting as a source of infection for other animals and humans.[15] Thus, we conclude that although the risk of pets-to-human transmission of bovine TB is very low, it cannot be overlooked, particularly considering people who are immunocompromised, have occupational exposure, and have close contact with their pets.

Evaluating TB infection in dogs and cats living on farms with *M. bovis*-infected cattle where TB control is being performed could be an interesting "active case-finding" screening. Farm cats and dogs are at very high risk of acquiring *M. bovis* infections from infected cattle. Snider[8] showed that 44.4% dogs and 41.1% cats were affected after exposure to infected cattle. Greene and Gunn-Moore[9] hypothesized that pets could play a role in the maintenance of *M. bovis* on a farm. Thus, systematic examination of farm pets should be performed to not only aid the bovine TB Eradication Program but also prevent the pets-to-human transmission of bovine TB.

Conflicts of interest

The authors declare no conflicts of interest.

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