Disseminated Tuberculosis in a Dog: a Putative Zooanthroponosis

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ABSTRACT

Background: Zooantropones that affect pets are rare. Microorganisms involved in human tuberculosis has great importance to public health and has potentiality to produce reverse zoonoses. Infection by Mycobacterium tuberculosis is in top of the most important infectious diseases related to public health. In dogs, the infection occurs through prolonged exposure to aerosols and respiratory secretions from humans. Canine tuberculosis is little reported and known in Brazil, this work aims to describe the anatomopathological findings of the first case of canine tuberculosis in a 5-year-old dog in the Federal District and alert to the presence of this disease in this region.

Case: The dog had history of close contact with its owner, who has been treated for pulmonary tuberculosis six months before. At necropsy scattered multifocal, firm, white to yellow-gray nodules, varying in size (3 mm to 10 mm) involving lungs, kidneys, heart, liver and spleen was observed. In frontal and occipital cerebral cortex, pale-gray circular lesions were detected with the same characteristics. The histological findings were, multifocal to coalescent granulomas, well organized with a central core of necrosis with foci of mineralization, surrounded by histiocytes, epithelioid macrophage, rare Langhans giant cells, some intact and degenerate plasma cells and neutrophils, and a rim of reactive fibroblasts producing fibrous connective tissue. Histological staining with ZN revealed free acid-fast bacilli and in the cytoplasm of macrophages and granulomas in all affected organs. The PCR allowed identification of DNA was extracted from formalin fixed paraffin embedded (FFPE) tissues using the ReliaPrep™ FFPE gDNA Miniprep System®, according to the manufacturer’s instructions (Promega, EUA). The Speed-Oligo Mycobacteria assay was used to identify the presence of M. tuberculosis complex DNA in tissue samples.

Discussion: Zooantropones should be considered as differential diagnosis of infectious diseases affecting companion animals. Tuberculosis is a prevalent and highly relevant disease that plagues humans and animals. Canine tuberculosis is neglected what become information on the epidemiological and clinical situation scarce and its diagnosis a challenge. The systemic form of M. tuberculosis infection is rare in dogs, it occurs by contact with secretions of actively infected humans or ingested contaminated food with the bacillus. In the present case the dog contracted the infection by direct contact with owner who had tuberculosis and presented acute clinical alterations resulting from the disseminated form of the disease. Transmission of M. tuberculosis from humans to dogs is considered a determinant in the epidemiological chain of infection in companion animals. Histological lesions in natural cases of tuberculosis in dogs and cats differ from typical tuberculoid granulomas found in cattle and humans. In this case of disseminated tuberculosis in the DF scarce Langhans giant cells in the granulomas was observed, which is a typical manifestation of the disease in the species. The coloration of Ziehl-Neelsen allows evidence of alcohol-acid-resistant bacilli, as seen in the present report. Health actions aiming prevention and control of tuberculosis should include investigation of the disease in companion animals in households where there are records of human tuberculosis. The anatomopathological and molecular findings confirmed the diagnosis of Mycobacterium tuberculosis infection transmitted by the owner, an uncommon disease in companion animals, a challenge to the clinical diagnosis, and previously unknown in the Federal District.

Keywords: canine, Mycobacterium tuberculosis, reverse zoonoses, Ziehl-Neelsen, Immunohistochemistry.
INTRODUCTION

Zooanthroponosis or reverse zoonoses in pets are rare. Parasitic and viral agents that cause disease in humans may eventually affect dogs and cats, producing clinical disease and death. Human tuberculosis (TB) is of particular importance for public health, as well as for its putative potential to cause infection in companion animals. Canine as well as human TB is caused mainly by *Mycobacterium tuberculosis* complexes, which produce granulomatous disease [1,3-5,10]. The disease is distributed worldwide and with approximately 8 million new human cases annually represents one of the most important public health problems [3,12]. In dogs infection can occur through prolonged exposure to aerosols and respiratory secretions from humans, the natural hosts to the microorganisms [2,4,12]. The objective of this paper is to present diagnostic and pathological findings and discuss the occurrence of the disease as a putative zooanthroponosis and public health management schemes.

CASE

A 5-year-old Yorkshire dog from Brasília, Federal District, Brazil, received emergency care due to convulsions, emesis, anorexia, and dehydration. With worsening of the clinical signs, the animal died during the night and was taken to necropsy. At necropsy, scattered multifocal, white-grayish to yellowish lesions with an irregular surface, measuring 3 mm to 10 mm were observed on the surface and in the parenchyma of the lungs, and in kidneys (Figure 1A), heart, liver, and spleen. In the central nervous system (CNS), the frontal (Figure 1B) and occipital cortex presented pale-grayish circular foci with the same characteristics as those observed in the other organs. Samples of CNS, heart, lung, liver, spleen and kidneys were collected and fixed in 10% buffered formalin solution, pH 7.0, paraffin embedded, cut into 5 μm sections and stained with hematoxylin and eosin (H&E) and Ziehl-Neelsen (ZN).

Microscopically, organs showed multifocal to coalescent lesions with central areas of necrosis containing cell debris, sometimes with foci of mineralization (Figure 2A), surrounded by a thin fibrous capsule and granulomatous inflammatory infiltrate consisting predominantly of histiocytes, epithelioid macrophages, rare Langhans giant cells, plasma cells and some intact and degenerate neutrophils. Histological slides stained with ZN revealed the presence of free acid-fast bacilli, and acid-fast bacilli within the cytoplasm of macrophages in granulomas of all affected organs (Figure 2B).

Immunohistochemistry (IHC) was performed as follows: Tissue sections were incubated with polyclonal anti-*Mycobacterium bovis* - Bacillus Calmette-Guérin, BCG primary antibodies (1:30,000 dilution) amplified with alkaline phosphatase (AP)-conjugated polymer (Histofine AP) and revealed using the warp red chromogen kit. Tissue sections in which the primary antibodies were replaced by non-immune serum served as negative controls. IHC detected myriad of *Mycobacteria* in the granulomas.

DNA was extracted from formalin fixed paraffin embedded (FFPE) tissues using the ReliaPrep FFPE gDNA Miniprep System, according to the manufacturer’s instructions. The Speed-Oligo Mycobacteria assay was used to identify the presence of *M. tuberculosis* complex DNA in tissue samples [9]. When the owner was informed about the disease status of his dog, he stated that he had contracted pulmonary tuberculosis and had been treated for the last six months. Diagnosis of human tuberculosis in the dog owner had been confirmed by radiographic identification of pulmonary lesions, direct bacilloscopy, culture, isolation, and identification of *M. tuberculosis* in sputum [7]. The animal cohabited in the same environment and had been kept in close contact with the owner since he was a puppy. The dog was kept with dry dog food only, had never been in contact with farms and did not receive milk derivatives or other products that could carry *M. bovis*.

DISCUSSION

In view of the growth of pet species populations, especially in large cities zooanthroponoses should be considered in the differential diagnosis of infectious diseases affecting companion animals. In Countries with high incidence rates of human tuberculosis such as Brazil (36.7 cases per 100,000 inhabitants in 2012-2013) [7], disease susceptibility of companion animals and the close coexistence with their owners facilitate potential transmission, and the putative infection of dogs should be investigated by surveillance animal and human health systems.

TB is a disease that has plagued humans and animals on all continents for centuries [2,3,8,11], and despite substantial developments in control and eradication measures in developing and emerging countries,
it still is a disease of great importance [7]. Relevance of canine tuberculosis and its role in the overall epidemiology of TB has been neglected, and only scarce information is available about clinical presentation, diagnosis and epidemiology.

The systemic form of *M. tuberculosis* infection is considered rare in dogs and is not usually associated with the presence of specific clinical signs [4,5]. The natural infection of companion animals, although uncommon, occurs through human reservoirs, when dogs cohabit and come in contact with secretions of actively infected human individuals or ingestion of contaminated food [4,5,12]. In the present case, the animal had close and continuous contact with its TB-infected owner before and during treatment period, during which the dog became infected. However, due to typically slow dissemination of the disease the dog only started to present acute clinical signs at least six month after the end of the treatment of the owner.

Asymptomatic animals with tuberculosis are of significant epidemiological importance because they expel bacilli through secretions that may infect other animals and humans [4,12]. The transmission of canine tuberculosis to humans is not well characterized, however, the zoonotic potential of these bacteria represents a risk of infection, especially for immunosuppressed individuals [3,8,10].

Previous necropsy findings in dogs with tuberculosis are similar to those observed in this case and are characterized by multiple granulomas disseminated in lungs, kidneys, heart and adjacent lymph nodes [4,2,11]. Unusual in dogs with the disseminated form are lesions of the central nervous system with subsequent severe neurological manifestations [1,3,12]. The spread of the bacillus to the brain may possibly be explained by prolonged exposure to the agent in the environment, allowing widespread dissemination.

Granulomas observed in this case with disseminated tuberculosis and in natural cases of tuberculosis in dogs and cats differ from other species such as cattle and humans, since they present marked granulomatous inflammation mainly composed of histiocytes, epithelioid macrophages and rare giant cells of Langhans surrounding the necrotic centers [4,10,11] and not typical tuberculous granulomas [3,12].

Ziehl-Neelsen staining of histological sections and cytological smears is a standard diagnostic method for identifying alcohol-acid-resistant bacilli in animal
tissues [10,11]. However, the taxonomic identification of *Mycobacterium* species based on PCR technique is currently used in humans and domestic animals [2,8]. The PCR assay promotes rapid identification of infectious agents in body fluids, and tissues [4,10], and allowed the confirmation of *Mycobacterium tuberculosis* complex infection in the dog. In addition, immunohistochemistry demonstrated the dissemination of the infectious agent in lesions of different tissues.

Cohabitation with TB infected dogs should be considered a putative health risk factor to immunodeficient people and animals [1,8]. In view of the risk of disease transmission by respiratory and intestinal secretions, it is recommended that these dogs be euthanized [8,10]. In most Countries, guidelines for the public health management of TB do not provide a protocol for the management of pets with close contact with human cases of tuberculosis. Anyway, it is mandatory to Veterinary Practitioners recognize suspected cases in dogs and notify surveillance services.

Considering its relevance for public health, the diagnosis of tuberculosis in dog owners should prompt the investigation of companion animals. Adequate public health procedures need to be in place to prevent and control spread of infection, including into other susceptible species. On the other hand, dogs exposed to the risk of tuberculosis infection by their owners are a challenge for clinical diagnosis and surveillance services and should be thoroughly investigated and kept in quarantine until diagnosis is concluded.

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REFERENCES


