Pulmonary *Mycobacterium tuberculosis* (Beijing strain) infection in a stray dog

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ABSTRACT

Mycobacterium tuberculosis infection in dogs is rarely reported and has not previously been documented in South Africa. A case of a stray Maltese crossbreed dog with extensive multifocal pulmonary tuberculosis due to *M. tuberculosis* is described. Pulmonary granulomas in this case were poorly encapsulated and contained large numbers of acid-fast bacteria, highlighting the potential for infected companion animals to excrete the pathogen. Treatment of canine tuberculosis is generally not advised, and for this reason, euthanasia of diseased animals must be advocated in most instances. Physicians and veterinarians must be aware that companion animals with active disease caused by *M. tuberculosis* could act as a potential source of infection.

Key words: canine, dog, *Mycobacterium tuberculosis,* tuberculosis, zoonosis.

Parsons S D C, Gous T A, Warren R M, van Helden P D **Pulmonary *Mycobacterium tuberculosis* (Beijing strain) infection in a stray dog**. *Journal of the South African Veterinary Association* (2008) 79(2): 95–98 (En.). DST/NRF Centre of Excellence for Biomedical TB Research/MRC Centre for Molecular and Cellular Biology/Division of Molecular Biology and Human Genetics Department of Biomedical Sciences, Faculty of Health Sciences, Stellenbosch University, PO Box 19063, Tygerberg, 7505 South Africa.

INTRODUCTION

Mycobacterium tuberculosis is one of a number of closely related intracellular bacterial pathogens, grouped together as the *M. tuberculosis* complex (MTC) which cause granulomatous disease in a broad range of host species. It is the principal cause of human tuberculosis and the extraordinary success of this pathogen is reflected by its distribution. It is believed to infect a third of the world's population, causing 8 million new cases of human tuberculosis each year[7]. Despite the widespread occurrence of *M. tuberculosis,* infection by this organism is rarely diagnosed or maintained in free-living non-human hosts[1]. The precise nature of this apparent host-adaptation is unresolved, presumably involving aspects of both host physiology and ecology[19].

'Spillover' of *M. tuberculosis* infection to animals requires prolonged and close contact between humans and susceptible animal species and this scenario is classically illustrated by the prevalence of *M. tuberculosis* infection in zoo animals[17]. Companion animals living in close contact with tuberculosis patients represent a group at particular risk of high levels of exposure to this bacterium; however, cases of canine and feline tuberculosis are rarely described[9,22]. South Africa currently experiences an extremely high incidence rate for all forms of TB (600/100 000), suggesting that the risk of spillover of disease to contact animals will be significant in this country[24]. As far as is known this is the 1st documented case of tuberculosis caused by *M. tuberculosis* in a dog in South Africa and the implications of this disease in this high-incidence setting are dicussed.

CASE HISTORY

An adult Maltese crossbreed dog was presented as a stray with an unknown history. The dog exhibited generalised alopecia and a multifocal superficial dermatitis. A skin scraping failed to identify a cause for the dermatitis. Clinical signs were judged as suggestive of *Sarcoptes scabei* infestation and the dog was treated with doramectin (Dectomax, Pfizer AH) at a dose of 200 μg/kg body weight. The following day the dog exhibited severe dyspnoea, of apparently acute onset, and died before further treatment could be implemented.

Gross abnormalities detected at *post mortem* examination were restricted to the respiratory tract. The lungs were generally congested and all lobes contained multifocal, pale grey areas of consolidation, 2–4 mm in diameter. A single tracheobronchial lymph node that measured 12 mm in diameter was firm and pale yellow in appearance. Specimens from the lungs and bronchial lymph nodes were collected for histopathological examination. Lung sections showed multifocal to confluent necrogranulomas consisting of central coagulative to occasionally liquefactive necrosis that were infiltrated by low numbers of neutrophils. Some necrotic foci showed mild central calcification. The necrotic areas were surrounded by a moderately developed granulomatous layer consisting of large numbers of macrophages and epithelioid cells, moderate numbers of lymphocytes and plasma cells, and low numbers of fibroblasts, with the formation of an indistinct and poorly developed outer fibrous capsule (Fig. 1). Scanty Langhans' multinucleated giant cells were present. The remainder of the lungs showed moderate numbers of small multifocal to confluent granulomas consisting of macrophages, as well as widespread alveolar and interstitial infiltration of numerous macrophages, lymphocytes and plasma cells, with moderate fibrinous oedema. There was mild to moderate epithelialisation of pneumocytes. The pleura was moderately thickened as a result of fibrosis and the infiltration of low numbers of macrophages, lymphocytes and plasma cells. Ziehl-Neelsen (ZN) staining revealed numerous acid-fast bacilli in the cytoplasm of macrophages and epithelioid cells of the necrogranulomas and granulomas, and in individual macrophages throughout the parenchyma (Fig. 2).

The tracheobronchial lymph node sampled showed effacement of the normal architecture, which was replaced by extensive caseous necrosis with prominent central calcification. The area of necrosis was surrounded by large numbers of macrophages and epithelioid cells, moderate numbers of lymphocytes and plasma cells, low numbers of fibroblasts, and scanty Langhans' multinucleated giant cells. Numerous acid-fast bacilli

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were demonstrated, using ZN staining, in the cytoplasm of macrophages and epithelioid cells, as well as in the caseous necrotic centre.

Samples of the tracheobronchial lymph node, mesenteric lymph node, and lung tissue were homogenised separately, decontaminated and subjected to culture in BACTEC mycobacterial growth indicator tube (MGIT) medium containing polymixin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (PANTA) (Becton Dickinson, USA) as previously described[23]. Acid-fast bacilli were identified in each culture. A multiplex polymerase chain reaction (PCR) test performed on heat-killed culture lysates, as previously described[23], identified the bacteria as *M. tuberculosis*. This isolate was shown to belong to the Beijing strain of *M. tuberculosis* by the IS*6110* restriction fragment length polymorphism (RFLP) genotyping technique, as previously described[18] (Fig. 3).

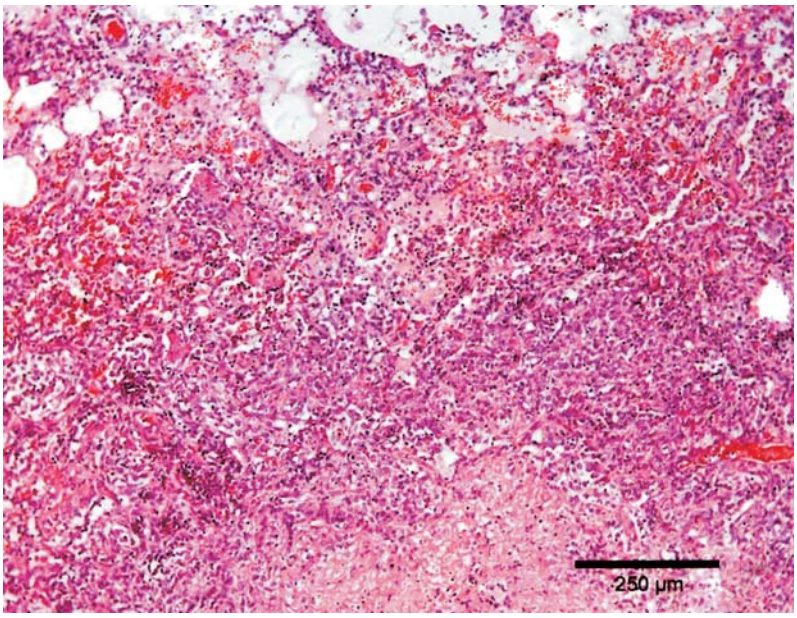

Fig. 1: **Periphery of a typical pulmonary granuloma showing an indistinct division between the lymphocyte cuff and surrounding lung tissue** (haematoxylin and eosin).

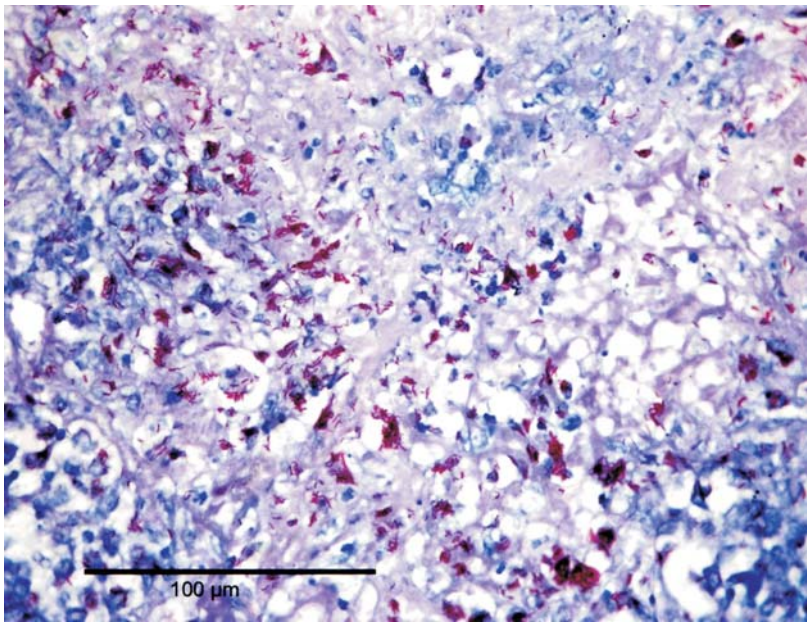

Fig. 2: **Pulmonary granuloma showing numerous acid-fast bacilli** (stained bright red)**, demonstrated by using Ziehl Neelsen staining.**

DISCUSSION

Canine tuberculosis is rarely diagnosed worldwide and as far as could be established has not previously been reported in South Africa. In the present case, as is most commonly found in reported cases of canine tuberculosis, the causative organism was *M. tuberculosis,* and such infections have been associated with close contact between companion animals and human tuberculosis patients[9,14]. Notably in this case, the infection was caused by a Beijing strain of the pathogen, a genotype associated with high transmission rates and pathogenicity[15].

An estimation of the prevalence of canine TB in South Africa must be speculative. Human tuberculosis is most prevalent in resource-poor environments in which veterinary services are also rarely readily available. Given that the diagnosis of canine TB is time-consuming and relies on sophisticated and costly procedures, it will remain under-diagnosed. Reports from the 1st half of the 20th century estimated prevalence rates of canine TB, based on necropsy studies, at between 0.1 and 6.7 % (median 1.9 %) in various European cities[20]. These figures may approximate the scenario in South Africa, where the national incidence of human pulmonary TB[24] is more than double that of the European settings described above[2]. In the modern setting, however, antibiotic treatment of human TB, by reducing the severity and chronicity of disease, will probably affect the likelihood of disease transmission to contact animals. Also, differences in pet population age structures and differences in pet-owner interactions will affect the probability of transmission and disease in different settings. Additionally, control of *Mycobacterium bovis* has reduced the contribution of this pathogen to the incidence of canine TB[8].

Ante mortem prevalence rates in dogs in the European settings described above are recorded as varying between 0.04 and 1 %[16] and 0.15 and 2 %[20].

These figures are spurious, however, as dogs often present with sub-clinical mycobacterial disease or with vague, non-specific symptoms which vary according to the organ systems affected. Clinical signs of canine tuberculosis are most commonly associated with respiratory disease and may include pyrexia, listlessness, inappetance, weight loss, a non-productive cough, retching, vomition and dyspnoea[16]. Non-respiratory signs can include diarrhoea, hepatomegaly, polyuria and polydypsia[16]. Radiographic evidence of canine TB can often be non-specific but may include signs of pleural and/or pericardial effusion, tracheobronchial lymph node enlargement, multifocal interstitial pneumonia (with a nodular to mixed nodular/alveolar pattern), hepatomegaly, splenomegaly and ascites[14].

The *ante mortem* diagnosis of canine TB is also complicated by the fact that dogs are poor responders to the intradermal tuberculin test commonly used in cattle and humans. In a recent study, 14 dogs

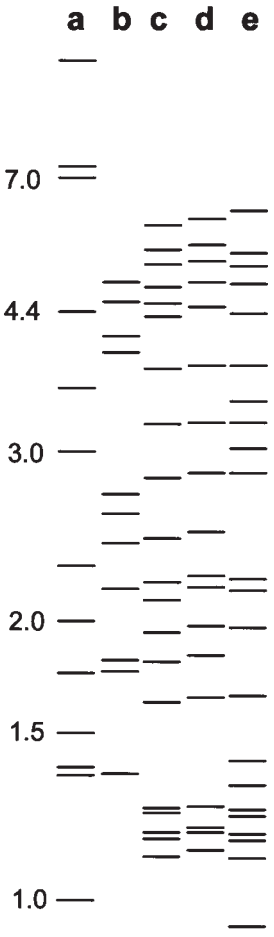

Fig. 3: **IS*6110* Restriction fragment length polymorphism patterns of** (a) **a reference strain of *Mycobacterium tuberculosis*** (Mt14323) **with selected fragment lengths indicated in kilobases,** (b) **a non-Beijing strain of *M. tuberculosis*,** (c,d) ***M. tuberculosis* Beijing strains isolated from human subjects, and** (e) **the *M. tuberculosis* strain isolated in this case.**

experimentally infected with *M. tuberculosis* failed to respond to intradermal injections of *M. tuberculosis* and *M. bovis* purified protein derivative (PPD) tuberculin[3]. The use of higher concentrations of *M. tuberculosis* PPD in the form of Old Tuberculin, as used in the diagnosis of primate TB, may be more appropriate for eliciting a skin response[21]. Alternative *in vitro* diagnostic assays are available for a wide variety of host species, but their use in dogs has not been reported[4].

Definitive diagnosis of tuberculosis relies on histopathology of affected tissues and culture of the causative bacterium. The lesions of tuberculosis in carnivores differ from those in other species. Nonspecific granulomatous inflammation comprising macrophages is the usual manifestation and the lesions often have a sarcomatous macroscopic appearance. Typical tubercles are not commonly found and caseation necrosis, calcification and giant cells are rare findings. Fibrous encapsulation and calcification are usually not present, while acid-fast bacilli may be numerous[6]. The present case generally exhibited similar pathology but microscopic caseation necrosis with calcification was quite prominent in the 1 tracheobronchial lymph node sampled for histopathology.

The treatment of canine tuberculosis must be considered in the light of the fact that diseased animals present a potential source of human infection. The transmission of *M. tuberculosis* from companion animals to humans has not been described; however, the potential risk for zoonotic disease is apparent. This report has shown that infected dogs may carry high mycobacterial loads and granulomas may be poorly contained in these animals, allowing for the excretion of large numbers of bacteria (Figs 1, 2). This is evidenced by the isolation of *M. tuberculosis* from laryngeal and rectal samples from 7 of 48 dogs and cats living in close contact with tuberculosis patients[13]. Also, Bonovska *et al.* (2005)[3] have shown transmission of *M. tuberculosis* from infected to healthy dogs under experimental conditions.

Multiple-drug therapy must always be used in the treatment of tuberculosis in order to reduce the potential for the development of bacterial drug resistance. Current recommendations are a 6 to 9 month regimen combining a fluoroquinolone (*e.g.* enrofloxacin or ciprofloxacin) (5–15 mg/kg *per os,* daily), clarithromycin (5–10 mg/kg *per os,* daily) and rifampicin (10–20 mg/kg *per os,* daily)[10]. Alternatively, a combination of rifampicin (10–20 mg/kg *per os,* daily) isoniazid (10–20 mg/kg *per os,* daily) and ethambutol (15 mg/kg *per os,* daily) given for 2 months followed by a combination of rifampicin and isoniazid for at least a further 4 months can be used[10].

Such lengthy treatments require intense commitment by the pet owner and this scenario presents a serious risk of noncompliance with treatment protocols. The dangers of this include the ongoing potential for zoonotic transmission of infection and importantly, the opportunity for the development of drug resistant mycobacterial strains. This is of particular importance for drugs such as the fluoroquinolones that are used in the treatment of recurrent and multi-drug resistant (MDR) TB in humans[5]. Additionally, treatment may require careful monitoring for the development of severe side-effects to drugs such as isoniazid[11] and rifampicin[12]. For these reasons, it is widely believed that the treatment of canine TB is ill-advised and euthanasia of diseased animals must be advocated in the majority of cases[8,21] (D Gunn-Moore, University of Edinburgh, pers. comm., 2007).

The implications of canine tuberculosis within the context of the South African human tuberculosis epidemic remain undefined. However, veterinarians and physicians must be aware of the potential for companion animals to act as reservoirs of *M. tuberculosis.* This is particularly true for veterinarians and veterinary staff working with animals from communities experiencing high levels of human tuberculosis.

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