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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Mycobacterium microti Infection in Two Meerkats (*Suricata suricatta*)

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Summary

Mycobacterium microti is a member of the *Mycobacterium tuberculosis* complex (MTC). *M. microti* is generally considered a pathogen of small rodents, although sporadic infections in a range of other mammals, including domestic animals and man, have been reported. While many human infections have been associated with immunosuppression, an increasing number of cases are being reported in immunocompetent patients. Two cases of *M. microti* infection in meerkats (*Suricata suricatta*) are reported. These are the first cases of mycobacterial disease to be described in meerkats outside Africa.

Keywords: meerkat; mycobacteriosis; *Mycobacterium microti*; *Mycobacterium tuberculosis* complex

Mycobacteria are obligate aerobic, weakly gram-positive, acid-fast, non-motile, non-spore-forming, rod-shaped bacteria belonging to the order Actinomycetales and suborder Corynebacterineae. They are therefore most closely related to *Corynebacterium*, *Nocardia* and *Rhodococcus* spp. All four genera have a similar complex cell wall containing a high percentage of lipids, including abundant large-branched mycolic acids. The cell wall is responsible for the acid-fast staining characteristics and also makes these organisms persistent in the environment and relatively impermeable to antibiotics (Quinn *et al.*, 1994). Mycobacteria of veterinary importance are generally divided into three groups: (1) obligate primary pathogens requiring a mammalian host to perpetuate their life cycle (including members of the *Mycobacterium tuberculosis* complex [MTC] such as *Mycobacterium microti*); (2) saprophytes that may become facultative pathogens (divided further into fast-growing and slow-growing opportunistic [or atypical] non-tuberculous mycobacteria); and (3) those which are difficult to grow in culture and have a poorly-defined environmental niche (Gunn-Moore, 2010).

The MTC includes *M. tuberculosis*, *Mycobacterium bovis*, *Mycobacterium caprae*, *Mycobacterium africanum*, *Mycobacterium pinnipedii* and *M. microti*. Despite exhibiting marked genetic homology, these species vary remarkably in their host range and pathogenicity. *M. microti* is generally considered a pathogen of small rodents (e.g. field voles, bank voles, wood mice and shrews) and poses little risk to other mammals, including man (Wells and Oxon, 1937; Wells, 1946; Reed, 1957; Cavanagh *et al.*, 2002; Burthe *et al.*, 2008). Indeed, during the 1950s, *M. microti* was used in large-scale human trials as an antituberculosis vaccine in the UK and Czechoslovakia. Although both attenuated and non-attenuated strains proved safe and effective, they were found to be no better than

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the established Bacille Calmette–Guérin (BCG) vaccine, which is based on an attenuated strain of *M. bovis* (Sula and Radkovsky, 1976; Hart and Sutherland, 1977; Smith *et al.*, 2009). Despite its specificity for small rodents, *M. microti* has been reported occasionally in a number of other species, including a badger, ferret (Xavier Emmanuel *et al.*, 2007), dog (Deforges *et al.*, 2004), rock hyrax (Lutze-Wallace *et al.*, 2006), calf (Jahans *et al.*, 2004), alpaca, llamas (Pattyn *et al.*, 1970; Oevermann *et al.*, 2004; Zanolari *et al.*, 2009), pigs (Huitema and Jaartsveld, 1967; Taylor *et al.*, 2006), domestic cats (Huitema and van Vloten, 1960; Huitema and Jaartsveld, 1967; Gunn-Moore *et al.*, 1996; Rüfenacht *et al.*, 2011), squirrel monkeys (Henrich *et al.*, 2007) and man (van Soolingen *et al.*, 1998; Xavier Emmanuel *et al.*, 2007; Smith *et al.*, 2009). While many of the human infections are attributed to immunosuppression (including patients infected with human immunodeficiency virus [HIV] or those receiving immunosuppressive therapy) (Foudraine *et al.*, 1998; van Soolingen *et al.*, 1998; Horstkotte *et al.*, 2001; Xavier Emmanuel *et al.*, 2007), an increasing number of case reports and small case series are being published describing *M. microti* infections in immunocompetent patients (Kremer *et al.*, 1998; van Soolingen *et al.*, 1998; Niemann *et al.*, 2000; Geiss *et al.*, 2005; Xavier Emmanuel *et al.*, 2007; de Jong *et al.*, 2009; Frank *et al.*, 2009; Smith *et al.*, 2009).

Members of the MTC are primarily identified by growth characteristics on solid egg- and liquid-based media, followed by molecular confirmation by polymerase chain reaction (PCR). Once an organism is identified as a member of the MTC, it can be distinguished using a combination of three genotyping techniques: spoligotyping, variable-number tandem-repeat (VNTR) typing and deletion typing (Smith *et al.*, 2006). Spoligotyping classifies members of the MTC based on polymorphisms within 43 spacer sequences (direct variant repeat [DVR] units) in the direct repeat (DR) region. Spoligotypes are designated based on loss (deletion) of single DVR units; these are given an international identifier by www.Mbovis.org. VNTR typing is a form of minisatellite typing at up to 30 polymorphic loci. Deletion assays evaluate a number of specific deletions (or regions of difference, RDs), which have been shown to be informative in the MTC. For example, RD4 is deleted in all strains of *M. bovis*, but is intact in strains of *M. microti*. Strains of *M. microti* are identified by the deletion of $RD1^{mic}$.

The meerkat or suricate (*Suricata suricatta*) is a burrow-dwelling, desert-adapted member of the mongoose family (Herpestidae) originating from the Kalahari Desert. They live in large groups (2–30 individuals) with a complex social structure. Meerkats are largely insectivorous, but may also consume arachnids, birds and small mammals (Mills and Bester, 2005). In 2009, a study of wild meerkats demonstrated large numbers of individuals infected with *M. bovis* (Drewe *et al.*, 2009b). In 2002, an epizootic in South Africa caused by *M. tuberculosis* was reported in meerkats and banded mongooses (*Mungos mungo*) (Alexander *et al.*, 2002). To our knowledge, *M. microti* infection has not been described previously in the meerkat. Furthermore, this is also the first published report of any mycobacterial disease occurring in meerkats outside Africa.

A 17-month-old, male meerkat from a private zoological collection was humanely destroyed after a 2-week history of lethargy, anorexia and failure to respond to antibiotic and supportive therapy. The other four meerkats in the enclosure appeared clinically normal at that time; however, 1 week later a second meerkat was found dead. One year later, the remaining three meerkats are clinically normal.

At post-mortem examination, both meerkats were in poor bodily condition (condition score 1/5) and presented with similar gross lesions. In the first meerkat, the abdominal cavity contained 9.5 ml of serosanguineous fluid. In the cranial abdomen, immediately caudal to the liver, there was a 1.5 cm, ovoid, pale yellow, firm mass (a markedly enlarged hepatic lymph node). Multifocal strong (fibrous) adhesions were present between the mass and the left limb of the pancreas, visceral surface of the liver, spleen and omentum. Delicate adhesions were also present between various organs and structures in the cranial abdominal cavity; however, these could be easily separated (fibrin). The spleen had multifocal to coalescing, pinpoint to 5 mm, pale yellow nodules and plaques over its entire surface, which extended into the parenchyma (Fig. 1). The degree of autolysis in the second meerkat precluded detailed examination; however, similar enlargement of the hepatic lymph node was observed in addition to multifocal nodules within the liver and spleen.

Sections of liver, gall bladder, spleen, lymph node, pancreas, adrenal gland, kidney, urinary bladder, testis, oesophagus, stomach, small and large intestines, trachea, lung, heart, thyroid gland, skeletal muscle, femur, brain, pituitary gland and eye were collected into 10% neutral-buffered formalin and used to generate paraffin wax-embedded sections for routine histopathological examination. Additional parallel samples of liver, spleen, lymph node and lung from both meerkats were submitted for routine aerobic/anaerobic and selective mycobacterial culture.

The spleen, liver and hepatic lymph node were affected by severe, multifocal to coalescing

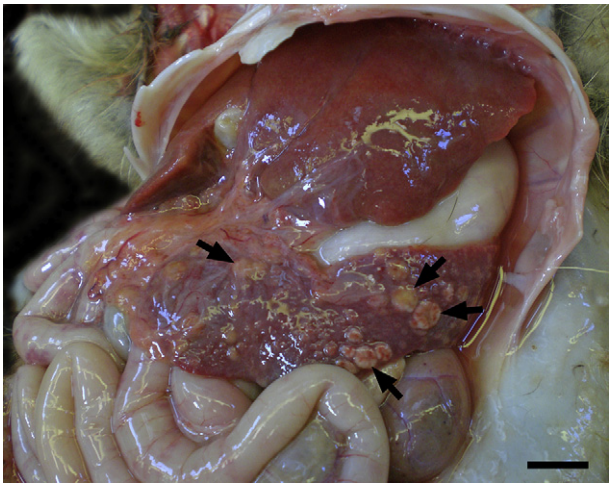

Fig. 1. Cranial abdomen of a 17-month-old meerkat (*Suricata suricatta*). The spleen contains numerous pinpoint to 5 mm in diameter, tan, roughly circular, nodular lesions protruding from the capsular surface, which extend into the parenchyma (arrows). Bar, 1 cm.

granulomatous inflammation. This was characterised by sheets of macrophages, which replaced up to 80% of the parenchyma (Fig. 2). In the spleen, the smooth muscle trabeculae and capsule were distorted by the expanding sheets of macrophages, giving the surface an undulating profile. The splenic and hepatic capsules were infiltrated multifocally and effaced by aggregates of macrophages, which penetrated through to the external surface and formed a thick mat admixed with lymphocytes, fewer plasma cells and fibrin. The hepatic lymph node was severely distended and almost completely effaced by sheets of macrophages and regionally extensive zones of coagulative necrosis. Occasional small remnants of attenuated lymph node capsule could be identified within the sheets of macrophages, which extended widely into the surrounding perinodal tissue; these were variably contained by layers of fibrous connective tissue. The submandibular lymph nodes contained a similar, but much less severe, infiltrate, which was confined to the sinus regions and did not efface the normal architecture. Within the myocardium, renal cortex and pulmonary interstitium there were rare, microscopical, poorly-defined foci of histiocytic and lesser neutrophilic infiltrates. Additional sections of spleen, liver and hepatic lymph node stained by the Ziehl–Neelsen (ZN) method revealed myriad intrahistiocytic acid-fast bacilli consistent with *Mycobacterium* spp. (Fig. 3). No acid-fast organisms were detected in the lung, myocardium and kidney. No significant findings were observed in the remaining tissues.

Routine aerobic/anaerobic microbiological culture of liver, spleen and lymph node failed to grow any microorganisms. Additional samples were submitted to the Scottish Mycobacteria Reference Laboratory (SMRL, Royal Infirmary of Edinburgh). Direct real-time PCR and MTBDR plus assay (Hain Lifescience GmbH, Nehren, Germany) confirmed the presence of a member of the MTC; no mutations indicating resistance to isoniazid or rifampcin were identified. Mycobacterial culture resulted in the growth of an organism with spoligotype pattern VLA type 34 (www.Mbovis.org SB0118) and extended VNTR profile 53562 26324 222_2. The isolate was confirmed as *M. microti* by deletion assay of

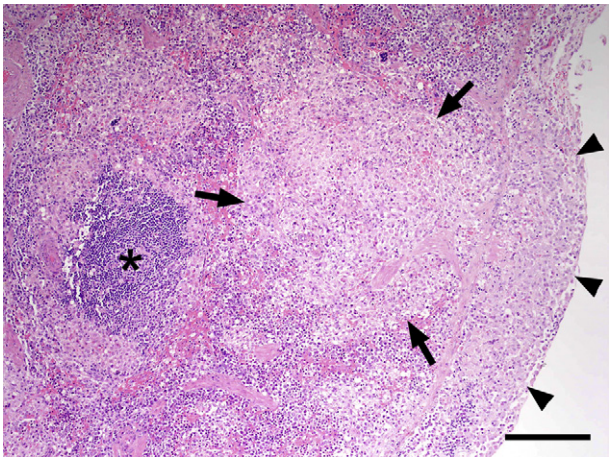

Fig. 2. Splenic tissue from a meerkat (*Suricata suricatta*). The spleen is affected by a multifocal to coalescing severe histiocytic infiltrate, which effaces the normal architecture (arrows) and penetrates multifocally through the capsule (arrowheads). Normal white pulp is present (asterisk). HE. Bar, 200 µm.

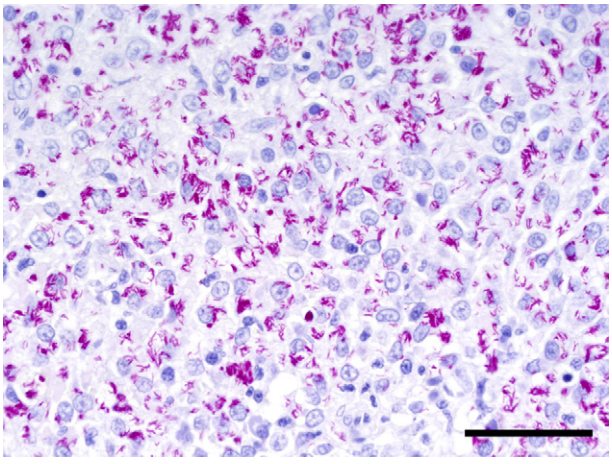

Fig. 3. Splenic tissue from a meerkat (*Suricata suricatta*). The spleen is effaced by a severe histiocytic infiltrate containing myriad intracytoplasmic, acid-fast, rod-shaped, bacteria, consistent with *Mycobacterium* spp. ZN. Bar, 50 µm.

RD1^{mic} (deleted) and RD4 (intact) and by the distinct 2.2 allele at the ETR-F VNTR locus (Smith *et al*., 2009). Strains of *M. microti* with this spoligotype have been recovered previously from cats in Southern Scotland and North-West England (Smith *et al*., 2009).

These are the first two cases of mycobacterial disease reported in meerkats outside Africa and are the only examples of *M. microti* infection reported in this species. In both cases the lesions were largely confined to the abdomen with no pulmonary involvement, suggesting an oral route of transmission. This is consistent with the report of *M. tuberculosis* in meerkats by Alexander *et al*. (2002), in which infection was also predominantly located in the abdominal organs. In contrast, Drewe *et al*. (2009b) reported a high incidence of pulmonary involvement in *M. bovis* infection and suggest that aerosol transmission represents the primary route of infection for this organism. This may also explain why close social interaction between meerkats is associated with higher transmission rates of *M. bovis* (Drewe, 2010). Together, these reports indicate that *M. microti* and *M. tuberculosis* infection may share a similar aetiology in meerkats compared with *M. bovis*.

Meerkats are found commonly in zoological collections and the increasing number of reported cases of *M. microti* in immunocompetent people may raise concerns regarding the zoonotic potential of this organism. However, as the lesions described here were primarily located in the abdomen, it would appear that the opportunity for aerosol transmission to people would be minimal. There are no clear epidemiological data to explain the origin of infection in this instance; however, llamas within the zoo were negative when tested for the MTC. At present, there are no validated in-vivo tests for *M. microti* in meerkats. Although tests for *M. bovis* have recently been evaluated in meerkats (Drewe *et al*., 2009a), it is unclear whether these would be able to detect *M. microti* and/or differentiate between members of the MTC.

Wild rodents, including field voles (*Microtus agrestis*), bank voles (*Myodes glareolus*, formerly *Clethrionomys glareolus*), wood mice (*Apodemus sylvaticus*) and shrews (*Sorex araneus*), are known to carry *M. microti* and are considered to be the primary reservoir of infection responsible for transmission to domestic animals (particularly cats) (Gunn-Moore, 2010; Rüfenacht *et al*., 2011). It is therefore possible that the meerkats in this study became infected in the same manner. In the wild, meerkat burrows are reported to be co-inhabited by a number of wild rodent species (Mills and Bester, 2005) and it is possible that these may also represent a potential reservoir of infection.

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