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Pathology of *Mycobacterium bovis* Infection in Wild Meerkats (*Suricata suricatta*)

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Summary

Pathological lesions associated with *Mycobacterium bovis* infection (bovine tuberculosis; bTB) in free-living meerkats (*Suricata suricatta*) in the Kalahari Desert of South Africa are described. The pathology of bTB in meerkats was determined through detailed post-mortem examinations of 57 animals (52 meerkats showing clinical signs of bTB, and five not showing signs of disease). Lymph nodes and tissue lesions thought to be associated with bTB were cultured for mycobacteria. All 52 bTB-infected meerkats showed gross or microscopical granulomatous lesions, but *M. bovis* was cultured from only 42% (22/52) of these animals. The majority (96%, 50/52) of diseased meerkats had lesions in multiple sites, the pattern of which suggested haematogenous spread of *M. bovis* infection in this species. The histological characteristics of the tuberculous lesions, together with the gross pathology and the wide range of body systems affected, indicate that infection in meerkats is acquired principally via the respiratory and oral routes, whereas excretion is most likely via the respiratory tract and suppurating skin wounds. Urine and faeces appear to be unlikely sources of infection. The findings of this study provide information on the transmission, pathogenesis and epidemiology of bTB in meerkats that is likely to be relevant to the understanding of *M. bovis* infection in other social mammal species such as the European badger (*Meles meles*).

Keywords: meerkat; *Mycobacterium bovis*; pathology; *Suricata suricatta*; tuberculosis

Introduction

The development of effective control programmes for bovine tuberculosis (bTB; *Mycobacterium bovis* infection) in domestic animals requires a thorough understanding of the epidemiology of the disease in free-living species of wildlife that may act as reservoirs of infection. Knowledge of the pathogenesis of the disease is an essential part of this understanding. In all species studied to date, bTB is a chronic, progressive disease with infection frequently lasting for many weeks and disease lasting for several months to years (Bengis, 1998). Animals appear clinically normal for the majority of the course of infection, with the most common clinical sign of disease — weight loss — occurring only in the late stages of infection (De Lisle *et al.*, 2002). However, a wide range of clinical signs, which vary between species, have been reported, making generalizations difficult. Routes of infection and excretion also vary according to the behaviour and ecology of individual species, and reflect differences in the pathogenesis of bTB between affected species.

Meerkats (*Suricata suricatta*) are small (<1 kg) southern African desert-adapted mongooses living in social groups of 6–40 animals. Groups typically include a dominant female and male and a variable number of other individuals of both sexes who assist with pup rearing. Meerkats are diurnally active and feed mainly on insects, arachnids and small vertebrates. By night they shelter as a group in elaborate burrow systems. A population of wild meerkats living in the Kalahari have been the focus of detailed

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behavioural ecology studies since 1993 (Clutton-Brock *et al*., 1998).

The apparent introduction of *Mycobacterium tuberculosis*, a primary human pathogen, into free-living meerkats and banded mongooses (*Mungos mungo*) in southern Africa was documented by Alexander *et al*. (2002). Prior to this *M. tuberculosis* had only been reported in humans and in captive animals in close contact with humans such as domestic cats and zoo animals (Michalak *et al*., 1998; Montali *et al*., 2001). The clinical signs in the single meerkat studied by Alexander *et al*. (2002) included cachexia and enlarged head lymph nodes, which ruptured and then discharged pus for two months, after which the lesion persisted as a non-healing wound. Gross post-mortem examination revealed the presence of abscesses in the parotid, axillary, pulmonary, hepatic and mediastinal lymph nodes, and in the pancreas. Granulomatous inflammation was identified by microscopical examination. The disseminated nature of the lesions, the single post-mortem examination and the pooling of tissues for culture meant that it was not possible to determine the likely pathogenesis of *M. tuberculosis* in meerkats from this previous study. The significance of *M. tuberculosis* in meerkats remains unclear, particularly since subsequent detailed studies on the same population have identified *M. bovis*, suggesting that historical cases may have been infected with *M. bovis* also. The present study reports the investigation of the pathological evidence of *M. bovis* in free-living meerkats and represents the first published report of *M. bovis* infection in this species.

Studies of the pathology of *M. bovis* infection in other wildlife species have demonstrated common routes of transmission between individuals. Inhalation of aerosols leading to respiratory infection occurs in both European badgers (*Meles meles*) in the UK (Clifton-Hadley *et al*., 1993; Gallagher *et al*., 1998; Delahay *et al*., 2000) and brushtail possums (*Trichosurus vulpecula*) in New Zealand (Cooke *et al*., 1995; Jackson *et al*., 1995a, b). In contrast, the alimentary route of infection is thought to be the primary source in feral ferrets (*Mustela furo*) in New Zealand (Lugton *et al*., 1997b). In those animals with lesions of the head lymph nodes (in particular the retropharyngeal nodes) determination of whether the infection was acquired through ingestion or inhalation is difficult. Although detailed anatomical studies of the lymphatic system in all these species are lacking, studies in the domestic dog have shown that the retropharyngeal lymph nodes drain areas common to both the respiratory and oral systems (Belz and Heath, 1995).

The aim of the present study was to determine the pathogenesis of naturally occurring *M. bovis* infection in wild meerkats in order to understand how the disease may be transmitted in this free-living species. A thorough understanding of the epidemiology of this disease is needed in order to establish the potential implications of bTB in meerkats to the infection in other wildlife species, domestic animals and humans.

Materials and Methods

Study Site and Animals

Data and samples were collected at the Kalahari Meerkat Project in the Northern Cape, South Africa (26°58′S, 21°49′E) which is an area consisting of dry riverbed, semi-arid herbaceous flats and sparsely grassed dunes surrounded by ranch land on which cattle, sheep, goats and ostriches are farmed. Around 300 wild meerkats living in 14 social groups were habituated to close observation and visited at least once every three days between April 2005 and May 2007. Detailed life histories were available for all meerkats, including date of birth, parentage, status in group, dates of departure and return to group (where this occurred), reproductive status and daily weight change. All research protocols were approved by the Research Ethics Committee of the University of Pretoria, and permission to conduct the research was granted by Northern Cape Nature Conservation Service.

Post-Mortem Examination

Fifty-two meerkats displaying signs consistent with terminal stages of bTB infection (submandibular swellings, emaciation and lethargy) were caught by gently but firmly lifting them by the tail base and immediately placing them in a dark-coloured cotton bag. Each meerkat was supported by placing an arm under the bag whilst it was carried approximately 100 m away from the rest of the group. Meerkats were anaesthetized using a combination of 10 mg/kg ketamine hydrochloride (Anaket-V™; Bayer Animal Health, Johannesburg, South Africa) and 0.07 mg/kg medetomidine hydrochloride (Domitor™; Pfizer Animal Health, Johannesburg, South Africa) injected intramuscularly, then killed by intracardiac injection of 170 mg/kg pentobarbitone sodium (Eutha-naze™; Centaur Labs, Johannesburg, South Africa). A further five meerkats were available for post-mortem examination; these did not show signs of bTB, having died from other causes (killed in road traffic accidents, $n = 4$; bitten by puff adder (*Bitis arietans*), $n = 1$). A standardized gross post-mortem examination of each meerkat was performed with samples of the major organs and suspected tuberculous lesions collected into 10% neutral buffered formalin.

Gross lesions were defined as any apparent abnormality and included organomegaly, presence and nature (purulent, caseous) of abscesses and inflammation

(including adhesions). Tissue samples (approximate size $10 \times 10 \times 5$ mm) of submandibular, medial retropharyngeal, sternal, mediastinal, mesenteric and inguinal lymph nodes, plus lung and spleen, were collected into sterile plastic containers and pooled for mycobacterial culture. In addition, any lesions observed outside of these organs were also sampled. Due to the remote location of the study site, samples for mycobacterial culture were frozen at −20°C before being transported to the laboratory in batches approximately every three months.

Histopathology

Tissues were available for microscopical examination from 89% (51/57) of the meerkats (46/52 with clinical signs suggestive of bTB; 5/5 without). The six meerkat cadavers not examined microscopically had been stored frozen, which may have disrupted cell architecture, and so these were excluded from the analysis. Formalin-fixed tissues were embedded in paraffin wax by standard procedures. Sections (3 μm) were stained with haematoxylin and eosin (HE) and by the Ziehl–Neelsen method.

Mycobacterial Culture

Pooled tissues from all 57 meerkats undergoing post-mortem examination were cultured. Tissues were homogenized in distilled sterile water and then divided equally into two vials. The contents of one vial were decontaminated with hydrochloric acid, whilst sodium hydroxide was added to the other vial to remove contaminating organisms. Samples were centrifuged at 1650*g* for 10 min then diluted with distilled sterile water and centrifuged again. The pellet that remained after decanting the supernatant was sown onto solid mycobacteria-selective culture media (Lowenstein–Jensen with pyruvate and Lowenstein–Jensen with glycerol). Media were incubated at 37°C for 10 weeks and examined weekly for growth. The organisms from colonies growing on the mycobacteria-selective media were confirmed as acid-fast bacilli by Ziehl–Neelsen staining and identified as *M. bovis* by polymerase chain reaction (PCR, Parsons *et al.*, 2002). Positive and negative controls were included in each batch of samples at all stages. Animals were considered positive for bTB infection if *M. bovis* was cultured and identified by PCR or if classical granulomatous lesions containing acid-fast bacilli were identified histologically.

Head Lymph Node Drainage

The lymphatic drainage pathways of the meerkat head were investigated immediately after euthanasia in 12 animals by injecting 0.2 ml of Indian ink through a 29 gauge needle placed just below the skin of the nares ($n = 8$) or the parenchyma of the palatine tonsil ($n = 2$) or the musculature of the tongue ($n = 2$). Since lymph is known to continue to flow for up to 4 h after death in the dog (Mendel and Hooker, 1902), ink was left to drain for 20 min before the skin of the chin and ventral neck was reflected and the submandibular and medial retropharyngeal lymph nodes on both sides were dissected out and examined visually. Presence of ink in a lymph node was considered to indicate lymph drainage from the site of injection to that node.

Statistical Analysis

Data were examined for normality using an Anderson–Darling test. Associations between disease status and age, plus number of lesions and sex, were analyzed using Mann–Whitney *U* tests. Magnitudes of association between anatomical locations of multiple lesions were calculated as relative risks using standard methods (Kleinbaum *et al.*, 1982). Where appropriate, 95% confidence intervals are presented with results.

Results

A positive diagnosis of bTB was made from culture or histology in all 52 meerkats displaying clinical signs. Of these, 26 were male and 26 were female, suggesting that both sexes are equally prone to bTB. Age at death ranged from 10 months to 7 years and 3 months (median 2 years and 1 month). No association was found between disease status (presence or absence of bTB) and age at death (Mann–Whitney *U* test, $U = 109$, $N_{\text{diseased}} = 52$, $N_{\text{non-diseased}} = 5$, $P = 0.554$).

Distribution of Tuberculous Lesions

The prevalence of gross and microscopical lesions by anatomical location is shown in Table 1. Data were not normally distributed (Anderson–Darling test, $P = 0.010$ for gross lesions only; $P = 0.011$ for gross and microscopical lesions). The number of organs with gross lesions per meerkat ranged from 1–14, with a median of 5.0 (Fig. 1). Additional microscopical examination of organs increased the total number of lesion sites detected per meerkat to a median of 6.0, with a range of 1–16. There was no significant difference between sexes in the number of lesions per meerkat (Mann–Whitney *U* test, $U = 266$, $N_{\text{male}} = 26$, $N_{\text{female}} = 26$, $P = 0.182$). Gross post-mortem examination revealed 88.9% (281/316) of all bTB lesions detected; the remaining 11.1% (35/316) of lesions were detected only microscopically.

Table 1
Prevalence of gross and microscopical lesions by anatomical location in 52 tuberculous meerkats

Anatomical location	*Lesion prevalence (%)*		*Number of meerkats examined*
	Gross lesions only	*Gross and microscopical lesions*	
Spleen	81	87	52
Head lymph nodes (submandibular and medial retropharyngeal)	77	77	52
Lungs	67	77	52
Liver	38	75	52
Mesenteric lymph nodes	53	53	49
Mediastinal lymph nodes	33	33	46
Skin	29	29	52
Muscle/subcutis	20	20	50
Sternal lymph nodes	14	14	51
Kidneys	0	13	52
Heart	10	13	52
Mammary gland	7	7	26
Inguinal lymph nodes	5	5	41
Axillary lymph nodes	5	5	41
Popliteal lymph nodes	2	2	41
Stomach	2	2	52
Small intestine	0	0	52
Large intestine	0	0	52
Bladder	0	0	52
Reproductive organs	0	0	52
Brain	0	0	1

The number of observations varies because not all lymph nodes were sampled in every post-mortem examination.

The spleen was the organ in which tuberculous lesions were most commonly observed, with 87% (45/52) of meerkats with bTB displaying gross or microscopical lesions in this organ (Table 1). The spleen and liver were the two organs most often concurrently affected, with lesions being present in at least one of these organs in 90% (47/52) of animals and lesions occurring in both organs in 71% (37/52) of animals. These two organs were considered together in subsequent analyses due to this high degree of co-involvement and because lesions in either liver or spleen may indicate generalized haematogenous spread of infection. The submandibular and medial retropharyngeal lymph nodes (hereafter referred to as 'head lymph nodes') were affected in 77% (40/52) of meerkats having bTB. Meerkats with head lymph node lesions were found to have lesions in at least one other organ 98% (39/40) of the time. Lung lesions were present in 75% (30/40) of meerkats with head lymph node lesions and in 83% (10/12) of meerkats without head lesions.

Concurrence of lesions in the most commonly affected organs is shown in Fig. 2. Associations between the occurrences of lesions in specific anatomical locations are presented as relative risks in Table 2. The presence of head lymph node lesions was found to be a strong indicator of pathology in other anatomical locations. Meerkats with lesions in the head lymph nodes were three times more likely to have lung lesions, and 19 times more likely to have lesions in the spleen and liver, than meerkats that did not have head lymph node lesions.

Gross Pathology

The number and appearance of gross lesions are summarized in Table 3. Photographs of the most frequently occurring lesions appear in Fig. 3.

Histopathology of Tuberculous Lesions

Granulomatous inflammation associated with acid-fast bacilli was present in tissues from 90% (46/51) of meerkats examined microscopically. The five

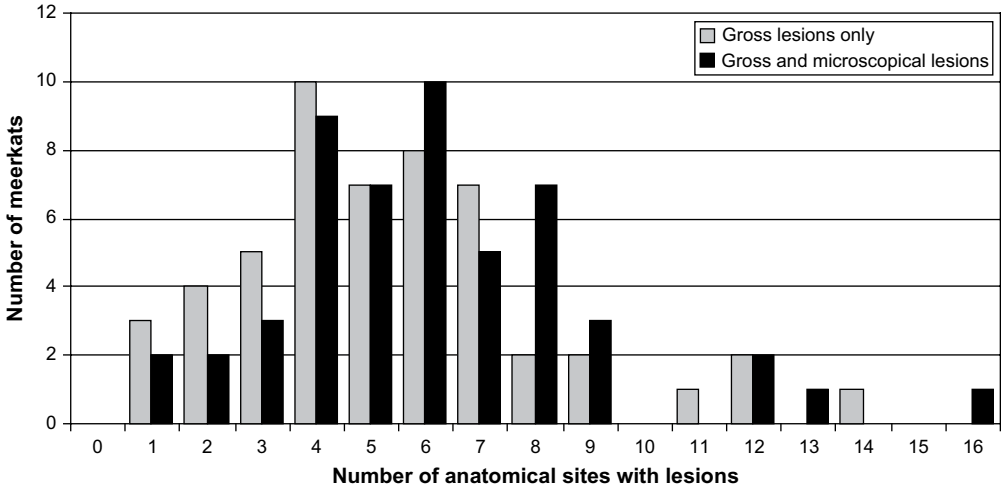


Fig. 1. Frequency and number of anatomical sites containing gross and microscopical lesions per individual in 52 tuberculous meerkats.

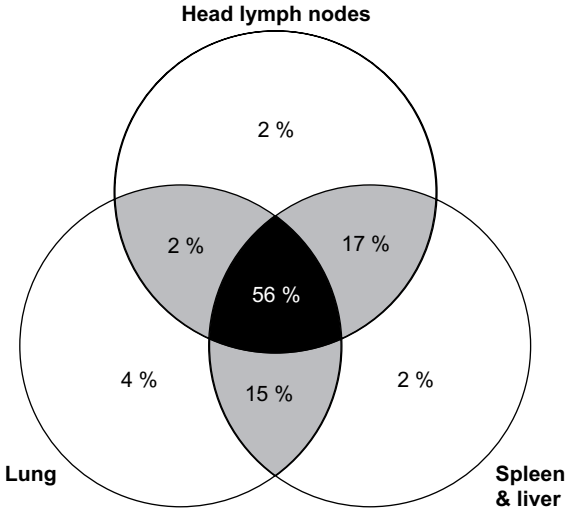


Fig. 2. Percentage co-involvement of the most commonly affected organs in 52 tuberculous meerkats. Only one meerkat did not have lesions in any of the locations shown.

animals without microscopical lesions were the five meerkats that died without any clinical signs suggestive of bTB.

Microscopically, lesions consisted of multifocal zones of granulomatous inflammation and lesions were generally similar between different organs. Epithelioid macrophages dominated the infiltrate, forming solid sheets, intermixed with low numbers of lymphocytes and less commonly neutrophils (Fig. 4). Multinucleate giant cells were seen rarely and significant peripheral fibrosis was not detected. In some of the larger lesions, central foci of caseous necrosis were detected. Lesions in the lung were generally randomly distributed within the parenchyma, only occasionally extending from peribronchiolar regions. Similarly, the lesions in the liver were randomly distributed within hepatic lobules, only occasionally involving portal areas. Splenic lesions involved both lymphoid follicles and red pulp. Affected lymph nodes were effaced by sheets of granulomatous inflammatory cells, with only small regions of normal cortex remaining at the periphery. In some of the submandibular nodes examined, the inflammation extended into nearby connective tissue, often closely adjacent to salivary glands. In a small number of animals the inflammation had extended from mesenteric nodes through to the peritoneal cavity with a layer of macrophages containing acid-fast bacilli present over the serosal surfaces (Fig. 4d). In samples from the skin lesions, extensive sheets of granulomatous inflammation extended from the deep dermis into the subcutis. All lesions contained moderate (1–2 per high power × 100 oil immersion field) to plentiful (>10 per high power field) intracellular acid-fast bacilli (Fig. 4 h). Most macrophages contained a single organism but occasionally multiple organisms were identified. Neither granulomatous inflammation nor acid-fast bacilli were detected in any of the sections of small and large intestine or bladder mucosa.

Table 2
Relative risk values for co-involvement of organs in tuberculous meerkats

Predictor variable	*Response variable*	*Relative risk*	*95% confidence interval*
Head lymph nodes*	Lung	3.00	2.39–3.43
Head lymph nodes*	Spleen and liver	19.0	16.7–19.7
Head lymph nodes*	Lung and spleen and liver	2.64	2.07–3.05
Lung	Spleen and liver	12.3	10.7–13.0
Spleen and liver	Lung	3.70	3.06–4.13

The data show that meerkats with lesions in the head lymph nodes were three times more likely to have lung lesions, and 19 times more likely to have lesions in the spleen and liver, than meerkats that did not have head lymph node lesions. Animals with lung lesions were 12.3 times as likely to also have spleen and liver lesions as animals without lung lesions.
*Refers to lesions in the submandibular and medial retropharyngeal lymph nodes.

Incidental microscopical findings included the presence of low numbers of adult nematodes in the small airways of several animals as well as occasionally in sections of small intestine. These were associated with only mild inflammatory changes at both locations. In the dermis and subcutis of one animal, several distended hair follicles contained numerous (10–20) mites with morphology consistent with *Demodex* spp.

Mycobacterial Culture

Based on the stated diagnostic criteria (positive *M. bovis* culture confirmed by PCR or microscopical identification of granulomatous lesions containing acid-fast bacilli), all 52 meerkats showing clinical signs of submandibular swellings, emaciation or lethargy at death were considered positive for bTB infection. *M. bovis* was cultured from 42% (22/52) of tissue samples taken from these animals. Tissue from 46 of these meerkats was examined microscopically and 100% (46/46) of these samples had evidence of granulomatous lesions. None of the five meerkats dying without clinical signs of bTB had gross or microscopical granulomatous lesions, or produced a positive *M. bovis* culture, and hence all five of these meerkats were considered negative for bTB infection.

Head Lymph Node Drainage

There were three submandibular lymph nodes and one medial retropharyngeal lymph node on each side of the head in all 52 meerkats examined

Table 3
Descriptions of gross lesions seen in 52 meerkats infected with *M. bovis*

Organ	*Number of gross lesions per organ*	*Description of gross lesions*
Lung	>100	Discrete, circular, white foci 0.5–1.0 mm diameter affecting all lobes, occasionally coalescing to form large caseating granulomas engulfing almost all the lung tissue (Fig. 3d). Granulomas were homogenous on cut surface, with central consistency ranging from liquid to caseous. Pleural adhesions were present in one meerkat.
Heart	1	Infiltrating large (10 mm diameter) irregularly shaped lesions in the pericardial and mediastinal tissues that were firm and homogenously caseous on cut surface. Due to the extensive infiltration of surrounding tissues, it was not possible to determine the origin of these lesions.
Liver	>100	Multiple, randomly spaced, white pin-point lesions throughout the parenchyma of all liver lobes (Fig. 3e). In some cases lesions were larger (up to 5 mm) and irregular in shape, creating a mottled effect. Hepatomegaly was occasionally evident.
Spleen	1–50	Multiple miliary granulomas, ranging 1–10 mm, were common in the spleen (Fig. 3f). Granulomas ranged from soft (liquefactive) to firm in texture. In most cases granulomas caused bulging of the splenic capsule.
Lymph nodes (submandibular, retropharyngeal)	1	Marked enlargement of these nodes was common, presenting as clearly visible swellings in the submandibular and neck area (Fig. 3a, c). Individual nodes measured over 20 mm in diameter (size in healthy meerkat = 3–4 mm). Affected nodes were caseous and necrotic, with central viscosity varying from fluid to solid. Sinus tracts draining to the skin surface were frequent.
Lymph nodes (other)	1	Other lymph nodes that were found to be enlarged and caseous were, in descending order of frequency: mesenteric; mediastinal; sternal; inguinal; axillary; and popliteal.
Skin	1–3	Chronic discharging skin lesions 2–4 mm diameter occurred in 15/52 tuberculous meerkats in the following locations (frequency in brackets): forelimb (5); muzzle (3); submandibular area (3) (Fig. 3b); mammary region (2); inguinum (1); tail (1). Those in the submandibular and mammary areas were due to infection in the underlying lymph nodes and mammary glands, respectively, whilst those in other locations may have been abrasions or bite wounds from other meerkats. Histopathological evidence of granulomatous inflammation with acid-fast organisms was present in 67% (10/15) of skin wounds.
Mammary gland	1	Gross lesions were present in the mammary glands of two meerkats. The caudal glands were enlarged (50 mm diameter), thickened and firm, but not grossly caseous, with abrasions in the overlying skin. Both meerkats had been lactating at the time of development of mammary lesions.

(Fig. 3c). No lateral retropharyngeal lymph nodes were detected in any of the animals. The drainage patterns of the head lymph nodes in meerkats as determined by injection of Indian ink into 12 cadavers immediately after death are shown in Fig. 5 and summarized in Table 4. Both the respiratory (nasal cavity) and alimentary (oral cavity) systems were found to drain to the submandibular and medial retropharyngeal lymph nodes, although only the respiratory system drained to the contralateral submandibular lymph nodes.

Discussion

Sampling fresh material from a well-characterized population of wild meerkats made it possible to study in detail the pathology of bTB at the gross and microscopical level. A wide range of disease manifestations, from single lesions through to disseminated tuberculosis, was revealed by post-mortem examination of 52 tuberculous meerkats. Distinct patterns in lesion location and co-involvement of organs were evident.

The results of the present study suggest that *M. bovis* infection in meerkats often occurs via the respiratory tract. Lung lesions were present in 77% (40/52) of tuberculous meerkats, with mediastinal lymph nodes also affected in 33% (15/46) of animals (Table 1). The extent and frequency of such lesions suggest that inhalation of aerosolized mycobacteria occurs commonly. This concurs with a high prevalence (84%) of lung lesions in tuberculous possums reported by Jackson *et al.* (1995a), which the authors concluded was probably of central importance to the pathogenesis of the disease in this species. Conversely, only 6% (4/64) of tuberculous ferrets were found to have lung lesions in a study by Lugton *et al.* (1997b), implying that respiratory transmission of infection is probably rare in ferrets. The majority (38/40) of meerkats with lung lesions also had lesions in at least one other organ, principally the head lymph nodes, spleen and liver (Fig. 2). Thus it is possible that thoracic infection

Fig. 3. Gross lesions of tuberculosis in meerkats. (a) Large submandibular swellings in a live meerkat. (b) Chronic discharging sinus tract (arrow) from underlying abscessated submandibular lymph node. (c) Ventral view of head and neck showing enlargement and abscessation of the submandibular (thin arrows) and medial retropharyngeal (thick arrow) lymph nodes. Skin covering the ventral neck and chin has been reflected. Scale in mm. (d) Multiple solid white granulomatous foci throughout the lungs. (e) Multiple white spherical lesions of varying sizes densely packed throughout liver parenchyma. (f) Miliary granulomas in spleen.

in meerkats is secondary to haematogenous spread of infection from alternative primary sites in some animals, particularly where lung lesions were diffuse and not limited to the cranial lobes. However, sole infection of the respiratory tract was evident in 4% (2/52) of meerkats with bTB, indicating that inhalation of the pathogen does occur.

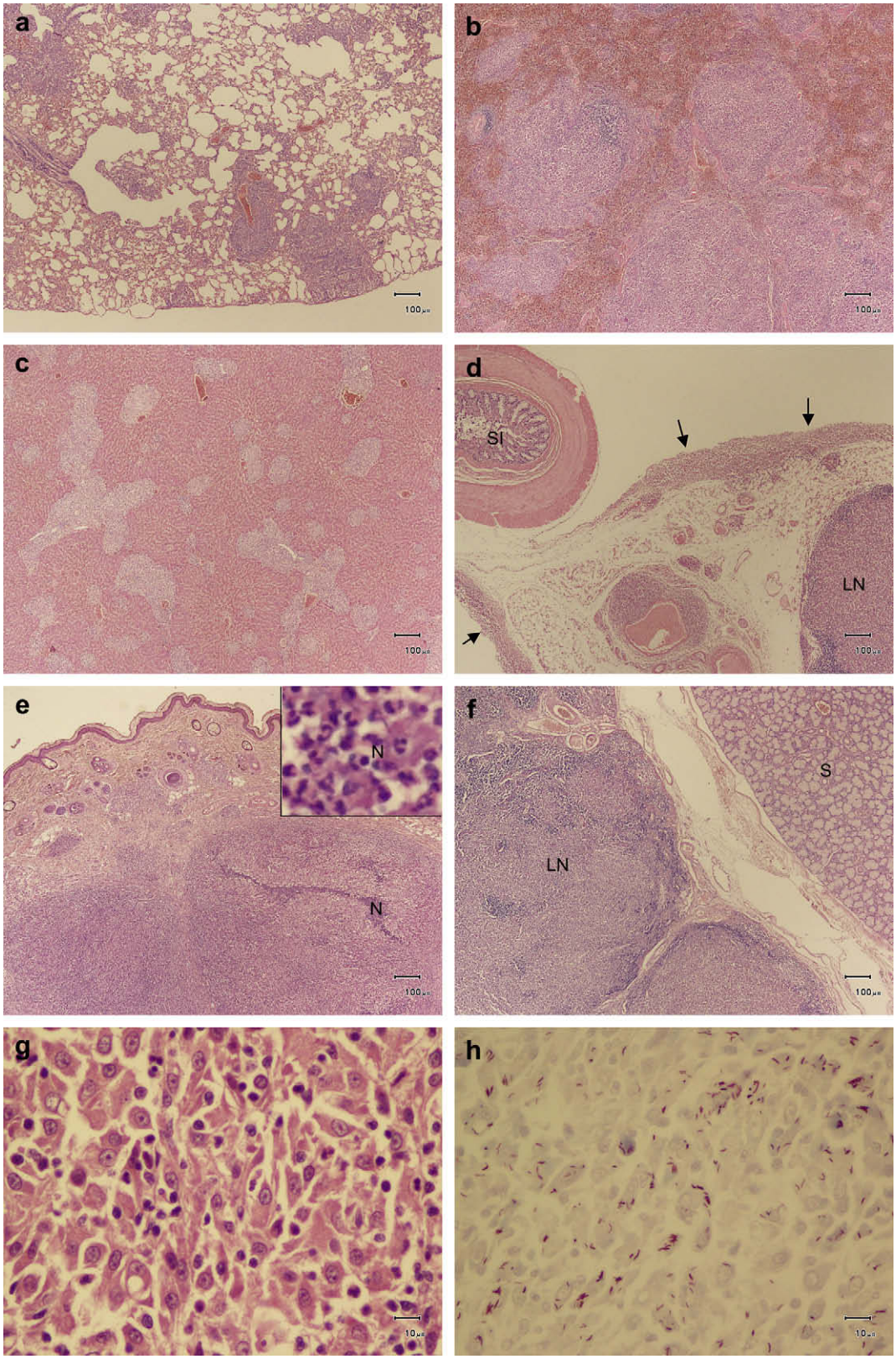
The results indicate that not all meerkats were infected through the respiratory tract, as 8% (4/52) of tuberculous meerkats had lesions in the mesenteric

lymph nodes without thoracic pathology, suggesting that infection may have entered via the oral route as occurs in other species (De Lisle *et al*., 2002). A significant number of meerkats (22/49) had mesenteric lesions in addition to lesions in other sites and some of these may represent primary alimentary infections. However, no gross or microscopical intestinal lesions were detected in any meerkat, although complete tracts were not examined microscopically, with only one section of duodenum and one section of colon being examined from every animal. Lesions in the respiratory and gastrointestinal tracts of tuberculous lions (*Pantera leo*) were reported to indicate both respiratory and oral routes of infection (Bengis, 1998). However, in their study of tuberculous possums, Jackson *et al*. (1995a) suggested that lesions of the lymph nodes draining the digestive system may be part of a generalized disease or secondary to lesions in the lung, rather than indicative of primary alimentary infection. Indeed, animals with active lung lesions that acquired infection via inhalation of aerosolized mycobacteria may well cough up and swallow organisms leading to gastrointestinal pathology. Nonetheless, the present study found the relative frequency of mesenteric lymph node involvement in meerkats (53%) to be much higher than has been reported in tuberculous badgers (less than 4%) (Gallagher and Clifton-Hadley, 2000), suggesting that even if infection did not enter via the alimentary route, excretion may occur this way in meerkats.

Evidence supporting haematogenous spread of bTB was found in the vast majority of tuberculous meerkats in this study. Lesions were present in either the spleen or liver or both in 90% (47/52) of tuberculous animals, and in 56% (29/52) of meerkats the spleen, liver, head lymph nodes and lungs were all affected together. Lesions in the liver, spleen and kidneys were considered by Jackson *et al*. (1995a) to indicate systemic haematogenous spread of infection due to the role of these organs in haemofiltration. This aspect of bTB pathology appears similar in meerkats and possums as 86% of the latter species displayed evidence of haematogenous spread (Jackson *et al*., 1995a). Lesions in sites other than the haemofiltration organs may have resulted from initial infection at that site or from lymphatic or haematogenous spread from another location. The distributions of the number of both gross and microscopical lesions per meerkat show a positive (right) skew (Fig. 1), indicating that most animals had fewer than the median number of lesions, but a small proportion had well above the median number of lesions. A similarly skewed distribution was found in tuberculous possums by Jackson *et al*. (1995a). Lesion location in those meerkats with a disproportionately high number of affected organs was similar to those animals with the median number of lesions, with the addition of several extra lymph nodes (principally the sternal, inguinal, axillary and popliteal lymph nodes). This suggests that lymphatic spread of bTB is important in meerkats, particularly in advanced stages of disease.

Gross post-mortem examination was very sensitive for detecting bTB in the study population, with 100% of tuberculous meerkats and 88.9% of lesions being detected grossly. The sensitivity of post-mortem examination for detecting bTB in the present study may have been expected to be lower if animals had been selected at random from the study population, rather than based on the presence of clinical signs, since meerkats with very early stages of bTB may not have developed clinical signs of disease. Indeed, this was the case in studies of bTB pathology in brushtail possums, in which gross post-mortem was found to have 87.2% sensitivity for the detection of tuberculosis in 78 out of 486 possums sampled (Jackson *et al*., 1995a). Similarly, in feral ferrets, gross post-mortem was 72.2% sensitive for the detection of tuberculosis in 72 out of 220 ferrets sampled (Lugton *et al*., 1997a). Jackson *et al*. (1995a) concluded that in possums, tuberculosis appears to commonly spread to multiple organs before lesions become visible at any body site. The absence of tuberculous meerkats without visible lesions in this study supports this finding and suggests that dissemination of infection occurs readily in meerkats. Rapid progression of tuberculosis has been reported in other social mammal species with high intraspecific contact rates, such as baboons (Tarara *et al*., 1985) and possums (Cooke *et al*., 1995). This suggests that host population structure plays an important role in bTB transmission and pathogenesis, particularly in social mammal species.

The pattern of lesions seen in this study suggests multiple routes for excretion of mycobacteria from infected meerkats. Exhalation of infected aerosols and discharging skin wounds appear to be most important based on the high frequency of active lesions in these sites. Faecal excretion is unlikely based on lack of significant intestinal pathology, but cannot be discounted given the observation of mesenteric lymph node lesions in some animals. A similar pattern was seen in infected possums (Cooke *et al*., 1995) and badgers, in which *M. bovis* was excreted by several routes including the respiratory, digestive, urinary and cutaneous routes (Gavier-Widen *et al*., 2001). An apparently major difference between badgers and meerkats, however, is the potential for infection to be transmitted through urine. Renal acid-fast organisms were identified in only 2% (1/52) of tuberculous meerkats, although inflammatory kidney lesions were found in 13% (7/52) of meerkats with bTB.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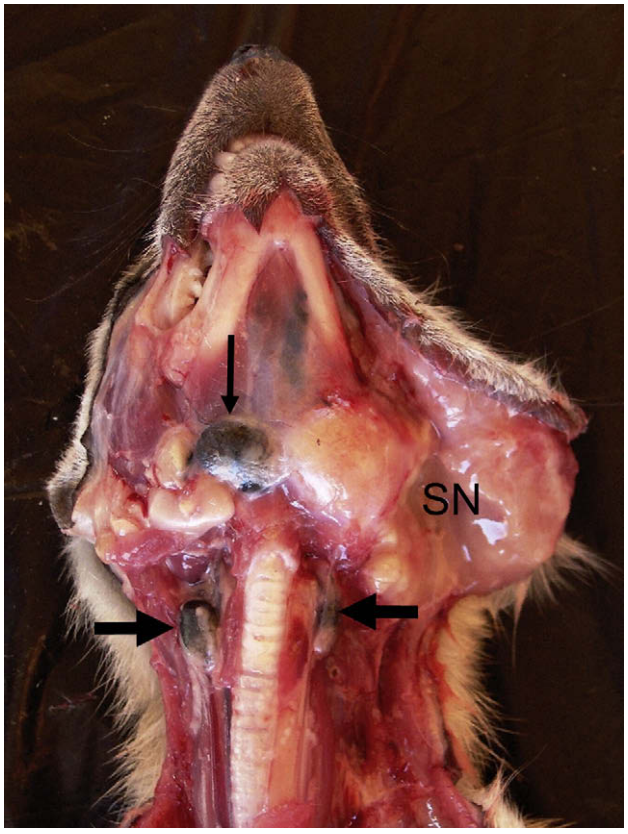

Fig. 5. Drainage patterns of head lymph nodes in the meerkat. Ventral view of head and neck showing presence of Indian ink in one submandibular (thin arrow) and both medial retropharyngeal (thick arrows) lymph nodes 20 min after injection of ink into the tongue immediately after death. Suppurative necrosis (SN) of the left submandibular lymph nodes is evident. Skin covering the ventral neck and chin has been removed.

Table 4
Lymph drainage patterns of the meerkat head

Anatomical location	*Drains to*			
	Submandibular lymph nodes		*Medial retropharyngeal lymph node*	
	Ipsilateral nodes	*Contralateral nodes*	*Ipsilateral node*	*Contralateral node*
Nares	+	+	+	+
Palatine tonsil	+	–	+	+
Tongue	+	–	+	+

Lymph flow determined by injection of Indian ink into the indicated anatomical locations in 12 cadavers immediately after death, (+) presence of ink indicating lymph flow to that node from the anatomical location injected, (–) absence of ink indicating no lymph flow to that node from the anatomical location injected.

Since focal chronic inflammation in the kidneys could be incidental in older animals, the true incidence of tuberculous kidney lesions, and therefore potential for urinary transmission in meerkats, appears to be very small. A similarly low level of renal involvement (7%) was found in tuberculous feral ferrets despite a high prevalence of hepatic lesions (93%) (Lugton *et al*., 1997a). The lack of a significant population of macrophages in the renal interstitium is likely to result in fewer pathogenic mycobacteria being entrapped in this organ compared with reticuloendothelial organs such as the liver and spleen. This contrasts with some studies of tuberculous badgers where renal lesions have been reported to be more severe than lung lesions (Gallagher *et al*., 1976) and large numbers of bacilli were found in the urine of infected badgers (MAFF, 1979). However, these findings were refuted in a recent study of 1166 tuberculous badgers (Jenkins *et al*., 2008) in which the prevalence of renal lesions was found to be only 13% (51/393 badgers with visible lesions), suggesting transmission via urine may actually be less important than previously thought. Additionally, a high prevalence of renal lesions does not necessarily indicate that urinary excretion occurs, for despite a 45% (33/73) prevalence of bTB lesions in kidneys of infected possums, Jackson *et al*. (1995a) failed to culture *M. bovis* from all of 38 urine samples tested.

An important means of bTB transmission in badgers is injection of infected saliva via bite wounds, and this has been linked to rapid haematogenous spread of infection (Clifton-Hadley *et al*., 1993). Meerkat saliva may occasionally be infectious due to infiltration of infection from nearby lymph nodes, although direct infection of salivary glands was not seen in this study. Meerkats show high levels of intraspecific aggression (Stephens *et al*., 2005), often inflicting severe bites on members of other social groups and occasionally on members of their own group. Skin wounds were seen in 29% (15/52) of tuberculous meerkats, with clear evidence of granulomatous inflammation being present in 67% (10/15) of these wounds. One third (5/15) of skin wounds were associated with sinus tracts

Fig. 4. Microscopical lesions in tuberculous meerkats. Multifocal granulomatous to pyogranulomatous inflammation in (a) lung, (b) spleen, (c) liver, (d) mesenteric lymph node, (e) skin and (f) submandibular lymph node. In (d), the granulomatous inflammation has extended to the peritoneal cavity with marked serositis (arrows); however, inflammation and acid-fast organisms are not present in the adjacent regions of small intestine. In the lesions in the skin (e), neutrophilic infiltration is evident in the centre of the granulomas (inset top right). At higher magnification (g), sheets of epithelioid macrophages are intermixed with low numbers of lymphocytes. Fibroblasts are uncommon, even at the periphery of lesions. Central regions of necrosis were occasionally present in larger lesions (not shown). In (h), high numbers of intracellular acid-fast bacilli are present within macrophages. LN = lymph node; N = neutrophils; S = salivary gland; SI = small intestine. (a)–(g) HE. (h) Ziehl–Neelsen stain.

draining from underlying lymph nodes or mammary glands. The remaining ten skin wounds were found particularly on the head and forelimbs, which are locations prone to bite wounding from other meerkats. Based on the heavy acid-fast bacterial loads often present in the skin wounds, evidence of rapid haematogenous spread of infection and the similarities with patterns of disease seen in badgers, it is likely that tuberculosis is transmitted via bite wounds in meerkats.

Tuberculous lesions were present in the mammary glands of two lactating meerkats. Excretion of mycobacteria from the mammary gland occurs in cattle in late stages of disease (Stamp, 1944). Pseudo-vertical transmission of bTB may be significant in meerkats, since multiple adult females in each group can lactate to pups of the dominant female (Scantlebury *et al.*, 2002). Transplacental infection would appear to be unlikely as no lesions were detected in four fetuses from tuberculous meerkats examined in the present study. The youngest animal in which lesions were found in this study was 10 months old, which may reflect an incubation period of several months following infection as a pup, or alternatively infection may have been acquired as a juvenile. Analysis of detailed behavioural interaction data and social networks to investigate these possibilities is currently being undertaken.

Granulomatous lesions were easily identified histologically in multiple organs and contained large numbers of intracellular acid-fast bacilli. The lack of fibrosis surrounding the granulomas is similar to histopathological findings described in badgers (Gavier-Widen *et al.*, 2001), possums (Cooke *et al.*, 1995) and ferrets (Lugton *et al.*, 1997a), but unlike tuberculous lesions in domestic cattle (Palmer *et al.*, 2007). This may reflect rapid development of the disease in the meerkat, with earlier lesions having less fibrosis (Wangoo *et al.*, 2005) or could relate to inherent differences in the type of immune response (Th1 *versus* Th2) and activation of anti-fibrotic mediators such as nitric oxide (Lousada *et al.*, 2006) between different species.

The results of the head lymph node drainage study (Table 4) indicate that there is a high degree of ipsi- and contralateral drainage from the submandibular lymph nodes to the medial retropharyngeal lymph nodes in meerkats. Both sets of nodes were found to drain similar areas of the head, although the medial retropharyngeal lymph nodes received afferent lymph from both sides of the head more frequently than the submandibular lymph nodes. This agrees with node location and findings in other more commonly studied species such as the dog (Evans, 1993) in which the retropharyngeal lymph nodes drain areas common to both the respiratory and oral systems (Belz and Heath, 1995). The drainage patterns from the nares and the tongue in meerkats were not sufficiently different to enable discrimination of route of infection (inhalation or ingestion) from the examination of head lymph node involvement. This would seem unsurprising given the high degree of cross-drainage seen. However, in a study of bTB in feral pigs (*Sus scrofa*) in Australia, Corner *et al.* (1981) considered submandibular lymph node lesions to be indicative of oral infection, seemingly because lung lesions were rare in that species. Primary lesions in the retropharyngeal lymph nodes of tuberculous ferrets were reported to indicate an alimentary route of infection (i.e. ingestion) by Lugton *et al.* (1997b), although evidence that these nodes do not also drain the nasal passages was not given, suggesting inhalation of bTB infection may also occur in this species. Whichever the route of infection, evidence was found in the current study that infection may bypass the head lymph nodes, since 25% (10/40) of meerkats with lung lesions did not have detectable lesions in the head lymph nodes. Thus it appears that a simple examination of the head lymph nodes is not sufficient to determine the route of infection of tuberculosis in meerkats.

The gross and microscopical pathology of naturally occurring *M. bovis* infection in wild meerkats reported in this study indicate that bTB in this species is a disseminated disease that develops rapidly and spreads to multiple organs via haematogenous and lymphatic routes. Whilst infection appears to be acquired principally via the respiratory and oral routes, excretion appears to be mainly from the respiratory tract and suppurating skin wounds. The highly social structure of meerkat societies means that infection is likely to spread rapidly through groups and co-operative behaviours such as cross-suckling may contribute to disease dissemination. Similarities in the pathology of bTB infection in other social mammal species such as ferrets and badgers exist, although several aspects of the disease in meerkats were found to differ from these species. The findings of the present study are relevant to understanding potential routes of transmission in meerkats and give some indication of which behaviours and social interactions may affect transmission of tuberculosis in this species. This research has aided our understanding of bTB epidemiology in a social mammal species and begins to establish the potential implications of bTB in meerkats to other wildlife species, domestic animals and humans.

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References

Alexander, K. A., Pleydell, E., Williams, M. C., Lane, E. P., Nyange, J. F. and Michel, A. L. (2002). *Mycobacterium tuberculosis*: an emerging disease of free-ranging wildlife. *Emerging Infectious Diseases*, **8**, 598–601.

Belz, G. T. and Heath, T. J. (1995). Lymph pathways of the medial retropharyngeal lymph-node in dogs. *Journal of Anatomy*, **186**, 517–526.

Bengis, R. G. (1998). Tuberculosis in free-ranging mammals. In: *Zoo & Wild Animal Medicine*, 4th Edit., M. E. Fowler and R. E. Miller, Eds, W.B. Saunders Company, Philadelphia, pp. 101–114.

Clifton-Hadley, R. S., Wilesmith, J. W. and Stuart, F. A. (1993). *Mycobacterium bovis* in the European badger (*Meles meles*): epidemiological findings in tuberculous badgers from a naturally infected population. *Epidemiology and Infection*, **111**, 9–19.

Clutton-Brock, T. H., Gaynor, D., Kansky, R., MacColl, A. D. C., McIlrath, G., Chadwick, P., Brotherton, P. N. M., O'Riain, J. M., Manser, M. and Skinner, J. D. (1998). Costs of cooperative behaviour in suricates, *Suricata suricatta*. *Proceedings of the Royal Society of London, Series B*, **265**, 185–190.

Cooke, M. M., Jackson, R., Coleman, J. D. and Alley, M. R. (1995). Naturally occurring tuberculosis caused by *Mycobacterium bovis* in brushtail possums (*Trichosurus vulpecula*): II. Pathology. *New Zealand Veterinary Journal*, **43**, 315–321.

Corner, L. A., Barrett, R. H., Lepper, A. W. D., Lewis, V. and Pearson, C. W. (1981). A survey of mycobacteriosis of feral pigs in the Northern Territory. *Australian Veterinary Journal*, **57**, 537–542.

De Lisle, G. W., Bengis, R. G., Schmitt, S. M. and O'Brien, D. J. (2002). Tuberculosis in free-ranging wildlife: detection, diagnosis and management. *Revue Scientifique et Technique de l'Office International des Epizooties*, **21**, 317–334.

Delahay, R. J., Langton, S., Smith, G. C., Clifton-Hadley, R. S. and Cheeseman, C. L. (2000). The spatio-temporal distribution of *Mycobacterium bovis* (bovine tuberculosis) infection in a high-density badger population. *Journal of Animal Ecology*, **69**, 428–441.

Evans, H. E. (1993). Lymph nodes and vessels of the head and neck. In: *Miller's Anatomy of the Dog*, 3rd Edit., W.B. Saunders, Philadelphia, pp. 724–729.

Gallagher, J. and Clifton-Hadley, R. S. (2000). Tuberculosis in badgers; a review of the disease and its significance for other animals. *Research in Veterinary Science*, **69**, 203–217.

Gallagher, J., Monies, R., Gavier-Widen, M. and Rule, B. (1998). Role of infected, non-diseased badgers in the pathogenesis of tuberculosis in the badger. *Veterinary Record*, **142**, 710–714.

Gallagher, J., Muirhead, R. H. and Burn, K. J. (1976). Tuberculosis in wild badgers (*Meles meles*) in Gloucestershire: pathology. *Veterinary Record*, **98**, 9–14.

Gavier-Widen, D., Chambers, M. A., Palmer, N., Newell, D. G. and Hewinson, R. G. (2001). Pathology of natural *Mycobacterium bovis* infection in European badgers (*Meles meles*) and its relationship with bacterial excretion. *Veterinary Record*, **148**, 299–304.

Jackson, R., Cooke, M. M., Coleman, J. D. and Morris, R. S. (1995a). Naturally occurring tuberculosis caused by *Mycobacterium bovis* in brushtail possums (*Trichosurus vulpecula*): I. An epidemiological analysis of lesion distribution. *New Zealand Veterinary Journal*, **43**, 306–314.

Jackson, R., Cooke, M. M., Coleman, J. D., Morris, R. S., de Lisle, G. W. and Yates, G. F. (1995b). Naturally occurring tuberculosis caused by *Mycobacterium bovis* in brushtail possums (*Trichosurus vulpecula*): III. Routes of infection and excretion. *New Zealand Veterinary Journal*, **43**, 322–327.

Jenkins, H. E., Morrison, W. I., Cox, D. R., Donnelly, C. A., Johnston, W. T., Bourne, F. J., Clifton-Hadley, R. S., Gettinby, G., McInerney, J. P., Watkins, G. H. and Woodroffe, R. (2008). The prevalence, distribution and severity of detectable pathological lesions in badgers naturally infected with *Mycobacterium bovis*. *Epidemiology and Infection*, **136**, 1350–1361.

Kleinbaum, D. G., Kupper, L. L. and Morgenstern, H. (1982). Measures of association. In: *Epidemiologic Research: Principles and Quantitative Methods*, John Wiley and Sons, Chichester, pp. 140–158.

Lousada, S., Florido, M. and Appelberg, R. (2006). Regulation of granuloma fibrosis by nitric oxide during *Mycobacterium avium* experimental infection. *International Journal of Experimental Pathology*, **87**, 307–315.

Lugton, I. W., Wobeser, G., Morris, R. S. and Caley, P. (1997a). Epidemiology of *Mycobacterium bovis* infection in feral ferrets (*Mustela furo*) in New Zealand: I. Pathology and diagnosis. *New Zealand Veterinary Journal*, **45**, 140–150.

Lugton, I. W., Wobeser, G., Morris, R. S. and Caley, P. (1997b). Epidemiology of *Mycobacterium bovis* infection in feral ferrets (*Mustela furo*) in New Zealand: II. Routes of infection and excretion. *New Zealand Veterinary Journal*, **45**, 151–157.

MAFF. (1979). *Third Report on Bovine Tuberculosis in Badgers*. HMSO, London.

Mendel, L. B. and Hooker, D. R. (1902). On the lymphagogic action of the strawberry, and on post-mortem lymph flow. *American Journal of Physiology*, **7**, 380–386.

Michalak, K., Austin, C., Diesel, S., Bacon, J. M., Zimmerman, P. and Maslow, J. N. (1998). *Mycobacterium tuberculosis* infection as a zoonotic disease: transmission between humans and elephants. *Emerging Infectious Diseases*, **4**, 283–287.

Montali, R. J., Mikota, S. K. and Cheng, L. I. (2001). *Mycobacterium tuberculosis* in zoo and wildlife species. *Revue Scientifique et Technique de l'Office International des Epizooties*, **20**, 291–303.

Palmer, M. V., Waters, W. R. and Thacker, T. C. (2007). Lesion development and immunohistochemical changes in granulomas from cattle experimentally infected with *Mycobacterium bovis*. *Veterinary Pathology*, **44**, 863–874.

Parsons, L. M., Brosch, R., Cole, S. T., Somoskovi, A., Loder, A., Bretzel, G., van Soolingen, D., Hale, Y. M. and Salfinger, M. (2002). Rapid and simple approach for identification of *Mycobacterium tuberculosis* complex isolates by PCR-based genomic deletion analysis. *Journal of Clinical Microbiology*, **40**, 2339–2345.

Scantlebury, M., Russell, A. F., McIlrath, G. M., Speakman, J. R. and Clutton-Brock, T. H. (2002). The energetics of lactation in cooperatively breeding meerkats *Suricata suricatta*. *Proceedings of the Royal Society of London, Series B*, **269**, 2147–2153.

Stamp, J. T. (1944). A review of the pathogenesis and pathology of bovine tuberculosis with special reference to practical problems. *Veterinary Record*, **56**, 443–446.

Stephens, P. A., Russell, A. F., Young, A. J., Sutherland, W. J. and Clutton-Brock, T. H. (2005). Dispersal, eviction, and conflict in meerkats (*Suricata suricatta*): an evolutionarily stable strategy model. *American Naturalist*, **165**, 120–135.

Tarara, R., Suleman, M. A., Sapolsky, R., Wabomba, M. J. and Else, J. G. (1985). Tuberculosis in wild olive baboons, *Papio cynocephalus anubis* (Lesson), in Kenya. *Journal of Wildlife Diseases*, **21**, 137–140.

Wangoo, A., Johnson, L., Gough, J., Ackbar, R., Inglut, S., Hicks, D., Spencer, Y., Hewinson, G. and Vordermeier, M. (2005). Advanced granulomatous lesions in *Mycobacterium bovis*-infected cattle are associated with increased expression of type I procollagen, γδ ($WC1^+$) T cells and $CD\ 68^+$ cells. *Journal of Comparative Pathology*, **133**, 223–234.

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