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DIAGNOSIS AND IMPLICATIONS OF *MYCOBACTERIUM BOVIS* INFECTION IN BANDED MONGOOSES (*MUNGOS MUNGO*) IN THE KRUGER NATIONAL PARK, SOUTH AFRICA

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ABSTRACT: Bovine tuberculosis (bTB) was first diagnosed in the Kruger National Park (KNP) in 1990. Research has since focused on the maintenance host, the African buffalo (*Syncerus caffer*) and clinically affected lion (*Panthera leo*). However, little is known about the role of small predators in tuberculosis epidemiology. During 2011–12, we screened banded mongooses (*Mungos mungo*) in the bTB high-prevalence zone of the KNP for *Mycobacterium tuberculosis* complex members. Fecal swabs, tracheal swabs, and tracheal lavages of 76 banded mongooses caught in cage traps within a 2-km radius of Skukuza Rest Camp were submitted for *Mycobacterium* culture, isolation, and species identification. Lesions and lymph node samples collected from 12 animals at postmortem examination were submitted for culture and histopathology. In lung and lymph nodes of two banded mongooses, well demarcated, irregularly margined, gray-yellow nodules of up to 5 mm diameter were identified with either central necrosis or calcification, characterized on histopathology as caseating necrosis with epithelioid macrophages or necrogranuloma with calcified centre. No acid fast bacteria were identified with Ziehl–Neelsen stain. We isolated *Mycobacterium bovis* from lung, lymph node, and liver samples, as well as from tracheal lavages and tracheal swab from the same two banded mongooses. Blood samples were positive by ElephantTB STAT-PAK® Assay for 12 and Enferplex™ TB Assay for five animals. Only the two banded mongooses positive on pathology and *M. bovis* culture were positive on both serologic assays. We provide evidence of bTB infection in banded mongooses in the KNP, demonstrate their ability to shed *M. bovis*, and propose a possible antemortem diagnostic algorithm. Our findings open the discussion around possible sources of infection and their significance at the human/wildlife interface in and around Skukuza.

Key words: Banded mongoose, Enferplex, Kruger National Park, *Mungos mungo*, *Mycobacterium bovis*, STAT-PAK.

INTRODUCTION

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, has been one of the globally most important infectious diseases in cattle (OIE 2009). Introduced into South Africa in the wake of British colonization (Smith 2012), bTB was reported as early as the 1920s in South African wildlife (Paine and Martinaglia 1929). Where susceptible wildlife species occur in high densities, *M. bovis* can circulate and persist in these wildlife populations, which develop into maintenance hosts, making disease control and eradication difficult (Palmer 2013). Examples of these wildlife maintenance host species are the European badger (*Meles meles*) in the British Isles (Garnetta et al. 2003), the brushtail possum

(*Trichosurus vulpecula*) in New Zealand (O'Brien et al. 2011), and the African buffalo (*Syncerus caffer*) in South Africa. With the African buffalo established as a maintenance host (Rodwell et al. 2001), bTB evaded country-wide eradication programs in cattle and persisted in wildlife conservation areas (Michel et al. 2009). Subsequent spillover infections were recorded in a broad spectrum of antelope, pig, primate, and predator species (Clifford et al. 2013; Michel 2015). The spillover pathway with the most prominent impact on wildlife is the infection of lion (*Panthera leo*) from African buffalo, which is especially evident in the Kruger National Park (KNP) in northeastern South Africa (Keet et al. 1996). Bovine tuberculosis was detected in the KNP for the first time in 1990 in an African buffalo (Bengis et al. 1996) and spread throughout the park within <2 decades of monitoring (Palmer 2013). At present, the highest prevalence of bTB in the KNP is still in the south (Cross et al. 2009).

With research on bTB in the KNP focused primarily on the buffalo as the maintenance host and lion as a clinically affected species, little is known about the role that small predators might play in the epidemiology of tuberculosis in the KNP. With *M. bovis* diagnosed in the large-spotted genet (*Genetta tigrina*) and the honey badger (*Mellivora capensis*) (Michel et al. 2006), small predators might contribute to the spread of bTB, similar to the European badger in the UK (Nolan and Wilesmith 1994; Garnetta et al. 2003). Other members of the *Mycobacterium tuberculosis* complex (MTC) (e.g., *Mycobacterium suricattae* in suricates [*Suricata suricatta*; Parsons et al. 2013] and *Mycobacterium mungi* in banded mongooses [*Mungos mungo*; Alexander et al. 2010]) caused rapidly progressive disease, further indicating susceptibility of small predators to mycobacteria and a potential role as spillover or even maintenance host. At the human-wildlife interface, *M. tuberculosis* constitutes a health threat to a wide variety of mammal species, and wildlife can be a sentinel for environmental contamination (Michel et al. 2013).

We evaluated whether the banded mongoose plays a role in bTB epidemiology in the KNP and whether *M. tuberculosis* transmission at the small predator–human interface of rest camps occurs. For this purpose, we screened the banded mongoose population in the vicinity of the Skukuza rest camp in the bTB high-prevalence zone for infection with *M. bovis* and other MTC members.

MATERIALS AND METHODS

Study animals and study site

Between December 2011 and November 2012, we examined 76 banded mongooses, 18 subadult (<12 mo) males, 22 adult males, 19 subadult females, and 17 adult females. All animals originated from within a 2-km radius of the Skukuza rest camp (SANParks 2016) in southwest KNP. Surrounded by natural bush veld and riverine vegetation, the three main capture areas consisted of residential areas, wild animal holding facilities, and a mosaic of dorm rooms, administrative and operational buildings, and the tourist day visitor area. In packs of approximately 30 animals, banded mongooses forage or hunt and opportunistically raid rubbish bins (Apps 2000).

Animal capture and anesthesia

Similar to the capture method described by Cant (2000) and de Luca and Ginsberg (2001), we baited cage traps (72×28×32-cm^3 Standard Humane Cage Trap AHATSD provided by SANParks, Scientific Services, Animal Handling Support Systems, Greenside, South Africa) with peanut butter and oats. Traps were set after sunrise in preferred foraging sites; checked regularly for captures, malfunction, or disturbance; and closed in late afternoon.

In 87 capture and recapture events, 76 individual banded mongooses were restrained in the cage traps with a wire-pane insert for intramuscular injection of an anesthetic cocktail. A combination of either ketamine, medetomidine, and butorphanol (n=36) (3.2 mg/kg ketamine, Kyron Laboratories, Benrose, South Africa; 0.2 mg/kg medetomidine, Domitor®, Pfizer, Sandton, South Africa; 0.39 mg/kg butorphanol, Kyron) or of Zoletil® and medetomidine (n=51) (2.86 mg/kg Zoletil®, Virbac, Centurion, South Africa; 0.14 mg/kg medetomidine) was used. The latter combination resulted in longer duration of anesthesia than the first. Where indicated, anesthetic depth was maintained by readministering 50% of the original dose. Atipamezole (Antisedan®, Pfizer) at five times the amount of

medetomidine and naltrexone (Kyron) at 20 times the amount of butorphanol were administered intramuscularly for animals to recover fully before release at the capture site.

Animal identification and clinical evaluation

Individuals were identified by ear notches and microchip transponders (Identipet® FDX-A 10-digit microchip, small TX1440L10S, Identipet [Pty] Ltd., Muldersdrift, South Africa). Sex, mass, body condition score, and dehydration status were recorded; injuries were scored; and external parasite loads were estimated. We estimated age using relative tooth wear (de Luca and Ginsberg 2001), coat color change from neonate red-brown to adult gray with black bands (Skinner and Chimimba 2005), tooth eruption, and maximum adult head measurements (Cant 2000).

Euthanasia, postmortem examination, and histopathology

Selection criteria for euthanasia and subsequent postmortem examination were advanced age (i.e., >6 yr, as indicated by heavy tooth wear [de Luca and Ginsberg 2001]; n=2), a positive reaction on STAT-PAK (n=7) (STAT-PAK Assay; Chembio Diagnostic Systems, Medford, New York, USA) or presence of clinical signs typically associated with mycobacterial disease (Alexander et al. 2002; Drewe et al. 2009; n=0). Animals were euthanatized via intracardial injection of pentobarbitone (Euthapent®, Kyron; 200 mg/kg) while still anaesthetized. An additional three animals were found dead and opportunistically included in the study.

At postmortem examination, animals (n=12) were examined externally and skinned to sample peripheral lymph nodes. The abdomen and thorax were opened to evaluate all inner organs and lymph nodes macroscopically. Aliquots of macroscopically visible organ and lymph node lesions were collected and stored frozen in 10% buffered formalin. Macroscopically normal lymph nodes were pooled as head (mandibular, parotid, retropharyngeal), peripheral (superficial cervical, axillary, inguinal, popliteal), thoracic (mediastinal, sternal, tracheobronchial), and abdominal lymph nodes (mesenteric, gastric, hepatic, renal) and stored at –80 C.

For histopathologic evaluation, the samples of macroscopic evident lesions stored in 10% buffered formalin were sectioned at 4–6 μm, stained with H&E and Ziehl–Neelsen stain and evaluated microscopically for tuberculosis-like lesions and acid fast bacilli by the Pathology Section, Department of Paraclinical Sciences, Faculty of Veterinary Science, according to standard operational procedures.

Mycobacterium culture

Laryngotracheal and fecal swab samples (n=73) were collected by brushing sterile swabs, wetted with 0.9% saline, over the laryngeal mucosa or inserting them into the rectum. After saturation with sterile 0.9% saline, swabs were stored in a 1.8-mL cryotube (Cryo.s^{TM}, Greiner Bio-One, Kremsmünster, Austria) at –80 C. Tracheal lavages (n=65) were collected with animals in sternal, slightly downward angled recumbency. Adapting from Drewe et al. (2009), using a sterile 3-mL syringe (Terumo®) and 18–22 gauge intravenous catheter (Jelco® IV Catheters, Smiths Medical UK, Ashford, UK) without stilette, 2 mL of sterile 0.9% saline was instilled into the trachea and immediately retrieved by gentle suction, then stored at –80 C. Fecal samples (n=9) were collected opportunistically from the capture cage.

Laryngotracheal swabs, tracheal lavages, fecal swabs, fecal samples, and frozen tissue samples were processed and cultured as described by Warren et al. (2006) using mycobacterial growth indicator tubes (BD Biosciences, San Jose, California, USA) together with the automated Bactec 960 TB system (BD Biosciences). Positive samples were screened for contamination using Ziehl–Neelsen staining (Kent and Kubica 1985). For mycobacterial identification, 1 mL of uncontaminated culture was boiled for 1 h at 95 C. For PCR targeting, 16S rRNA and *gyr*B gene, 1 μL of DNA template, 2.5 μL of 10× PCR buffer, 2 μL 25 mM $MgCl_2$, 1 μL 10 mM dNTPs, 5 μL Q-buffer, 0.5 μL of each primer (50 pmol/μL) (16S rRNA forward AGA GTT TGA TCC TGG CTC AG, 16S rRNA reverse GCG ACA AAC CAC CTA CGA G, *gyr*B forward TCG GAC GCG TAT GCG ATA TC, *gyr*B reverse ACA TAC AGT TCG GAC TTG CG), 0.125 μL HotStarTaq DNA polymerase (Qiagen, Hilden, Germany), and 12.375 μL double distilled water were used. DNA template from *M. tuberculosis* H37Rv served as positive control, and no template was added as negative control. For amplification, *Taq* polymerase was activated at 95 C for 15 min, followed by 45 cycles of 94 C for 30 s, 60 C for 1 min, and 72 C for 30 s and final elongation at 72 C for 10 min. As previously described, the 16S rDNA (Harmsen et al. 2003) and *gyr*B gene (Huard et al. 2006) were sequenced by the Central Analytical Facility of Stellenbosch, South Africa.

Serologic analysis

A maximum of 1.5 mL of blood was collected aseptically from the brachycephalic vein with a 23- to 21-gauge needle (Terumo, Somerset, New Jersey, USA) into a serum and lithium heparin MiniCollect® tube (Greiner Bio-One). After

centrifugation for 10 min at 1,008 × G (DYNAC 10,000 rpm Centrifuge 420101, Clay Adams, Becton Dickinson and Company, Franklin Lakes, New Jersey, USA), serum and plasma were harvested in 60–100-µL aliquots and stored at –80 C.

As per test kit instructions, the lateral flow immunochromatographic ElephantTB STAT-PAK Assay (Chembio Diagnostic Systems 2007) (n=75) was used on freshly sampled lithium heparin blood, detecting antibodies to early secretory antigenic target 6 kDa (ESAT-6), culture filtrate protein 10 kDa (CFP10), and mycobacterial protein bovis 83 (MPB83) (Lyashchenko et al. 2012). Plasma of STAT-PAK Assay–positive animals (n=12) was tested with the dual-path platform Chembio DPP® VetTB Assay for elephants (DPP; Chembio Diagnostic Systems 2012), detecting antibodies for antigen MPB83 in test area 1 (DPP 1) and CFP10/ESAT-6 fusion protein in test area 2 (DPP 2). Reactions were graded as very strong positive (3+, test stronger than control line), strong positive (2+, test as strong as control line), positive (1+, test weaker than control line after 20 min), weak positive (+, test weaker than the control line at 30 min), and negative (only control line at 30 min).

Serum samples (n=74) were analyzed by Enfer Scientific (Newhall, Naas, County Kildare, Ireland) using the Enferplex™ Bovine TB Assay (Whelan et al. 2008), a multiplex enzyme-linked immunosorbent assay using ESAT-6, ESX-1 secretion system protein (Rv3616c), MPB83, CFP-10, mycobacterial protein bovis 70 (MPB70), and MPB70 peptide. To adjust for the small sample volume compared with bovine samples, banded mongoose sample analysis was modified as follows: 1:500 serum sample diluted with Enfer sample dilution buffer (Enfer Scientific), 50 µL diluted sample added per microplate well precoated with the multiple antigens (Enfer Scientific), 60 min of incubation at 25 C, washing with and removal of Enfer wash buffer, 50 µL of Protein G (Pierce® Protein Biological Products, Thermo Fisher Scientific Inc., Rockford, Illinois, USA) in detection antibody dilution buffer (1:5,000) added, 30 min incubation at 25 C, washing as above, and 50 µL chemiluminescent substrate–diluent mixture (50:50) added. Chemiluminescence signals of 45 s were captured by a Quansys Biosciences Imaging system (Quansys Biosciences, Logan, Utah, USA). Data were extracted as relative light units with custom software (Quansys Q-View software, version 2.0) and analyzed as described by Whelan et al. (2008). A positive result was defined as a reaction above the threshold toward at least one antigen.

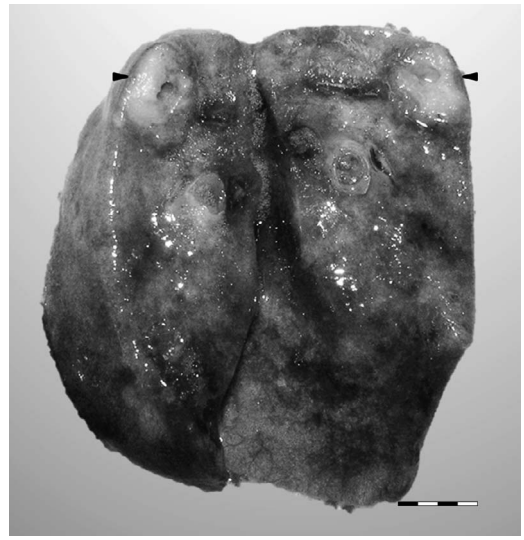

FIGURE 1. Arrowheads (▶) indicate a granulomatous lesion with central necrosis in the right caudal lung lobe (cut surface view) of a banded mongoose (*Mungos mungo*) from Kruger National Park, South Africa. Bar=4 mm.

Statistical data analysis

Categorical data were described as frequencies and percentages; quantitative data were described as medians and ranges. The bTB-positive category included animals with any positive serologic test result or a culture positive for *M. bovis*. Categorical variables were compared using chi-square and Fisher's exact tests and quantitative variables using Mann-Whitney *U*-tests. Statistical analysis was performed using IBM SPSS Statistics Version 22 (IBM Corp., Armonk, New York, USA) and Epi Info, version 6.04, (Centers for Disease Control, Atlanta, Georgia, USA) at the Department of Production Animal Studies of the Faculty of Veterinary Science. Statistical significance was set at $P<0.05$.

RESULTS

Macro- and histopathology

On postmortem examination, 1 (A56) of 12 animals had nine and one (A57) animal had one granulomatous lesion. The lesions consisted of multifocal, well demarcated, round, irregularly margined, gray-yellow nodules of up to 5 mm diameter, which were predominantly located in the caudal lung lobes (Fig. 1) but also in the retropharyngeal, tracheobron-

chial, and superficial cervical lymph node (LN) (Table 1). The larger lesions showed central necrosis or calcification. Histopathologically these lesions were scattered caseating foci of necrosis associated with epithelioid macrophages or scattered foci of mucosal calcification in the lumen of some bronchioles, as well as necrogranuloma with calcified center in the LNs. Ziehl–Neelsen staining did not detect acid fast organisms in any specimens. The majority of animals (75%) examined on postmortem examination had multifocal to miliary (1–3 mm), well demarcated, crème-colored, spherical foci of necrosis in all liver lobes (Table 1).

Mycobacterium culture

We isolated *M. bovis* from 2 (3%) of 76 banded mongooses (Table 2; animals A56 and A57). Various nontuberculous mycobacteria (NTMs) were isolated from 48 (63%) individuals, whereas no mycobacteria were recovered from 28 (37%) individuals. We isolated *M. bovis* from one tracheal swab (A56), two tracheal lavages (A56, A57), and two abdominal (A56, A57), one retropharyngeal (A56), and one tracheobronchial LN (A57) (Table 2), as well as one liver (A57) and one lung sample (A57) (Tables 1, 2).

The two *M. bovis*–infected banded mongooses (A56 and A57) were concurrently infected with members of five *Mycobacterium* groupings, namely the *M. simiae* group (from pooled head and a retropharyngeal LNs), *M. avium* complex (from tracheobronchial, mandibular, retropharyngeal, and cervical superficial LNs), *M. szulgai* (from pooled peripheral LNs), and *M. parascrofulaceum* (from mandibular and peripheral LNs) (Table 1). Members of the *M. fortuitum* group were isolated from a fecal swab (Table 1).

Serologic analysis

The STAT-PAK assay was performed for 75 banded mongooses. Twelve individuals (16%) were positive, with reaction intensities from weak positive (+: n=5), positive (1+: n=4), strong positive (2+: n=1), to very strong positive (3+: n=2) (Table 3). Stored heparin plasma of these 12 individuals also were positive for test line one (DPP 1) coated with protein MPB83 (Table 3) when using DPP VetTB Assay. Only 10 of the 12 samples were positive for test line two (DPP 2) (Table 3) coated with CFP10/ESAT-6 fusion protein. When comparing the reaction intensities of STAT-PAK and DPP tests, only two individuals (A56 and A57) showed a very strong reaction (3+) and one (A1) a strong reaction (2+) on both STAT-PAK and DPP 1. Because the DPP test, which is considered confirmatory to the STAT-PAK assay, did not rule out any of the weak or very weak positive STAT-PAK results, all 12 STAT-PAK results were considered positive.

The Enferplex assay was performed on 74 serum samples and revealed five (7%) positive reactors to antigens MPB83 (n=4) and MPB70 peptide (n=1) (Table 3). All samples reacted to one antigen only, and no antibodies were detected against antigens ESAT-6, Rv3616c, CFP-10, or MPB70.

Statistical evaluation of serologic test performance was not possible because of the small number of *M. bovis* culture–positive animals.

Comparison of demographics and clinical findings with *Mycobacterium* status

The bTB status was evaluated statistically with the quantitative variables of age and weight and categorical variables location, signalment, health, and coinfection with NTMs. The only significant findings were that bTB-positive banded mongooses were older (P=0.025) and weighed more (P=0.008) than bTB-negative animals.

Both animals from which *M. bovis* was cultured, originated from the troop frequenting the staff dorm rooms, administrative and operational buildings, and tourist day visitor area, but there was no statistical association of the capture location with the bTB-positive group (P=0.463).

In summary, two banded mongooses were antibody positive on all three serologic assays. These were the only two animals from which *M. bovis* was isolated from antemortem and postmortem samples and that had lesions of

TABLE 1. *Mycobacterium* culture results for antemortem samples (tracheal lavage, tracheal swab, fecal swab) and postmortem lesions (lymph node [LN], lung, liver) of South African banded mongooses (*Mungos mungo*) A56 and A57 compared with macropathology and histopathology. Dash = no sample.

ID[a]	Tracheal lavage	Tracheal swab	Fecal swab	LN head	LN retropharyngeal	LN tracheobronchial	LN abdominal	LN peripheral	LN superficial cervical	Lung	Liver
A56											
C	*M. bovis*	*M. bovis*	*M. fortuitum* group	*M. simiae* group	*M. bovis*	*M. avium* complex	*M. bovis*	*M. szulgai*	Negative	Negative	Negative
M	—	—	—	Negative	Granuloma calcified	Granuloma calcified	Negative	Negative	Granuloma calcified	Granuloma calcified	Moderate liver necrosis
H	—	—	—	—	Necrogranuloma calcified[b]	Necrogranuloma calcified[b]	—	—	Necrogranuloma calcified[b]	Mucosa calcified	Moderate liver necrosis
A57											
C	*M. bovis*	Negative	Negative	*M. intracellulare*, *M. parascrofulaceum*[c]	*M. simiae*, *M. intracellulare*	*M. bovis*	*M. bovis*	*M. parascrofulaceum*	*M. intracellulare*	*M. bovis*	*M. bovis*
M	—	—	—	Hyperplasia[c]	Hyperplasia	Negative	Negative	Negative	Hyperplasia	Granuloma	Severe liver necrosis
H	—	—	—	Negative[c]	—	—	—	—	—	Caseating necrosis, epithelioid macrophages[b]	Severe liver necrosis

[a] ID = study animal number; C = culture; M = macropathology; H = histopathology.

[b] Negative for acid fast bacteria.

[c] Mandibular LN.

TABLE 2. *Mycobacterium* culture results by sample type for 76 banded mongooses (*Mungos mungo*) from Kruger National Park, South Africa, 2011–12.[a]

	Sample type									
	Tracheal swab	Tracheal lavage	Fecal swab	LNa	LNh	LNp	LNt	Liver	Lung	Spleen
M. bovis	1	2	0	2	1	0	1	1	1	0
NTM	19	13	42	6	9	9	7	0	2	2
Culture negative	52	50	31	4	2	3	4	7	5	7
Total no. of animals	72	65	73	12	12	12	12	8	8	9

[a] NTM = nontuberculous *Mycobacterium*; LN = pooled lymph nodes; a = abdomen; h = head; p = periphery; t = thorax.

granulomatous character. No other members of the MTC were detected.

DISCUSSION

Mycobacterial culture of ante- and postmortem samples confirmed that banded mongooses in the vicinity of the Skukuza Rest Camp in the southern KNP were infected with *M. bovis*. The most likely mode of transmission in these banded mongooses seemed to be intraspecies aerosol transmission, because lesions only occurred in the lungs or lung and head lymph nodes, and because the two *M. bovis*–infected animals came from the same troop. These findings are comparable to those in the European badger, brushtail possum (Palmer 2013), and suricate (Drewe et al. 2011), with respiratory shedding the main mode of intraspecific transmission, facilitated by contact during nursing, intensive grooming, or when sleeping in confined spaces (Drewe et al. 2009, 2011).

Isolation of *M. bovis* from abdominal LNs and liver samples indicated some degree of oral pathogen transmission. Alternatively, *M.*

TABLE 3. Comparison of banded mongooses (*Mungos mungo*) with positive STAT-PAK, DPP, or Enferplex test result.[a]

	STAT-PAK	DPP		Enferplex		
ID	(ESAT-6+CFP10+MPB83)	DPP1 (MPB83)	DPP2 (CFP10/ESAT-6)	MPB70 peptide	MPB83	Other
A56	3+	3+	1+	−	+	−
A57	3+	3+	2+	−	+	−
A1	2+	2+	−	−	−	−
A8	1+	3+	1+	−	−	−
A29	1+	2+	1+	−	−	−
A24	1+	2+	(+)	−	−	−
A5	1+	(+)	(+)	−	−	−
A52	(+)	2+	2+	−	−	−
A22	(+)	2+	−	−	−	−
A63	(+)	1+	2+	−	−	−
A31	(+)	1+	2+	−	−	−
A2	(+)	1+	1+	−	−	−
A49	−	NT	NT	−	+	−
A78	−	NT	NT	−	+	−
A70	−	NT	NT	+	−	−

[a] ID = study animal number; DPP 1 = test line one of DPP assay; DPP 2 = test line two of DPP assay; Other = ESAT-6, Rv3616c, CFP-10, MPB70; (+) = weak positive; 1+ = positive; 2+ = strong positive; 3+ = very strong positive; − = negative; + = positive; NT = not tested.

bovis isolated from the mesenteric LNs could have originated from swallowed organisms shed from the respiratory tract. Hematogenous spread after respiratory infection seemed unlikely because lesions were not as widely distributed throughout the body as described by Alexander et al. (2002). The oral route of infection from infected food, as seen in lions and leopards (Keet et al. 1996, 2010), but also from urine, feces, or sputum-contaminated food or water (Michel et al. 2006), indicates possible interspecies transmission of *M. bovis*. Whereas banded mongooses possibly become infected orally, failure to isolate *M. bovis* from fecal swabs or fecal samples may indicate a lower risk of fecal-oral compared with aerosol spread discussed earlier.

We saw no indication of percutaneous infection for the banded mongooses in this study. We noted no nasal lesions, as described by Alexander et al. (2010) for infection of banded mongooses with *M. mungi*, and no skin lesions resembling bite wounds, and *M. bovis* was not isolated from peripheral LNs. This is contrary to reports from Drewe et al. (2009) for suricates, where intraspecific grooming led to percutaneous infection and the development of draining fistulas in superficial LNs. This might be because of a lack of grooming injuries in the KNP's banded mongooses or due to shedding loads being too small for transdermal infection while reaching the threshold dose for aerosol transmission, the latter being the smallest when compared with oral or transdermal infection (Bengis 1999).

With regard to possible sources of infection, we believe that the buffalo population around Skukuza with an estimated bTB prevalence of 38.2% (Rodwell et al. 2001) constitutes the most likely direct or indirect source of *M. bovis* to banded mongooses. Through their scavenging behavior, banded mongooses may be exposed to alimentary or respiratory infection with *M. bovis* from contaminated feces (Skinner and Chimimba 2005), carcasses, or abattoir scraps from the Skukuza Game Processing Plant, as postulated for a resident troop of baboons (*Papio ursinus*) (Keet et al. 2000). Contemporary outbreaks of bTB had been recorded in the study area in the local baboon troop, as well as resident warthogs (*Phacochoerus africanus*) (De Klerk pers. comm.). Interestingly, the two infected banded mongooses of this study roamed and slept in proximity to the bTB infected baboon troop (Keet et al. 2000; De Klerk pers. comm.), raising the question of infection through a common source or the possibility of a new spillover host relationship between baboon and banded mongoose.

We regarded the human-wildlife interface as the less likely source of infection, because no *M. tuberculosis* or other member of the MTC causing TB in humans were isolated in this study.

Infection of banded mongooses and their potential for shedding *M. bovis* as demonstrated by its isolation from tracheal samples raised the question of survival time and persistence in the environment. Supported by recent evidence of predominantly indirect transmission of bTB between the European badger and cattle in the UK (Drewe et al. 2013), environmental contamination might play an important and as yet underestimated role in bTB epidemiology at the wildlife interface in South Africa, highlighting the potential threat of *M. bovis* spillover to threatened or endangered wildlife species. Whereas survival times of *M. bovis* in the environment for up to 15 mo after host removal have been recorded in the UK (Sweeney et al. 2007), environmental conditions in South Africa might differ. Further studies, in addition to those conducted by Tanner and Michel (1999), are needed to elucidate the South African situation more accurately. Novel techniques such as immunomagnetic capture using a monoclonal antibody against MPB83, enabling *M. bovis* isolation directly from environmental samples, or real-time PCR detecting and quantifying *M. bovis* contamination (Sweeney et al. 2007) would shed light on the role of environmental contamination in bTB epidemiology in the KNP.

Histopathology confirmed the macroscopic lung and LN lesions to be caseating or calcifying necrogranulomas, which, associated

with epithelioid macrophages, are a typical manifestation of *Mycobacterium* infection (Dahme and Weiss 1999). Mucosal calcification of bronchioles, however, is rarely diagnosed in domestic animals and, in the banded mongoose, might be secondary to dystrophic processes of unknown etiology (Stünzi and Weiss 1990).

We considered the liver necrosis to be unrelated to mycobacterial infection, because 9 of 12 animals examined postmortem showed similar macroscopic lesions, whereas only one of nine samples of liver lesions was *M. bovis* culture positive. A possible parasitic origin of liver necrosis could not be confirmed because no parasites were detected in the liver, and the only three animals with histologically evident intestinal helminth nodules of unidentified species had developed no or only mild miliary liver necrosis.

Our failure to demonstrate acid fast bacilli with Ziehl–Neelsen staining suggests a paucibacillary process in the banded mongoose, which might explain why some granulomatous lesions yielded a negative *M. bovis* culture result and is consistent with low screening sensitivity of macro- and histopathology previously described by O'Brien et al. (2013) for routine LN sampling (Schmitt et al. 2002).

Comparing serologic test results, antibodies predominantly against MPB83, correlating with *M. bovis* antibody reaction (Lyashchenko et al. 2012), were detected with STAT-PAK, DPP, and Enferplex. Only the two animals with macroscopic lesions yielded *M. bovis* on culture, both from lesions as well as antemortem sampled tracheal lavage and swab, suggesting that animals with negative antemortem *Mycobacterium* culture are false-positive serologic test results. Only serial interpretation of STAT-PAK and Enferplex together or a high test reaction intensity would correlate with a positive *M. bovis* culture result. We therefore concluded that only a combination of STAT-PAK or DPP with Enferplex interpreted in series seemed to identify *M. bovis*–infected animals correctly.

Even though isolation of MTC organisms remains the gold standard for TB diagnosis (OIE 2009), our serologic diagnosis was confirmed by *M. bovis* recovery from tracheal lavage and swab. We therefore recommend this test combination for nonlethal sampling and monitoring purposes, which also enhances the likelihood of making the correct diagnosis (Drewe et al. 2009). From a practical perspective, limited availability of serologic test kits might restrict monitoring programs in South Africa to rely on culture of antemortem samples.

In conclusion, *M. bovis* infection in the banded mongoose population in the vicinity of the Skukuza Rest Camp was detected by serology and macro- and histopathology and confirmed by culture. A test combination consisting of STAT-PAK, Enferplex, and *Mycobacterium* culture from tracheal lavage and swabs for bTB monitoring has potential and warrants further investigation.

The way forward would be to confirm the source and route of infection, (e.g., by identifying the *M. bovis* genotype) in order to elucidate the epidemiology of bTB in these small predators and their role in bTB epidemiology in the KNP.

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