Tuberculosis infection in wildlife from the Ruaha ecosystem Tanzania: implications for wildlife, domestic animals, and human health

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SUMMARY

Mycobacterium bovis, a pathogen of conservation, livestock, and public health concern, was detected in eight species of wildlife inhabiting protected areas bordering endemic livestock grazing lands. We tested tissues from 179 opportunistically sampled hunter-killed, depredation, road-killed, and live-captured wild animals, representing 30 species, in and adjacent to Ruaha National Park in south-central Tanzania. Tissue culture and PCR were used to detect 12 (8·1%) *M. bovis*-infected animals and 15 (10·1%) animals infected with non-tuberculosis complex mycobacteria. Kirk's dik-dik, vervet monkey, and yellow baboon were confirmed infected for the first time. The *M. bovis* spoligotype isolated from infected wildlife was identical to local livestock, providing evidence for livestock–wildlife pathogen transmission. Thus we advocate an ecosystem-based approach for bovine tuberculosis management that improves critical ecological functions in protected areas and grazing lands, reduces focal population density build-up along the edges of protected areas, and minimizes ecological stressors that increase animals' susceptibility to bovine tuberculosis.

Key words: One health, tuberculosis (TB), veterinary epidemiology and bacteriology, wildlife disease, zoonoses.

INTRODUCTION

Tuberculosis (TB) is one of the most widespread infectious diseases and a leading cause of death for adults worldwide [1]. Although much attention has focused on treatment and prevention of human TB caused by *Mycobacterium tuberculosis*, zoonotic TB due to

Mycobacterium bovis, known as bovine tuberculosis (bTB), has become an important re-emerging public health concern in developing countries. A large number of livestock keepers (pastoralists) combined with poor public health infrastructure, limited bTB control measures for cattle and animal products, and a large immunocompromised human population due to HIV/AIDS make Africa particularly vulnerable to the health impacts of bTB [2]. Zoonotic bTB infection is significant, as *M. bovis* is naturally resistant to

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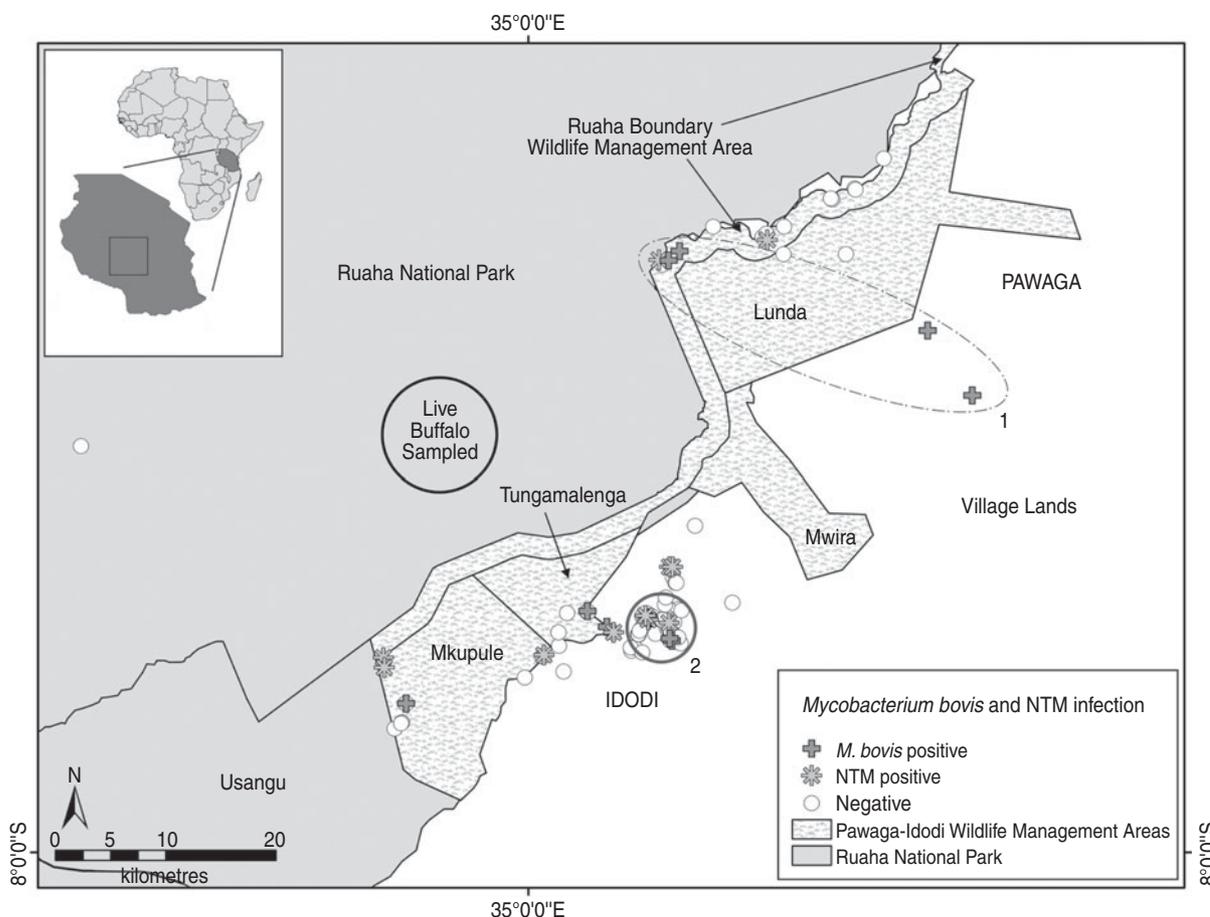
Fig. 1. Spatial distribution of *M. bovis* and non-tuberculosis complex mycobacteria (NTM) infected and uninfected wildlife carcasses ($n = 121$), and the approximate sampling area where 30 live buffalo were tested for bovine tuberculosis in a livestock–wildlife interface area in the southern portion of the Ruaha ecosystem, south-central Tanzania. Although not statistically significant, an elliptical (no. 1, $P = 0.057$) and circular (no. 2, $P = 0.066$) shaped region of a higher than expected number of *M. bovis* cases (nos. 1 and 2), as indicated by spatial scan statistics are shown. The geopolitical division of village lands (Idodi or Pawaga) is shown in capital letters.

pyrazinamide, a first-line TB treatment drug [1]. Both of these TB-complex mycobacteria have demonstrated the ability to move between animal (including wildlife) and human populations [3, 4].

Bovine TB has also emerged as a disease of concern for wildlife conservation. Spillover from bTB-infected cattle herds was thought to have resulted in bTB infections in African buffalo (*Syncerus caffer*) inhabiting the southern portion of Kruger National Park (KNP), South Africa (Kloeck, 1998 cited in [5]). Within 15 years of the discovery of the first cases in KNP in 1990, bTB had spread through most of park's buffalo herds [6], and infections were documented in 10 other species [5]. Reported impacts of bTB in KNP and other South African parks included decreased body condition and drought tolerance of infected buffalo [7], lowered buffalo reproductive success [8], and mortality and disruption of pride

dynamics in infected lion prides [5]. In East Africa, bTB infections have been documented since the 1960s in buffalo in Uganda [9, 10] and recently in wildebeest (*Connochaetes taurinus*), topi (*Damaliscus lunatus*), and one lesser kudu (*Tragelaphus imberbis*) in northern Tanzania [11]. However, little is known about whether or not bTB is present in other ecosystems, and population-level impacts of bTB have not been characterized.

The Ruaha ecosystem of south-central Tanzania encompasses vast wildlife protected areas of high conservation value bordered by lands inhabited by large numbers of livestock. Farmers, traditional livestock keepers, livestock, and wildlife inhabiting the southern portion of the Ruaha ecosystem all depend upon water from the Great Ruaha River and its tributaries (Fig. 1). Over time, human migration due to protected area creation and other government resettlement

programmes [12] coupled with the seasonal drying of the Great Ruaha River largely due to upstream diversion for agriculture [13] has altered the distribution of people, livestock, and wildlife. Heightened competition for land and water resources has increased wildlife conflict and concerns that increased overlap among human, wildlife, and livestock populations in Ruaha may be increasing the risk of zoonotic disease transmission including bTB [14, 15].

Despite data indicating widespread bTB infection in cattle surrounding Ruaha's wildlife protected areas, it was not known if wildlife in the Ruaha ecosystem had been infected. Prevalence of bTB in cattle grazing the southeastern portion of the Ruaha ecosystem was estimated to be 13%, with 51% of sampled herds containing one or more positive reactors [16]. Previous wildlife data from Ruaha were limited to results from a sample of six wild animals [five elands (*Taurotragus oryx*) and one roan antelope (*Hippotragus equinus*)] that were serologically negative by enzyme immunoassay [11]. Accordingly, we set out to determine if bTB was present in Ruaha's wildlife as part of a large-scale project assessing the impact of zoonotic diseases at the rapidly changing environmental interface of human, livestock, and wildlife populations in the Ruaha ecosystem.

We hypothesized that given widespread bTB presence in cattle, bTB would also be present in wildlife species inhabiting the livestock–wildlife interface areas south of Ruaha National Park (RNP) and that wildlife species would be infected with the same strain of *M. bovis* found in local cattle. We also examined the species and spatial distribution of *M. bovis* wildlife infections to determine potential for pathogen persistence in wildlife maintenance hosts, and identify potential high-risk areas for transmission that could be targeted as part of an ecosystem-based approach to reduce transmission of disease at the livestock–wildlife interface and improve animal and human health.

METHODS

Study area

The southern extent of the Ruaha ecosystem lies in northern Iringa District, south-central Tanzania (07°19′S to 07°36′S and from 35°05′E to 35°29′E). It covers about 30 000 km^2 of different rangeland-use areas, RNP, the Rungwa, Kisigio and Muhesi game reserves, the Lunda-Mkwambi Game Controlled Area, the recently formed community-based Pawaga–Idodi

Wildlife Management Area (PIWMA), and village lands. The area is internationally significant in terms of biodiversity conservation because it contains the only protected area system covering the transition between the vegetation communities of the Sudanian *Acacia-Commiphora* zone of East Africa and the *Brachystegia* (miombo) woodlands of southern Africa [12]. Our study was concentrated in the southernmost portion of the Ruaha ecosystem, comprised of rural villages, the PIWMA, and RNP (Fig. 1). Habitat consisted of patchily distributed semi-arid woodland and brushland, and active and fallow agriculture fields. Lands bordering the wildlife protected areas are heavily grazed, as evidenced by denuded vegetation, bare patches of soil, and the presence of many livestock and livestock faeces.

Ethics statement

All research activities in Tanzania were reviewed, approved, and permitted by the Tanzania Commission on Science and Technology (COSTECH), the Tanzania Wildlife Research Institute (TAWIRI), Tanzania National Parks (TANAPA), and University of California Davis Institutional Animal Care and Use Protocols nos. 12394 and 15919. No animals were killed for the purposes of this study.

Animal sampling

From 2006–2010, lungs, mediastinal and mesenteric lymph nodes, and other tissue samples from hunter-killed wildlife, opportunistically found carcasses, and wildlife depredated for causing crop damage in and around the PIWMA and RNP were collected by community game scouts trained to safely collect specimens, project veterinarians (D. Clifford, H. Sadiki), or technicians. The date, sex, age, and species were recorded for each animal sampled. Whenever possible the location of the carcass (latitude and longitude in decimal degrees, World Geographic System 1984 datum) was recorded using a handheld global positioning system (GPS) unit. For carcasses where GPS locations were not recorded, the nearest village or a locally known place name within the protected area was recorded by the scout. Collected tissues were examined for gross lesions and subsectioned: the outer surface of the tissue was seared, and then a sample for culture was collected using forceps that were chemically disinfected and a new sterile disposable scalpel blade for each animal. Specimens for

culture were placed into sterile Whirlpak® plastic collection bags (Nasco, USA). Samples were frozen on average within 24 h of collection and then stored frozen at −20 °C until testing.

Additionally, we received heparinized blood samples from 30 live African buffalo immobilized in RNP in 2011 during foot-and-mouth disease (FMD) surveillance efforts conducted by the South African Development Community and the Tanzanian Ministry for Livestock. Buffalo belonging to the Msembe herd that ranges in the southern portion of RNP were opportunistically immobilized via dart gun delivered from a helicopter. Additional buffalo herds inhabiting other areas of RNP were not sampled. Only buffalo aged 2–4 years were captured and sampled as they were the target age group for FMD testing.

Diagnostic testing

Culture and molecular diagnostics – wildlife tissues

Frozen tissues were thawed to room temperature, then pooled lung and lymphoid tissues were homogenized, decontaminated, and neutralized using standard methods [17]. Resulting sediments were inoculated onto Lowenstein–Jensen media with pyruvate and Lowenstein–Jensen media with glycerol and incubated at 37 °C for up to 12 weeks. Positive cultures with appropriate colony morphology [18] were subcultured onto another set of the same media for 3–4 weeks to obtain pure culture and then examined by microscope for the presence of acid-fast-bacilli (AFB) using Ziehl−Neelsen stain.

Heat-killed AFB-positive samples were further characterized by multiplex polymerase chain reaction (PCR), using primers to the 16S rRNA gene specific for the *Mycobacterium* genus and able to distinguish between *M. avium* and *M. intracellulare*, and primers aimed at the MPB70 gene of *M. tuberculosis* complex (MTC) organisms [19]. Samples with an amplification product of 1030 bp indicative of the genus *Mycobacterium* and of 372 bp were considered positive signals for MTC.

Spoligotyping was used to delineate the *Mycobacterium* species for animals with MTC and distinguish unique strain types. Briefly, PCR products representing all the spacer sequences in an isolate's genome were amplified using primers specific for the direct-repeat sequences of the direct repeat locus of the MTC chromosome. Hybridization of the amplification product mixture against a membrane to which the 43 individual spacer sequences were covalently linked was used to generate a specific pattern of positive and negative hybridization signals [20]. The absence of spacers 3, 9, 16, and 39–43 was used to classify isolates as *M. bovis* [20, 21]. The *M. bovis* spoligotype patterns from wildlife samples were coded [22] and compared to spoligotypes SB0133 and SB0425 which were dominant in the cattle isolates from a study in an adjacent area in Mbeya and Iringa regions [23], and in 2007 from a cow from a village in Pawaga Division on the southern border of the PIWMA and RNP (Fig. 1).

Serology – live buffalo samples

Blood samples from live-captured buffalo were tested for bTB infection using a commercially available *M. bovis* gamma interferon (INF-γ) test kit, that utilizes a monoclonal antibody-based sandwich enzyme immunoassay to detect the production of INF-γ (Bovigam®, Prionics, Switzerland). Within 8 h of collection, heparinized blood samples from each animal were subdivided into three aliquots that were mixed with phosphate-buffered saline (nil antigen), bovine and avian purified protein derivative (tuberculin; Veterinary Laboratory Agency Weybridge, UK), respectively, and then incubated for approximately 20 h at 37 °C. After incubation, plasma was harvested by centrifugation, frozen at −20 °C, and transported to Sokoine University of Agriculture where the Bovigam assay was performed according to the manufacturer's instructions. All samples were tested in duplicate and positive and negative controls supplied by the manufacturer included on each 96-well plate. Animals were classified as positive bTB reactors if $OD_{bovine} − OD_{control}$ was ≥ 0.049 and if OD_{bovine} was greater than OD_{avian} according to Whipple *et al.* [24], with additional consideration given to samples with $OD_{bovine} \geq 0.385$, as this value optimized test predictive value for sampled buffalo in South Africa [25].

Data analysis

Field and laboratory data were entered into an electronic database (Microsoft Excel, USA). The proportion of AFB-positive, *M. bovis*-infected, and non-TB complex mycobacteria (NTM) infected animals was calculated for all animals tested and for each species. Associations between *M. bovis* and

NTM infection proportions, species group (defined as hoofstock, carnivores, primates, or small mammals), age (young *vs.* adult), sex, and sampling location (inside *vs.* outside of a wildlife protected area) were examined using Fisher's exact tests using Stata version 11.2 software (StataCorp., USA).

For carcasses that had known locations, a spatial scan statistic was used to determine if *M. bovis* infections were distributed randomly over space in our sampling area, and if not, to evaluate any spatial infection clusters for statistical significance (SaTScan version 9.1.1, M. Kulldorff, Harvard Medical School, Boston, MA, USA) [26]. A Bernoulli model utilizing case-control (0/1) data was used, as it does not assume a homogenous distribution of the underlying population. A circular or elliptically shaped moving window was used to scan for spatial clusters encompassing from zero to not more than 50% of the locations by comparing the observed infections inside *vs.* outside the window using likelihood functions [27]. Both the circular and elliptical window options were evaluated because the elliptical window allows for a better fit to linear geographical features, including roads and rivers, which may be associated with the location of carcass recovery due to ease of access [28]. Maximum-likelihood estimates were generated by Monte Carlo simulations of 999 iterations and clusters evaluated for significance with $P = 0.05$. Spatial scan statistics were not used to evaluate clusters of NTM infections as location data was not known for all NTM-infected individuals.

RESULTS

Culture and molecular diagnostics – wildlife tissues

Tissue samples from 149 animals comprising at least 30 different species were collected. The exact number of species represented in the sample was not known as the specific species was not identified for three squirrels and 10/11 sampled mongooses. Kirk's dik-dik (*Madoqua kirkii*) and impala (*Aepyceros melampus*) were the two most commonly sampled species, comprising 36% of the total sample (Table 1). Hoofstock species comprised 59% of the sample ($n = 88$), with the remainder of sampled animals comprised of 24% carnivores ($n = 36$), 12% primates ($n = 18$), 4% small mammals ($n = 6$) and a single elephant. Adult animals were most commonly sampled ($n = 125$, 84%); 15 young animals were sampled, and age was not recorded for nine animals. Male animals

comprised 70% of the animals sampled ($n = 105$); 33 females and 11 animals not having sex identified comprised the remainder of the sample. Variable numbers of samples were collected each year [2006 ($n = 19$); 2007 ($n = 20$), 2008 ($n = 6$), 2009 ($n = 96$), 2010 ($n = 8$)]. Of 124 animals whose exact or approximate locations were known, 38 (31%) were located within a wildlife protected area [PIWMA ($n = 37$), RNP ($n = 1$)]; while the remaining 86 individuals were located outside protected areas on village lands. Hoofstock were more likely to be sampled within protected areas than carnivores (one-sided Fisher's exact $P = 0.048$) and primates (one-sided Fisher's exact $P = 0.009$). All small mammals were sampled outside of protected areas.

Cultures from 34 animals had positive AFB growth and were further characterized using the mycogenus PCR (Table 1). Of the 27 samples that produced amplification products, 12 belonged to the MTC, and 15 were NTM. *M. avium* and *M. intracellulare* were not identified. Yellow, white, or grey nodules were noted on gross examination of tissues from eight animals, but only one animal with gross lesions, an African lion (*Panthera leo*), had AFB growth on culture (Table 1).

Spoligotyping allowed classification of all 12 MTC isolates as *M. bovis* as they lacked spacers 3–7, 9, 16, and 39–43 (Table 2) [20, 21]. *M. bovis* was isolated from 8% (12/149) of individuals sampled comprising eight species (Table 1). All 12 *M. bovis* isolates were spoligotype pattern SB0133 (Table 2), a previously recognized pattern in the *M. bovis* online spoligotype database belonging to the African 2 clonal complex of *M. bovis* [29]. The spoligotype pattern of wildlife samples was the same as those previously isolated from cattle sampled in Iringa and adjacent Mbeya region from 1993 to 1995 [23], and the single positive cow from our study area that was slaughtered and sampled in 2007 (Table 2).

Although *M. bovis* infection was documented in 17% (3/18) of sampled primates, the primate infection proportion was not significantly greater than that of sampled hoofstock (8%, 7/88, one-sided Fisher's exact $P = 0.227$), or carnivores (6%, 2/36, one-sided Fisher's exact $P = 0.200$). *M. bovis* infection proportions were equivalent between sampled males (9%) and females (9%, Fisher's exact $P = 1.00$) and there was no difference in *M. bovis* infection proportions between animals sampled inside (8%, 3/38) and outside (10%, 9/86) wildlife protected areas (Fisher's exact $P = 0.754$). Infection with *M. bovis* was only detected in adult animals.

Table 1. *Results of acid-fast bacilli culture and mycobacterium PCR for 149 wild animals sampled from 2006 to 2010 in a livestock–wildlife interface area in and around the Pawaga–Idodi Wildlife Management Area and Ruaha National Park, Iringa Region, south-central Tanzania*

Species	No. tested	No. AFB positive	No. NTM	No. MTC
African buffalo (*Syncerus caffer*)	5	2 (40)	1 (20)	1 (20)
Bushbuck (*Tragelaphus scriptus*)	5	2 (40)	0 (0)	0 (0)
Bush duiker (*Sylvicapra grimmia*)	2	0 (0)	0 (0)	0 (0)
Eland (*Taurotragus oryx*)	1	0 (0)	0 (0)	0 (0)
Greater kudu (*Tragelaphus strepsiceros*)	3	1 (33·3)	1 (33·3)	0 (0)
Kirk's dik-dik (*Madoqua kirkii*)	30	4 (13·3)	2 (6·7)	2 (6·7)
Impala (*Aepyceros melampus*)	24	9 (37·5)	3 (12·5)	3 (12·5)
Lesser kudu (*Tragelaphus imberbis*)	1	1 (100)	0 (0)	1 (100)
Sable antelope (*Hippotragus niger*)	2	0 (0)	0 (0)	0 (0)
Waterbuck (*Kobus ellipsiprymnus*)	1	1 (100)	1 (100)	0 (0)
Bush pig (*Potamochoerus larvatus*)	6	1 (16·7)	1 (16·7)	0 (0)
Common warthog (*Phacochoerus africanus*)	2	0 (0)	0 (0)	0 (0)
Giraffe (*Giraffa camelopardalis*)	2	0 (0)	0 (0)	0 (0)
Common zebra (*Equus quagga*)	4	0 (0)	0 (0)	0 (0)
African elephant (*Loxodonta africana*)	1	0 (0)	0 (0)	0 (0)
Vervet monkey (*Chlorocebus pygerethrus*)	11	2 (18·2)	0 (0)	2 (18·2)
Yellow baboon (*Papio cynocephalus*)	7	2 (28·6)	0 (0)	1 (14·3)
Aardwolf (*Proteles cristata*)	1	0 (0)	0 (0)	0 (0)
African civet (*Civettictis civetta*)	2	0 (0)	0 (0)	0 (0)
Black-backed jackal (*Canis mesomelas*)	1	0 (0)	0 (0)	0 (0)
Blotched genet (*Genetta tigrina*)	11	3 (27·3)	2 (18·2)	1 (9·1)
Caracal (*Felis caracal*)	1	1 (100)	1 (100)	0 (0)
Leopard (*Panthera pardus*)	2	1 (50)	0 (0)	0 (0)
Lion (*Panthera leo*)	3	1 (33·3)	1 (33·3)	0 (0)
Mongoose (*Herpestes* or *Mungos* spp.)	11	2 (18·2)	1 (9·1)	1 (9·1)
Spotted hyena (*Crocuta crocuta*)	1	0 (0)	0 (0)	0 (0)
Zorilla (*Ictonyx striatus*)	3	0 (0)	0 (0)	0 (0)
Squirrel spp. (Sciuridae)	3	1 (33·3)	1 (33·3)	0 (0)
Porcupine (*Hystrix* spp.)	1	0 (0)	0 (0)	0 (0)
Spring hare (*Pedetes capensis*)	2	0 (0)	0 (0)	0 (0)
Total	**149**	**34 (22·8)**	**15 (10·1)**	**12 (8·1)**

AFB, Acid-fast bacilli; NTM, non-tuberculosis complex mycobacteria; MTC, *Mycobacterium* tuberculosis complex.
The number of animals positive/total tested (% positive) for acid-fast bacilli growth on culture, and tuberculosis and NTM is reported. All tuberculosis complex mycobacteria isolated were classified as *Mycobacterium bovis* by spoligotyping.

NTM infection was detected in 10% (15/149) of sampled animals belong to 11 species (Table 1). There was no difference in NTM infection proportion in species groups: 17% (1/6) of small mammals, 14% (5/36) of carnivores, and 10% (9/88) of hoofstock sampled were infected. No NTM infections were documented in sampled primates. NTM infection proportions were similar between sampled males (10%) and females (12%, Fisher's exact $P = 0·758$), and between sampled adults (12%) and young animals (15%, Fisher's exact $P = 0·677$). There was also no difference in NTM infection proportion between animals sampled inside (16%, 6/38) and outside (12%, 7/86) protected areas (Fisher's exact $P = 0·215$).

Exact locations for 12 *M. bovis*-infected animals and 109 uninfected animals were available for spatial analyses. A geographical cluster of four *M. bovis*-infected animals was detected inside a 143 km^2 elliptical area that extended along the road from grazing lands through the Lunda portion of the PIWMA and up to the PIWMA/RNP border (no. 1, Fig. 1), but was not statistically significant ($P = 0·057$). A second smaller 10·9 km^2 circular geographical cluster of three *M. bovis*-infected animals was detected in village lands (no. 2, Fig. 1), but was also not statistically significant ($P = 0·066$). Although neither cluster was statistically significant, less than one infected animal would be expected to have

Table 2. *Spoligotype pattern (SB0133; www.mbovis.org) of 12 Mycobacterium bovis isolates obtained from a sample of 149 wild animals and a domestic cow inhabiting a livestock–wildlife interface area in the southern portion of the Ruaha ecosystem, south-central Tanzania*

Species	Location	Spacers 1–7	8–38	39–43
African buffalo	PIWMA(Lunda)	■■■■■■■	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■	
Kirk's dik-dik	Village (Idodi)	■■■■■■■	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■	
Kirk's dik-dik	Village (Lunda)	■■■■■■■	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■	
Impala	PIWMA (Lunda)	■■■■■■■	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■	
Impala	PIWMA (Mkpule)	■■■■■■■	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■	
Impala	Village (Tungamalenga)	■■■■■■■	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■	
Lesser kudu	Village (Lunda)	■■■■■■■	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■	
Vervet monkey	Village (Idodi)	■■■■■■■	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■	
Vervet monkey	Village (Idodi)	■■■■■■■	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■	
Yellow baboon	Village (Tungamalenga)	■■■■■■■	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■	
Blotched genet	Village (Idodi)	■■■■■■■	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■	
Mongoose sp.	Village (Tungamalenga)	■■■■■■■	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■	
Domestic cow	Village (Pawaga)	■■■■■■■	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■	

The approximate location of each case; Pawaga–Idodi Wildlife Management Area (PIWMA) or village lands (Village), and the nearest place name (in parentheses) with reference to Figure 1 is specified.

occurred in each of these areas if *M. bovis* infections were randomly distributed throughout the area where carcasses were found.

Serology – live-sampled buffalo

All 30 sampled buffalo were noted to be apparently healthy at the time of capture. Three buffalo (10%, 2 male, 1 female) were positive reactors according to test criteria established by Whipple *et al.* [24]. Two of three positive reactors also had mean OD_{bovine} values $\geqslant 0.385$ and thus would be considered positive using an alternate cut-off designed to maximize test predictive value in African buffalo developed by Michel *et al.* [25].

DISCUSSION

We document that bTB is present in wild animals inhabiting protected areas and village lands in the Ruaha ecosystem of south-central Tanzania. Our findings demonstrating *M. bovis* infection in 8% of sampled wild animals are consistent with findings from northern Tanzania [11]. However, the opportunistic nature of the sampling effort in this study limits our ability to estimate the true prevalence of bTB in this population. Although weaker animals may be more likely to be successfully hunted, opportunistically found, or depredated, and thus potentially more likely to be infected than the general population, this potential bias in our data may be balanced by the fact that infected wildlife sampled in this study lacked clinical bTB lesions, and that trophy hunters prefer to select larger healthy males.

An unexpected finding was the documentation of bTB infection in eight different species occupying different ecological niches. To our knowledge, this is the first published report of *M. bovis* infection in free-ranging vervet monkey, yellow baboon, and Kirk's dik-dik in Africa, and the first published isolation of *M. bovis* in African buffalo and impala in Tanzania. *M. bovis* infection has been documented previously in chacma baboons (*Papio ursinus*) in South Africa [5] and in olive baboons (*Papio anubis*) from Kenya that were feeding on slaughterhouse offal from *M. bovis*-infected cows [30, 31]. Neither of these baboon species is present in the Ruaha ecosystem, but the yellow baboon fills a similar niche, being abundant both in protected areas and in village lands where they are adept at utilizing anthropogenic food sources. No published record of *M. bovis* infection was found for vervet monkeys, but *M. tuberculosis* infection has

been reported in vervet monkeys held at a wildlife rehabilitation centre in South Africa [32].

Spoligotype similarity between wildlife sampled in this study and local livestock supports the hypothesis that livestock and wildlife are sharing pathogens. Infection of small carnivores and primates may indicate more recent spillover transmission from cattle as these species often live close to human settlements, have limited potential to maintain bTB in the absence of an alternate infection source (i.e. dead-end hosts), and often have short duration of illness [33]. The infected mongoose, blotched genet, vervet monkeys, yellow baboon, and Kirk's dik-dik in this study were all sampled near villages.

In addition to spillover, documentation of *M. bovis* infection in three species of large bodied, long-lived, gregarious herbivores (African buffalo, lesser kudu, impala) emphasizes the possibility for pathogen persistence in one or more wildlife maintenance hosts. Buffalo are the major wildlife bTB maintenance host in Africa, transmitting bTB within their herds without repeated spillover from cattle and serving as a source of bTB to other sensitive species, especially large carnivores [34]. Although there is no evidence indicating lesser kudu and impala are maintenance hosts, greater kudu with advanced bTB can shed large numbers of bacteria and may be able to maintain a separate infection cycle, as documented in KNP [33]. Once established, bTB infection in wildlife maintenance hosts can be a source of infection for livestock, thereby complicating control efforts or resulting in the re-emergence of bTB in livestock populations where the disease was formerly eradicated [35, 36].

Our documentation of infected buffaloes in the community-based wildlife management area and in RNP has conservation and economic significance. Large herds of healthy buffalo attract revenue from both non-consumptive tourists and hunters coming to the game reserves surrounding RNP. Seasonal drying of the Great Ruaha River may reduce the spatial distribution of buffalo and cattle, compressing herds into a smaller area where potential for disease transmission is higher and forage competition is more severe. In addition, forage limitation, due to increased frequency and severity of bush fires, may be an ecological stressor that acts synergistically to cause disease (P. Coppolillo, unpublished data).

We did not identify a unique buffalo-only strain of *M. bovis* in Ruaha's buffalo, thus it is possible that the infections found were solely due to spillover from livestock. However, the fact that 3/30 young buffalo from a single herd sampled inside RNP were serological reactors strongly suggests that bTB is being transmitted buffalo-to-buffalo. Given the continued ecological threats, a wider systematic buffalo health assessment of multiple herds and age groups, coupled with updated herd demographic and spatial distribution data is needed to estimate the population prevalence of bTB, determine if bTB is widespread in buffalo herds in Ruaha, and determine if bTB or other disease could be contributing to any spatial range contraction or demographic changes.

In addition to conservation impact, bTB infection in wildlife has implications for human health. Buffalo, kudu, and impala are important hunted species, and dik-dik are commonly consumed bushmeat. Although the risk of contracting bTB from consumption of well-cooked meat is minimal for most people, organ meat (including lungs) is commonly consumed and most carcasses are processed in the field with little or no sanitary precautions. These factors coupled with a relatively high prevalence of infection with HIV/AIDS may put people processing and consuming hunted wildlife at greater risk of contracting and developing clinical TB [1].

Implications for *M. bovis* control at the wildlife–livestock interface

We provide supportive evidence of a multi-host transmission cycle of *M. bovis* in the Ruaha ecosystem, involving spillover between livestock and wildlife, as well as pathogen persistence in wildlife maintenance hosts. Unfortunately, eradication strategies in and around other protected areas where similar pathogen dynamics have been documented involve resource-intensive actions, such as fencing, culling, or large-scale test-and-slaughter programmes, which are not compatible with larger ecosystem conservation goals. Even with intense interventions, these efforts have met with limited success due to logistical challenges and the existence of other possible maintenance hosts that preclude a single-species intervention approach [33].

Perhaps what is most achievable in an ecosystem like Ruaha is to recognize that bTB is a multi-host disease affecting both wildlife and livestock and that a myriad of factors including spatio-temporal population overlap, animal density, drought, human-induced habitat change, and cultural practices will affect bTB transmission and should be considered when designing efforts to control it. An alternative approach to control and better understand bTB

in this resource-poor system could include targeting testing of cattle and wildlife in shared grazing lands to identify areas or sites with increased spillover risk for management. For example, spatial analysis revealed two areas of higher than expected numbers of *M. bovis* infections within our sampling area. Even though these infection clusters were not statistically significant, potentially due to small sample size and low infection proportion, they may indicate a localized region within our sampling area that warrants additional field investigation. If resources were available to support more systematic bTB surveillance in both wildlife and livestock, focused testing in high-risk areas identified by spatial statistics could reveal a source of spillover or a highly infected local livestock or wildlife population for targeted intervention. Additionally, a better understanding of livestock grazing strategies, locations, and densities would also help identify transmission risk and control points.

At this time, there is no large-scale control or eradication programme for bTB in Tanzanian cattle or wildlife. Accordingly, the best approach to control the disease may be to focus on preserving the ecological functions of both protected and grazing areas to minimize both species overlap and the ecological stressors that increase an animal's susceptibility to bTB. For example, provision of adequate grazing and water resources for livestock would help minimize livestock incursions into wildlife protected areas during times of resource scarcity and would reduce interspecies transmission of not only bTB but potentially many other diseases. Programmes to facilitate market access for rural cattle could be coupled with efforts to promote sustainable livestock herd densities to optimize health, carcass condition, and efficient use of grazing resources. For wildlife population resilience, restoration of sustainable agricultural practices could support adequate dry-season water resources and minimize habitat degradation and encroachment.

CONCLUSION

A cattle strain of *M. bovis* infects multiple wildlife species inhabiting protected areas at the livestock–wildlife interface in the Ruaha ecosystem of Tanzania. Determining the species and spatial distribution of infection in both wildlife and livestock could enable an ecosystem-based approach to reduce disease transmission and improve opportunities for conservation interventions, tourism growth, livestock productivity, and livestock and human health security.

DECLARATION OF INTEREST

None.

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