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# Tuberculosis in Tanzanian Wildlife

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ABSTRACT: Bovine tuberculosis, caused by *Mycobacterium bovis,* is a pathogen of growing concern in free-ranging wildlife in Africa, but little is known about the disease in Tanzanian wildlife. Here, we report the infection status of *Mycobacterium bovis* in a range of wildlife species sampled from protected areas in northern Tanzania. *M. bovis* was isolated from 11.1% (2/18) migratory wildebeest (*Connochaetes taurinus*) and 11.1% (1/9) topi (*Damaliscus lunatus*) sampled systematically in 2000 during a meat cropping program in the Serengeti ecosystem, and from one wildebeest and one lesser kudu (*Tragelaphus imberbis*) killed by sport hunters adjacent to Tarangire National Park. A tuberculosis antibody enzyme immunoassay (EIA) was used to screen serum samples collected from 184 Serengeti lions (*Panthera leo*) and 19 lions from Ngorongoro Crater sampled between 1985 and 2000. Samples from 212 ungulates collected throughout the protected area network between 1998 and 2001 also were tested by EIA. Serological assays detected antibodies to *M. bovis* in 4% of Serengeti lions; one positive lion was sampled in 1984. Antibodies were detected in one of 17 (6%) buffalo (*Syncerus caffer*) in Tarangire and one of 41 (2%) wildebeest in the Serengeti. This study confirms for the first time the presence of bovine tuberculosis in wildlife of northern Tanzania, but further investigation is required to assess the impact on wildlife populations and the role of different wildlife species in maintenance and transmission.

*Key words:* Bovine tuberculosis, *Mycobacterium bovis,* Serengeti, Tanzania, wildlife.

*Mycobacterium bovis* infects a wide range of species and is increasingly recognized as an important pathogen of free-ranging African wildlife (Keet et al., 2000a; de Vos et al., 2001; Keet et al., 2001; de Lisle et al., 2002; Michel, 2002). Wildlife tuberculosis is a potential source of infection for domestic livestock and humans (Cleaveland et al., 2002; Michel, 2002), and the disease poses a threat to valuable and endangered wildlife species. For example, *M. bovis* in the Kruger National Park, South Africa, has resulted in mortality and morbidity in buffalo (*Syncerus caffer*; Keet et al., 1996), lion (*Panthera leo*; Keet et al., 2000b), and cheetah (*Acinonyx jubatus*; de Lisle et al., 2002).

Although *M. bovis* has been reported for many years in Tanzania (Markham, 1952; Chillaud, 1995), recent studies have focused on infection in cattle (Kazwala, 1996) and very little is known about the extent or impact of bovine tuberculosis (bTB) in wildlife. In the Serengeti National Park, bTB was first recorded in wildlife with acid-fast bacilli detected in granulomatous lesions of giraffe (*Giraffa camelopardalis*) and eland (*Taurotragus oryx*) (Roelke-Parker and Parker, 1994). However, culture and molecular characterization of *Mycobacteria* species were not possible until the establishment of containment laboratories and molecular diagnostic facilities at the Sokoine University of Agriculture (SUA) in 1997. Here, we report results on the bTB infection status of different wildlife species in Tanzania, using results from culture and serologic testing of lions and wild ungulates. The identification of other *Mycobacteria* species also is reported.

For culture and polymerase chain reaction (PCR) analysis, samples were obtained from a range of sources, including: 1) samples collected opportunistically from carcasses with tuberculous lesions detected at post-mortem examination in



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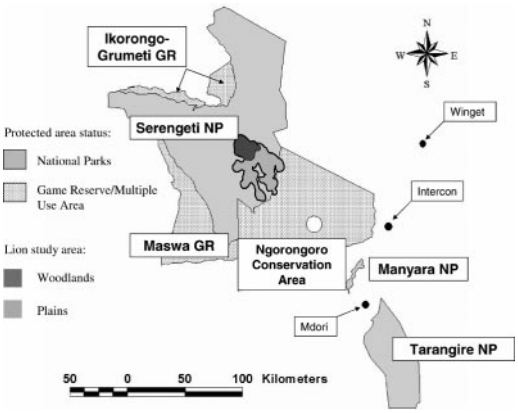



FIGURE 1. Map showing location of study sites in northern Tanzania, including the three hunting camps, Winget, Intercon, and Mdori (black circles), Game Reserves (GR), and National Parks (NP). The Ngorongoro Crater is represented by the large open circle.

the Serengeti National Park between 1999 and 2001 (33°50′ to 36°E, 1°30′ to 3°7′S) (Fig. 1); 2) pooled samples of lung and retropharyngeal, mediastinal, mesenteric, and prescapular lymph nodes from wildebeest (*Connochaetes taurinus*) and topi (*Damaliscus lunatus*) killed during local meat cropping programs in the Ikorongo–Grumeti Game Reserve, adjacent to the Serengeti National Park, in 2000 (33°57′ to 34°52′E, 1°50′ to 2°13′S) (Fig. 1); and 3) samples of lung and retropharyngeal, mediastinal, mesenteric, and prescapular lymph nodes showing gross visible lesions collected from animals killed by sport hunters in areas adjacent to Tarangire National Park and in Monduli and Longido hunting areas in northern Tanzania between 1999 and 2001 (Fig. 1). Samples from all wildebeest and topi sampled at Ikorongo–Grumeti Game Reseve were cultured irrespective of whether visible lesions were observed. Lesions in hunter-killed animals were detected by game skinners who had been trained in meat inspection by SUA staff.

Samples were stored at −20 C, transported to the laboratory in cool boxes, and processed according to the Scottish Mycobacterium Reference Laboratory protocol (Watt et al., 1993). After neutralization, sediments were inoculated onto two slants of Lowenstein–Jensen medium, one containing pyruvate and one containing glycerol. Cultures were incubated at 37 C for a maximum of twelve weeks and positive cultures subcultured onto the same type of medium for identification by growth characteristics and standard biochemical tests. A proportion of culture-positive samples were tested using PCR to differentiate *M. bovis* from other species in the *M. tuberculosis* complex.

A tuberculosis antibody enzyme immunoassay (EIA) (CSL Limited, Melbourne, Victoria, Australia) was carried out on serum samples collected from: 1) ungulate species sampled for rinderpest surveillance activities and as part of the year 2000 Ikorongo–Grumeti meat cropping program (Table 1), and 2) 184 Serengeti lions (*Panthera leo*) and 19 lions from the nearby Ngorongoro Crater collected between 1984 and 2000 as part of long term ecological and epidemiological studies. Samples collected between 1984 and 1993 were part of a genetic survey and thus constitute a random sample with respect to tuberculosis. Beginning in 1994, additional samples were collected from individuals showing clinical signs of disease as part of a veterinary surveillance program conducted by Tanzania National Parks.

The procedures for preparing the tuberculosis EIA materials are described by Jones et al. (1992). Briefly, antibodies in undiluted sera were allowed to react simultaneously with MPB70 (Fifis et al., 1989) antigen (5 μg/mL) bound onto microtiter plates and with conjugate (MPB-70 labelled with horseradish peroxidase) in the liquid phase for 60 min at room temperature. Following removal of unbound material by washing and incubation with enzyme substrate (30 min), color development was measured spectrophotometrically. All sera were assayed in duplicate, and sera rendering a positive result (OD>0.1) were subjected to repeat testing for confirmation. Enzyme immunoassay

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TABLE 1. Geographic origin of ungulate sera analysed for tuberculosis antibodies by EIA.

| Species | Serengeti* | Tarangire | Ruaha | Rungwa | Katavi | Mikumi | Mkomazi | Myowosi | Ugalla | Totals |
|---|---|---|---|---|---|---|---|---|---|---|
| Buffalo (*Sycerus caffer*) | 30 | 17 | | 7 | 19 | 10 | 2 | 9 | 13 | 107 |
| Wildebeest (*Connochaetes taurinus*) | 41 | | | | | | | | | 41 |
| Zebra (*Equus burchelli*) | 18 | | | | | | | | | 18 |
| Elephant (*Loxodonta africana*) | 3 | | | | | | | | | 3 |
| Thomson's gazelle (*Gazella thomsoni*) | 2 | | | | | | | | | 2 |
| Hartebeest (*Alcelaphus buselaphus*) | 1 | | | | | | 5 | | | 6 |
| Topi (*Damaliscus lunatus*) | 10 | | | | | | | | | 10 |
| Impala (*Aepyceros melampus*) | 4 | | | | | | | | | 4 |
| Giraffe (*Giraffa camelopardalis*) | 3 | | | 1 | | | | | | 4 |
| Eland (*Taurotragus oryx*) | 4 | | 5 | | 4 | | | | | 13 |
| Lesser Kudu (*Tragelaphus imberbis*) | | | | | | | 1 | | | 1 |
| Roan Antelope (*Hippotragus equinus*) | | | 1 | | 1 | | | | | 2 |
| Sable Antelope (*Hippotragus niger*) | | | | 1 | | | | | | 1 |
| Totals | 116 | 17 | 6 | 9 | 24 | 10 | 8 | 9 | 13 | 212 |

* The Serengeti samples include sera collected from the Ikorongo-Grumeti game reserves, which are part of the Serengeti ecosystem.

positive control serum consisted of pooled bovine serum from cattle experimentally infected with *M. bovis* (Fifis et al., 1994). Each EIA assay was valid if $OD_{450nm}$ of the negative control (pooled normal bovine serum) and positive control were $<0.1$ and $>0.75$, respectively.

Individual lions within the Serengeti study area (Fig. 1) have been monitored from birth as part of a long term demographic research program (Packer et al., 1999). For lions sampled outside the study area, ages were estimated from body size, mane development (for males), and nose coloration (Whitman et al., 2004). Age-prevalence curves were calculated to investigate age-related patterns of infection.

*Mycobacterium bovis* was cultured from 2/18 (11%) carcasses of apparently healthy wildebeest, and from 1/9 (11%) topi culled as part of a meat cropping program in the Ikorongo-Grumeti game reserves adjacent to the Serengeti National Park. None of these carcasses showed visible lesions on post-mortem examination carried out by qualified veterinarians. Of the carcasses with gross lesions sampled in the sport hunting camps, *M. bovis* was isolated from one out of three wildebeest and the one kudu (*Tragelaphus imberbis*) from Mdori, adjacent to the Tarangire National Park. No *Mycobacteria* species were cultured from any of the lesion-positive samples collected in the Winget (Longido) or Intercon hunting areas (Monduli District) (Table 2, Fig. 1).

Several *Mycobacteria* species other than tuberculosis were isolated from the Serengeti ungulate samples collected during the meat cropping program. These included *M. terrae,* which was isolated from one wildebeest (5.6%), and *M. phlei* from one topi (11.1%), neither with any apparent clinical or post-mortem signs. In the Serengeti, *M. avium* was isolated from one buffalo, one wildebeest, and one topi. The buffalo had been killed and partly consumed by lions, but showed extensive granulomatous lesions in the cervical lymph nodes. Caseous subcutaneous ab-

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TABLE 2. Frequency of isolation of *M. bovis* from wildlife samples submitted for culture at Sokoine University of Agriculture.

| Species | Serengeti ecosystem | Winget (Longido) | Mdori (Tarangire) | Intercon (Monduli) |
|---|---|---|---|---|
| Wildebeest (*Connochaetes taurinus*) | 2/18 (11%) | 0/3 | 1/3 | |
| Topi (*Damaliscus lunatus*) | 1/9 (11%) | | | |
| Buffalo (*Syncerus caffer*) | 0/1 | 0/1 | | |
| Kudu (*Tragelaphus* spp.)* | | 0/2 | 1/1 | |
| Thomson's gazelle (*Gazella thomsoni*) | | 0/2 | | 0/1 |
| Hartebeest (*Alcelaphus buselaphus*) | | 0/2 | 0/1 | |
| Other herbivore species# | 0/1 | 0/10 | | |
| Lion (*Panthera leo*) | 0/4 | | | |
| Total | 33 | 20 | 5 | 1 |

* Kudu not distinguished by species.

# Other herbivore species comprise the following: Serengeti: Zebra (*Equus burchelli*) (1); Winget: Eland (*Taurotragus oryx*) (2), Grant's gazelle (*Gazella granti*) (2), Impala (*Aepyceros melampus*) (3), Oryx (*Oryx gazella*) (2), Steinbuck (*Raphicerus campestris*) (1).

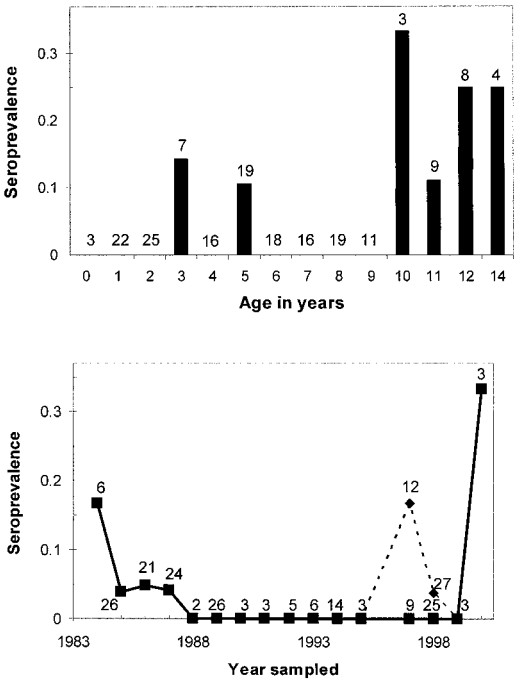



FIGURE 2. Seroprevalence of Serengeti lions to the MPB70 antigen in relation to age (a) of individual and (b) year sampled. The solid lines represent animals sampled as part of a genetic study and thus constitute a random sample with respect to tuberculosis. The overall seroprevalence is shown by the dotted lines and includes animals sampled because they were showing clinical signs of disease.

scessation was detected in the nuchal area of the wildebeest, but no visible lesions were seen in any of the lymph nodes.

The five *M. bovis* isolates identified by biochemical tests also tested positive for *M. bovis* by PCR. These isolates all produced a 245bp DNA product from the IS986 insertion sequence but failed to generate a 396bp amplification product from the mtp40 genomic fragment, identifying the strains as *M. bovis* (Sinclair et al., 1995).

Overall, eight of 184 (4%) Serengeti lions were seropositive for tuberculosis (Fig. 2). Although seroprevalence was highest in recent years, the difference was not significant ($\chi^2=1.69$, d.f.=1, $p=0.19$), with four of 45 (9%) animals seropositive since 1997 and four of 139 (3%) seropositive from 1984–1996. Furthermore, the latter period included six animals that were sampled because they showed obvious clinical signs of disease. Including only those lions sampled "randomly" as part of genetic studies, the seroprevalence was similar in both periods, with one of 39 (3%) seropositive animals detected after 1997 ($\chi^2=0.58$, d.f.=1, $p=0.45$). The Ngorongoro lions were all sampled between 1984 and 1991, and none were seropositive. However, with an overall low seroprevalence of 4% in the Serengeti, the sample

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size from the Crater population might have been too small to detect positive individuals.

Five of the eight seropositive lions were >10 yrs of age (Fig. 2). Four of the seropositive lions were obviously ill when sampled, and a fifth became ill within 2 months; none of these individuals survived for more than 6 months after sampling. Clinical signs in these individuals included dyspnea, bilateral sub-mandibular swelling, ataxia, hypermetria, and debilitation. The other three all appeared to be in good health when sampled, and their subsequent survival was 6 months, 18 months, and 37 months. Although seropositive animals had somewhat shorter survival than seronegative animals of the same age, the sample size is too small to detect statistical significance.

Only two of 212 (0.9%) ungulate samples were seropositive for tuberculosis, including one of 41 (3%) wildebeest in the Serengeti and one of 17 (6%) buffalo in the Tarangire ecosystem.

This study provides the first confirmation of *Mycobacterium bovis* in Tanzanian wildlife, with infection detected in wildebeest, topi, and kudu. Serological results indicate that buffalo might also be infected with *M. bovis.* Although sample sizes were small, the isolation of *M. bovis* from 11% of culled wildebeest suggests that infection might be widespread because the Serengeti wildebeest population exceeds one million individuals (Tanzania Wildlife Conservation Monitoring, 2000). These infected wildebeest are part of migratory herds that range throughout the Serengeti ecosystem, including the Masai Mara National Reserve, Kenya.

None of the *M. bovis*-infected wildebeest showed detectable lesions at postmortem examination and were therefore unlikely to be excreting high levels of *Mycobacteria* in aerosol, urine, or feces, as has been described for wildlife maintenance hosts of *M. bovis* in other areas (de Lisle et al., 2002). Nonetheless, it is possible that the disease could be transmitted to carnivores through the consumption of infected tissue. Migratory wildebeest spend considerable periods outside the protected areas of the Serengeti National Park, and transmission of *M. bovis* between livestock and wildlife might also be possible. Intradermal skin testing of Masai cattle has demonstrated a prevalence of bovine tuberculosis of 2% in villages on the southern and eastern Serengeti plains, where wildebeest graze in the wet season (Shirima, unpubl. data).

Serological tests have often been considered too insensitive to detect tuberculosis in cattle as well as in wildlife (Goodger et al., 1994; Costello et al., 1997). It has been shown in previous investigations in the Kruger National Park that the EIA used in this study has a very high specificity and can reliably detect *M. bovis* infection at a population level. Evaluation of this EIA in comparison with bacterial culture and histopathology in buffalo in the Kruger National Park demonstrated a sensitivity of 10.4% and a false positive reactor rate of 1%. In lions, the sensitivity of the EIA was 25% with no false positive reactions in culture-negative specimens (Michel, unpubl. data). Similarly, the EIA detected 27% of deer with gross lesions and culture-confirmed *M. bovis* (Jones, unpubl. data).

Due to the limited and variable sensitivity of the EIA, the true prevalence of infection in different species is difficult to determine, and seroprevalence estimates reported here are likely to provide only an indication of minimum prevalence. Furthermore, the detection of antibodies to the MPB70 antigen indicates exposure to *Mycobacteri*a species in the *M. tuberculosis* complex, but does not discriminate between different species in the group, which is comprised of *M. tuberculosis, M. bovis, M. microti, M. africanum,* and *M. canettii* (Brosch et al., 2002). However, with the isolation of *M. bovis* in common prey species of lions, we consider it likely that seropositivity in lions reflects exposure to *M. bovis* through consumption of infected prey.

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Despite the limitations of the EIA, this assay proved a useful tool in this study, indicating, first, the presence of *Mycobacteria* infection in the Serengeti lion population, second, that infection has been present in the lion population since at least 1984, and third, the presence of infection in buffalo (for which culture-positive samples have not yet been obtained).

As with wild ungulates, we do not yet know the impact of tuberculosis on the Serengeti lions. Preliminary data from a study currently carried out in the Kruger National Park indicate that tuberculosis spreads rapidly in lion populations; 90% of lions monitored in an area with a high herd prevalence of *M. bovis* in buffalo have become infected (South Africa Veterinary Foundation, http://www.savf.org.za/rhino.htm). Over 97% of the animals sampled in this study were known to be positive for feline immunodeficiency virus, FIV (Packer et al., 1999). However, FIV is absent in the lions of Hluhluwe–Umfolozi Park (Van Vuuren et al., 2003), yet bTB has caused severe pathology in the HUP lions (Dave Cooper, pers. comm.), suggesting that FIV might not play an important role in the pathogenicity of infection.

Other *Mycobacterium* species isolated from wildlife in this study included *M. avium, M. phlei,* and *M. terrae,* which are in a group described as non-tuberculous mycobacteria. *Mycobacterium avium* can cause clinical disease in some ungulate species (Thorel et al., 2001) and, in this study, was associated with pathology in the infected buffalo in the Serengeti. *Mycobacterium phlei* and *M. terrae* generally are considered non-pathogenic in most species, but in recent studies in Tanzania these *Mycobacteria* species have been associated with granulomatous lesions in both cattle and humans (Kazwala et al., 2002). Transmission of non-tuberculous *Mycobacteria* is generally considered to occur through environmental contamination. However, the isolation of *M. phlei* and *M. terrae* from lesions in the lung and thoracic lymph nodes in cattle raises the possibility of direct animal-to-animal transmission.

It has been suggested that bTB has the potential to have a major impact on the wildlife of Kruger National Park because it is an alien pathogen that has only recently been introduced into naïve wildlife populations (de Vos et al., 2001). Results from this study indicate that bTB has been present in the Serengeti for several decades, reinforcing earlier findings from Uganda and Kenya, which have demonstrated bTB as a disease problem in buffalo in Uganda (Woodford, 1982a, b) and in baboons (*Papio cynocephalus*) in the Masai Mara, Kenya (Tarara et al., 1985). In southern Africa, livestock and wildlife are usually separated by fences, and the intensive control measures in South African livestock might have limited the opportunities for transmission of bTB to wildlife. In contrast, the continued practice of traditional (fenceless) livestock husbandry in East Africa is likely to have facilitated the spread and establishment of infection in wildlife over a much longer period, perhaps permitting a more stable endemic pattern of infection.

Consumption of infected buffalo is considered the predominant route by which lions in the Kruger National Park become infected with *M. bovis* (Keet et al., 2000b). With *M. bovis* confirmed in the wildebeest population, Serengeti lions also are clearly at risk of infection through eating infected prey. We suggest that bovine tuberculosis has the potential to be a mortality factor in Serengeti lions because seropositivity was associated with clinical signs of disease and shortened survival times in several individuals. However, clinical signs associated with seropositivity were inconsistent with those recorded in tuberculosis-infected lions in the Kruger National Park (Keet et al., 2000b) and this hypothesis can only be examined when post-mortem and culture findings become available for Serengeti lions and the data linked with survival data. Without longitudinal data on known individuals, it is also impossible to assess

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the severity of infection in ungulate populations.

In summary, *M. bovis* has been confirmed in a range of wild ungulate species in the Serengeti and Tarangire ecosystems in northern Tanzania. Serological surveys also demonstrate exposure of Serengeti lions to *M. bovis* since at least 1984. The limitations of this data set do not allow an accurate estimate of the prevalence or impact of infection in different species. Further investigation is clearly needed to determine the impact on tuberculosis on wildlife populations in the Serengeti and the role of different species in maintenance and transmission of infection.

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