BOVINE TUBERCULOSIS IN ETHIOPIAN WILDLIFE

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ABSTRACT: Bovine tuberculosis (BTB) is endemic in Ethiopian cattle. However, the status of the disease in wildlife populations that often share habitat with livestock is unknown. We screened for BTB in wildlife in five regions in Ethiopia. Blood and tissue samples from 133 mammals of 28 species were collected from 2006 to 2008. We used a rapid serology test (RT) based on lateral flow technology, and performed culture of lymph node specimens inoculated onto Lowenstein-Jensen and Middlebrook 7H11 media. Acid-fast colonies were further analyzed by molecular typing. Sera from 20 of 87 animals (23%) were positive for BTB by RT; acid-fast bacilli were cultured from 29 of 89 animals (32.5%). None of the positive cultures yielded mycobacteria from the Mycobacterium tuberculosis complex but many environmental mycobacteria were isolated. Among these, Mycobacterium terrae was the most common. We demonstrated a high prevalence of environmental mycobacteria in wildlife, the role of which is unknown. Flagship rare endemic species such as the mountain nyala (Tragelaphus buxtoni) and the Ethiopian wolf (Canis simensis) may be at risk for BTB. We also assessed the utility of RT for field purposes.

Key words: Bovine tuberculosis, Ethiopia, Mycobacterium bovis, Mycobacterium spp., wildlife.

INTRODUCTION

Bovine tuberculosis (BTB) is a multi-host disease caused by Mycobacterium bovis. This pathogen belongs to the Mycobacterium tuberculosis complex, a group of genetically closely related mycobacteria, which includes the human pathogen, M. tuberculosis (Brosch et al., 2002; Mostowy et al., 2005). Cattle are considered to be the main host for M. bovis and the disease has considerable economic impact on the agricultural and trade sector (Zinsstag et al., 2006). BTB is a potential zoonotic threat to humans who may be infected by consuming raw meat and milk products from BTB-infected cattle (Grange, 2001; Neill et al., 2005). Wildlife is also increasingly described as a source for BTB in humans that have close contact with infected animals, such as hunters (in North America) and game farmers (Liss et al., 1994; Wei et al., 2004; Baker et al., 2006; Wilkins et al., 2008).

The list of wildlife species around the world from which M. bovis has been isolated is long and reports in the literature of new susceptible species have increased in recent years. Some wildlife species have long been known to be maintenance hosts (i.e., wildlife species that can maintain the disease in the absence of infected cattle). Classic examples of maintenance hosts include the brushtail possum (Trichosurus vulpecula) in New Zealand (Coleman and Cooke, 2001), the white-tailed deer (Odocoileus virginianus) in the USA (O’Brien et al., 2002), the Eurasian badger (Meles meles) in the UK and Ireland (Griffin et al., 2005), the African buffalo (Syncerus caffer) in South Africa (de Vos et al., 2001; Rodwell et al., 2001), and more recently described, the wild boar (Sus scrofa) in Spain (Naranjo et al., 2008) and wood bison (Bison bison) in Canada (Nishi et al., 2006). These maintenance hosts are a source of infection for livestock, thus hampering costly national control and eradication programs in developed countries. For example, the incidence of BTB...
in the UK is increasing by 18% per year and the existence of an uncontrolled BTB wildlife reservoir is a strong contributing factor. Control of the disease in livestock costs the British government as much as 74 million pounds sterling per year (Mathews et al., 2006).

These maintenance hosts are a source of infection for other wildlife species sharing the same habitat and have the potential to threaten valuable or endangered species. This was shown recently in Kruger National Park, Republic of South Africa (RSA), where lions preyed on BTB-infected buffaloes, leading to severe mortality in the park’s lion population (Keet et al., 2000; De Vos et al., 2001). Wildlife species other than maintenance species are spillover or dead-end hosts that cannot sustain the disease in the absence of an infectious source. However, their role in the complex epidemiology of BTB is unknown, especially as more species are detected with BTB and new interactions and new maintenance hosts are described (Renwick et al., 2007).

In sub-Saharan Africa, the most detailed data on BTB in wildlife has been collected in southern Africa (Bengis et al., 1996; Keet et al., 2001; Michel et al., 2006, 2009), Kenya (Tarara et al., 1985; Sapolsky and Else 1987), Uganda (Woodford, 1982a, b), and Tanzania (Cleaveland et al., 2005). No data are available regarding BTB infection in Ethiopian wildlife. Ethiopia is a country known for its rich biodiversity and varied ecosystems and is home to 255 mammal species, of which 31 are endemic and 38 are listed on the International Union for Conservation of Nature’s Red List of Threatened Species (IUCN, 2008). The rapid intensification of the human-livestock-wildlife interaction in Ethiopia is fueled by rapid human population growth, massive land degradation, and recurrent drought and presents a potential risk of disease transmission.

The primary aim of this study was to assess the prevalence of BTB infection in wildlife species from Ethiopia using serology, isolation in culture, and molecular typing. Our second aim was to assess the utility of a rapid serologic test for field use.

MATERIALS AND METHODS

Study sites

This study was conducted in close collaboration with the Ethiopian Wildlife Conservation Authority, which approved and supported the research. The study was carried out in five regions in Ethiopia: Welega (Oromia), Awash (Afar), Babille (Harari), Bale Mountains (Oromia), and South Omo (Southern Nations, Nationalities and Peoples Region; Fig. 1), between latitudes 5°–11°30′N and longitudes 35°–43°E, thus covering several ecologic zones, with altitudes ranging from 400 m to 3,800 m above sea level. The majority of samples were collected in hunting-controlled areas (74%), followed by the Bale Mountains National Park (19.5%) and the Babille elephant sanctuary (6.5%).

Sample collection

Samples from wildlife were collected between 2006 and 2008. Animal tissues were obtained from harvests by licensed hunters and opportunistically from fresh carcasses (e.g., road kills). No animals were killed for the purpose of the study. Animals were examined and the body condition assessed. Mediastinal, mesenteric, and submandibular lymph nodes were collected, as well as suspect tuberculous lesions from any organ, if present; tonsils were collected from carnivores. Specimens were collected using a different set of disposable, sterile surgical instruments (forceps, scalpel blades) for each animal and kept in Falcon® tubes (50ml; Becton Dickinson Labware, Franklin Lakes, New Jersey, USA) containing sterile phosphate-buffered saline (pH 7.2).

Blood was collected either from fresh carcasses or from anaesthetized live animals (captured, anaesthetized, and released in association with other projects for other purposes). For live animals, blood was drawn by venipuncture (choice of vein and syringe and needle size was species dependent). Blood from carcasses was collected after opening the carcass, either by heart puncture or by severing a large vessel (e.g., vena cava) and collecting the blood in a sterile collecting tube.

Blood tubes were stored upright for 6–12 hr and sera were pipetted from the tubes and transferred into 1-ml cryotubes. Tissue and serum samples were maintained at 4°C until
transported to the Armauer Hansen Research Institute laboratory in Addis Ababa, Ethiopia, where they were stored at −20°C until further processing.

Sample analysis

Serology for specific antibody detection was performed using the rapid test (RT; Chembio Diagnostic Systems, Inc., Medford, New York, USA). The RT is a colored, latex-based lateral flow technique using a cocktail of selected *M. bovis* antigens including ESAT-6, CFP10, and MPB83 (Lyashchenko et al., 2008). Results were read 20 min after adding the RT buffer solution to the serum. Animals were considered positive if any visible line appeared in the test area of the RT in addition to the control line. Results were negative if no band was visible in the test area in addition to the visible control band.

Submandibular and mediastinal lymph nodes were pooled; mesenteric lymph nodes were processed and cultured separately. After homogenization and neutralization according to standard methods (Roberts et al., 1991), sediments were inoculated onto three different media slants: Lowenstein-Jensen media supplemented with glycerol, the same media supplemented with pyruvate, and Middlebrook 7H11 medium supplemented as previously described (Gallagher and Horwill, 1977). Slants were incubated horizontally at 37°C for 1 wk then vertically for 5 wk. Cultures were considered negative if no visible growth was seen after 6 wk of incubation. Positive cultures were stained according to the Ziehl-Neelsen method and examined under the microscope for acid-fast bacilli (AFB).

Molecular typing

Heat-killed AFB-positive samples were investigated by multiplex polymerase chain reaction (PCR), using primers specific for the genus *Mycobacterium* and for the *M. tuberculosis* complex (Wilton and Cousins, 1992). Strains were investigated for the *M.
tuberculosis complex by PCR using protocols for RD4 typing (Gordon et al., 1999) and RD9 typing (Berg et al., 2009). Isolates that did not belong to the M. tuberculosis complex but were positive for the genus were sequenced over the 16S rRNA locus (Han et al., 2002). Sequence analysis was performed by basic local alignment search tool search (Altschul et al., 1997) of databases at the National Center for Biotechnology Information and the Ribosomal Differentiation of Medical Microorganisms (Harmsen et al., 2002). Sequences of the variable Region A and Region B (Kirschner et al., 1993) were especially considered when determining the Mycobacterium species.

Data analysis

Data were double-entered in an Access database and validated using Epi Info (version 3.3.2; Centers for Disease Control and Prevention, Atlanta, Georgia, USA). All statistical descriptive analysis was done using software package STATA 10.1 (StataCorp, College Station, Texas, USA). Molecular typing results of the isolated strains were used as a basis for estimating disease prevalence in wildlife.

RESULTS

Samples from 133 mammals of 28 species were investigated. The majority were male (n=116; 87%) and adults (n=128; 96%). Body condition was good in 124 animals (93%); eight animals (6%) were lean; one (1%) was emaciated.

Serology

The RT was performed on sera collected from 87 animals (13 live animals and 74 fresh carcasses). Results from the serologic tests are shown in Table 1. Twenty animals (23%), of 10 species, had detectable antibody by RT. The lymph nodes of 47 of these animals were further processed and cultured (see below).

Clinical examination and gross visible lesions

Of 133 animals sampled, six showed various clinical signs: one Grant’s gazelle (Nanger granti) was lame due to an old injury and five animals (gerenuk [Litocranius walleri], warthog [Phacochoerus africanus], Grant’s gazelle, and hartebeest [Alcelaphus buselaphus]) had enlarged head lymph nodes. On examination of the carcasses, gross visible nodules (pea size to 5 cm diameter) were seen in five animals. Nodules in the lung were seen in Menelik bushbuck (Tragelaphus scriptus) and hartebeest. Multiple nodules were observed on the liver in a warthog and on the mesenteries in a Grant’s gazelle.

Culturing and molecular typing

Lymph nodes from 89 animals (67%) were subjected to culture. Forty slants representing 29 animals (32.5%) showed colony growth. AFB-positive samples were further analyzed by Mycobacterium genus and deletion typing (Table 1). None of the samples belonged to the M. tuberculosis complex. However, 17 strains were identified as nontuberculous mycobacteria (NTM) with Mycobacterium terrae complex being the most prevalent agent (n=19; 65.5%) followed by NTM strains other than M. terrae complex and Mycobacterium avium complex (MAC; n=9; 31%). Strains of MAC were isolated from two Grants’ gazelles, and one isolate from a gerenuk was typed as Nocardia sp. Animals that had nodules on postmortem examination were all culture-negative. Among the 47 animals examined by serology and tissue culture, 15 were AFB-positive and 16 were serology-positive. However, only five of these animals were positive by both serology and culture.

DISCUSSION

This study provides the first data on tuberculosis in Ethiopian free-ranging wildlife. In our study, no M. bovis was isolated from wildlife. In contrast M. bovis was reported in bufaloes in Uganda (Woodford, 1982a, b) and in baboons in Kenya (Tarara et al., 1985; Sapolsky and Else, 1987). Bovine tuberculosis was also prevalent in the Serengeti-Ngorongoro ecosystem in Tanzania, affecting bufaloes, lions and wildebeest (Cleaveland et al., 2005).

Because wildlife habitats in Ethiopia are not fenced, there is intensive interaction
between a fast-growing human population and livestock and wildlife competing for scarce grazing land. Livestock also graze in various protected habitats, especially during times of fodder shortage (Jacobs and Schloeder, 2001; Stephens et al., 2001). In our study areas, wildlife remained geographically localized with no major migration patterns. Bovine tuberculosis is endemic in the Ethiopian cattle population, with prevalence ranging from 0.8% to 13.5% in rural areas (Ameni et al., 2007; Berg et al., 2009; Tschopp et al., 2009). Wildlife and, in particular, herbivores sharing pastures with cattle might therefore be at risk for BTB transmission.

The "classical" African species described in the literature as being reservoirs of Mycobacterium bovis at the wild herbivore-herbivore interface are the African buffalo (Syncerus caffer), the Greater kudu (Tragelaphus strepsiceros), the Bushbuck (Tragelaphus scriptus), and the Waterbuck (Kobus ellipsiprymnus). Other species that have been shown to be positive for Mycobacterium tuberculosis in Ethiopia include the densely populated wild hog (Potamochoerus larvatus), the Rock hyrax (Procavia capensis), and the Rock hyrax (Procavia capensis) (see Table 1).

### Table 1. Results of serology, culture for acid-fast bacilli, and molecular typing for bovine tuberculosis and other mycobacteria in wildlife in Ethiopia, 2006–08. Number of animals positive/total tested (% positive). a

<table>
<thead>
<tr>
<th>Species</th>
<th>No. tested</th>
<th>Positive serology</th>
<th>AFB-positive cultures</th>
<th>Molecular typing (16S rDNA gene sequencing)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bushbuck (Tragelaphus scriptus)</td>
<td>11</td>
<td>1/4 (25%)</td>
<td>2/10 (20%)</td>
<td>MTC</td>
</tr>
<tr>
<td>Soemmering gazelle (Gazella soemmeringi berberana)</td>
<td>1</td>
<td>0/1 (0%)</td>
<td>0/1 (0%)</td>
<td></td>
</tr>
<tr>
<td>Guenther’s dik-dik (Madoqua gunther)</td>
<td>8</td>
<td>1/3 (33%)</td>
<td>3/8 (37%)</td>
<td>MTC, Mycobacterium moriokaense</td>
</tr>
<tr>
<td>Grant’s gazelle (Nanger granti)</td>
<td>17</td>
<td>2/12 (17%)</td>
<td>10/17 (59%)</td>
<td>MTC, Mycobacterium wolinsky, MAC, Mycobacterium avium, M. moriokaense</td>
</tr>
<tr>
<td>Gerenuk (Litocranius walleri)</td>
<td>5</td>
<td>0/4 (0%)</td>
<td>3/5 (60%)</td>
<td>MTC, Nocardia testacea</td>
</tr>
<tr>
<td>Greater kudu (Tragelaphus strepsiceros)</td>
<td>1</td>
<td>NA</td>
<td>0/1 (0%)</td>
<td></td>
</tr>
<tr>
<td>Lesser kudu (Tragelaphus inberbis)</td>
<td>7</td>
<td>3/5 (60%)</td>
<td>3/7 (43%)</td>
<td>MTC, Mycobacterium flavescens spp.</td>
</tr>
<tr>
<td>Tiang ( Damaliscus lunatus)</td>
<td>4</td>
<td>0/3 (0%)</td>
<td>2/4 (50%)</td>
<td>MTC</td>
</tr>
<tr>
<td>Hartebeest (Alcelaphus buselaphus)</td>
<td>4</td>
<td>0/3 (0%)</td>
<td>1/2 (50%)</td>
<td>MTC</td>
</tr>
<tr>
<td>Mountain nyala (Tragelaphus buxtoni)</td>
<td>11</td>
<td>2/5 (40%)</td>
<td>1/9 (11%)</td>
<td>Mycobacterium vaccae, Mycobacterium vanbaalenii</td>
</tr>
<tr>
<td>Buffalo (Syncerus caffer)</td>
<td>5</td>
<td>1/3 (33%)</td>
<td>1/3 (33%)</td>
<td>Mycobacterium gordoniae, M. vaccae, M. vanbaalenii</td>
</tr>
<tr>
<td>Bohor reedbuck (Redunca redunca)</td>
<td>3</td>
<td>0/1 (0%)</td>
<td>1/2 (50%)</td>
<td></td>
</tr>
<tr>
<td>Waterbuck (Kobus ellipsiprymnus)</td>
<td>2</td>
<td>1/2 (50%)</td>
<td>0/2 (0%)</td>
<td></td>
</tr>
<tr>
<td>Bush duiker (Sylvicapra grimmia)</td>
<td>1</td>
<td>NA</td>
<td>0/1 (0%)</td>
<td></td>
</tr>
<tr>
<td>Warthog (Phacochoerus africanus)</td>
<td>6</td>
<td>0/2 (0%)</td>
<td>0/6 (0%)</td>
<td></td>
</tr>
<tr>
<td>Bushpig (Potamochoerus larcaus)</td>
<td>1</td>
<td>NA</td>
<td>0/1 (0%)</td>
<td></td>
</tr>
<tr>
<td>Giant forest hog (Hyl肇ocerus meinertzhageni)</td>
<td>2</td>
<td>0/2 (0%)</td>
<td>0/2 (0%)</td>
<td></td>
</tr>
<tr>
<td>Elephant (Loxodonta africana)</td>
<td>8</td>
<td>2/8 (25%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Rock hyrax (Procavia capensis)</td>
<td>1</td>
<td>0/1 (0%)</td>
<td>0/1 (0%)</td>
<td></td>
</tr>
<tr>
<td>Hippopotamus (Hippopotamus amphibius)</td>
<td>1</td>
<td>0/1 (0%)</td>
<td>0/1 (0%)</td>
<td></td>
</tr>
<tr>
<td>Ethiopian wolf (Canis simensis)</td>
<td>5</td>
<td>0/5 (0%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Serval cat (Leptailurus serval)</td>
<td>1</td>
<td>NA</td>
<td>1/1 (100%)</td>
<td>MTC, Mycobacterium gilvum</td>
</tr>
<tr>
<td>Leopard (Panthera pardus)</td>
<td>3</td>
<td>0/1 (0%)</td>
<td>0/2 (0%)</td>
<td></td>
</tr>
<tr>
<td>Black-backed jackal (Canis mesomelas)</td>
<td>1</td>
<td>0/1 (0%)</td>
<td>1/1 (100%)</td>
<td>MTC</td>
</tr>
<tr>
<td>Anubis baboon (Papio anubis)</td>
<td>3</td>
<td>NA</td>
<td>0/3 (0%)</td>
<td></td>
</tr>
<tr>
<td>Black-clawed brush-furred rat (Lophuromys melanonyx)</td>
<td>10</td>
<td>0/14 (0%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Blick’s grass rat (Arvicanthis blicki)</td>
<td>7</td>
<td>1/7 (14%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>133</td>
<td>20/87 (23%)</td>
<td>29/89 (32.5%)</td>
<td></td>
</tr>
</tbody>
</table>

* AFB = acid-fast bacilli; NA = not analyzed; MTC = Mycobacterium terrae complex (includes Mycobacterium priferae, Mycobacterium confluents, Mycobacterium brasiliensis, Mycobacterium semenuis, M. terrae, Mycobacterium kumamotoense, Mycobacterium arupense); MAC = Mycobacterium avium complex.
for BTB, such as buffaloes in the RSA, Uganda, and Tanzania (Woodford, 1982a, b; Cleaveland et al., 2005; Michel et al., 2007), or potential reservoirs, such as kudus (Bengis et al., 2001), exist in large numbers at our study sites. *Mycobacterium bovis* was not isolated from any of these species, suggesting that they may not be major reservoirs for the pathogen in Ethiopia. However, a definitive conclusion is not possible because of the small number of animals examined in this study.

The concern regarding potential disease transmission from livestock to high-profile species such as the mountain nyala (*Tragelaphus buxtoni*) and the Ethiopian wolves (*Canis simensis*) is high. Both species are listed in the IUCN Red List of Endangered Species and often share the same habitat as humans and livestock. Mountain nyalas belong to the *Tragelaphus* family, like the kudu, which was shown to be a susceptible and potential maintenance species for BTB (Bengis et al., 2001; Keet et al., 2001). In our study, 40% (2 of 5) of mountain nyalas were antibody-positive by RT. However, only a very small number of animals were tested and further surveillance is warranted. Twenty rodent species from the Web Valley (Bale Mountains National Park) were examined for BTB and one grass rat (*Arvicanthis blicki*) was antibody-positive; unfortunately isolation by culture was not performed due to logistic constraints (including remoteness and lack of cold chain) to confirm the serologic finding. This result may be important from a conservation point of view because Ethiopian wolves mainly prey on afro-alpine rodents (Siller-Zubiri and Gottelli, 1995). Various rodents have been shown to host BTB in the UK (Matthews et al, 2006; Delahay et al., 2007). As seen in Kruger National Park, carnivores might be at high risk for contracting BTB if the disease is present in their prey (Michel et al., 2006).

Ethiopia is characterized by small wildlife populations, a high number of rare endemic species, and remoteness and inaccessibility of wildlife areas. Under these conditions, the RT would be a practical, easy, and inexpensive tool to screen live animals in a way that is not constrained by the difficult logistics of collecting lymph nodes. Nevertheless, the sensitivity of serologic tests is generally poor (Cleaveland et al., 2005). Lyashchenko et al (2008) found an overall sensitivity of the RT of 77%, which increased to 97% in white-tailed deer. However, their study only included four species: white-tailed deer, wild boar, brushtail possum, and Eurasian badger. The RT has not been used for most African species. Although our initial aim was to validate the RT kit for wildlife species, the number of animals sampled was too small for conclusive results. Lyashchenko et al (2008) found that sensitivity increased with the presence of gross lesions. The fact that we found few “tuberculosis-like” gross lesions in the lymph nodes of animals tested could partly explain the low sensitivity of the RT. The performance of the test also seemed to vary with species tested. We used the RT in 22 species and nine species had positive reactions. Twenty of the tested animals (23%) were reactive by RT. The highest positive rates were found in Guenther’s dik-diks (*Madoqua gunther*; 33%), lesser kudus (*Tragelaphus imberbis;* 60%), bushbucks (25%), buffaloes (33%) and mountain nyalas (40%). Two of eight elephants (*Loxodonta africana*) tested (25%) were RT-positive, which is worrisome because the test has been validated for elephants (Lyashchenko et al., 2006). However, no postmortem examinations of elephants were conducted in this study. Bovine tuberculosis is an increasing problem in domestic elephants in the Indian subcontinent (Sreekumar et al., 2007). In Ethiopia, only a small population of elephant remains in the wild, and it cannot be ruled out that BTB is prevalent in this endangered population.

Large numbers of atypical mycobacteria were isolated by culture. It is possible that
some of these isolates cross-reacted with the antigens contained in the RT, producing false positive results, which could explain the high prevalence of BTB antibody. However, experimental infection with *Mycobacterium paratuberculosis*, *M. avium*, and other NTM (Lyashchenko et al., 2008) did not show any reaction in the RT, suggesting that our positive serology results may represent infection by species of the *M. tuberculosis* complex. No strains of the *M. tuberculosis* complex were isolated from samples that were processed for culture. Only 47 of the samples tested by serology were matched with the gold standard method of culturing. It is therefore possible that some antibody-positive animals, for which no culture was done, were indeed positive for BTB. Furthermore, we may have failed to detect some infected animals, which could have been in the early stage of infection with no visible lesions or with lesions in tissues that were not examined. This could explain the high antibody prevalence while cultures were negative. Serology was performed on samples from a large number of species but only on a few animals per species. Sensitivity and specificity of the RT for a particular species therefore could not be evaluated. More sensitive and specific tests are needed to screen African wildlife and future research on test validation should probably focus on possible maintenance species or highly endangered species.

The isolation of 17 strains of NTM is an important result. *Mycobacterium terrae* was one of the most frequently isolated species. It was also isolated in wildlife in Tanzania (Cleaveland et al., 2005) and in the RSA (Michel et al., 2007). These reports suggest that *M. terrae* may be a ubiquitous NTM across Africa. This is in contrast to other NTM strains that may be more region-specific. In RSA, reports indicate the presence of *Mycobacterium vaccae*, *Mycobacterium engbaeckii* (Michel et al., 2007) and *Mycobacterium goodie* (Van Helden et al., 2008) in wildlife, whereas in Tanzania, *Mycobacterium phlei* and MAC were the NTM isolated in addition to *M. terrae* (Cleaveland et al., 2005). None of these NTM, with the exception of *M. vaccae*, were isolated in our study. Although in the published reports mentioned above, only a few NTMs were isolated from wildlife, a wide range of distinct strains were isolated in our study. Some of these NTMs have been described previously in captive wildlife, such as *Mycobacterium asiaticum* in a red-handed tamarin (*Saguinus midas*; Siegal-Willott et al., 2006), and *Mycobacterium gordonae* and MAC in captive pumas (*Felis concolor*; Traversa et al., 2009). We isolated *M. asiaticum* (a human lung pathogen; Taylor et al., 1990) from free-ranging Grant’s gazelle. In Tanzania and Ethiopia, *M. terrae* was associated with granulomatous lesions in cattle and humans (Kazwala et al. 2002; Berg et al., 2009). Most atypical mycobacteria in our study were isolated from mesenteric lymph nodes, suggesting environmental exposure via fodder or water. However, they were also isolated from lungs in half of the animals (*n* = 16), suggesting direct animal-to-animal aerosol transmission, possibly associated with behavior. The transmission pathway of NTM between domestic livestock, humans, and wildlife is not clear.

In conclusion, results of this first study of the prevalence of tuberculosis in Ethiopian wildlife suggest that BTB may not be endemic. However, despite possible false positive results, the serology suggested that BTB may be prevalent in wildlife. Therefore, we cannot rule out the possibility that BTB occurs in wildlife in Ethiopia. This study highlights the need for complementary diagnostics, especially if tissue culture as gold standard cannot be performed (e.g., on live animals). It also underscores the need for further research to increase sensitivity and specificity of serologic tests and to validate an RT for individual African wildlife species.

The high numbers of NTM found in the tissue samples warrant further investiga-
tion regarding their pathogenicity, their effect on the animal’s immune system, and their role and possible interaction with *M. bovis*.

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**LITERATURE CITED**


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