

Original Article

Mycobacterium tuberculosis at the human/wildlife interface in a high TB burden country

Short title: **Mycobacterium tuberculosis in wildlife**

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Summary

This study reports on an investigation of M. tuberculosis cases in mostly captive wild animals using molecular typing tools (Variable Number of Tandem Repeat typing and Restriction Fragment Length Polymorphism typing). The investigation included cases from 1.

the National Zoological Gardens of South Africa (NZG) recorded between 2002 and 2011, 2. the Johannesburg Zoo: tuberculosis was first diagnosed in 2007 and has since been detected in three antelope species. 3. a rehabilitation center for vervet monkeys (*Chlorocebus pygerythrus*) in which M. tuberculosis was diagnosed in 2008, 4. incidental cases in other facilities including a sable antelope (*Hippotragus niger*), two unrelated cases in chacma baboons (*Papio ursinus*) (one of which was from a free-ranging troop) and a colony of capuchin monkeys (*Cebus capucinus*). Identical genetic profiles of the latter three isolates indicated the persistence of a single M. tuberculosis strain in this population since at least 2006. Results of the outbreak investigation in the captive vervet monkey colony indicate that it was caused by two unrelated strains, while all 13 M. tuberculosis isolates from 11 animal species in the NZG showed different VNTR patterns. A substantial increase in tuberculosis cases of 60% was recorded in the NZG, compared to the previous reporting period 1991 – 2001, and may indicate a countrywide trend of increasing spillover of human tuberculosis to wild animals. South Africa ranks among the countries with the highest tuberculosis burden worldwide, complicated by an increasing rate of multidrug resistant strains. Exposure and infection of captive wildlife in this high prevalence setting is therefore a growing concern for wildlife conservation but also to human health through potential spillback.

Keywords: Mycobacterium, Tuberculosis, molecular epidemiology, wildlife/human interface, transmission

Introduction

Tuberculosis is one of the most common infectious diseases and causes of death of captive wildlife, especially non-human primates in zoological collections worldwide (Montali et al. 2001). Prior to the introduction of effective control and eradication schemes for bovine

tuberculosis, zoo wildlife was probably equally often affected by Mycobacterium bovis and Mycobacterium tuberculosis, although the exact cause was difficult to establish due to the lack of methods of differentiation at the time (Kovalev 1980). Aggravating factors were often poor hygiene as well as lack of adequate veterinary care, overcrowding, unsuitable environments and close contact to visitors (Kohn 1994).

A molecular study of tuberculosis cases caused by *Mycobacterium tuberculosis* in the National Zoological Gardens of South Africa (NZG) was conducted between 1991 and 2001. The high genetic diversity between strains led to the conclusion that the visiting public was the most likely source of infection to captive wildlife in the NZG (Michel et al. 2003). This observation suggested a change in the drivers of tuberculosis from mainly internal infection determinants, such as poor hygiene and animal husbandry practices, to external sources, i. e. the incidence of infection in the human population. Given the high infection pressure in the human population in South Africa, it may be speculated that this shift is likely to lead to a net increase in the incidence of M. tuberculosis in captive animal populations in South Africa.

The aim of the present study was to conduct a follow-up investigation on the tuberculosis cases in the NZG since 2002, including an investigation of recent M. tuberculosis outbreaks in other wildlife facilities.

Materials and methods

Animals

National Zoological Gardens (NZG):

Between 2002 and 2011, macroscopic lesions consistent with tuberculosis were recorded in a total of 16 animals from 12 species, including 1 chimpanzee (*Pan troglodytes*), 2 warthogs

(*Phacochoerus africanus*), 1 bongo (*Tragelaphus eurycerus*), 1 babirusa pig (*Babyrousa babyrussa*), 1 Brazilian tapir (*Tapirus terrestris*), 1 Malayan tapir (*Tapirus indicus*), 2 beavers (*Castor canadensis*), 3 Patas monkeys (*Erythrocebus patas*), 1 nyala (*Tragelaphus angasii*), 1 lesser kudu (*Tragelaphus imberbis*), 1 gorilla (*Gorilla gorilla*) and 1 eland (*Tragelaphus oryx*). On *post mortem* and histopathological examination typical lesions indicative of tuberculosis were detected in all cases. Intradermal tuberculin testing was only performed in the nyala (TB6622), which yielded a negative result.

Johannesburg Zoo:

In 2007 the first suspect case of tuberculosis occurred in a scimitar horned oryx (*Oryx dammah*). Acid-fast organisms were observed on microscopic examination. Post mortem examinations conducted in 2008 on a waterbuck (*Kobus ellipsiprymnus*) and a nyala revealed calcified tuberculous lung lesions and granulomatous pneumonia, respectively.

Vervet Monkey Rehabilitation Center (VRHC)

Following the sporadic death of several vervet monkeys (*Chlorocebus pygerythrus*) at the VRHC (Limpopo Province of South Africa) and the suspected diagnosis of tuberculosis in 2008, tissue samples from a total of 17 euthanized monkeys (Table 1) were submitted to the ARC-OVI Tuberculosis laboratory for routine diagnosis (van Zijll Langhout et al., 2009).

Incidental cases:

TB 5232. An adult female pregnant sable antelope (*Hippotragus niger*) died on a private game farm with severe respiratory symptoms soon after purchase at a game auction in the Limpopo Province. Post mortem findings included multifocal granulomatous, partially calcified lesions in the lungs, thoracic lymph nodes, on the thoracic pleura and pericardium as well as throughout the spleen

TB 6767. Tuberculosis was suspected as possible cause of disseminated nodular lesions on the abdominal pleura and granulomatous lesions in the uterus as well as in multiple lymph nodes of a chacma baboon (*Papio ursinus*) kept in an approved animal research facility in the Gauteng Province of South Africa.

TB 5628, TB 6536, TB 6954. A Capuchin monkey died in a private zoo in the Northwest Province of South Africa. Lung lesions consistent with tuberculosis were detected and submitted for tuberculosis culture in 2006. Samples from two additional Capuchin monkeys with similar signs were submitted in 2007 and 2008, respectively.

TB 7167. A chacma baboon from a free-ranging troop in the Limpopo Province was spotted along a public road showing severe neurological signs. The compromised animal was captured by a veterinarian and euthanized when treatment proved unsuccessful. On post mortem examination general tuberculosis with granulomatous lesions in the lungs, liver, spleen and multiple lymph nodes was observed. Bilateral post-ocular soft tissue abscesses were also noted.

Bacterial isolation and genetic characterization of M. tuberculosis isolates

Tissue samples from a total of 39 animals were processed according to standard methods for decontamination and culture of M. tuberculosis complex organisms and inoculated onto Löwenstein Jensen medium with supplements as described previously (Alexander et al. 2002). M. tuberculosis isolates were genetically characterized by IS6110 restriction fragment length polymorphism typing and Variable Number of Tandem Repeat (VNTR) Typing using the ETR loci A-F , as described previously (Frothingham et al., 1998, Michel et al. 2008).

The tissue samples included 14 animals (11 animal species) from the NZG, 1 waterbuck and 1 nyala from the Johannesburg Zoo, 17 vervet monkeys from the VRHC, 3 Capuchin

monkeys from a private zoo, 1 captive and 1 free-ranging chacma baboon and 1 semi-free-ranging sable antelope).

In the case of the scimitar horned oryx (Lab number 030507) no unpreserved tissue samples had been collected and DNA was extracted from sections cut from formalin-fixed, paraffin-embedded tissues by boiling (Sethusa 2006), followed by PCR amplification of a M. tuberculosis complex specific 123 base pair sequence of the *IS6110* gene (Miller et al., 1997)

Results

The results of bacterial isolation and where applicable, *IS6110*-RFLP and VNTR typing, are listed in Table 1.

Bacterial isolation

For the group of 16 animals from the NZG, M. tuberculosis infection was confirmed by culture in 13 animals (11 animal species). In the Johannesburg Zoo, M. tuberculosis was isolated from a waterbuck (TB 7033).

VNTR analysis

VNTR typing using the ETR A-F loci yielded a unique profile for each of the 13 culture positive animals from the NZG. VNTR typing of 17 M. tuberculosis isolates from the captive vervet monkey colony revealed two unrelated strains present in 14 and 3 animals, respectively (Table 1).

Capuchin monkeys: VNTR typing of TB 6536 showed a unique profile. Although only ETR loci B – F were successfully amplified for the cases TB5628 and TB6954, their VNTR patterns matched that of TB6536 and it was concluded that they belonged to the same VNTR type.

The M. tuberculosis isolate from the sable antelope (TB5232) yielded a unique VNTR profile unrelated to the other profiles in the study.

The VNTR profile obtained from the free-ranging baboon (TB 7067) was found to be identical to the profile of the waterbuck (TB7033) (Table 1).

IS6110-RFLP analysis

Seven isolates (TB6340, TB6622, TB6828, TB5628, TB6536, TB6954, TB6767) were typed using IS6110-RFLP analysis, resulting in 3 unique banding patterns for the lesser kudu, nyala and Patas monkey, respectively (NZG), which were unrelated to TB6767 (captive baboon) as well as the RFLP profile shared by the three Capuchin monkeys (TB5628, TB6536, TB6954).

A M. tuberculosis complex specific DNA product of 123 base pairs was successfully amplified from the DNA extracted from preserved tissues of a scimitar horned oryx, confirming infection with M. tuberculosis complex organisms.

Discussion

The first review of the molecular epidemiology of tuberculosis in the NZG covered an 11-year period from 1991 to 2001 and described 11 outbreaks in eight wildlife species (Michel et al., 2003). During the 10-year period of the present follow up investigation the number of outbreaks has increased by 60% to 16. The number of affected species increased from 8 in the previous study to 11, including 7 new and 4 previously affected species (chimpanzee, lesser kudu, Malayan tapir and Brazilian tapir). No new cases were observed in members of the other 4 previously affected species and we conclude that the diseased animals were dead end hosts and probably did not show extensive shedding of tubercle bacilli sufficient for intra-species transmission. Similarly, in the present study we report that two beavers

(TB3950 & TB4041), which died within less than one year from each other, were infected with two different strains of M. tuberculosis.

As in the earlier study the findings of the follow up investigation showed a high degree of genetic diversity between the 13 M. tuberculosis strains, which is a strong indication that the animals contracted the infections from external rather than from internal sources (Michel et al 2003). In the case of the Johannesburg Zoo, this study has provided the first confirmation of M. tuberculosis infection in the zoo's ungulate population. In both zoos strict quarantine protocols and programmes to monitor the health of staff are in place to prevent, amongst others, the introduction of *M. tuberculosis* with infected animals or staff into the collections.

Tuberculosis mainly affects wild animals if they are exposed to humans and their civilization (Griffith 1928, Epstein and Price 2009). The risk for contracting human tuberculosis, in particular, is assumed to be highest in zoological collections in metropolitan areas, e.g in the NZG. In contrast to large zoos, however, the transmission of M. tuberculosis in private wildlife facilities is predominantly from a single human or animal source to members in the same unit, resulting in the propagation of a single M. tuberculosis strain in the captive population, as shown in the Capuchin monkey colony in a private zoo. The fact that the same strain was repeatedly isolated over a 3-year period further suggests that the infection was either maintained within the Capuchin monkey colony or by a closely associated person, such as a caretaker. A higher risk of introducing infectious diseases, and in particular tuberculosis, exists in the rehabilitation center for vervet monkeys every time a new monkey with unknown tuberculosis status is allowed to join the colony. It is therefore not surprising that two unrelated M. tuberculosis strains (e.g. TB7554 & TB7374) were isolated, confirming at least two independent introductions of the disease into the centre.

Mycobacterium tuberculosis is not considered a primary animal pathogen and does not appear to have an indigenous animal reservoir (Montali et al. 2001). According to Kovalev (1980) epizootiological outbreaks of tuberculosis take a milder course among free-living animals than among those in captivity (Kovalev 1980). Our diagnosis of generalised tuberculosis in a chacma baboon from a free-ranging troop in a rural region of the Limpopo Province of South Africa, could, however, suggest that captivity may not in all cases be the most important determinant of disease severity. Pathogen related factors such as the strain of M. tuberculosis, infectious dose, drug resistance profiles, and repeated or prolonged exposure may be equally or more important (Lécu and Ball 2011).

The findings from this study also demonstrate that unless the source of infection is known, the measures applied to control the outbreak might prove ineffective. While quarantine and sanitary measures would be suitable to control the outbreaks in the vervet monkey and capuchin colonies, they are not likely to lead to an elimination of tuberculosis from the NZG. M. tuberculosis disease in wild animals appears to occur more frequently in South Africa than currently known and is not restricted to zoological collections and research facilities, as demonstrated by the cases in the sable antelope and the free-ranging chacma baboon reported here. Previously M. tuberculosis had been isolated from semi-free ranging bontebok (*Damaliscus pygargus pygargus*) and springbok (*Antidorcas marsupialis*) in the Western Cape Province and a vervet monkey from Kwazulu- Natal Province of South Africa (Michel, unpublished data).

Overall we are of the opinion that the findings of this investigation should no longer be considered the result of incidental spillover of human tuberculosis to wild animals. The human tuberculosis epidemic in South Africa is among the worst in the world with an incidence of *M. tuberculosis* of 948/100000 per annum (WHO, 2007). It has probably reached a threshold) with spillover to both captive and free-ranging wild animals as an

inevitable consequence. An increasing human population and the associated encroachment of previously uninhabited wildlife habitats give rise to a competition for space and human/wildlife conflict as a result. Under these circumstances wildlife can be considered sentinels for human zoonotic pathogens and a warning system for critical disease thresholds in public health management. The apparent increase in the occurrence of this human pathogen is of great concern considering the high diversity of wild animal/human interactions in South Africa and the associated health risk both in terms of wildlife conservation as well as of potential spillback to humans.

The 'One Health' approach is therefore better suited than any other strategy to exploit human and animal health information towards the implementation of integrated control measures to the health benefit of both populations. It will therefore be valuable to compare the strain profiles retrieved from wildlife with those of human M. tuberculosis isolates especially in Gauteng and other provinces involved to elucidate a human/wildlife link.

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