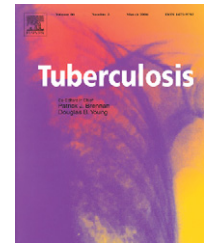# Pulmonary infection due to the dassie bacillus (*Mycobacterium tuberculosis* complex sp.) in a free-living dassie (rock hyrax—*Procavia capensis*) from South Africa

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Summary
We report a case of extensive necrogranulomatous pneumonia due to infection with the dassie bacillus (*Mycobacterium tuberculosis* complex sp.) in a free-living pregnant adult female dassie (rock hyrax—*Procavia capensis*). A juvenile female dassie from the same colony also showed a focal lesion in the lungs suggestive of mycobacterial pneumonia. Our findings indicate the widespread occurrence of the dassie bacillus in free-living dassies and suggest very high infection rates in some populations. The introduction of South African dassies into novel environments should be considered in this light.
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In June 2006, two female dassies (*Procavia capensis*) were randomly chosen and euthanized by an accredited government conservation official on Dasklip Pass, Grootwinterhoek Mountains, Western Cape, South Africa (S32″53′ 18.45;

E19″01′ 49.36), as part of an infectious disease survey of dassies. The dassies were sampled from a colony approximately 10 km from Porterville, the nearest human settlement. The animals were apparently healthy with no external injuries noted. Due to size difference and wearing of the teeth, it was determined that one dassie was an adult female while the other was a juvenile (under 3 years). Body condition was good in each female, weight was within the reported range for female *P. capensis* and all other signs were apparently normal. The adult female was identified as being pregnant with three fetuses.
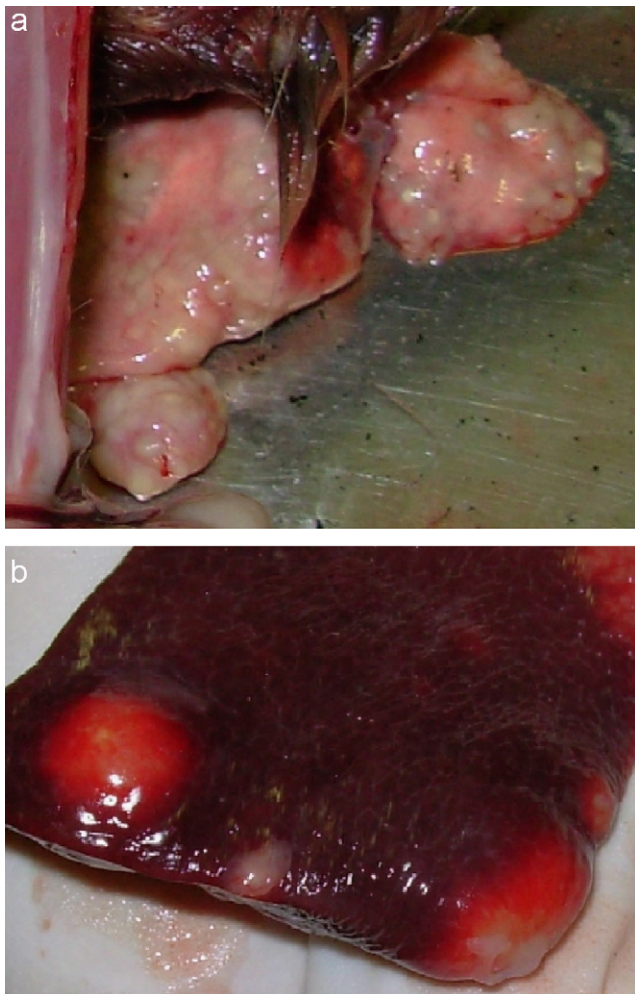
Both were subjected to post-mortem examination. Macroscopic lesions in the lungs of the pregnant adult female dassie consisted of numerous multifocal to confluent, round to irregular, 1–20 mm, dull whitish nodules distributed throughout the lung parenchyma with little normal lung tissue remaining (Figure 1a). The pleura was covered with similar slightly raised nodules and plaques. Many lesions showed central caseous necrosis with mild calcification. A few nodules showed mild central liquefactive necrosis with pus formation. The spleen showed similar lesions (Figure 1b). The liver and placenta had a few small, multifocal, round, raised, dull whitish nodules, ≤1 mm. The lungs of the juvenile dassie contained a focal nodular lesion

Figure 1 Extensive necrogranulomatous lesions observed in (a) the lungs and (b) the spleen of the adult female dassie.

of 2 mm in the cranial lobe of the left lung similar in appearance to the adult dassie. No other macroscopic lesions were detected in both animals. Samples from the lungs, spleen, liver, kidney and heart were collected from the adult dassie in 10% buffered formalin for microscopical examination. Fresh samples from the lung were collected aseptically from this animal for mycobacterial culture. No samples for histopathological examination or mycobacterial culture were collected from the juvenile dassie.
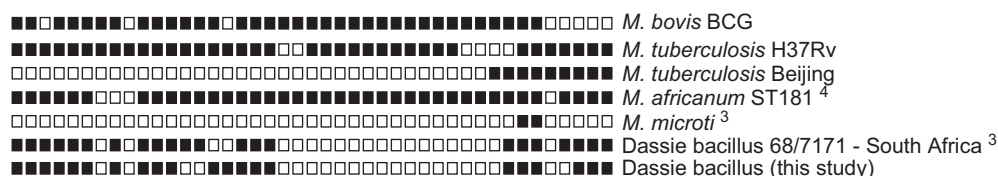
Microscopically, the lungs of the adult dassie showed numerous multifocal to confluent necrogranulomas throughout the parenchyma and the pleura. They consisted of a central area of caseous necrosis that was occasionally calcified. This was surrounded by a rim of moderate numbers of macrophages and epithelioid cells, and low numbers of multinucleated giant cells. Many macrophages and epithelioid cells contained single to multiple, small to large, round to oval, clear intracytoplasmic lipid vacuoles. There was an outer layer of lymphocytes and plasma cells within a mildly developed fibrous capsule. Numerous smaller, multifocal to confluent, often indistinctly outlined granulomas, without necrosis and calcification, consisting of vacuolated and normal macrophages and epithelioid cells, surrounded by lymphocytes and plasma cells were also visible. Some granulomas and necrogranulomas showed central infiltration of low to moderate numbers of neutrophils. Similar microscopic lesions were present in the spleen. The liver showed a few multifocal granulomas that consisted of moderate to large numbers of vacuolated and normal macrophages and epithelioid cells, surrounded by low to moderate numbers of lymphocytes and plasma cells. The center of the liver granulomas showed a small area of coagulation necrosis that was infiltrated by low numbers of neutrophils.

Ziehl–Neelsen staining of the lung and spleen tissue revealed scanty medium-sized, slender, acid-fast bacilli in the cytoplasm of macrophages and epithelioid cells comprising the smaller granulomas, and those along the edge of the necrotic center of necrogranulomas. Affected lung tissue was prepared and cultured in triplicate with the BACTEC MGIT culture system (Becton Dickinson, USA) as previously described.[1] A multiplex polymerase chain reaction (PCR) test was performed on heat-killed culture lysates as previously described,[1] and identified the organism as a member of the *Mycobacterium tuberculosis* complex with genomic characteristics consistent with the dassie bacillus. A subsequent PCR to detect a novel deletion in the dassie bacillus genome, RD1das, using previously published primers,[2] confirmed the isolation of this organism. Spoligotyping showed a pattern similar to that of a previous isolate of the dassie bacillus[3] and dissimilar to *M. tuberculosis* H37Rv, *Mycobacterium bovis* BCG, *Mycobacterium microti*[3] and *Mycobacterium africanum* subtype I[4] (Figure 2).

The *Mycobacterium* sp. defined as the dassie bacillus was first isolated from the lungs of a free-living dassie from Nieu Bethesda, in the Great Karoo, Eastern Cape, South Africa in the 1950s.[5] It is a member of the *M. tuberculosis* complex closely related to *M. microti* and *M. africanum* subtype Ia.[6–8] It has been more recently isolated from a few captive dassies and a single captive suricate (*Suricata suricatta*) all of which originated from unknown locations in South Africa (Göran Bolske, personal communication[2,5,7]), but it has not

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Figure 2 Spoligotype pattern for the dassie bacillus isolate is similar to a previously reported dassie bacillus spoligotype pattern and distinct from *M. tuberculosis*, *M. bovis* BCG, *M. microti* and *M. africanum* subtype I.

been found elsewhere. There are also no further reports of it being found in the wild since the original isolation, although no surveys have been conducted since then. It appears to have little virulence in rabbits or guinea pigs, species which are normally highly susceptible to pathogenic *Mycobacteria*.[5,9] The genome of the dassie bacillus has nine major regions of difference (RD) to that of *M. tuberculosis*, of which five are shared with *M. microti* and *M. africanum* subtype I.[2,8] A probably crucial difference is the small RD1[das] deletion in the ESX-1 region. This region, which contains the immunopathologically important T-cell antigens of the ESAT-6 gene family,[10,11] has been extensively studied and all evidence suggests that it is implicated in virulence.[12–15] Other mycobacterial species which show virulence in limited hosts, e.g. *M. microti* in voles, also have RD1 deletions in the ESX-1 region.[15,16]

Here we report that the dassie bacillus can be a pulmonary pathogen in free-living dassies. Whether this organism is sufficiently pathogenic to contribute in a meaningful way to the ecology of the dassie is currently unknown, but a study in this regard is probably justified as a general decline in dassie numbers has been observed over the last few years (unpublished observations). These animals live in colonies of up to 80 individuals, which is divided into smaller groups headed by one male and consisting of up to 20 females with their young.[17] The large numbers living in close proximity in natural crevices of rocks or boulders, together with the fact that they spend a significant proportion of their time huddling together for warmth (they have a poorly developed internal temperature regulation), probably provides ample opportunity for the spread of the bacillus. The dassie has a relatively slow reproduction rate, giving birth to only two or three young after a 6–7 month gestation period.[17] Young are only sexually mature after 16 months, reach adult size at 3 years, and typically live about 10 years. The pregnant dassie in this case was severely infected despite previous observations that this mycobacterial species did not appear significantly virulent. This could perhaps be attributed to immune suppression during pregnancy, although more work would need to be done in this area. It may be significant, as it would specifically cause a decrease in the breeding population of dassies, with a subsequent decline in numbers, as has been observed over the last few years (unpublished observations). Future studies aimed at elucidating the association between the decline in the population and the prevalence of the dassie bacillus are currently planned. It is clear from the captive suricate example that interspecies transmission of the dassie bacillus is possible. Whether the dassie bacillus can infect and affect predator species (of the dassie or suricate), such as eagles, caracal (*Felis caracal*) and leopard

(*Panthera pardus*), has never been reported, although it seems possible as dassies and suricates are important prey species for these animals.

The identification of the bacillus in an additional geographically distinct population of dassies (situated approximately 600 km from the original isolation in Nieu Bethesda) suggests the widespread occurrence of this organism in South Africa. The fact that random sampling of two free-living dassies revealed infection in one and suspected lesions in the other, also suggests high rates of infection in at least the Dasklip population. These findings contrast markedly with the results of the Nieu Bethesda survey in which only 4 of 86 animals were found to have granulomatous lung lesions. Of these, the dassie bacillus was isolated from only a single animal.[5] Importantly, these findings, combined with the isolation of the organism from captive animals originally from South Africa, highlight the need for vigilance in preventing the translocation of mycobacterial pathogens during the movement of host species. No skin test assays have been done on dassies before, so we do not know whether this can be used as a diagnostic or survey instrument. However, given the limited genetic differences between the dassie bacillus and the rest of the members of the *M. tuberculosis* complex, the use of bovine or tuberculosis PPD would probably be justified. Further studies are underway to determine the prevalence of this organism in the dassie population.

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