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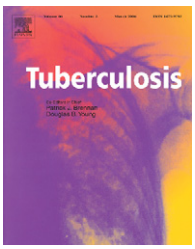

Pulmonary infection due to the dassie bacillus (*Mycobacterium tuberculosis* complex sp.) in a free-living dassie (rock hyrax—*Procavia capensis*) from South Africa

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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;

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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, $\leqslant$1 mm. The lungs of the juvenile dassie contained a focal nodular lesion 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.

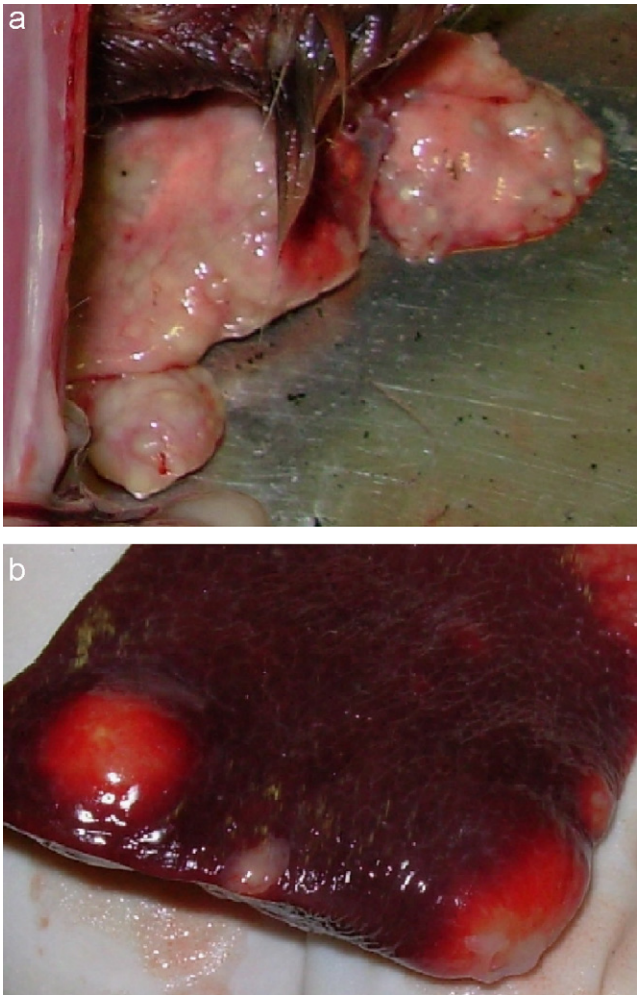

Figure 1 Extensive necrogranulomatous lesions observed in (a) the lungs and (b) the spleen of the adult female dassie.

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, RD1^{das}, 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