Novel Cause of Tuberculosis in Meerkats, South Africa

Sven D.C. Parsons, Julian A. Drewe, Nicolaas C. Gey van Pittius, Robin M. Warren, and Paul D. van Helden

The organism that causes tuberculosis in meerkats (Suricata suricatta) has been poorly characterized. Our genetic analysis showed it to be a novel member of the Mycobacterium tuberculosis complex and closely related to the dassie bacillus. We have named this epidemiologically and genetically unique strain M. suricattae.

Tuberculosis (TB) is caused by a group of distinct mycobacterial strains that might have evolved as host-adapted ecotypes (1) and that are collectively named the Mycobacterium tuberculosis complex (MTC) (2). In southern Africa, M. tuberculosis and M. bovis cause TB in numerous animals (3), the dassie bacillus infects rock hyraxes (dassies, Procavia capensis), and M. mungi infects banded mongooses (Mungos mungo) (4–6).

TB in free-living meerkats (Suricata suricatta) from the Kalahari Desert, South Africa, was first reported in 2002 (7), and its epidemiology and pathology have been comprehensively described (8,9). Mycobacterial strains isolated from these animals have been described as M. tuberculosis (7), M. bovis (8), and as a “member of the animal-adapted lineage of the MTC” (10), raising concerns that the occurrence of TB in these meerkats resulted from anthropogenic exposure to these pathogens and that affected meerkat populations could pose an infection risk to other wildlife, domestic animals, and humans (8). However, these studies used genetic analyses designed to differentiate between M. tuberculosis and M. bovis (7) and between these and M. africanum, M. canetti, M. microti, and M. bovis BCG (8) but not between these strains and the dassie bacillus or M. mungi. To gain greater insight into the etiology of this disease, we conducted a more comprehensive genetic analysis of mycobacterial isolates from this meerkat population.

The Study

Permission to sample meerkats was obtained from the University of Pretoria Animal Ethics Committee. Post-mortem examinations were performed on 4 meerkats from the Kalahari Meerkat Project (26°58′S, 21°49′E) that had shown visible disease. Samples from lesions typical of TB in this species (8) were used to establish mycobacterial cultures in the BD BACTEC MGIT 960 Mycobacterial Detection System (Becton Dickinson, Franklin Lakes, NJ, USA) (11). Four cultures originating from 3 animals were positive by Ziehl-Neelsen stain and were grown further on Difco Middlebrook 7H10 Agar supplemented with 10% OADC Enrichment (Becton Dickinson) for 6–8 weeks, after which DNA was extracted (11). However, only 1 isolate (MK172) yielded sufficient DNA for DNA fingerprinting by the IS6110 method (12). PCRs were conducted by using either heat-killed liquid cultures or purified DNA as a template.

Isolates were screened for the presence or absence of 4 phylogenetically informative genomic regions of difference (RDs) (11), and all showed deletion of RD9 but not of RD1, RD4, and RD12. This genotype is shared by M. africanaum, M. orygis, and the dassie bacillus (2,11); isolates were therefore analyzed for the presence or absence of RD1das, a genetic marker specific for the dassie bacillus (5). Because this RD was deleted in all isolates, these were subsequently analyzed for the presence or absence of N-RD25das, RD5das, and RDVirSdas (5); a G→A single-nucleotide polymorphism (SNP) in Rv1510 (Rv1510129); and a single-nucleotide deletion in Rv0911 (Rv09111389) (2). For all isolates, N-RD25das, RD5das, and RDVirSdas were deleted and Rv1510129 and Rv0911389 were present, consistent with the dassie bacillus genotype (2,5). However, although the RD5das deletion in this bacillus has been caused by the insertion of an inverted IS6110 sequence (5), for the meerkat strain, sequencing of the RD5das PCR product showed this region to be occupied by an IS6110 sequence in a forward orientation, followed by a proline-proline-glutamate gene homologue.

Spoligotyping was performed according to the internationally standardized method (13). However, we repeatedly obtained no amplification of any spacer included in this array. We investigated the possible deletion of the direct-repeat region, the genomic region analyzed by spoligotyping, by attempting to amplify by PCR selected genetic sequences upstream and downstream thereof (Table 1). This analysis confirmed that much, if not all, of the direct-repeat region had been deleted in these isolates, together with ≈3,500 bp upstream and up to 1,700 bp downstream of this region (Table 1).

Additionally, genetic characterization was done by sequencing of fragments of the gyrB gene (2) and 16S rDNA (14). For all isolates, the gyrB gene was consistent with
that of *M. africanaum*, *M. pinnipedii*, and the dassie bacillus (2). However, the 16S rDNA sequence differed from that of all other MTC members by having a T→G SNP at position 214 (16S rDNA). Analysis by mycobacterial interspersed repetitive unit–variable number tandem repeats (15) identified 2 strain variants in our sample set (Table 2); IS6110 DNA fingerprint analysis (12) of isolate MK172 showed it to contain 21 copies of the IS6110 insertion sequence element (Figure 1).

### Conclusion

We genetically characterized the causative pathogen of meerkat TB as a novel MTC strain that has several genetic features typical of the dassie bacillus and *M. mungi* (Figure 2). However, this pathogen differs from the closely related dassie bacillus in its mycobacterial interspersed repetitive unit–variable number tandem repeats patterns (Table 2) by being a unique RD5 variant and by containing 21 copies of the IS6110 insertion element (Figure 1) (compared with 10–15 copies in the dassie bacillus (2)). This evidence of IS6110 copy number expansion might indicate involvement of this insertion sequence in the occurrence of other genetic deletions in this strain, including those in the direct-repeat region. Notably, in addition to the novel SNP 16S rDNA, the loss of the direct-repeat region spacers, which are routinely screened for by spoligotyping, distinguishes this strain from all other MTC members (1,13,14). As evidenced by their shared RDs and SNPs, the genetic homogeneity of multiple isolates of this distinctive strain suggests that it has undergone selective evolution, possibly through adaptation to its meerkat host (1). It is highly pathogenic in this species and seems to be substantially more virulent than the genetically similar dassie bacillus (4,5). As such,

<table>
<thead>
<tr>
<th>Locus</th>
<th><em>M. africanaum</em></th>
<th><em>M. mungi</em>†</th>
<th><em>M. suricattae</em></th>
<th>Dassie bacillus†</th>
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<tr>
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†MIRU-VNTR, mycobacterial interspersed repetitive unit–variable number tandem repeats; †From Alexander et al., 2010 (4).
‡Copy number of 3 isolates from 2 meerkats, including MK172.
§Copy number of a fourth isolate from a third meerkat.
confirms the shared SNP Rv15101129 as a genetic marker. bacillus share a unique common progenitor (2); our study
budasse bacillus, which have been isolated from animals
express our gratitude to Tim Clutton-Brock and the Kalahari Meerkat Project for providing the meerkat carcasses.
This study demonstrates that the occurrence of TB in the Kalahari meerkats might not be indicative of an external infectious source of Mycobacterium tuberculosis or M. bovis, as has been reported (6,8). Rather, our findings suggest that the disease is caused by an indigenous MTC member, which we have named Mycobacterium suricattae. Our limited sample set precludes a detailed analysis of the epidemiology of this
distinguish this epidemiologically unique strain from other MTC members, we have named it Mycobacterium suricattae after the host species from which it has been isolated.
pathogen; however, the identification of this strain and the characterization of several of its discriminatory genetic markers will be useful for future investigations of the ecology and evolution of the African animal–adapted members of the MTC.

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Dr Parsons is a postdoctoral fellow in the Faculty of Medicine and Health Sciences, Stellenbosch University. His research interests include the diagnosis, epidemiology, and control of TB in wildlife.

References

Figure 1. IS6110 restriction fragment length polymorphism patterns of A) a reference strain of Mycobacterium tuberculosis (M14323) with selected fragment lengths indicated in kilobases, and B) Mycobacterium suricattae (isolate MK172).

Figure 2. Phylogeny of the Mycobacterium tuberculosis complex (MTC) detailing relevant genetic regions of difference (RDs) and single-nucleotide differences that distinguish between Mycobacterium africanum subtype 1 and the small African mammal–adapted members of these strains.
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