

'Emerging' mycobacteria in South Africa

P D van Helden^{a*}, S D C Parsons^a and N C Gey van Pittius^a

ABSTRACT

Disease can be caused by various species of the genus *Mycobacterium*. A number of reports, both published and unpublished, of rarely reported mycobacteria have surfaced in South Africa in the last few years. Some unusual hosts have also been involved, causing concern in some quarters. These include reports on *Mycobacterium goodii* in a spotted hyaena (*Crocuta crocuta*), *M. xenopi* in a ruffed lemur (*Varecia variegata*), *M. intracellulare* in wild-caught chacma baboons (*Papio ursinus*), the 'dassie bacillus' in free ranging rock hyrax (dassies; *Procavia capensis*) the 'oryx bacillus' from free-ranging buffalo (*Syncerus caffer*) and *M. tuberculosis* in suricates (*Suricata suricatta*), a domestic dog and in baboons. In this article it has been attempted to put these in context and show how improved surveillance and technologies have allowed mycobacteria to be identified to species level more easily. Most of the unusual mycobacterial species have most likely been present in the region for many years and have probably caused disease episodes before, but have been misdiagnosed. Each case must be evaluated carefully with respect to the animal species involved, the environment in which the host is found and the mycobacterial species, and operational decisions made accordingly.

Keywords: animalia, Mycobacteria, speciation, tuberculosis.

van Helden P D, Parsons S D C, Gey van Pittius N 'Emerging' mycobacteria in South Africa. *Journal of the South African Veterinary Association* (2009) 80(4): 210–214 (En.). DST/NRF Centre of Excellence for Biomedical TB Research/MRC Centre for Molecular and Cellular Biology/Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, Faculty of Health Sciences, Stellenbosch University, PO Box 19063, Tygerberg, 7505 South Africa

INTRODUCTION

A number of incidents (both published and unpublished) regarding the unusual occurrence of tuberculosis (TB) and TB-like disease in animals in South Africa have emerged recently, highlighting infections caused by mycobacterial species.

These incidents include reports on *Mycobacterium goodii* in a spotted hyaena (*Crocuta crocuta*)²⁴, *M. xenopi* in a ruffed lemur (*Varecia variegata*) (E P Lane, National Zoological Gardens, unpubl. data), *M. intracellulare* in healthy wild-caught chacma baboons (*Papio ursinus*)¹, the so-called 'dassie bacillus' in a free-ranging rock hyrax (*Procavia capensis*)¹⁷, as well as another hyrax from the Western Cape (PDvH, unpubl. data). In addition, reports on the 'oryx bacillus' from an African buffalo (*Syncerus cafer*) in KwaZulu-Natal (Gey van Pittius, unpubl. data), *M. bovis* from a black rhinoceros (*Diceros bicornis*) belonging to the National Zoo (I Espie, National Zoological Gardens, pers.

comm., 2009), *M. tuberculosis* in suricates (*Suricata suricatta*) in the northern Cape (A Michel, University of Pretoria, pers. comm., 2009), *M. tuberculosis* in a domestic dog from Cape Town¹⁶, and *M. tuberculosis* in chacma baboons¹⁵. None of these is necessarily surprising or alarming for a variety of reasons. This communication is an attempt to put these in context.

THE MYCOBACTERIA

The genus *Mycobacterium* currently consists of 128 species and 5 subspecies, some of which are fast growers and mostly non-pathogenic, while others are slow growers and usually pathogenic³. This latter branch includes those in the *M. tuberculosis* complex (MTC), such as *M. canettii*, *M. tuberculosis*, *M. africanum* (subtype Ia and Ib), dassie bacillus, oryx bacillus, *M. microti* (the vole bacillus), *M. pinnipedii* (the seal bacillus), *M. caprae* (the goat bacillus), *M. bovis* (the bovine bacillus) and *M. bovis* BCG (see Fig. 1)⁷. Those mycobacterial species that are not usually pathogenic may become pathogens under unusual circumstances. In humans, these circumstances may include HIV infection, diabetes and steroid treatment, which suppresses the immune system and can

allow usually non-pathogenic organisms to become pathogens¹⁸.

The mycobacteria are regarded as very successful organisms and occupy a wide range of niches in the environment (soil and water) as well as being pathogens in some cases¹⁴. Some mycobacterial species have even been found to colonise extremely harsh environments, such as acidic hot springs in Yellowstone National Park with extreme acidity (pH 3.0) and temperature (56 °C)²¹. In fact, 37 % of gene clones analysed from a highly acidic (pH 1.0) volcanic environment were found to originate from previously unidentified mycobacterial species²⁶, signifying their ability to survive under extreme conditions. New species of mycobacteria are constantly being identified (9 new species were described in 2006 alone) and 52 % of known species have been identified in the last 20 years (data not shown). At least 5 new species have been found recently from human sampling in the Western Cape in South Africa, which are in the process of being characterised by one of the authors (NCGvP).

The mycobacteria that are able to colonise animals have evolved in such a way that many specialise, forming host-adapted ecotypes with preferred hosts²². These include *M. tuberculosis* and *M. africanum*, which tend to be found almost exclusively in humans. *M. bovis*, on the other hand, has a very broad range of hosts, although it is found primarily in bovines. It is interesting to note that although many of these species that are pathogenic to mammals have evolved from a common ancestor (see Fig. 1) they are rarely, under natural circumstances, found outside of a defined host or host family (e.g. *M. microti* in voles, *M. pinnipedii* in seals, and dassie bacillus in hyraxes). In addition, there is strong evidence for an original home range or home population for some species or subspecies²². It should be noted that even *M. tuberculosis* has evolved extensively, leading to various strains and sub-strains that have individual differentiating characteristics. Some strain types are seen in abundance in certain populations of people or geographical regions and are absent or almost absent in other regions. For example, the well-known 'Beijing' strains of *M. tuberculosis* that are highly

^aDST/NRF Centre of Excellence for Biomedical TB Research/MRC Centre for Molecular and Cellular Biology/Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, Faculty of Health Sciences, Stellenbosch University, PO Box 19063, Tygerberg, 7505 South Africa

*Author for correspondence. E-mail: pvh@sun.ac.za

Received: June 2009. Accepted: November 2009.

abundant in Asia and have now spread worldwide. This strain family is highly successful^{6,23}, prone to becoming drug resistant^{8,9}, and is increasing in numbers in the Western Cape relative to other strains²³. On the other hand, the so-called KZN (XDR) TB strain, which caused much publicity recently with high numbers of deaths in Tugela ferry⁴, is not a new strain at all, but is part of a family of *M. tuberculosis* strains found all over South Africa²³. This XDR strain variant of *M. tuberculosis* has found a vulnerable human population in an extremely high HIV incidence area and has thus been able to cause increased mortality in this population⁴.

NEW TECHNOLOGIES HIGHLIGHT EMERGING DISEASE

While many basic microbiological techniques to define mycobacterial species were developed decades ago, these are extremely labour intensive and cannot discriminate all species or subspecies and certainly not strain types. However, these techniques were able to define important pathogens such as *M. tuberculosis* and *M. bovis* in the late 1800s and even less important ones such as the dassie bacillus in the late 1950s. The dassie bacillus (which has never received a 'proper' name), was found first in rock hyraxes from the Nieu Bethesda area, was tested for virulence by deliberate infection of a number of TB-susceptible animal species, and was found to be non-pathogenic in all of them.

However, newly developed molecular-based (genome) diagnostic and typing techniques allow one to identify species and subspecies more easily and more accurately. Such techniques, which include PCR-sequencing of target genes, as well as line probe hybridisation assays, have been successfully applied to the mycobacterial field. These are usually based on detection of known specific DNA sequences and include the newly-developed Hain Genotype product series which is used to determine the species of some mycobacteria and to detect certain drug resistance markers²⁰. These easy to use, fast and accurate techniques have allowed an explosion of new investigations, aimed at re-examining many of the old established dogmas in mycobacterial disease. A vast body of this type of work is done in research laboratories, where traditionally, research results often do not reach a wider audience. This paradigm is shifting, with researchers now putting more effort into translational research and dissemination of information. Some effort has also been directed to check that past species identification is in fact correct. This was not possible in the past because

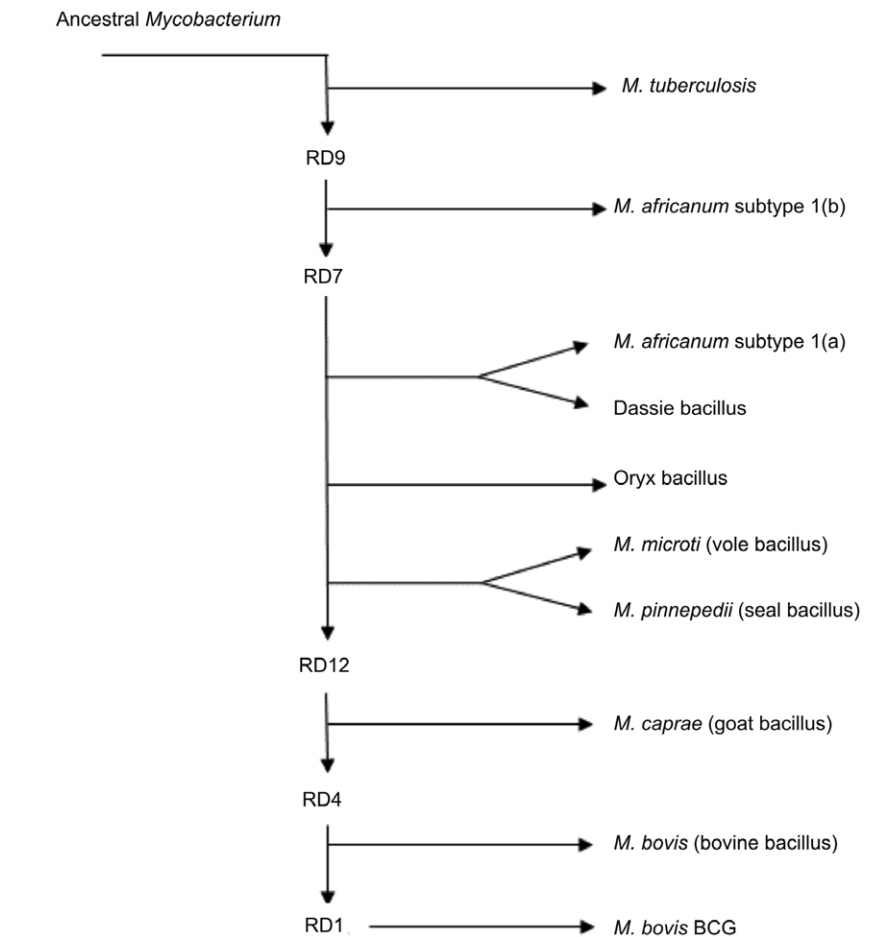


Fig. 1: Phylogenetic tree of the members of the *Mycobacterium tuberculosis* complex. 'RD' denotes specific 'regions of difference' detected between the genomes of different species.

of old and crude techniques that were not adequately discriminatory or simply too labour intensive and expensive to use.

It should be noted that with the advent of antibiotics (mostly from the late 1950s up to the mid-1960s), TB incidences largely stabilised or declined in the human population in many countries¹¹. Similarly, in the veterinary field, prevalence of *M. bovis* declined, since skin testing and slaughter policies have been quite effective¹⁹. The consequence was that TB was thought to be conquered and it became a neglected area of research for quite some time. This is nowhere more clearly illustrated than in the words of the Nobel prize winner Selman A. Waksman (discoverer of over 20 antibiotics) who in 1964 wrote that '...the ancient foe of man, known as consumption, the great white plague, tuberculosis, or by whatever other name, is on the way to being reduced to a minor ailment of man. The future appears bright indeed, and the complete eradication of the disease is in sight'²⁵.

THE 'NEW' TB EPIDEMIC

For reasons that are not clear, the incidence of *M. tuberculosis* in the South African population increased dramatically from

the mid-1970s even prior to the introduction of HIV (South African National Department of Health). Similar increases were noted worldwide, even in developed countries such as the USA where socio-economic conditions were good and healthcare was improving steadily. This prompted new investment and a resurgence of research in TB, with the additional development of new technology.

Using these new sensitive tools, it is now possible to find new mycobacterial species in previously unidentified niches. It is likely that many of these were present in the past, but simply not identified or misidentified. Essentially, we find them because we are now actively looking for them and because recent research has allowed more detailed characterisation of species. A number of these rare species have been identified in the laboratory at Stellenbosch University, but this is part of an advanced research programme and such sophistication should not be expected from a routine diagnostic laboratory. A large TB prevalence survey has been embarked on in humans in the Western Cape, and many individuals with *M. tuberculosis* infection were found who were asymptomatic, as expected, as many who were infected by non-tuberculous

mycobacteria (NCGvP, unpubl. data) were young. Some of these mycobacteria are known pathogens, but no signs of clinical disease were evident in the individuals infected by them. The impact of these infections is not known and to understand this will require extensive research. Some of these individuals were followed up to ascertain whether disease developed, but no symptoms are yet evident in those checked (M Muyoyeta, University of Zambia School of Medicine, unpubl. data).

Additionally, because of 'failure to cure', *M. intracellulare* has been diagnosed in 2 human cases recently (NCGvP, unpubl. data). If these samples had not been sent to the research laboratory, they would have been classified as drug resistant TB and treated accordingly, or the patient may have died. Both patients were of advanced age, suggesting that *M. intracellulare* may primarily be a pathogen of immunocompromised individuals.

RELEVANCE TO ANIMALS

The 'new or emergent' TB in animals should be seen in a similar light. There have been many cases of cross-species infection from zoos. The most common perhaps is *M. tuberculosis* moving into animals from handlers or the viewing public¹³, or *M. bovis* moving between animals or to human handlers from the animal. *M. tuberculosis* has been found in captive baboons¹⁵ and vervet monkeys in South Africa and even in free living baboons in close contact with farm workers or farmyards. This is not at all surprising, given the cavalier attitude to TB evinced by many South Africans, with spitting and unsanitary behaviour being common, and a TB incidence rate which is arguably the highest in the world.

The dassie bacillus has more recently been isolated in Australia from captive hyraxes originating in South Africa². We were therefore eager to obtain a rock hyrax carcass that showed visible lesions associated with this species in South Africa to determine if it could be 'rediscovered' since it was not possible to obtain a culture from outside South Africa for our basic research. This opportunity occurred recently when 2 apparently healthy hyraxes were shot as part of a leopard research project in the Winterhoek Mountains of the Western Cape. The hyrax had extensive necrogranulomatous pneumonia due to infection with the dassie bacillus¹⁷. Since previous reports of the dassie bacillus do not point to the Western Cape, this most likely shows that it is widespread in South Africa. However, this needs to be confirmed, since there is no definitive proof of its distribution.

A case of *M. bovis* infection in a black rhinoceros held in one of the facilities of the National Zoological Gardens was recently diagnosed (I Espie, National Zoological Gardens, pers. comm., 2009). It should be noted that this rhinoceros was old and in poor condition. It cannot be said unequivocally that *M. bovis* caused the poor condition of the rhinoceros at all, since only 2 relatively small lesions were found at necropsy.

It is perhaps pertinent to note at this point that tuberculosis in humans is a complex disease. It is known that after infection there are 3 possible outcomes: 1) the person does not develop an infection at all and the bacteria are killed, 2) a primary infection is established, but contained, and no symptoms occur, 3) there is immediate progression to disease. In the absence of immunosuppression, only 5 % of infected individuals fall into the last category. In developed countries such as Europe and the USA, one can see this phenomenon in the elderly once the immune system functionality declines.

It is not known what the situation is in different animal species, or whether different mycobacteria behave in the same way as *M. tuberculosis* in humans. However, it is highly likely that a similar situation prevails in many animals, perhaps with quantitative differences in the relative proportions that progress to active disease soon after infection compared with those that can be latently infected with the potential for later activation.

The recent 'oryx bacillus' event in which an oryx bacillus was isolated from a healthy, skin test positive African buffalo on a farm known to have previously imported buffalo from a zoo in Portugal can be speculatively explained (NCGvP, unpubl. data). The oryx bacillus was originally isolated from Arabian oryx and camelids, and so far appeared to be restricted to the northern hemisphere. Buffalo captive in a zoo in Portugal were probably in contact with Arabian oryx, which allowed infection. The oryx bacillus infection was presumably kept in check by the innate or adaptive immune system of the buffalo. Some buffaloes from the zoo in Portugal were then transferred to South Africa approximately 20 years ago, in all likelihood bringing the dormant oryx bacillus in at least 1 animal. It is unlikely that such an animal has been sampled, but transmission between animals without observed clinical signs might have occurred and it is detected in a 2nd- or 3rd-generation animal. Given the rare reactions seen in this herd, as well as the lack or apparent absence of disease noted over nearly 20 years, as far as our information extends, it is likely that this is

not a serious pathogen in buffalo. However, this has not been proven and it is also not known what could happen if it were to be excreted by the buffalo and were to infect other wildlife. The pathogenicity of the organism in the Southern African oryx species called the gemsbok (*Oryx gazella*) has never been established, but given their relationship to the Arabian oryx, the preferred host species of this organism, it can be hypothesised that it would also be pathogenic for gemsbok.

Possibly the oryx bacillus (like the dassie bacillus) is less virulent than *M. tuberculosis* or *M. bovis*. (Virulence is not understood at all well in mycobacteria, particularly when the species is not in its preferred host.) Buffalo may even have reasonable innate resistance to oryx bacillus, although clearly not to *M. bovis*. Note that *M. tuberculosis* infection is extremely rare in bovids and that transfer of *M. tuberculosis* from one bovid to another does not occur, as far as is known. One should not lose sight of the fact that *M. bovis* is probably a far more serious mycobacterial pathogen than the oryx bacillus. It is not even known whether buffalo infected by the oryx bacillus will mount a positive skin test response in all cases. The only way to know is to test this experimentally.

From a management point of view, this is a difficult problem. One cannot know whether the oryx bacillus has crossed into another host with potential to infect buffalo again. Therefore, to slaughter all buffalo on the affected property may not work. On the other hand, a typical response to a BTB herd would be recommended, *i.e.* initial test and slaughter, with regular follow up if possible.

Finally, it should be noted that in many instances, cases of TB in animals do not involve the lungs and therefore unless the animal is eaten uncooked (*e.g.* by a predator), it presents only a remote chance for transmission, particularly to humans.

CANINE TUBERCULOSIS

In a recent case¹⁶, a stray Maltese cross-breed domestic dog with extensive multifocal pulmonary tuberculosis due to *M. tuberculosis* was detected as part of an intensive research project being done in our centre to investigate innate resistance to TB. The rationale is that if innate or acquired resistance could be understood, it may be possible to develop entirely new therapies. Since TB has been reported very rarely in dogs, it is hypothesised that dogs have innate resistance to TB. In order to do this study, we selected dogs from communities in Cape Town that rank among those with the highest reported TB incidence in the world. It is known that at least 80 % of adults in these

communities show infection with *M. tuberculosis*¹⁰ and therefore the dogs are almost certainly also exposed to TB infection. Owing to poverty and many other social factors, hundreds of dogs from such communities are euthanased by animal welfare organisations every year and these animals provide an important resource for biosurveillance of zoonotic diseases such as TB. A limited *post mortem* survey of 100 stray dogs, using the 'gold standard' of diagnosis, namely culturing, detected 1 animal with pulmonary tuberculosis, and infection in 3 others that showed no gross or histopathological signs of disease. Apart from bacterial culture, there is no validated test for TB infection in dogs, but a high prevalence of immunological sensitivity to *M. tuberculosis* antigens has been detected among dogs living with TB patients, suggesting a high level of transmission of this pathogen from owners to their pets. Our interpretation of these early findings is that despite frequent transmission of *M. tuberculosis* to dogs, a relatively small number of infected animals progress to active TB and contribute little to the national TB epidemic. Therefore, our current data suggest no need for a concerted intervention in this regard. However, there may be particular circumstances where the potential for pets to act as reservoirs of infection should be carefully considered. HIV positive individuals are highly susceptible to TB and, where possible, companion animals living in close contact with immunocompromised individuals should be screened for all zoonotic diseases. Animals that have potentially been infected by multi drug-resistant (MDR) strains of *M. tuberculosis* warrant concern. The management of MDR-TB requires enormous resources and in extreme cases this disease may be virtually untreatable. Companion animals infected with these strains therefore potentially pose a significant danger to human health. However, since only 1 case of TB with fulminating lesions has been found in a dog after 3 years of working in a high risk environment, transmission of TB from dogs can be regarded as highly unlikely in most cases. Given the infection pressure of *M. tuberculosis* from human to human, particularly in South Africa where the disease is so prevalent, dog-to-human transmission risk is almost certainly low to vanishing point. However, it may be of interest to practising veterinarians, who may suspect TB occasionally in a dog or related wild animal.

WHY WERE THESE UNUSUAL EVENTS NOT FOUND BEFORE?

It should be noted that new technology,

particularly in genomics, has made identification easier and more accurate and more mycobacterial species are differentiated and described every year. Mycobacteria are common in the environment¹² and under the right circumstances can become pathogens. We may therefore expect more, but probably rare, interesting and unusual cases to emerge over time.

It is estimated that there are approximately 4–6 nonillion ($4-6 \times 10^{30}$) bacteria on earth²⁷ and that only approximately 4000 prokaryotic species have been described to date, with recent estimates for the number of species in this domain of life ranging from 10^5 to 10^7 (ref. 5). Clearly, identification and description of these is beyond the remit and capabilities of routine laboratories. It is also costly to establish advanced technology, particularly for rare events, and therefore not within the boundaries of most routine investigations. Most practising clinicians and veterinarians cannot be expected to be aware of the complexities of the genus *Mycobacteria*, or of what might constitute an appropriate request for an advanced level test or be an appropriate response to an unusual result. This is still a highly specialised research area. Our laboratory has developed a very deep and thorough species characterisation technology^{8,24} involving genomics and bioinformatics in order to be able to identify the exact species of mycobacteria infecting the host. It does not offer a routine diagnostic service, but a research based collaborative service. We deliberately search for interesting cases and mycobacterial species as part of our focus and mandate. Even this has only very recently been made possible by increased funding and new technologies that have been developed.

CONCLUSION

Much research still needs to be done to understand TB in humans and animals. Vigilance needs to be exercised, but not at the cost of trying to keep in check the major epidemics of *M. tuberculosis* in humans in SA and *M. bovis* in domestic stock and wildlife. We are increasingly finding cases of TB-like disease due to species of mycobacteria not within the *M. tuberculosis* complex, but this may simply represent the success in searching deliberately for them. Many of them were almost certainly present before recent reports, but remained undetected because of rare occurrence, or the absence of clinical disease and of tools or funding to identify them. At this stage, it is unlikely that they represent a serious threat to animal or human health other than at an occasional individual level. Nevertheless,

many of the mycobacteria can be regarded as 'alien' to South Africa and as such should be kept under surveillance. Any veterinarian or biologist who finds an 'unusual' case that is a suspect *Mycobacterium* sp. infection should contact a specialised laboratory

REFERENCES

- Chege G K, Warren R M, Gey van Pittius N C, Burgers W A, Wilkinson R J, Shephard E G, Williamson A L 2008 Detection of natural infection with *Mycobacterium intracellulare* in healthy wild-caught chacma baboons (*Papio ursinus*) by ESAT-6 and CFP-10 IFN-ELISPOT tests following a tuberculosis outbreak. *BMC Microbiology* 8: 27
- Cousins D V, Peet R L, Gaynor W T, Williams S N, Gow B L 1994 Tuberculosis in imported hyrax (*Procavia capensis*) caused by an unusual variant belonging to the *Mycobacterium tuberculosis* complex. *Veterinary Microbiology* 42: 135–145
- Euzéby J P List of Prokaryotic names with Standing in Nomenclature (LPSN). Online at: <http://www.bacterio.cict.fr/> (accessed January 2010)
- Gandhi N R, Moll A, Sturm A W, Pawinski R, Govender T, Lalloo U, Zeller K, Andrews J, Friedland G 2006 Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet* 368: 1575–1580
- Hammond P M, Allsopp D, Colwell R R, Hawksworth D L 2009 *Microbial diversity and ecosystem function*. CABI Publishers, Wallingford: 29–71
- Hanekom M, van der Spuy G D, Gey van Pittius N C, McEvoy C R, Ndabambi S L, Victor T C, Hoal E G, van Helden P D, Warren R M 2007 Evidence that the spread of *Mycobacterium tuberculosis* strains with the Beijing genotype is human population dependent. *Journal of Clinical Microbiology* 45: 2263–2266
- Huard R C, Fabre M, de Haas P, Lazzarini L C, van Soolingen D, Cousins D, Ho J L 2006 Novel genetic polymorphisms that further delineate the phylogeny of the *Mycobacterium tuberculosis* complex. *Journal of Bacteriology* 188: 4271–4287
- Johnson R, Warren R, Strauss O J, Jordaan A M, Falmer A A, Beyers N, Schaaf H S, Murray M, Cloete K, van Helden P D, Victor T C 2006 An outbreak of drug-resistant tuberculosis caused by a Beijing strain in the western Cape, South Africa. *International Journal of Tuberculosis and Lung Disease* 10: 1412–1414
- Johnson R, Warren R M, van der Spuy G D, Gey van Pittius N C, Theron D, Streicher E M, Bosman M, Coetzee G J, van Helden P D, Victor T C 2010 Drug-resistant tuberculosis epidemic in the Western Cape driven by a virulent Beijing genotype strain. *International Journal of Tuberculosis and Lung Disease* 14: 119–121
- Mahomed H, Hughes E J, Hawkrigde T, Minnies D, Simon E, Little F, Hanekom W A, Geiter L, Hussey G D 2006 Comparison of mantoux skin test with three generations of a whole blood IFN-gamma assay for tuberculosis infection. *International Journal of Tuberculosis and Lung Disease* 10: 310–316
- McCray E, Weinbaum C M, Braden C R, Onorato I M 1997 The epidemiology of

- tuberculosis in the United States. *Clinics in Chest Medicine* 18: 99–113
12. Michel A L, de Klerk L M, Gey van Pittius N C, Warren R M, van Helden P D 2007 Bovine tuberculosis in African buffaloes: observations regarding *Mycobacterium bovis* shedding into water and exposure to environmental mycobacteria. *BMC Veterinary Research* 3: 23
 13. Michel A L, Venter L, Espie I W, Coetzee M L 2003 *Mycobacterium tuberculosis* infections in eight species at the National Zoological Gardens of South Africa, 1991–2001. *Journal of Zoo and Wildlife Medicine* 34: 364–370
 14. Narang R, Narang P, Mendiratta D K 2009 Isolation and identification of nontuberculous mycobacteria from water and soil in central India. *Indian Journal of Medical Microbiology* 27: 247–250
 15. Parsons S D C, Gous T A, de Villiers C, Warren R M, Seier J V, van Helden P D 2009 Detection of *Mycobacterium tuberculosis* infection in chacma baboons (*Papio ursinus*) using the QuantiFERON-TB Gold (In-Tube Method) assay. *Journal of Medical Primatology* 38: 411–417
 16. Parsons S D C, Gous T A, Warren R M, van Helden P D 2008 Pulmonary *Mycobacterium tuberculosis* (Beijing strain) infection in a stray dog. *Journal of the South African Veterinary Association* 79: 95–98
 17. Parsons S, Smith S G D, Martins Q, Horsnell W G C, Gous T A, Streicher E M, Warren R M, van Helden P D, Gey van Pittius N C 2008 Pulmonary infection due to the dassie bacillus (*Mycobacterium tuberculosis* complex sp.) in a free-living dassie (rock hyrax – *Procavia capensis*) from South Africa. *Tuberculosis* 88: 80–83
 18. Petrini B 2006 Non-tuberculous mycobacterial infections. *Scandinavian Journal of Infectious Diseases* 38: 246–255
 19. Reichel R 1995 South Africa. In Thoen C O, Steele J H (eds) *Mycobacterium bovis infection in animals and man*. Iowa State University Press, Ames: 285–292
 20. Richter E, Weizenegger M, Fahr A M, Rusch-Gerdes S 2004 Usefulness of the GenoType MTBC assay for differentiating species of the *Mycobacterium tuberculosis* complex in cultures obtained from clinical specimens. *Journal of Clinical Microbiology* 42: 4303–4306
 21. Santos R, Fernandes J, Fernandes N, Oliveira F, Cadete M 2007 *Mycobacterium parascrofulaceum* in acidic hot springs in Yellowstone National Park. *Applied Environmental Microbiology* 73: 5071–5073
 22. Smith N H, Kremer K, Inwald J, Dale J, Driscoll J R, Gordon S V, van Soolingen D, Glyn Hewinson R, Maynard Smith J 2006 Ecotypes of the *Mycobacterium tuberculosis* complex. *Journal of Theoretical Biology* 239: 220–225
 23. Van der Spuy G D, Kremer K, Ndabambi S L, Beyers N, Dunbar R, Marais B J, van Helden P D, Warren R M 2009 Changing *Mycobacterium tuberculosis* population highlights clade-specific pathogenic characteristics. *Tuberculosis* 89: 120–125
 24. Van Helden P D, Gey van Pittius N C, Warren R M, Michel A, Hlokwe T, Morar D, Godfroid J, du Plessis E C, Bengis R 2008 Pulmonary infection due to *Mycobacterium goodii* in a spotted hyena (*Crocuta crocuta*) from South Africa. *Journal of Wildlife Diseases* 44: 151–154
 25. Waksman S A 1964 *The conquest of tuberculosis*. University of California Press, Berkeley and Los Angeles
 26. Walker J J, Spear J R, Pace N R 2005 Geobiology of a microbial endolithic community in the Yellowstone geothermal environment. *Nature* 434: 1011–1014
 27. Whitman W B, Coleman D C, Wiebe W J 1998 Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences USA* 95: 6578–6583