Transboundary and Emerging Diseases

Transboundary and Emerging Diseases

REVIEW ARTICLE

Mycobacteria and Disease in Southern Africa

L. Botha, N. C. Gey van Pittius and P. D. van Helden

DST/NRF Centre of Excellence for Biomedical Tuberculosis Research/Medical Research Council (MRC) Centre for Molecular and Cellular Biology, Division of Molecular Biology and Human Genetics, Faculty of Health Sciences, Stellenbosch University, Tygerberg, South Africa

Keywords:
Mycobacterium tuberculosis complex; *M. bovis*; NTM; southern Africa

Correspondence:
P. D. van Helden, DST/NRF Centre of Excellence for Biomedical Tuberculosis Research/ Medical Research Council (MRC) Centre for Molecular and Cellular Biology, Division of Molecular Biology and Human Genetics, Faculty of Health Sciences, Stellenbosch University, PO Box 19063, Tygerberg 7505, South Africa.
Tel.: +21 938 9124; Fax: +21 938 9863;
E-mail: pvh@sun.ac.za

Received for publication August 1, 2013

doi:10.1111/tbed.12159

Summary

The genus *Mycobacterium* consists of over 120 known species, some of which (e.g. *M. bovis* and *M. tuberculosis*) contribute extensively to the burden of infectious disease in humans and animals, whilst others are commonly found in the environment but may rarely if ever be disease-causing. This paper reviews the mycobacteria found in southern Africa, focussing on those in the *M. tuberculosis* complex as well as the non-tuberculous mycobacteria (NTM), identifying those found in the area and including those causing disease in humans and animals, and outlines some recent reports describing the distribution and prevalence of the disease in Africa. Difficulties in diagnosis, host preference and reaction, immunology and transmission are discussed.

Introduction

Tuberculosis (TB) commonly occurs in humans and animals throughout the world, with Africa and particularly South Africa being a very high burden region, with the incidence of *M. tuberculosis* in humans averaging 913/100 000 per annum (World Health Organization). Detailed and thorough prevalence surveys are rarely carried out, but the few that have been performed show that infection with *M. tuberculosis*, the causative agent for TB in humans, is far more common than estimated from passive case finding (den Boon et al., 2007). South Africa currently has the highest global burden of human disease from *M. tuberculosis*. It is not the purpose of this manuscript to reiterate that situation, other than to remark that the situation is complex, given that we know that the genus *Mycobacterium*, to which *M. tuberculosis* belongs, consists of a larger group of species, most of which have been shown to be able to cause different forms of mycobacterial disease under appropriate conditions (Phillips and von Reyn, 2001; Petrini, 2006; Warren et al., 2006; Moore et al., 2010; Simons et al., 2011). The fast-growing species (<7 days in culture) are often non-pathogenic, whereas slow-growing species (more than 7 days in culture) are mostly pathogenic (see Fig. 1). The slow-growing species include the members of the *M. tuberculosis* complex. Other species outside the complex are known as the non-tuberculous mycobacteria (NTM). Prevalence surveys, where mycobacterial speciation has been carried out, have shown that NTM may be up to three times more commonly found in humans than *M. tuberculosis* (Muyoyeta et al., 2010 and our own unpublished data). Similarly, although *M. bovis* is the most common cause of tuberculosis in animals, it cannot always be assumed to be the causative agent of tuberculosis in animals.

The genus *Mycobacterium* currently consists of around 128 validly published species and five subspecies with at least a further 34 species not fully described or named (van Helden et al., 2009). They are commonly encountered in the environment, where niches include water, soil, protozoans, domestic and wild animals, invertebrates, and milk and food products (Holland, 2001; Michel et al., 2007; Falkinham, 2010). Some are obligate or opportunistic pathogens, but many are saprophytes. Apart from the common and well-known pathogenic mycobacteria that are primar-

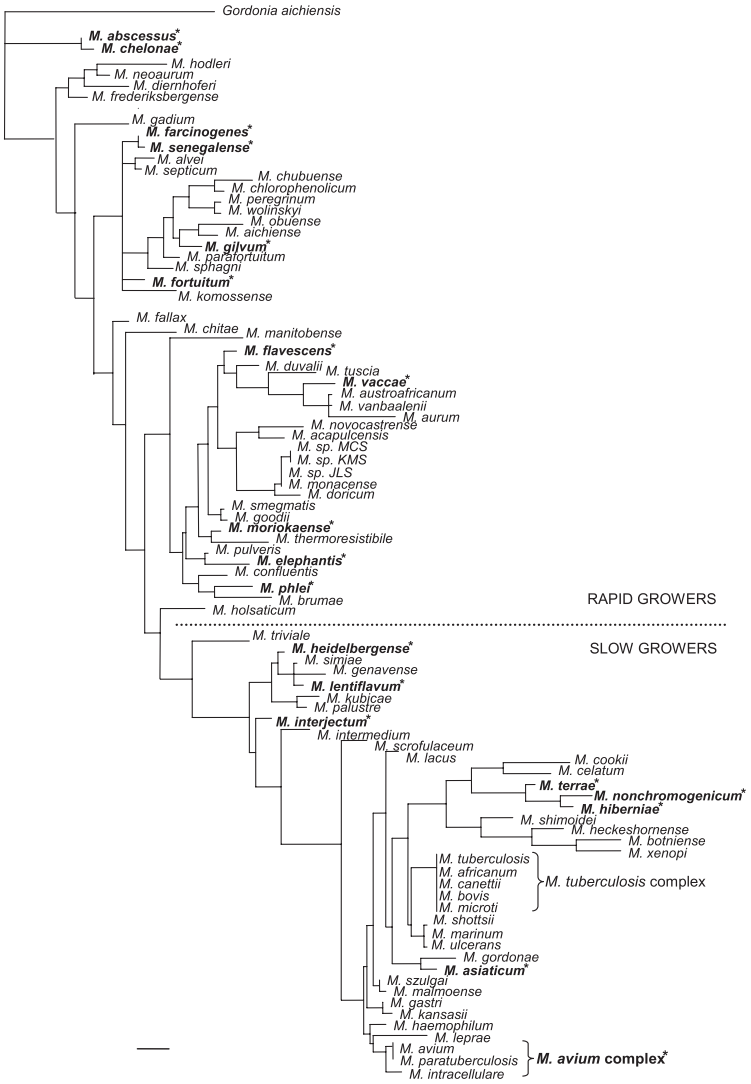

Fig. 1. The genus *Mycobacterium*. Well-known pathogens are mostly slow growers. The species marked in bold and with a * are NTM isolated in South Africa from animals. Figure adapted from (Gey van Pittius et al., 2006).

ily transmitted between hosts, the environment can be a source of exposure to mycobacteria. We have investigated water as a source for such and found *M. terrae*, *M. vaccae* (or *vanbaalenii*), *M. engbaekii* and *M. thermoresistibile* (Michel et al., 2007). Although not extensively investigated, there is clearly regional variation in the occurrence of mycobacterial species, for example, *M. ulcerans* has not (yet) been found in southern Africa. However, with the movement of animals (and birds) often uncontrolled, one can expect the unexpected (e.g. the occurrence of *M. orygis* in South Africa (Gey van Pittius et al., 2012a,b).

Mycobacterium tuberculosis complex members

Mycobacteria grouped in the *Mycobacterium tuberculosis* complex (MTBC) are characterized by what is regarded as more than 99% similarity at the nucleotide level and identical 16S rRNA sequences. The traditional members of the *Mycobacterium tuberculosis* complex include the following four species: *Mycobacterium tuberculosis*, *Mycobacterium africanum*, *Mycobacterium microti* and *Mycobacterium bovis*. Newly recognized additions to the MTBC include *Mycobacterium caprae*, *Mycobacterium pinnipedii* and *Mycobacterium canettii*, as well as potentially rarer or perhaps more geographically restricted MTBC variants, such as the so-called dassie (Parsons et al., 2008a) and oryx bacillus, now known as *Mycobacterium orygis* (Gey van Pittius et al., 2012a,b; van Ingen et al., 2012b) and *Mycobacterium mungi*, which causes TB in the banded mongoose (Alexander et al., 2010).

Infection of animals or humans by the members of the MTBC, most particularly *M. tuberculosis* and *M. bovis,* is

clearly of concern, as these are the commonly recognized causes of the massive global burden of TB. Standard molecular tools used to delineate and speciate the MTBC are IS*6110* restriction fragment length polymorphism (RFLP) analysis; spacer oligonucleotide typing (spoligotyping); regions of difference (RD) analysis and MIRU-VNTR typing. These tools can be used to position the different members of the MTBC into the phylogenetic tree of the complex (Fig. 2).

Our work shows that by far the majority of human tuberculosis cases (from *M. tuberculosis*) in South Africa originate from active and ongoing transmission and not reactivation disease and that this dynamic is valid for both antibiotic-sensitive and resistant *M. tuberculosis* cases. (Hanekom et al., 2007). Despite this enormous infection pressure, there have been very few cases of confirmed anthropozoonotic TB in South Africa specifically, viz. a single case of *M. tuberculosis* in a dog (Parsons et al., 2008b) and one of *M. tuberculosis* in a zoo elephant (Tordiffe A et al., unpublished). However, a number of *M. tuberculosis* cases in livestock have been reported in Ethiopia and other African countries (Gumi et al., 2012).

Whilst the incidence and prevalence of *M. bovis* cases in humans is far lower than *M. tuberculosis*, this pathogen is problematic in various settings in animal and human

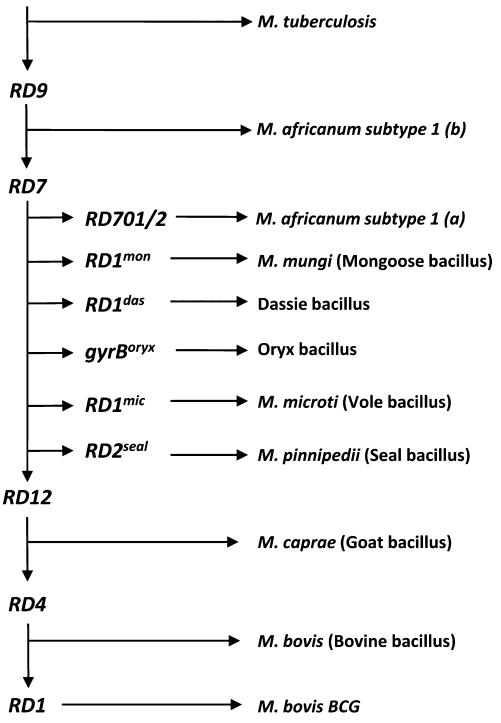

Fig. 2. The phylogeny of the *Mycobacterium tuberculosis* complex (MTBC) members based on genome differences, which can form the basis of differential diagnostics. Figure adapted from (Gey van Pittius et al., 2012a,b).

populations in the different countries. However, in southern African countries (here defined as South Africa, Namibia, Zimbabwe, Zambia, Tanzania, and Mozambique), there are some considerable differences. In South Africa, almost all *M. bovis* occurs in the wildlife population, due to good disease control practice in the past, but historical invasion into the African buffalo (Michel et al., 2007, 2009). Whilst it has spread into at least 12 mammal species, it occurs predominantly in the wildlife parks, and there is as yet no evidence to suggest spread across the interface into humans or domestic livestock (Michel et al., 2010). There are sporadic occurrences of *M. bovis* in domestic herds, but these are apparently well controlled by state veterinarians. Zimbabwe claimed to have no *M. bovis* (in the past), whereas Zambia, Mozambique and Tanzania do. In Zambia, *M. bovis* commonly occurs in wildlife in Kafue (specifically lechwe) (Munyeme et al., 2009). In Mozambique, *M. bovis* is found in a number of provinces in livestock, even up to a prevalence of 60% based on skin testing in some districts (A Machado, personal communication). In Tanzania, there is evidence for *M. bovis* in wildlife and domestic stock (up to 13% positivity) and a few cases of zoonotic *M. bovis* in humans have been reported (Katale et al., 2012). As none of these countries can claim a full diagnostic service with speciation, the true picture with respect to zoonotic TB is unknown.

The social behaviour in captive and free-living animal herds in African wildlife parks provide favourable conditions for *M. bovis* transmission to members of the same herd, for example buffalo in South Africa (Michel et al., 2007, 2009) and lechwe in Zambia (Munyeme et al., 2009). Transmission of the *Mycobacterium tuberculosis* complex members is also effective in species that maintain social or familial groups in underground dens. Drewe et al. conducted a study in a South African meerkat population and found that grooming (both giving and receiving) was more likely than aggression to be correlated with mycobacterial disease transmission and that groomers were at higher risk of infection than groomees (Drewe et al., 2011).

Although respiratory transmission is probably the most important route of infection in groups of animals that remain in close contact, indirect transmission via food is another important route (Kaneene and Thoen, 2004). For oral transmission to occur, an uninfected animal has to consume feed or water contaminated with mucous or nasal secretions, faeces, or urine that contain the infective organisms or receive milk from an infected dam; therefore, the mycobacterium must be able to survive outside an infected host for sufficient time to be ingested by another animal. Research conducted in South Africa and elsewhere suggests that infected buffalo serve as a source of direct oral infection to large predators such as lions and scavenging omnivores such as warthogs (Michel et al., 2007, 2009).

Non-tuberculous mycobacteria

Most of the species included in the genus *Mycobacterium* are distinguished from the *Mycobacterium tuberculosis* complex members by the fact that they are not obligate pathogens, but are inhabitants of the environment. Although these mycobacteria are classified as saprophytes and live freely in the environment, they can be opportunistic pathogens, particularly in immunocompromised individuals. This latter factor is of major concern in high HIV prevalence settings, such as those found in many parts of Africa. For such individuals, there may be no such thing as a 'non-pathogenic' mycobacterium.

Unlike tuberculosis caused by *M. tuberculosis*, which is spread from human–human or human–animal or *vice versa*, non-tuberculous mycobacterial infections have not been considered to be particularly contagious (Brown, 1985). There is little or no evidence that the infection can be transmitted from one person to another. Several reports have debated the possibility of co-infection with both *M. tuberculosis* and NTM in individuals (Shamaei et al., 2010). The role of NTM, particularly when public health issues are concerned, should nevertheless not be ignored, despite the fact that no definitive route of transmission is yet proposed. NTM and the pathologies they cause have received increasing attention worldwide during the past decade (Griffith et al., 2007), despite the fact that the reported cases are still relatively few in numbers. Therefore, any disease episode, especially those presenting with clinical manifestations during their involvement merits being reported, along with the major mycobacterial pathogens, such as *M. tuberculosis, M. leprae, M. avium* and *M. bovis* (Griffith et al., 2007).

About one-third of the NTM have thus far been associated with disease in humans and can cause localized disease in the lungs, lymph glands, skin, wounds or bone (Katoch, 2004). From a human and clinical perspective, amongst the most important slow-growing species are *Mycobacterium avium* and *Mycobacterium intracellulare* (the *M. avium complex* (MAC).

A recent clinical study in humans (Simons et al., 2011) based on data from a number of geographically distant regions in Asia showed that the *M. avium* complex was responsible for 56% of clinically relevant pulmonary disease (range 40–81%), *M. abscessus* for 35%, *M. chelonae* for 31%, *M. kansasii* and *scrofulaceum* for 17%, *M. celatum* 9%, *M. szulgai* 6%, *M. fortuitum* 5%, *M. gordonae* 2% and *M. terrae* 1%. It is important to define what infection means, illustrated by the following example: in Denmark (Andrejak et al., 2010), 1282 adult humans were diagnosed as NTM-positive, of which 26% had definite disease, 19% possible disease and 55% colonization only. Five-year mortality after definite NTM disease was 40.1%. Infection with *Mycobacterium xenopi* was found to be associated with worse prognosis than *Mycobacterium avium* complex. Amongst 283 subjects studied in the USA (Yew et al., 2011), 47% of them met American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) pulmonary NTM disease criteria for a minimum overall 2-year period prevalence of 8.6/100,000 persons, and 20.4/100 000 in those at least 50 years of age. The prevalence ranged from 1.4 to 6.6 per 100 000. The prevalence of NTM lung disease globally appears to be increasing (Phillips and von Reyn, 2001; Petrini, 2006; Iseman and Marras, 2008; Moore et al., 2010; Prevots et al., 2010) by over 2.8% per year, mostly amongst persons older than 60 years of age, although in most parts of the world, there is insufficient data to know how universal this may be. Thus, although the prevalence of disease from NTM may be low, the presence of NTM species is associated with a high likelihood of disease and thereafter mortality.

From such detailed reports in human studies, one may reasonably assume that most if not all of these species could cause TB-like disease in animal species and reports suggest that the *Mycobacterium avium* complex (MAC, which includes *Mycobacterium intracellulare*), *Mycobacterium kansasii*, *Mycobacterium malmoense, Mycobacterium xenopi, Mycobacterium ulcerans, Mycobacterium fortuitum, Mycobacterium abscessus* and *Mycobacterium chelonae* are indeed found in a significant number of animals such as cattle, deer, sheep and goats, as well as wild and domesticated birds, fish, reptiles and amphibians (Karne et al., 2012) (Bercovier and Vincent, 2001). However, it is important to note that the presence of an NTM does not necessarily imply an active disease process.

There are very few reports of NTM isolated from animals, especially from Africa. This is partly because of the perception that NTM rarely cause disease (with the exception of *M. avium* subsp *paratuberculosis*) and partly because identification and speciation of NTM is relatively complex. However, mycobacterial species (whether in the MTBC or NTM) that have been identified to be disease causing in African animals include *M. goodii, M.orygis and M. mungi*, as well as a new species from the meerkat (*Suricata suricatta*), as yet undescribed (Parsons et al., 2013).

The following NTM have been found to cause disease in Africa: *M. avium* is the most frequent bacterial opportunistic infection of AIDS (Karne et al., 2012); *M. fortuitum* is most frequently isolated from fish and was found to produce a high mortality rate in South African fish farms (Bragg et al., 1990); *M. senegalense* has been implicated in tuberculosis-like pathology of cattle in Africa (Bercovier and Vincent, 2001). Whether a single agent is responsible for bovine farcy is still unclear, although Chamoiseau considered *M. farcinogenes* var. *senegalense* and *M. farcinogenes*

var. *tchadense* to be the sole agents of bovine farcy because of the isolation of these species from the lesions (Chamoiseau, 1973). *M. kansasii* has been detected in humans as well as in wild animals such as deer, camels, birds, monkeys and in water (which may be the natural reservoir) and was one of the first species shown to be responsible for non-tuberculous pulmonary infection (Bercovier and Vincent, 2001). The impact of this important pathogen in human infection is regularly reported and is considerable; however, it is a relatively rare pathogen of animals (Bercovier and Vincent, 2001).

Over the past few years, we have prepared various animal samples for mycobacterial culture analysis when necropsy has been carried out as part of routine practice. Cultures obtained from bronchial washes, lymph nodes and lung material include the following species identified by 16S rRNA sequence analysis: *M. abcessus*, *M. asiaticum*, *M. avium*, *M. brasilienses*, *M. chelonae*, *M. elephantis, M. engbackii, M. farcinogenes, M. fortuitum, M. gilven, M. gordonae, M. heraklionense, M. hiberniae, M. intracellulare, M. interjectum, M. lentiflavum, M. marseillense, M. moriokaense, M. nonchromogenicum, M. palustre, M. palveris, M. paraffinicum, M. phlei, M. senegalense, M. simiae, M. sherrisii, M. sphagni, M. terrae* and *M. vulneris.* Animal species examined were lions, rhinos, banded mongooses, cattle, baboons, elephants and monkeys. The presence and isolation of these mycobacteria does not imply active disease in each case. However, should any animal become immunocompromised, many species could be pathogenic. Thus, with rare exceptions, we have observed that mycobacterial species other than *M. tuberculosis*, *M. bovis* and *M. avium* subsp. *paratuberculosis*, seldom cause extensive disease, and very few reports exist in the literature.

Geographical distribution and environmental determinants

There is a reasonable body of literature regarding the distribution of the MTBC, but little concerning NTM. These will both be discussed briefly below. Regional variations in the geographical distribution of mycobacteria in Asia are reported, and it is reasonable to expect a similar variation across Africa (Simons et al., 2011). Certainly, for example, this is supported by the localized occurrence of mycobacterial species such as *M. cannetti, M. ulcerans* and subtypes of *M. bovis.* Specifically, *M. ulcerans* and *M. bovis* Af1 subtypes are found predominantly in west Africa (Niemann et al., 2000), *M cannetti* in the Horn of Africa (Pfyffer et al., 1998), *M. bovis* Af2 subtype in east Africa (Niemann et al., 2000), *M. mungi* in Botswana (Alexander et al., 2010) and the dassie bacillus and *M. suricattae* thus far in South Africa only (Parsons et al., 2008a, 2013). Factors playing a role in distribution may include initial host range and topography, host specificity and host susceptibility. Environmental factors leading to previously unrecorded species appearing sporadically in other regions can be as a result of travel of animals, humans or birds, for example (De Groote and Huitt, 2006; Griffith et al., 2007). Alternatively, the apparent absence of a species from an area may simply be because no survey or relevant diagnostic work has been done there.

With the exception of *M. ulcerans*, these are all members of the *M. tuberculosis* complex, and this biodiversity suggests a possible origin for the *M. tuberculosis* complex to be 'Out of Africa', with regional evolution to explain the emergence of different strains or species of mycobacteria. Further evidence to support this 'Out of Africa' suggestion comes from work done in Ethiopia, where a new *M. tuberculosis* lineage has been found, which is restricted to the Ethiopian highlands (Berg et al., 2011).

The distribution of NTM may be regional or global: for example, the *M. avium* complex, *M. abscessus, M. scrofulaceum, M. marinum* and *M. fortuitum* are encountered globally, whilst *M. malmoense* is found mainly in Scandinavia (Griffith et al., 2007) and *M. ulcerans* mainly in Australia, Africa and South-East Asia (the tropics) (Ablordey et al., 2005). In Chad and Nigeria, the *M. fortuitum* complex was most frequently isolated from cattle and pigs (respectively) when they conducted a study to investigate tuberculosis (Bercovier and Vincent, 2001). Similarly, pathogenic NTMs isolated from the pastoral ecosystems of Uganda included the *M. avium* complex (Muller et al., 2008).

Because of the presence of NTM in the environment, human activities have had direct impacts on their ecology and hence their epidemiology (Kankya et al., 2011) as humans have very substantially altered the living environment (Falkinham, 2010). This may provide new niches that some mycobacteria can exploit and thereby increase our risk of exposure. An example of this is water supply systems, where mycobacteria can easily form biofilms, which are almost impossible to remove (Falkinham, 2011). In all the habitats, where NTM have been recovered (Michel et al., 2007), the mycobacteria are part of the normal flora, existing as stable, resident and growing populations. An exception may be *M. avium* subsp. *paratuberculosis,* where growth and persistence in the environment has not been reported, despite being prevalent in Bovidae worldwide. Farms or settlements with persistent infection have been described as sources of infection in this case (Whittington et al., 2005).

Compared with other bacteria, NTM are relatively disinfectant, heavy metal, and antibiotic resistant. Thus, the use of any anti-microbial agent selects for mycobacteria. Employment of disinfectants for drinking water treatment

leads to selection and enrichment of mycobacteria in distribution systems in the absence of disinfectant-sensitive competing micro-organisms (Falkinham, 2010, 2011). Non-tuberculous mycobacteria selection may also occur as a consequence of the presence of antibiotics in drinking water and drinking water sources. Likewise, pollution, large scale agriculture, human and animal movement and a myriad of activities can provide micro-organisms in general and mycobacteria specifically with a rich opportunity to move and exploit their environment (Falkinham, 2010, 2011).

Discovery that human behaviours lead to selection and proliferation of NTM in habitats occupied by both humans and NTM, creates the dilemma that human actions taken to reduce pathogen exposure (i.e. water disinfection), lead to possible increased NTM disease (Petrini, 2006; Moore et al., 2010; Prevots et al., 2010; Yew et al., 2011).

Hosts of mycobacteria

Some individuals (and species or strains of animals) are more susceptible to mycobacterial disease than others. For example, in cattle, it has been shown that various breeds have differing susceptibility to bovine TB (Ameni et al., 2007). Additionally, there is evidence to suggest that different populations of hosts may also select for certain pathogen types or subtypes (Hanekom et al., 2007).

Although it is largely *M. tuberculosis* that is found in active TB humans and *M. bovis* in terrestrial animals, Mycobacterium species can cross the species barrier in both the zoonotic and anthropozoonotic directions. Thus, cases of *M. bovis* in humans are well known, but less commonly, cases of *M. tuberculosis* have been reported in animals such as cats, dogs and elephants (Parsons et al., 2008b; Angkawanish et al., 2010; LoBue et al., 2010), where the sources of these infections have commonly been traced to infected humans. However, there are many cases of *M. tuberculosis* in animal collections, where it is arguably an important cause of morbidity and mortality from infectious disease in captive wildlife (Montali et al., 2001). Transmission of *M. tuberculosis* from animal to animal has not been conclusively shown, although extensive transmission is found with the animal-adapted species of the complex, such as *M. bovis*. Parsons et al. showed that *M. tuberculosis* antigens were found in 50% of dogs living in close contact with sputum smear-positive TB patients (Parsons et al., 2012). As South Africa is rated as a high TB incidence and risk setting, it is not surprising that companion animals living in such environments will be exposed to this pathogen. Whether these animals can be reservoirs for human disease, in turn, is not known. Despite these cases of *M. tuberculosis* in animals, most free-living animals diagnosed as tuberculous are reported or regarded to be infected with *M. bovis*; therefore, infection with *M. bovis* is of public-health and economic importance.

However, the complex members also cause TB; even if they show limited host or geographical association (see Table 1). For example, the recently described *M. orygis* (Gey van Pittius et al., 2012a,b; van Ingen et al., 2012b) is apparently host-adapted to the antelope and found almost exclusively in the Arabian oryx (*Oryx leucoryx*) in the Middle East. However, the first isolation of this species in South Africa was from an African buffalo (Gey van Pittius et al., 2012a,b), although the history of this animal suggests that contact with Arabian oryx was highly likely at some stage. This finding shows that infected animals with host-adapted pathogens which may at first appear to be host specific can sometimes infect a new host species.

Clinical signs and diagnoses

Rapid diagnoses of animals infected with *M. tuberculosis or M. bovis* are performed using two primary immunological tests, namely the *in vitro* tuberculin skin test (TST) and the *in vivo* interferon-gamma (IFN-γ) release assay (IGRA). These tests typically rely on the detection of antigen-specific T lymphocyte-mediated responses as surrogate markers of infection by the causative organism. The IGRAs now include proteins largely specific to *M. tuberculosis* and *M. bovis*, such as early secretory antigenic target 6 kDa (ESAT-6) and culture filtrate protein 10 kDa (CFP-10), for better diagnoses. These tests generally indicate infection and not active disease and are also not able to speciate (differentiate) the pathogen or definitively diagnose an infection or disease caused by an NTM. However, once a positive culture of NTM has been obtained, the Capilia TB assay was found to be a quick and easy test to use for differ-

Table 1. *Mycobacterium tuberculosis* complex species and their host association

Species	Host (s)	Found in southern Africa
M. tuberculosis	Humans	Yes
M. canetti	Humans	No
M. africanum	Humans	No
M. mungi	Banded mongoose	Yes
Dassie bacillus	Dassies	Yes
M. orygis	Arabian oryxes	Yes
M. microti	Voles, mice, shrews	No
M. pinnipedii	Sea lions, fur seals	No
M. caprae	Goats	No
M. bovis	Bovids mainly, array of mammalian hosts	Yes

Note: this table is based on published data and is accurate as of June 2012.

entiation between the *Mycobacterium tuberculosis* complex and NTM culture isolates (Kaufmann, 2002; de la Rua-Domenech et al., 2006; Muyoyeta et al., 2010).

Because of the high incidence of *M. bovis* detected in a wide variety of animal species (and very high incidence of *M. tuberculosis* in humans) in Africa, it is not surprising that less emphasis is put on the diagnoses of NTM infection. This is the case in animals, even if the animal has tested positive for PPD^{Avium}. It is particularly true in areas of high *M. tuberculosis* complex prevalence. In the face of a high disease burden from *M. tuberculosis* and *M. bovis,* many underdeveloped nations with inadequate health budgets do not devote any attention to NTM infection, where the literature mostly reports an NTM incidence rate of less than 5/100 000. However, recent studies in South and southern Africa show that one is likely to isolate NTM from people suspected to have TB, at a rate at least equal to that with which *M. tuberculosis* is found. In fact, in some communities with a high prevalence of TB, far more NTM than *M. tuberculosis* have been isolated from subjects (Muyoyeta et al., 2010; Chihota et al., 2012). From the disease and infection control perspective, it is important to know whether one is dealing with disease due to *M. tuberculosis* complex or NTM, as antibiotic susceptibility and control may differ. For example, in animal health work, the discovery of TB-like lesions in a livestock animal during necropsy and the easily derived but possibly incorrect diagnosis of *M. bovis* infection (Muller et al., 2011) may have devastating and unnecessary consequences for the herd owner.

However, NTM are increasingly identified due to better detection and speciation by PCR (Warren et al., 2006) or target gene sequencing. Clinical signs of NTM disease manifestation vary depending on the extent and location of the lesions. NTM disease most commonly presents as pulmonary manifestations (Cook, 2010). However, lymph node, skin and soft tissue involvement, as well as disseminated disease, are of clinical importance. Clinical presentation of pulmonary disease due to NTM may be similar to tuberculosis with the following clinical signs: low fever, night sweats, anaemia and weight loss, malaise, anorexia, diarrhoea and painful adenopathy in humans and in animals (Katoch, 2004). Clinical diagnosis of tuberculosis is usually possible only after the disease has reached an advanced stage and is dependent on the site of lesions. At the time of diagnosis, most infected hosts may have shed bacilli and have been or are a potential source of infection for other hosts.

As it is relatively difficult to isolate, culture and speciate mycobacteria, as well as decide whether the presence of that organism is simply colonization, or infection and disease, the literature on many of the complex species and for NTM is not definitive. Isolation may simply represent recent acquisition, colonization without disease or infection with disease implying clinical manifestation of tissue damage (at either micro or visible scale). Additionally, as NTM are ubiquitous, great care must be exercised with sampling, as environmental contamination could occur (Hatherill et al., 2006). It is clear that care must be taken in interpretation of diagnostic reports: if the only laboratory-based test used is a ZN (Ziehl-Neelsen) smear, then acid fastness may be over-interpreted as TB positive (*M. tuberculosis* or perhaps *M. bovis*) and dealt with accordingly, but incorrectly under the directly observed therapy, short-course (DOTS) programme. Directly observed therapy, short-course treatment for human pulmonary tuberculosis is started only on the basis of sputum microscopy results, which has an inherent possibility of misdiagnosing NTM disease, with subsequent incorrect treatment. For example, it has been noted that some of the cases that are identified as anti-TB treatment failure or suspected as drug resistance are actually due to NTM (Griffith et al., 2007).

Conclusion

Although the burden of disease from *M. tuberculosis* or *M. bovis* is apparently orders of magnitude higher than that from NTM, the latter can potentially carry a relatively high burden of morbidity and mortality as they may be difficult to diagnose and treat (van Ingen et al., 2012a). For the purposes of mycobacterial disease control, care must be exercised where diagnosis is based only on macroscopic examination and perhaps smear or simple culture positivity or immunological reactivity. We do not yet clearly understand the disease-causing potential of the various mycobacteria in different animal species or the risk factors and drivers that may promote such disease, as opposed to exposure and infection. Our lack of such knowledge impacts on our ability to control these diseases and generate useful efficacious vaccines. The public-health threat of tuberculosis in Africa requires urgent investigation on this topic through collaborative veterinary and medical research programmes.

Conflicts of Interest

All three authors have no conflicts of interest.

References

Ablordey, A., J. Swings, C. Hubans, K. Chemlal, C. Locht, F. Portaels, and P. Supply, 2005: Multilocus variable-number tandem repeat typing of *Mycobacterium ulcerans*. *J. Clin. Microbiol.* 43, 1546–1551.

Alexander, K. A., P. N. Laver, A. L. Michel, M. Williams, P. D. van Helden, R. M. Warren, and N. C. Gey van Pittius, 2010:

Novel *Mycobacterium tuberculosis* complex pathogen, *M. mungi*. *Emerg. Infect. Dis.* 16, 1296–1299.

Ameni, G., A. Aseffa, H. Engers, D. Young, S. Gordon, G. Hewinson, and M. Vordermeier, 2007: High prevalence and increased severity of pathology of bovine tuberculosis in Holsteins compared to zebu breeds under field cattle husbandry in central Ethiopia. *Clin. Vaccine Immunol.* 14, 1356–1361.

Andrejak, C., V. O. Thomsen, I. S. Johansen, A. Riis, T. L. Benfield, P. Duhaut, H. T. Sorensen, F. X. Lescure, and R. W. Thomsen, 2010: Nontuberculous pulmonary mycobacteriosis in Denmark: incidence and prognostic factors. *Am. J. Respir. Crit. Care Med.* 181, 514–521.

Angkawanish, T., W. Wajjwalku, A. Sirimalaisuwan, S. Mahasawangkul, T. Kaewsakhorn, K. Boonsri, and V. P. Rutten, 2010: *Mycobacterium tuberculosis* infection of domesticated Asian elephants, Thailand. *Emerg. Infect. Dis.* 16, 1949–1951.

Bercovier, H., and V. Vincent, 2001: Mycobacterial infections in domestic and wild animals due to *Mycobacterium marinum*, *M. fortuitum*, *M. chelonae*, *M. porcinum*, *M. farcinogenes*, *M. smegmatis, M. scrofulaceum, M. xenopi, M. kansasii, M. simiae* and *M. genavense*. *Rev. Sci. Tech.* 20, 265–290.

Berg, S., M. C. Garcia-Pelayo, B. Muller, E. Hailu, B. Asiimwe, K. Kremer, J. Dale, M. B. Boniotti, S. Rodriguez, M. Hilty, L. Rigouts, R. Firdessa, A. Machado, C. Mucavele, B. N. Ngandolo, J. Bruchfeld, L. Boschiroli, A. Muller, N. Sahraoui, M. Pacciarini, S. Cadmus, M. Joloba, D. van Soolingen, A. L. Michel, B. Djonne, A. Aranaz, J. Zinsstag, P. van Helden, F. Portaels, R. Kazwala, G. Kallenius, R. G. Hewinson, A. Aseffa, S. V. Gordon, and N. H. Smith, 2011: African 2, a clonal complex of *Mycobacterium bovis* epidemiologically important in East Africa. *J. Bacteriol.* 193, 670–678.

den Boon, S., S. W. van Lill, M. W. Borgdorff, D. A. Enarson, S. Verver, E. D. Bateman, E. Irusen, C. J. Lombard, N. W. White, C. de Villiers, and N. Beyers, 2007: High prevalence of tuberculosis in previously treated patients, Cape Town, South Africa. *Emerg. Infect. Dis.* 13, 1189–1194.

Bragg, R. R., H. F. Huchzermeyer, and M. A. Hanisch, 1990: *Mycobacterium fortuitum* isolated from three species of fish in South Africa. *Onderstepoort J. Vet. Res.* 57, 101–102.

Brown, T. H., 1985: The rapidly growing mycobacteria–*Mycobacterium fortuitum* and *Mycobacterium chelonei*. *Infect. Control* 6, 283–288.

Chamoiseau, G., 1973: "*Mycobacterium farcinogenes*" causal agent of bovine farcy in Africa (author's transl). *Ann. Microbiol. (Paris)* 124, 215–222.

Chihota, V. N., B. Muller, C. K. Mlambo, M. Pillay, M. Tait, E. M. Streicher, E. Marais, G. D. van der Spuy, M. Hanekom, G. Coetzee, A. Trollip, C. Hayes, M. E. Bosman, N. C. Gey van Pittius, T. C. Victor, P. D. van Helden, and R. M. Warren, 2012: Population structure of multi- and extensively drug-resistant *Mycobacterium tuberculosis* strains in South Africa. *J. Clin. Microbiol.* 50, 995–1002.

Cook, J. L., 2010: Nontuberculous mycobacteria: opportunistic environmental pathogens for predisposed hosts. *Br. Med. Bull.* 96, 45–59.

De Groote, M. A., and G. Huitt, 2006: Infections due to rapidly growing mycobacteria. *Clin. Infect. Dis.* 42, 1756–1763.

Drewe, J. A., K. T. Eames, J. R. Madden, and G. P. Pearce, 2011: Integrating contact network structure into tuberculosis epidemiology in meerkats in South Africa: implications for control. *Prev. Vet. Med.* 101, 113–120.

Falkinham, J. O., 2010: Impact of human activities on the ecology of nontuberculous mycobacteria. *Future Microbiol.* 5, 951–960.

Falkinham, J. O., 2011: Nontuberculous mycobacteria from household plumbing of patients with nontuberculous mycobacteria disease. *Emerg. Infect. Dis.* 17, 419–424.

Gey van Pittius, N. C., S. L. Sampson, H. Lee, Y. Kim, P. D. van Helden, and R. M. Warren, 2006: Evolution and expansion of the *Mycobacterium tuberculosis* PE and PPE multigene families and their association with the duplication of the ESAT-6 (esx) gene cluster regions. *BMC Evol. Biol.* 6, 95.

Gey van Pittius, N. C., K. D. Perrett, A. L. Michel, D. F. Keet, T. Hlokwe, E. M. Streicher, R. M. Warren, and P. D. van Helden, 2012a: Infection of African buffalo (*Syncerus caffer*) by oryx bacillus, a rare member of the antelope clade of the *Mycobacterium tuberculosis* complex. *J. Wildl. Dis.* 48, 849–857.

Gey van Pittius, N. C., P. D. van Helden, and R. M. Warren. 2012b: Characterization of *Mycobacterium orygis*. *Emerg. Infect. Dis.* 18, 1708–1709.

Griffith, D. E., T. Aksamit, B. A. Brown-Elliott, A. Catanzaro, C. Daley, F. Gordin, S. M. Holland, R. Horsburgh, G. Huitt, M. F. Iademarco, M. Iseman, K. Olivier, S. Ruoss, C. F. von Reyn, R. J. Jr Wallace, K. Winthrop, 2007: An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am. J. Respir. Crit. Care Med.* 175, 367–416.

Gumi, B., E. Schelling, S. Berg, R. Firdessa, G. Erenso, W. Mekonnen, E. Hailu, E. Melese, J. Hussein, A. Aseffa, and J. Zinsstag, 2012: Zoonotic transmission of tuberculosis between pastoralists and their livestock in South-East Ethiopia. *EcoHealth* 9, 139–149.

Hanekom, M., G. D. van der Spuy, N. C. Gey van Pittius, C. R. McEvoy, S. L. Ndabambi, T. C. Victor, E. G. Hoal, P. D. van Helden, and R. M. Warren, 2007: Evidence that the spread of *Mycobacterium tuberculosis* strains with the Beijing genotype is human population dependent. *J. Clin. Microbiol.* 45, 2263–2266.

Hatherill, M., T. Hawkridge, A. Whitelaw, M. Tameris, H. Mahomed, S. Moyo, W. Hanekom, and G. Hussey, 2006: Isolation of non-tuberculous mycobacteria in children investigated for pulmonary tuberculosis. *PLoS ONE* 1, e21.

van Helden, P. D., S. D. Parsons, and N. C. Gey van Pittius, 2009: 'Emerging' mycobacteria in South Africa. *J. S. Afr. Vet. Assoc.* 80, 210–214.

Holland, S. M., 2001: Nontuberculous mycobacteria. *Am. J. Med. Sci.* 321, 49–55.

van Ingen, J., M. J. Boeree, D. van Soolingen, and J. W. Mouton, 2012a: Resistance mechanisms and drug susceptibility testing

of nontuberculous mycobacteria. *Drug Resist Updat* 15, 149–161.

van Ingen, J., Z. Rahim, A. Mulder, M. J. Boeree, R. Simeone, R. Brosch, and D. van Soolingen, 2012b: Characterization of *Mycobacterium orygis* as *M. tuberculosis* complex subspecies. *Emerg. Infect. Dis.* 18, 653–655.

Iseman, M. D., and T. K. Marras, 2008: The importance of nontuberculous mycobacterial lung disease. *Am. J. Respir. Crit. Care Med.* 178, 999–1000.

Kaneene, J. B., and C. O. Thoen, 2004: Tuberculosis. *J. Am. Vet. Med. Assoc.* 224, 685–691.

Kankya, C., A. Muwonge, B. Djonne, M. Munyeme, J. Opuda-Asibo, E. Skjerve, J. Oloya, V. Edvardsen, and T. B. Johansen, 2011: Isolation of non-tuberculous mycobacteria from pastoral ecosystems of Uganda: public health significance. *BMC Public Health* 11, 320-2458-11-320.

Karne, S. S., S. A. Sangle, D. S. Kiyawat, S. N. Dharmashale, D. B. Kadam, and R. S. Bhardwaj, 2012: *Mycobacterium avium-intracellulare* brain abscess in HIV-positive patient. *Ann. Indian. Acad. Neurol.* 15, 54–55.

Katale, B. Z., E. V. Mbugi, S. Kendal, R. D. Fyumagwa, G. S. Kibiki, P. Godfrey-Faussett, J. D. Keyyu, P. Van Helden, and M. I. Matee, 2012: Bovine tuberculosis at the human-livestock-wildlife interface: is it a public health problem in Tanzania? A review. *Onderstepoort J. Vet. Res.* 79, E1–E8.

Katoch, V. M., 2004: Infections due to non-tuberculous mycobacteria (NTM). *Indian J. Med. Res.* 120, 290–304.

Kaufmann, S. H., 2002: Protection against tuberculosis: cytokines, T cells, and macrophages. *Ann. Rheum. Dis.* 61(Suppl 2), ii54–ii58.

LoBue, P. A., D. A. Enarson, and C. O. Thoen, 2010: Tuberculosis in humans and animals: an overview. *Int. J. Tuberc. Lung Dis.* 14, 1075–1078.

Michel, A. L., L. M. de Klerk, N. C. Gey van Pittius, R. M. Warren, and P. D. van Helden, 2007: Bovine tuberculosis in African buffaloes: observations regarding *Mycobacterium bovis* shedding into water and exposure to environmental mycobacteria. *BMC Vet. Res.* 3, 23.

Michel, A. L., M. L. Coetzee, D. F. Keet, L. Mare, R. Warren, D. Cooper, R. G. Bengis, K. Kremer, and P. van Helden, 2009: Molecular epidemiology of *Mycobacterium bovis* isolates from free-ranging wildlife in South African game reserves. *Vet. Microbiol.* 133, 335–343.

Michel, A. L., B. Muller, and P. D. van Helden, 2010: *Mycobacterium bovis* at the animal-human interface: a problem, or not? *Vet. Microbiol.* 140, 371–381.

Montali, R. J., S. K. Mikota, and L. I. Cheng, 2001: *Mycobacterium tuberculosis* in zoo and wildlife species. *Rev. Sci. Tech.* 20, 291–303.

Moore, J. E., M. E. Kruijshaar, L. P. Ormerod, F. Drobniewski, and I. Abubakar, 2010: Increasing reports of non-tuberculous mycobacteria in England, Wales and Northern Ireland, 1995–2006. *BMC Public Health* 10, 612-2458-10-612.

Muller, B., B. Steiner, B. Bonfoh, A. Fane, N. H. Smith, and J. Zinsstag, 2008: Molecular characterisation of *Mycobacterium bovis* isolated from cattle slaughtered at the Bamako abattoir in Mali. *BMC Vet. Res.* 4, 26-6148-4-26.

Muller, B., L. M. de Klerk-Lorist, M. M. Henton, E. Lane, S. Parsons, N. C. Gey van Pittius, A. Kotze, P. D. van Helden, and M. Tanner, 2011: Mixed infections of *Corynebacterium pseudotuberculosis* and non-tuberculous mycobacteria in South African antelopes presenting with tuberculosis-like lesions. *Vet. Microbiol.* 147, 340–345.

Munyeme, M., L. Rigouts, I. C. Shamputa, J. B. Muma, M. Tryland, E. Skjerve, and B. Djonne, 2009: Isolation and characterization of *Mycobacterium bovis* strains from indigenous Zambian cattle using spacer oligonucleotide typing technique. *BMC Microbiol.* 9, 144-2180-9-144.

Muyoyeta, M., P. E. de Haas, D. H. Mueller, P. D. van Helden, L. Mwenge, A. Schaap, C. Kruger, N. C. Gey van Pittius, K. Lawrence, N. Beyers, P. Godfrey-Faussett, and H. Ayles, 2010: Evaluation of the Capilia TB assay for culture confirmation of *Mycobacterium tuberculosis* infections in Zambia and South Africa. *J. Clin. Microbiol.* 48, 3773–3775.

Niemann, S., E. Richter, and S. Rusch-Gerdes, 2000: Differentiation among members of the *Mycobacterium tuberculosis* complex by molecular and biochemical features: evidence for two pyrazinamide-susceptible subtypes of *M. bovis*. *J. Clin. Microbiol.* 38, 152–157.

Parsons, S., S. G. Smith, Q. Martins, W. G. Horsnell, T. A. Gous, E. M. Streicher, R. M. Warren, P. D. van Helden, and N. C. Gey van Pittius, 2008a: Pulmonary infection due to the dassie bacillus (*Mycobacterium tuberculosis* complex sp.) in a free-living dassie (rock hyrax-*Procavia capensis*) from South Africa. *Tuberculosis (Edinb)* 88, 80–83.

Parsons, S. D., T. A. Gous, R. M. Warren, and P. D. van Helden, 2008b: Pulmonary *Mycobacterium tuberculosis* (Beijing strain) infection in a stray dog. *J. S. Afr. Vet. Assoc.* 79, 95–98.

Parsons, S. D., R. M. Warren, T. H. Ottenhoff, N. C. Gey van Pittius, and P. D. van Helden, 2012: Detection of *Mycobacterium tuberculosis* infection in dogs in a high-risk setting. *Res. Vet. Sci.* 92, 414–419.

Parsons, S. D. C., J. A. Dewe, N. C. Gey van Pittius, R. M. Warren, and P. D. van Helden, 2013: A novel pathogen, *Mycobacterium suricattae*, is the cause of tuberculosis in meerkats (*Suricata suricatta*) in South Africa. *Emerg. Infect. Dis.* 19, (in press).

Petrini, B., 2006: *Mycobacterium abscessus*: an emerging rapid-growing potential pathogen. *APMIS* 114, 319–328.

Pfyffer, G. E., R. Auckenthaler, J. D. van Embden, and D. van Soolingen, 1998: *Mycobacterium canettii*, the smooth variant of *M. tuberculosis*, isolated from a Swiss patient exposed in Africa. *Emerg. Infect. Dis.* 4, 631–634.

Phillips, M. S., and C. F. von Reyn, 2001: Nosocomial infections due to nontuberculous mycobacteria. *Clin. Infect. Dis.* 33, 1363–1374.

Prevots, D. R., P. A. Shaw, D. Strickland, L. A. Jackson, M. A. Raebel, M. A. Blosky, R. Montes de Oca, Y. R. Shea, A. E. Seitz, S. M. Holland, and K. N. Olivier, 2010: Nontuberculous mycobacterial lung disease prevalence at four integrated

health care delivery systems. *Am. J. Respir. Crit. Care Med.* 182, 970–976.

de la Rua-Domenech, R., A. T. Goodchild, H. M. Vordermeier, R. G. Hewinson, K. H. Christiansen, and R. S. Clifton-Hadley, 2006: Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. *Res. Vet. Sci.* 81, 190–210.

Shamaei, M., M. Marjani, P. Farnia, P. Tabarsi, and D. Mansouri, 2010: Human infections due to *Mycobacterium lentiflavum*: first report in Iran. *Iran. J. Microbiol.* 2, 27–29.

Simons, S., J. van Ingen, P. R. Hsueh, N. Van Hung, P. N. Dekhuijzen, M. J. Boeree, and D. van Soolingen, 2011: Nontuberculous mycobacteria in respiratory tract infections, eastern Asia. *Emerg. Infect. Dis.* 17, 343–349.

Warren, R. M., N. C. Gey van Pittius, M. Barnard, A. Hesseling, E. Engelke, M. de Kock, M. C. Gutierrez, G. K. Chege, T. C. Victor, E. G. Hoal, and P. D. van Helden, 2006: Differentiation of *Mycobacterium tuberculosis* complex by PCR amplification of genomic regions of difference. *Int. J. Tuberc. Lung Dis.* 10, 818–822.

Whittington, R. J., I. B. Marsh, and L. A. Reddacliff, 2005: Survival of *Mycobacterium avium* subsp. *paratuberculosis* in dam water and sediment. *Appl. Environ. Microbiol.* 71, 5304–5308.

Yew, W. W., G. Sotgiu, and G. B. Migliori, 2011: Update in tuberculosis and nontuberculous mycobacterial disease 2010. *Am. J. Respir. Crit. Care Med.* 184, 180–185.

