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Evidence of increasing intra and inter-species transmission of *Mycobacterium bovis* in South Africa: Are we losing the battle?

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ABSTRACT

Tuberculosis caused by *Mycobacterium bovis* is recognized worldwide as a significant health risk in domestic cattle, farmed and wild animal species as well as in humans. We carried out spoligotyping and variable number of tandem repeat (VNTR) typing methods to characterize 490 *M. bovis* isolates from livestock (cattle, $n = 230$; pig $n = 1$) and wildlife species ($n = 259$) originating from different farms and regions in South Africa, with the aim to further establish the genetic diversity of the isolates, study the population structure of *M. bovis* and elucidate the extent of interspecies transmission of bovine tuberculosis. A total of ten spoligotype patterns were identified, two of which were novel (SB2199 and SB2200) and reported for the first time in the literature, while VNTR typing revealed a total of 97 VNTR profiles. Our results showed evidence of clonal expansion for some ancestral strains as well as co-infections with two or three *M. bovis* strains on some of the cattle and game farms, which suggested independent introductions of infected animals from epidemiologically unrelated sources. Five spoligotypes and nine VNTR profiles were shared between cattle and wildlife. Our findings showed that besides cattle, at least 16 different animal species in South Africa are infected with bovine tuberculosis, and highlight a strong evidence of inter and intra-species transmission of *M. bovis*. Infection of the blue wildebeest (*Connochaetes taurinus*) with *M. bovis* is described for the first time in this report. This update in epidemiological information raises concerns that bovine tuberculosis has increased its spatial distribution in South Africa and is also affecting an increasing number of wildlife species compared to ten years ago.

1. Introduction

Bovine tuberculosis (BTB) is still recognized worldwide as a significant animal health risk, primarily in domestic cattle and wildlife. The causative agent, *Mycobacterium bovis*, has a wide host range which includes farmed and wild animals as well as humans (Neill et al., 2005). *M. bovis* is a member of the *Mycobacterium tuberculosis* complex,

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which includes mycobacterial species that cause tuberculosis in animals and humans (Brosch et al., 2002; Huard et al., 2006). In South Africa, tuberculosis in cattle and free ranging wildlife species caused by *M. bovis* is well documented (Bengis et al., 2001; Michel et al., 2008, 2009; Hlokwe et al., 2011). The prevalence of the disease in commercial cattle was reported to be less than 1% in 1995, owing to the implementation of national BTB control and eradication scheme in 1969. The prevalence of the disease in communal cattle is currently unknown. Bovine tuberculosis in wildlife in the Kruger National Park (KNP) is endemic, with the highest disease prevalence in buffalo herds in the southern zone of the park. A single *M. bovis* strain was responsible for the epidemic and has subsequently spread progressively moving in a northern direction. It has infected at least 12 other wildlife species (Michel and Bengis, 2012) and has undergone evolutionary changes as described (Michel et al., 2009; Hlokwe et al., 2013). More recently, an epidemiological link between the KNP and the Gonarezhou National Park was confirmed, which has negative implications for the Greater Limpopo Transfrontier National Park (GLTFNP), De Garine-Wichatitsky et al., 2010; Hlokwe et al., 2013).

Bovine TB in the Hluhluwe-iMfolozi Park (HiP), which is geographically and epidemiologically distinct from KNP, is caused by at least three distinct *M. bovis* strains (Hlokwe et al., 2011). The prevalence of the disease in free ranging wildlife not associated with KNP or HiP, i.e. in private game reserves and game farms is currently unknown. Bovine tuberculosis in KNP and HiP was introduced by cattle from nearby communal farms and the persistence of the disease in these conservation areas as well as in communal farms pose a risk for ongoing transmission of the disease between wildlife and livestock. The situation may worsen if BTB prevalence in these ecosystems rises, since the disease in wildlife is generally not easy to control (Corner, 2006). Of further concern is that bovine tuberculosis poses a zoonotic risk, particularly in high HIV endemic communities surrounding the conservation areas (Thoen et al., 2006; Michel et al., 2010). An important factor for successful bovine tuberculosis control and eradication programs is contact tracing and point source identification as unregulated and illegal movement of infected animals is considered the major constraint in such control strategies (Aranaz et al., 2004).

Molecular methods have become very tightly integrated with traditional epidemiological tracing of tuberculosis and provide a paradigm for such integration at both local and international levels (Achtman, 2001). In addition to traditional methods, typing methods such as IS*6110* restriction fragment length polymorphism (RFLP) and polymorphic G–C rich sequences RFLP, spoligotyping and variable number of tandem repeat typing (VNTR) analyses are applied to characterize *M. bovis* isolates (Durr et al., 2000). Previous studies conducted in the KNP have shown that spoligotyping could not differentiate the parent C8 strain from its variants strains (Michel et al., 2009; Hlokwe et al., 2013) because of the slow evolutionary rate of the direct repeat region targeted. In addition, it generally has a lower discriminatory power for South African isolates as compared to other typing methods, i.e. IS*6110* typing, PGRS typing (Michel et al., 2008). Very recently, VNTR loci were assessed for their discriminatory power on isolates from South Africa. The results of this study led to a selection of a 13 locus VNTR panel for isolates from this region (Hlokwe et al., 2013).

The aim of the current study was to use spoligotyping and VNTR typing as described to characterize *M. bovis* isolated from livestock and wildlife species in South Africa to further establish their genetic diversity and assess the extent of intra- and inter-species transmission of bovine tuberculosis. The study also aimed to utilize the typing data to elucidate the population structure of *M. bovis* and generate a database to form the basis of back and forward tracing of sources of infection for improved surveillance and control of the disease in the country.

2. Materials and methods

2.1. Sample collection

The samples used in this study were received between 2002 and 2013 in the Tuberculosis Laboratory of the Onderstepoort Veterinary Institute for routine mycobacterial culture. Samples were collected from animals on livestock farms throughout South Africa and from different wildlife species from the KNP, HiP as well as private game ranches. They included tissue samples from lymph nodes, organs, and bronchial fluids. The majority of the samples were collected from: (i) tuberculin skin test and gamma interferon test positive animals at slaughter, (ii) gamma interferon test was conducted together with skin test in buffaloes from game farms/reserves. In some cases, buffaloes testing positive in the gamma interferon assay but negative in the skin test were slaughtered and samples collected for culture, (iii) lesions detected in healthy cattle during routine slaughter from abattoir tuberculosis suspect animals, (iv) as part of passive TB surveillance which was based on necropsy of all wild animals found dead in game parks/reserves and the collection of tissues showing pathological changes for specific testing. If tuberculous like lesions were found, the specimens were sent for tuberculosis culture. Bovine tuberculosis has been documented in all provinces of South Africa with a sporadic occurrence, irrespective of the size or density of the cattle population. Routine submissions formed part of the State Veterinary Service's strategy for confirming *M. bovis* infection in either skin test positive reactor cattle or slaughter cattle with suspect tuberculous lesions. All samples were accompanied by sample submission forms with information relating to the animal, owner and precise location. In case where additional information was required, the responsible state veterinarian assisted with back tracing of animals and contacts. An additional two tissue samples from cattle originating from two different regions (i.e. Chimoio district in Manica Province and Guvuru district in Inhambane Province) in Mozambique were included for comparison purposes. The different animal species sampled as well as their locations are indicated in Table 1.