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## Molecular epidemiology of *Mycobacterium bovis* isolates from free-ranging wildlife in South African game reserves

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### ABSTRACT

Bovine tuberculosis is endemic in African buffalo and a number of other wildlife species in the Kruger National Park (KNP) and Hluhluwe-iMfolozi Park (HiP) in South Africa. It was thought that the infection had been introduced into the KNP ecosystem through direct contact between cattle and buffalo, a hypothesis which was confirmed in this study by IS6110 and PGRS restriction fragment length polymorphism (RFLP) typing. The molecular characterisation of 189 *Mycobacterium bovis* isolates from nine wildlife species in the HiP, including three smaller associated parks, and the Kruger National Park with adjacent areas showed that the respective epidemics were each caused by an infiltration of a single *M. bovis* genotype. The two *M. bovis* strains had different genetic profiles, as demonstrated by hybridisation with the IS6110 and PGRS RFLP probes, as well as with regard to evidence of evolutionary changes to the IS profile. While the *M. bovis* type in HiP was transmitted between buffaloes and to at least baboon, bushpig and lion without obvious genetic changes in the RFLP patterns, in the KNP a dominant strain was represented in 73% of the *M. bovis* isolates, whilst the remaining 27% were variants of this strain. No species-specific variants were observed, except for one IS6110 type which was found only in a group of five epidemiologically related greater kudu. This finding was attributed to species-specific behaviour patterns rather than an advanced host–pathogen interaction.

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### 1. Introduction

In 1986 and 1990 bovine tuberculosis (BTB) was first diagnosed in African buffalo (*Syncerus caffer*) in two of South Africa's largest free-ranging conservation areas, the Hluhluwe-iMfolozi Park (HiP) and Kruger National Park (KNP)

(Bengis et al., 1996; Michel et al., 2006). HiP and other game reserves in KwaZulu-Natal are entirely surrounded by mostly communal farmland. BTB most probably entered the KNP ecosystem during the 1950s–1960s before the KNP's southern boundary was fully fenced. Extensive surveys in the KNP revealed that most buffalo herds in the southern region were infected with BTB (De Vos et al., 2001). A stratified, two-stage cluster sampling method, showed that the disease spread in a northern direction, such that the incidence of BTB in the southern region had possibly attained a prevalence of over 90% in some individual buffalo herds (De Vos et al., 2001; Rodwell et al., 2001; Thrusfield, 1995).

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Between the southern and central region the incidence increased from 4% to 16% while the initially BTB free area to the north showed an overall prevalence of 1.5% in 1998.

With the increasing *Mycobacterium bovis* infection rate in the buffalo population, the infection spilled over into other wildlife species (Keet et al., 1996). In subsequent years, transmission of *M. bovis* to an additional seven wildlife species including predators, herbivores and omnivores was demonstrated (Keet et al., 2000; Bengis et al., 2002; Michel et al., 2006). The spatial spread of BTB within and between species in the KNP was mainly northwards but occurred also outwards into neighbouring private game reserves as wildlife can move freely between the parks belonging to the greater KNP complex (GKNPC) (De Vos et al., 2001).

It was reported by De Vos et al. (2001) and Michel (2002) that most of the *M. bovis* genotypes seen in isolates from the buffalo were identical, and that variants were >70% homologous to this genotype. In this initial study, only one genotyping probe (IS6110) was used for analysis, and since *M. bovis* has few IS6110 copies, this analysis was indicative of a single introduction event of *M. bovis*, but could not provide unequivocal proof that no other events had taken place. In this paper we report on an integrated approach of genotyping and spatial analysis to study the BTB epidemic in the KNP by characterizing *M. bovis* isolates from wild animals and comparing them with those recently characterised from domestic cattle (Michel et al., 2008) in South Africa. We show that we can use molecular epidemiological techniques to establish the extent of *M. bovis* strain diversity in isolates collected in the KNP and HiP, including associated game parks. From these data, we recreate a putative evolutionary history of these *M. bovis* isolates, thereby providing evidence that the present BTB epidemics in KNP and HiP have been caused by two different progenitor strains.

## 2. Materials and methods

### 2.1. Animals and tissue samples

#### 2.1.1. Kwazulu-Natal Parks

Twenty-eight tissue samples had been collected during BTB surveys from tuberculin skin test and/or interferon gamma test positive buffaloes in the Spioenkop Nature

Reserve (SP;  $n = 2$ ), in western Kwazulu-Natal and the Hluhluwe-iMfolozi Park (HiP;  $n = 14$ ), Munyawana Game Reserve (MGR;  $n = 6$ ) and Eastern Shores of Lake St Lucia (at present part of the iSimangaliso Wetland Park) (ESL;  $n = 6$ ) in northern Kwazulu-Natal (Table 1). All samples were collected between 1992 and 2000. Buffalo populations in the latter two parks originated from HiP and were translocated in 1977 and 1997–2000, respectively. Four additional samples were collected during *ad hoc* post mortem examinations of one bushpig (*Potamochoerus larvatus*) and one baboon from HiP, one lion from MGR (TB 1199) and one lion (TB 613) which was translocated from HiP to the National Zoological Gardens in Pretoria (NZG) where it succumbed to tuberculosis and was euthanased. The last sample originated from a buffalo that was moved from HiP to a reserve in the Northern Cape province, where it was destroyed after both the tuberculin test and interferon gamma tests showed positive test results.

#### 2.1.2. Greater Kruger National Park Complex

Standard sets of tissue samples from the head, thoracic and mediastinal lymph nodes and, where applicable, lesions from any other affected tissues were collected as described by Bengis et al. (1996). A total of 122 animals in KNP, 17 in two private reserves within the GKNPC and adjacent to the western boundary of the central region of KNP and 17 animals on four properties neighbouring the southern region were sampled as listed in Table 1. The majority of buffalo were sampled during BTB surveys while other species were subject to passive surveillance only and tissue samples were collected at *ad hoc* post mortem examinations. All samples which became available during these operations between 1994 and 2003 were examined and no selection took place.

### 2.2. Bacteriology and genetic characterisation of *M. bovis* isolates

Tissue samples were processed and cultured, followed by species identification, as reported by Bengis et al. (1996) and Alexander et al. (2002). *M. bovis* isolates were genetically characterised using international consensus

**Table 1**

Summary of wildlife species and numbers of samples analysed from Kwazulu-Natal parks (KZN) and the Greater Kruger National Park Complex (GKNPC)

Species	KZN				GKNPC			Total
	HiP	MGR	ESL	SP	KNP	Properties west	Properties south	
Buffalo	15	6	6	2	82	5	14	130
Lion	1	1			22	5		29
Kudu					7	2	1	10
Baboon	1				8		1	10
Leopard					2	1		3
Cheetah					1	1		2
Hyena						3		3
Warthog							1	1
Bushpig	1							1
Total	18	7	6	2	122	17	17	189

HiP, Hluhluwe iMfolozi Park; KNP, Kruger National Park; MGR, Munyawana Game Reserve; ESL, Eastern Shores of Lake St. Lucia; SP, Spioenkop Nature Reserve.

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