



## The epidemiology of tuberculosis in free-ranging African buffalo (*Syncerus caffer*) in the Kruger National Park, South Africa

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### ABSTRACT

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The presence of bovine tuberculosis (*Mycobacterium bovis*) in the Kruger National Park (KNP) was determined for the first time in 1990. It was diagnosed in an African buffalo (*Syncerus caffer*) bull, which was found recumbent and in an emaciated and moribund state near the south-western boundary fence. This prompted an investigation into the bovine tuberculosis (BTB) status of the KNP, with emphasis on its epidemiological determinants and risk factors. This report documents the findings of surveys that were conducted from 1990 to 1996.

It was found that BTB had entered the KNP ecosystem relatively recently ( $\pm$  1960), and has found favourable circumstances for survival and propagation in a fully susceptible and immunologically naive buffalo population. Indications are that it entered the KNP from across the southern river boundary, where the presence of infected domestic cattle herds had been documented. From there the infection spread through the southern buffalo population and is currently spreading in a northward direction. It was estimated that this northward spread took place at a rate of about 6 km per year; the prospect being that, if this rate of spread is maintained, the entire KNP may be affected in less than 30 years from now.

Spillover from buffalo had already occurred in species such as chacma baboon (*Papio ursinus*), lion (*Panthera leo*), cheetah (*Acinonyx jubatus*), kudu (*Tragelaphus strepsiceros*) and leopard (*Panthera pardus*). Although there is no indication yet that these species act as maintenance hosts, the possibility is raised that these, or an as yet overlooked species, might assume such a role in future.

In the KNP, BTB manifests itself as a chronic and predominantly subclinical disease in buffalo. It may take years for clinical signs to develop, and then only at a terminal stage, when emaciation is a constant feature. It is suspected that the time from infection to death is variable and dependent on the animal's immune response, which can be weakened by such factors as stress, old age or droughts. It was found that, in the interim, buffalo have a normal reproductive life.

On necropsy, buffalo show almost exclusively lung and upper respiratory tract involvement, pointing to an aerogenous mode of transmission. Histologically, little sign of encapsulation of lesions was detected, which suggests that they are exceptionally susceptible to BTB and that most lesions are *open* and infectious and progressive, leading ultimately to death of the individual.

Evidence also indicates that BTB is progressive within the herd context (92 % being the highest prevalence rate thus far determined in a buffalo herd) as well as progressive within the KNP buffalo population (the implication being that virtually all buffalo herds in the KNP will eventually be infected).

Preliminary data suggest a positive correlation between disease prevalence and mortality, with potential mortality reaching up to 10 % in buffalo herds having BTB prevalence rates of 50 % and higher. Only the future will tell what the effect of the disease on the population dynamics of buffalo will be.

**Keywords:** African buffalo, baboon, cheetah, epidemiology, Kruger National Park, kudu, leopard, lion, *Mycobacterium bovis*, South Africa, *Syncerus caffer*, tuberculosis

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## INTRODUCTION

The host range of mycobacteria in non-domestic animals appears to be unlimited (Francis 1958). Tuberculosis has become recognized as a common disease of wild animals, particularly those closely associated with man, such as in zoological gardens and primate colonies (Francis 1958; Thoen & Himes 1981). A few such cases have been diagnosed in captive wild animals in southern Africa, viz. springbok (*Antidorcas marsupialis*) (Robinson 1953; Hofmeyr 1956), giraffe (*Giraffa camelopardalis*) (Martinaglia 1930; Basson, McCully, Kruger, Van Niekerk, Young, De Vos, Keep & Ebedes 1971), black rhinoceros (*Diceros bicornis*), African buffalo, nyala (*Tragelaphus angasi*) (Hofmeyr 1956) and primates (Fourie 1983).

Paine and Martinaglia (1929) were the first to report an outbreak of mycobacteriosis in free-ranging wild animals in southern Africa. They described the disease in kudu and duiker (*Sylvicapra grimmia*), the causal organism being *Mycobacterium bovis*. Evidence of deaths and the presence of abscesses amongst springbok, bushbuck (*Tragelaphus scriptus*) and hare (*Lepus* spp.) in the vicinity, was also found. Martinaglia (1930) also described a strain of "*Mycobacterium tuberculosis (bovis)*", which was isolated from a free-ranging giraffe. A disease condition in kudu that was known for many years in the farming community of the Grahamstown bush veld area, was later diagnosed as tuberculosis by Thorburn and Thomas (1940). Robinson (1944) showed that a strain of *M. bovis* was involved in this outbreak. Keep and Basson (1973) reported a case of mycobacteriosis in free-ranging black rhino from the Hluhluwe Game Reserve. More recently, the buffalo populations from Umfolosi and Hluhluwe game reserves in Natal were also found to be infected (Cooper 1998).

Further afield in Africa, tuberculosis has also been reported from free-ranging lechwe (*Kobus leche kafuensis*) in Zambia (Gallagher, MacAdam, Sayer & Van Lavieren 1972; Clancey 1977; Krauss, Roettcher, Weiss, Danner & Hübschle 1984). The first occurrence of tuberculosis in free-ranging African buffalo was reported by Guilbride, Rollinson, McAnulty, Alley & Wells (1963) in Uganda. They found eight animals with tuberculosis among 13 free-living buffalo shot in the Queen Elizabeth National Park (also known as Ruwenzori National Park). Thurlbeck, Butas, Mankiewicz & Laws (1965), later confirmed the diagnosis by isolating *M. bovis* in one out of five thin buffalo. More recently, Woodford (1982 a,b) surveyed the Queen Elizabeth National Park for tuberculosis and found that the disease had become endemic and was responsible for an annual mortality of about 1% of the 18000 buffalo inhabiting the Park. The disease was also found to have spilled

over into warthog (*Phacochoerus aethiopicus*) populations.

In the Kruger National Park (KNP), mycobacteriosis in wild animals was first described in November 1967 in an impala (*Aepyceros melampus*) from the Crocodile Bridge area south of the Sabie River (De Vos, McCully & Van Niekerk 1977). No isolation of the causative organism was attempted and, on histopathological grounds alone, a diagnosis of the avian form was made.

The disease entity caused by *Mycobacterium bovis*, and known as bovine tuberculosis (abbreviated as BTB), was first detected in the KNP in July 1990 (Bengis, Kriek, Keet, Raath, De Vos & Huchzermeyer 1995). The disease was diagnosed on necropsy of a two-year-old buffalo bull, which had been found in an emaciated and moribund state near the south-western boundary fence. This prompted an investigation into the BTB status of the rest of the buffalo population, and its significance as a disease entity in the KNP. This report documents the findings of the surveys that were conducted from 1990–1996.

## MATERIALS AND METHODS

### Study area

The KNP is an elongated nature reserve which is situated along the north-eastern border of South Africa. It comprises about two million ha of gently undulating hills and doleritic dykes, grassy plains, parkland savannah, dry deciduous forest and thornbush, and is inhabited by 132 free-ranging mammal species of which 20 can be classified as "large mammals" (Pienaar, Joubert, Hall-Martin, De Graaff & Rautenbach 1987). A buffalo population of 29 500 was documented during the 1991 aerial census. The eastern, southern and western boundaries of the KNP are fenced in by a game deterrent fence. Due to flooding, efforts to fence the extreme northern tip bordering on Zimbabwe, have been largely unsuccessful. In recent years most of the western fence between the Sabie and Olifants Rivers has been removed to allow free movement of wildlife between the KNP and the adjoining private nature reserves. For all practical purposes it shifts the western boundary south of the Olifants River about 20 km further west and adds a further 220 000 ha to the Greater KNP complex, with an additional 2 800 buffalo. The remaining southern and western areas bordering on the KNP are used mainly for agricultural purposes. Since the early 1970s the emphasis on livestock and irrigation farming to the west of the KNP has shifted gradually towards private game farming. In some of these areas livestock, especially cattle, are still found in the proximity of the boundary fence. On the Mozambique side subsistence agricultural practices predominate.

## Surveys

During 1991/1992 use was made of the ongoing buffalo population refuction (culling) operations for the post mortal surveillance of BTB. However, as a result of a natural die-off of buffalo in response to severe drought, culling operations on buffalo terminated in 1993. A total of 1974 buffaloes, culled in June to July and October to November of 1991, May to August of 1992 and November 1993, were utilized for the BTB survey. On average, 35 animals were killed per herd. Random sampling, as described by De Vos, Bengis & Coetzee (1983), was practiced. For the evaluation of ante mortem BTB diagnostic tests, a further 86 buffalo in groups of 8–11, were examined in 1991, 1992 and 1993 from known positive BTB herds in the southern sector and known negative herds in the far northern sector of the KNP (The results of these tests will be reported on elsewhere). Of these, 45 were selected for poor body condition, or lagging when chased by helicopter. When these tests were concluded the animals were slaughtered and necropsied. In 1996, in order to ascertain whether BTB had crossed the Olifants River, and to establish the disease's progress within three identifiable herds since 1991/1992, a further 163 buffaloes from six herds were sampled and screened for BTB. These were made up of three herds north of the Olifants River and three herds, or 96 animals, that were taken randomly from three known herds, which, in 1991/1992 had high, medium and low BTB prevalence values.

In 1993, a sample of 20 warthog and 20 impala were slaughtered and screened for tuberculosis. They came from the Mpanamana/Nhlanganzwane area, in close vicinity of the buffalo herds which previously had been shown to have the highest BTB prevalence.

Between 1991 and 1994 a total of 606 African elephant (*Loxodonta africana*) were slaughtered during population reduction exercises. Of these, 400 came from the southern half, a known BTB infected area of the KNP. All of them were scrutinized for possible tuberculosis lesions.

Field observations for possible BTB cases on clinical grounds, were intensified. Suspect cases were slaughtered and screened for tuberculosis. Specific BTB surveys on possible associated hosts, such as lion, baboon and kudu were also initiated, but are reported on elsewhere (Bengis & Keet 1998; Keet, Kriek, Penrith, Michel & Huchzermeyer 1996; Keet, Kriek, Penrith & Michel 1998; Keet, Kriek, Bengis, Grobler & Michel 2000).

## Clinical evaluation and necropsy procedures

The survey for BTB on all species screened relied mainly on necropsy procedures in order to determine

prevalence rates. Sex and age were recorded. Age was determined from tooth eruption patterns and wear of the teeth from the lower jaw. Whenever buffalo herds were observed, which was on an *ad hoc* basis, abnormal behavioural characteristics that could be associated with tuberculosis, such as coughing and lagging behind the herd, were noted and recorded. Gross abnormalities, such as emaciation, were noted.

Full standard necropsies were performed on 111 of the 1 122 buffalo carcasses that were screened for BTB. After having established the fact that lesions were mainly confined to the head and thoracic organs (Kriek, De Vos, Bengis, Huchzermeyer, Keet & Raath 1992) the survey technique concentrated predominantly on the lungs, the bronchial, mediastinal, retropharyngeal, parotid, mandibular, inguinal and prescapular lymph nodes and the tonsils. The lungs were first palpated for the presence of nodules before incisions into them were made. The lymph nodes were screened for gross abnormalities, such as enlargement, and by routinely making three to four incisions into them. During the 1996 survey the lymph nodes were serially sliced at about 1 mm thickness and examined for possible tuberculous lesions. In one herd, the Mpanamana herd, which has the highest BTB prevalence, the results of gross inspections were compared to a combination of gross, histological and bacteriological examinations on the same animals. In order to improve the keeping qualities of the carcasses for human consumption and in compliance with standard abattoir procedures (De Vos *et al.* 1983), they were eviscerated in the field, where the abdominal organs were scanned for gross lesions. The udder and associated lymph nodes were also removed and examined in the field. The plucks were left in the carcasses and only removed for examination after arrival at the abattoir. In addition, the reproductive status, judged by pregnancy and lactation, was recorded for all buffalo cows.

## Isolation and histological procedures

Initially, during the 1991/1992 survey, samples from lesions grossly suspected to be tuberculous, were further subjected to Ziehl-Neelsen-smear and histopathological examinations and bacteriological isolation procedures. For histopathological examination, samples were fixed in formal-saline and routinely processed. Tissue sections were stained by the haematoxylin and eosin, and Ziehl-Neelsen methods. Routine bacteriological techniques, as described by Bengis *et al.* 1995, were used to isolate and identify the causal organisms. Subsequently, after a pattern had been established, only selected cases were examined histologically and bacteriologically.

During the 1996 survey all buffalo carcasses, whether it appeared infected or not, were subjected to a more

intensive survey method of examination, as described above. This was combined with routine bacteriological isolation procedures on material from upper respiratory tract and thoracic lymph nodes.

### Genomic fingerprinting

Genomic fingerprinting, using the standardized method of Van Embden, Cave, Crawford, Dale, Eisenach, Gicquel, Herman, Martin, McAdam, Shinnick & Small (1993), was performed by the Onderstepoort Veterinary Institute on *M. bovis* isolates from buffalo in the KNP and cattle bordering on the KNP.

### Demographic data

Relevant census data for buffalo in the KNP were taken from the 1991, 1992 and 1993 census reports (Whyte & Wood 1994). Rainfall figures were obtained from the KNP climatological data bank.

## RESULTS

### Confirmation of the causal agent

The existence of mycobacteriosis was confirmed by smear and tissue examination. Culture attempts were made on samples from 179 buffalo and *Mycobacterium bovis* was isolated from 84 samples (47%).

### Genomic fingerprinting

A total of 17 genotype patterns with varying homology was identified among the infected species in the KNP. The majority of *M. bovis* isolates represent an identical genotype, designated ZA-01. In 1993, genotype ZA-01 was isolated from infected cattle slaughtered on a farm bordering the KNP in the south. The remaining genotypes mostly appear to share a homology of > 70% with genotype ZA-01.

### Clinical and necropsy findings

In most infected buffalo, BTB was found to be sub-clinical with clinical manifestations, such as debilitation, poor body condition or emaciation and lagging when chased by helicopter, being associated only with advanced cases with disseminated lung lesions or miliary disease. By selecting animals on the basis of these criteria during 1991/1992, a BTB prevalence value of 61.4% (27/45) was achieved in herds which shortly before, on random sampling, showed a BTB prevalence value of 44.3% (58/131); an increase of 17.1%.

In contrast to *healthy* herds, coughing was found to be a distinct and regular feature in buffalo herds with a high BTB prevalence.

TABLE 1 Correlation between bovine tuberculosis status and age in buffalo in the KNP (Chi-square = 14.0742 (3 d.f.)  $P = 0.0028$ )

Age in years	Tuberculosis status	
	Infected	Non-infected
0 +	6	46
1-3	57	143
4-8	49	116
9-20	18	20

No obvious difference could be found in the reproductive performances of BTB-infected and non-infected buffalo cows (Table 2).

As has been separately reported (Kriek *et al.* 1992), the BTB lesions in buffalo varied from small primary lesions, or tubercula in the lungs, to extensive caseous bronchopneumonia. The primary lesion appeared to be slowly progressive and was not well encapsulated. Lesions were almost exclusively restricted to the lymph nodes of the head, tonsils, lungs and associated lymph nodes of the thorax. In advanced cases, the lungs were most severely affected. Lesions in the lungs occurred particularly in the caudo-dorsal portions of the caudal lobes before progressing to the rest of the lungs. Infection of the mesenteric lymph nodes was found to be a rare occurrence, and only in individuals with severe pulmonary lesions. No involvement of the mammary gland was found.

A total of 14 out of 1 150 (1.2%) buffalo in the sample suffered from extensive caseating tuberculous pneumonia, characterized by multifocal caseous necrotic granulomatous lesions in which the exudate may liquify and coalesce to affect large portions of both lungs, or generalized tuberculosis, where lesions extend to the pleural and peritoneal surfaces, intestinal tract, parenchymatous organs, and other peripheral and visceral lymph nodes.

### Distribution and prevalence

The mean BTB macro prevalence rates for all infected buffalo herds combined during 1991/92, was found to be 23.9% (266 positive cases out of 1 122), with herd prevalence varying from 2-67%. Fig. 1 provides a graphic representation of the buffalo distribution by herd sizes in the KNP for 1992, and BTB prevalence values by herd, and includes all the surveys from 1991-1996.

From all the above-mentioned BTB-infected herds combined, 0.8% (9/1 125) had clinically advanced BTB lesions. In herds with prevalence rates of 40% and over, 6.6% (8/120) had advanced lesions. A simple regression analysis also indicates a strong posi-

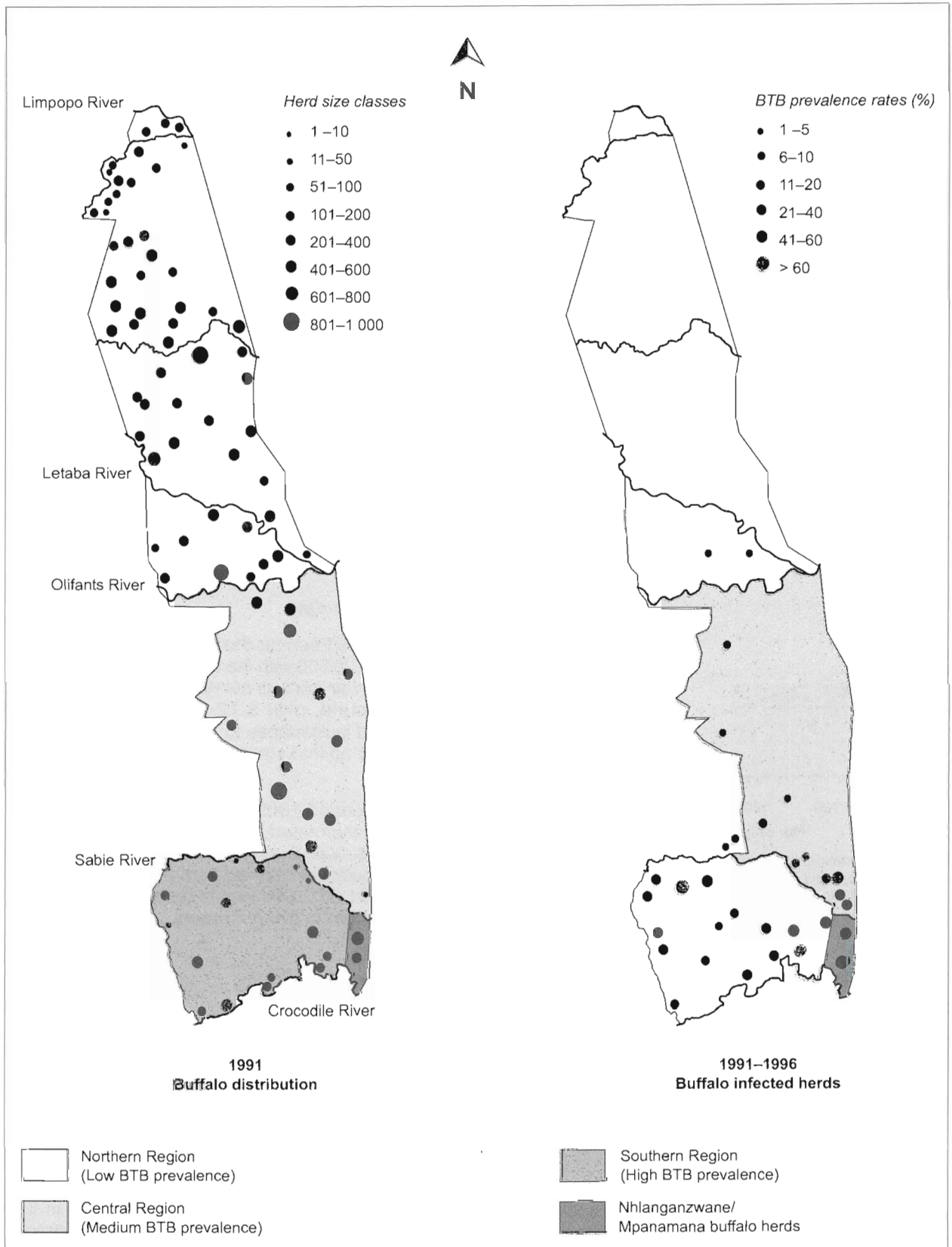


FIG. 1 Distribution of buffalo herds and bovine infected buffalo herds, indicating herd sizes and tuberculosis prevalence rates in the Kruger National Park

TABLE 2 Reproductive performance of tuberculosis infected to non-infected buffalo in the KNP

Tuberculosis status	Number	Reproductive status		
		Active		Inactive
		Pregnant	Lactating	
Infected	71	66.2 %	15.5 %	18.3 %
Non-infected	196	61.8 %	21.0 %	17.3 %

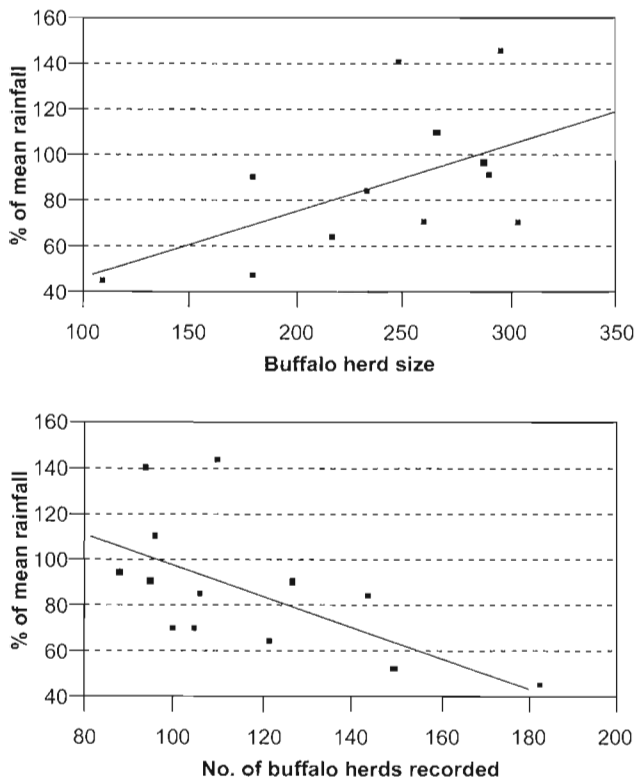


FIG. 2 The relationship between recorded rainfall and (a) mean buffalo herd size the following year and (b) the number of buffalo herds. Herd sizes and number of herds recorded during aerial censuses: 1980–1982

tive correlation between increasing prevalence values and the number of advanced BTB (potential mortality) cases. The correlation coefficients were found to be  $R^2 = 67\%$ .

The BTB prevalence values of three identifiable herds showed the following changes over a four year period (1992–1996): Low prevalence herd (Piet Grobler Dam) 4–3%; medium prevalence herd (Lower Sabie) 21–44%; high prevalence herd (Mpanamana) 67–67%. Combining the gross screening technique with a more intensive survey technique (thin slicing and bacteriological culturing of lymph nodes), the prevalence ratings increased from 67–92% in the Mpanamana herd, a difference of 25%, or an increase of 37.3%.

As depicted in Table 2, a positive correlation was found between advancing age and bovine BTB status (by prevalence) for the 1991/1992-survey period. No correlation could, however, be found between BTB prevalence rates and gender in buffalo during the 1991/92 survey (Chi-square = 1.45839;  $P = 0.00227$ ).

No signs of tuberculosis could be found in elephant, warthog and impala that were necropsied in the vicinity of the high BTB-prevalent buffalo herds. Incidental observations on other associated species however, revealed a few positive *M. bovis* cases in kudu, lion, cheetah, baboon and leopard as reported by Bengis & Keet 1998, Keet *et al.* 1996, Keet *et al.* 1998 & Keet *et al.* 2000.

**Demographic data**

During the 1991 census the buffalo population for the KNP totaled 29500 with the average buffalo herd size over a 22-year census survey period being 244. It was also found, over a 12-year period, that herd sizes varied in response to rainfall, with herds tending to break up into smaller units during dry periods (Fig. 2).

During the period 1969 to 1991, the buffalo population was manipulated by culling to maintain a level of 22 000–30 000. During the period 1992–1995, the buffalo population crashed from 29 359 to 14 123, a decline of 51.9%. This was in response to the most severe drought in living memory.

**DISCUSSION**

**Causal agent**

The majority of *M. bovis* isolates from the KNP was represented by an identical genotype (ZA-01), meaning that this genotype has not only spread through all infected species but throughout the entire infected area of the KNP, forming the main thrust of the outbreak. The same genotype (ZA-01), which was isolated from cattle near the southern boundary of the KNP, supports the belief that infected cattle in that region were the source of infection to the buffalo in the KNP. This is further emphasized by the fact that this genotype has not yet been found elsewhere in South Africa. The high (> 70%) homology of the other

genotypes with genotype ZA-01 suggests that they might have originated from ZA-01 by mutation. Mutation as a fairly recent event could explain the limited occurrence of these other genotypes, which could otherwise be the consequence of a reduced virulence or transmissibility of the genomic types.

### Hosts

To date, the buffalo population in the KNP, by virtue of its relatively high numbers, apparent high susceptibility and vulnerability and highly successful transmission between individuals and herds, has been found to be a highly effective maintenance host and reservoir of BTB in the KNP. The few *M. bovis* cases which were found in kudu, lion, cheetah, baboon and leopard point to incidental spillover of infection from the primary infection in buffalo to susceptible species, which thus far have not shown the ability to act as maintenance hosts (Bengis, personal communication; Bengis & Keet 1998; Keet *et al.* 1996; Keet *et al.* 1998; Keet *et al.* 2000).

### Disease manifestations

Clinical manifestations of BTB in buffalo correspond closely to those in cattle as described by Huchzermeyer, Brückner, Van Heerden, Kleeberg, Van Rensburg, Thomson & Tustin 1994. The chronic, and often subclinical manifestation of BTB in buffalo makes it virtually impossible to recognize the disease during the sub-terminal phases of the disease on clinical grounds only. Even the reproductive performance does not seem to be impaired by BTB infection (Table 1).

On gross necropsy examination, it was found that about 37% of infected animals show no macroscopically detectable lesions (Results). This is in accord with findings by Lugton, Wilson, Morris & Nugent (1998), who found 25% macroscopically undetected BTB lesions in red deer (*Cervus elaphus*) in New Zealand.

It is considered that this inapparent nature of BTB is responsible for the presence of the disease only being detected as long as about 30 years after its presumed entrance into the KNP. It was an emaciated and moribund animal in an advanced stage of BTB that first drew attention to the presence of the disease. Selective identification of infected animals is therefore only successful when advanced, recognizable cases are present, which is usually the case in high BTB prevalent herds. This was demonstrated during the 1991 survey when a 17.1% higher prevalence rate was achieved by selection on clinical appearance in a herd with known high BTB prevalence (see Results). Krauss *et al.* (1984) were more successful. They found that out of 41 lechwe, selectively shot because they were suspected to be suffering from BTB, 33 (80.5%) had advanced pulmonary tu-

berculosis. The selective removal of infected buffalo on clinical grounds is, therefore, of limited use as a disease control measure, especially in an essentially natural environment which tends to eliminate diseased animals.

A great deal of variation (from 2–67%) was detected in prevalence rates on gross examination of carcasses for BTB in the various infected buffalo herds (Fig. 1). Herds in the south, where BTB presumably gained entrance to the KNP, and which represent the oldest infection, had higher prevalence rates than the herds further away towards the periphery of the outbreak. This suggests that the rate of infection, as represented by prevalence rates, is a function of the length of exposure. This hypothesis is further supported by a highly significant correlation between disease prevalence and advancing age in buffalo (see Results). It indicates that the older the animal, and therefore, theoretically the longer the exposure to BTB, the higher the chances of being infected. Fuller (1966) and Woodford (1972) came to the same conclusion for bison in Wood Buffalo Park, Canada, and for African buffalo in Queen Elizabeth National Park, Uganda, respectively.

It is virtually impossible to provide a time span for the disease from its inception to a terminal stage. With the exception of a 2-year-old bull, all animals in an advanced tuberculous state were adult and past prime. Although this single case provides us with a period of 2 years or less, there are many possible factors, which may affect the progress of the disease. It is conceivable that the course of the disease in individuals, herds or the population may be influenced by high predator pressure and stressful events such as malnutrition, weaning, trauma or other infectious diseases, which could have an immunosuppressive effect on the host. This has been documented for domestic cattle (Thorns & Morris 1983; Waddington 1967). Seen from another perspective, major stressful events could also terminate advanced cases of tuberculosis at a relatively early stage. Judging from the results of the survey of 1992 and follow-up in 1996 on the Mpanamana herd, the prevalence rates remained the same, but the ratio of advanced cases to infected animals decreased (see Results). This decrease can probably be explained by the drought which occurred from 1992 to 1994, when 51.9% of the buffalo population in the KNP died out. It is believed that all sick and debilitated animals, including those in the advanced stages of BTB, were eliminated.

The pathological findings, varying from small primary lesions, or tubercula, to extensive caseous bronchopneumonia, are very similar to the description that Huchzermeyer *et al.* (1994) gave for the disease in cattle. As in cattle (Collins & Grange 1983), the primary BTB lesion in buffalo appears to be slowly progressive and in most cases is not fully encapsu-

lated with no indication of spontaneous healing, such as often happens in humans. This is indicative of low immunosuppression, and relatively high susceptibility and naïvety towards the disease.

There seemed to be no gender differences in BTB infection rates for buffalo in the KNP. This was also reported by Kriek *et al.* (1992).

### Transmission

The almost exclusive lung and upper respiratory tract involvement, points to an aerogenous mode of transmission. The alimentary canal seems to be incidentally infected from the respiratory tract (coughing and swallowing of infective material), with a negligible potential for the transmission of the disease. Similar observations were also made by Guilbride *et al.* (1963), Thurlbeck *et al.* (1965) and Woodford (1982a; b) for free-ranging buffalo in Uganda and by Gallagher *et al.* (1972) and Krauss *et al.* (1984) for lechwe in Zambia. Fuller (1966) recovered a ratio of respiratory to alimentary infection of 20:1 in wild American bison (*Bison bison*) in the Wood Buffalo National Park, Canada. This is also in agreement with findings in domestic cattle, where the lungs are affected in 90–95% of cases (Plum 1939; Cohrs 1967; Collins & Grange 1983).

Buffalo are extremely gregarious animals (Grimsdell 1969) and herd formation plus close social contact is probably a major factor in the transmission of BTB within herds. Census results show that the average buffalo herds size in the KNP, over a 22-year census survey period, was 244. Guilbride *et al.* (1963) and Woodford (1982a) also ascribed the success of transmission of BTB to the gregarious nature of buffalo in Ruwenzori National Park, Uganda. For spread by the respiratory route to take place, close contact in confined spaces is not a prerequisite. Bovine tuberculosis has been found in extensive cattle breeding areas in southern Africa where ranching conditions prevail and in which there is little or no close contact between animals in confined spaces for prolonged periods (Huchzermeyer *et al.* 1994). Apparently short periods of mingling, such as occur in a herd, is enough for the disease to be transmitted. Under similar conditions in Australia, BTB in extensively-kept cattle is thought to be due to their habit of congregating daily in large numbers at watering points under dusty conditions (Lepper & Pearson 1973).

The lack of encapsulation of lung BTB lesions suggests that lung lesions, even very small, are *open* and infectious. This possibly implies that buffalo can be infectious from a very early stage of infection and will remain so for the rest of their lives. In advanced cases where extensive areas of lung tissue are affected; these must be considered a formidable source of contamination and infection. The elimina-

tion of these advanced cases in nature by predation or stressful events, such as drought, can be expected to have an inhibitory effect on the spread of the disease, but on the other hand, it exposes carnivores to large numbers of *M. bovis* organisms which facilitates the initiation of infection in them. There are indications that this is currently happening to lions, the top member of the food chain in the KNP (Keet *et al.* 1998).

Huchzermeyer *et al.* (1994) maintain that the primary mode of spread of BTB between cattle herds is by the introduction of infected animals into non-infected herds. This is also probably the primary mode of spread of the disease between buffalo herds in the KNP. As illustrated (Fig. 2) KNP buffalo herds are dynamic and at times break up into smaller units, and at others amalgamate to form bigger herds, mostly in response to rainfall, which reflects the availability of water and food. The latter often involves the mixing of animals from different herds. It has also been found that groups of bulls, which are usually small, have a transient relationship with bigger mixed herds in response to rutting activities, or to accompany them to a drinking spot (De Vos, unpublished observations). Sometimes an individual buffalo, which has been disturbed and has lost contact with the herd might travel long distances to join up with other herds, thus making it possible for the infection to be transmitted over relatively long distances,

From annual reports of the State Veterinarian, Barberton/Nelspruit it can be determined that significant outbreaks of BTB occurred in cattle on farms near Hectorspruit along the Crocodile River which forms the southern boundary of the KNP, during the early 1960s and early 1980s (Bengis *et al.* 1995). During both periods, outbreaks of Corridor disease (*buffalo-associated theileriosis*) also occurred in cattle in this area, and indicates that close contact between cattle and buffalo occurred, which would have provided opportunities for BTB transmission. The exclusive occurrence of the same genotype (ZA-01) strain of *M. bovis* in both infected animals in the KNP and domestic cattle close to the southern boundary, lends further support to the belief that cattle along the southern border were the main source of BTB for the buffalo population of the KNP. Guilbride *et al.* (1963) also blamed cattle for the outbreak of BTB in buffalo in the Queen Elizabeth National Park.

### Temporal distribution

It is unlikely that BTB existed in South Africa prior to the importation of European breeds of cattle towards the end of the eighteenth century (Henning 1956). In the KNP, evidence, albeit circumstantial, seems to indicate a more recent entry. Although the first confirmed case of BTB was detected in 1990 near the south-western boundary of the KNP, subsequent surveys (1991/1992) showed that virtually all of the



buffalo herds, south of the Sabie River, were already infected by that date. Earlier, in 1966, a total of 100 buffalo from the area south of the Sabie River and east along the Lebombo mountains, mostly from the Nhlanguanzwane/Mpanamana herds, were slaughtered and full survey necropsies performed. In two cases lymph nodes showed slight granulomatous lesions which resembled BTB, but no acid-fast organisms could be demonstrated (Basson, McCully, Kruger, Van Niekerk, Young & De Vos 1970). In light of findings by Kriek *et al.* (1992) which indicate that large numbers of bacteria are present in liquifying, caseous exudates, whereas few, if any, are detected in lesions where necrosis is scant, it was decided in retrospect that these lesions could well have been the result of early *M. bovis* infection. If this assumption is correct, a 2% BTB prevalence rate already prevailed in the buffalo herds of that area, which means that the disease must have entered the KNP prior to 1966.

In 1977, a mycobacteriosis case was diagnosed in an impala from the same area. Isolation of the causal organism was not attempted and it was speculated on histopathological features only, that it could have been due to avian mycobacteriosis (De Vos *et al.* 1977). In retrospect, the possibility of BTB cannot be excluded. Nevertheless, it set off an intensive survey on 27 939 animals, mostly buffalo, impala and African elephant, which were slaughtered as part of a population reduction exercise. This met with negative BTB results. Routine meat inspections, from 1970–1980, of over 8 000 buffalo carcasses, of which about 3 000 came from south of the Sabie River, failed to detect the disease in spite of the fact that it must have been present. The reason for this can only be speculated that the disease was, typically of the growth of an epidemic (Wilson & Burke 1942), still in its initial phase of low-grade infection, and that standard meat inspection procedures failed to detect it. This provides a period of at least 20 years for the initial slow accretion phase of an expected epidemic curve. From 1980 until the first case of BTB in buffalo was diagnosed in 1990, viscera and plucks were left in the field without meat inspection being performed on them (Bengis *et al.* 1995). It must have been during that time that BTB in the buffalo population south of the Sabie River went through most of the acceleration (or exponential) phase of epidemic growth, with the Mpanamana herd reaching an asymptote of 67% prevalence rate in 1992 and again in 1996. It must, however, be borne in mind that a prevalence rate of 67% on gross inspection was found to be equivalent to 92% on more intensive and close examination (see Results). Judging from the experience in the Mpanamana herd, the asymptote point of a BTB outbreak in a buffalo herd is expected to be reached in about 30–40 years, making it a very slow and chronic disease in buffalo. It also means that the disease is expected to progress very slowly

in buffalo herds which are still in the accretion phase and herds which are nearing the asymptote of the epidemic (mostly herds south of the Sabie River and north of the Olifants River, respectively). On the other hand, the infection is expected to progress relatively fast in herds between the two extremes; i.e. herds, which are in the progressive phase of the expected epidemic curve (mostly in the central region, north of the Sabie River and south of the Olifants River). This hypothesis was proven correct in 1998 when a subsequent survey on 600 buffalo indicated significant BTB prevalence increases of 41% (from 27.1–38.2%) south of the Sabie River and 263.6% (from 4.4–16%) between the Sabie and Olifants Rivers. North of the Olifants River it increased from 0–1.4%, but due to a large sampling error the increase is not considered significant (Rodwell 1999).

Since BTB entered the KNP from the extreme south, it took about 30 years to reach the Olifants River, which is roughly the middle of the KNP, a distance of about 180 km. This provides a rough rate of spread of about 6 km per year in a northward direction. If this rate of spread is maintained, it can be expected that the forefront of BTB in buffalo herds, will reach Pafuri, the northern-most point of the KNP, in about another 30 years. However, with the present higher buffalo densities than was encountered 30 years ago, the rate of spread can be expected to be faster.

### Spatial distribution

Indications are that BTB entered the KNP buffalo herds from across the southern boundary, and since then has spread progressively in an outward and northward ripple-like fashion to other herds, with a gradient of declining infection, as reflected by prevalence rates, from south to north (Fig. 1). As sampling methods were not aimed at establishing the northern-most point of infection, it is quite conceivable that low-grade infection can already be north of the point indicated in Fig. 1.

### Risk assessment

Aberrancy in host selection, that is, spread of a parasite to a new host species, gives rise to a new imbalance that is likely to cause disease (Bang 1969). All indications are that BTB, which is a new (in evolutionary terms) disease to the KNP ecosystem, is causing imbalance which has taken on virulent epidemic proportions. Another evolutionary generalization in the case of diseases, is that the evolution of a disease tends toward development of a symbiotic relationship (Bang 1969). If it is taken into account that the KNP is an essentially natural area where such evolutionary relationships are allowed to develop without interference by man, there is a distinct possibility that adaptation will eventually take place. However, being a slow and chronic disease and with

its hosts showing a relatively low reproductive turnover, no dramatic change is expected in the foreseeable future.

The success of buffalo as a BTB maintenance host conveys to the KNP, a BTB endemic status. With little or no immediate prospect of eradicating the disease from the KNP, this status is expected to be maintained for the foreseeable future. This is seen as a complication to the current Bovine Tuberculosis Scheme in South Africa, which was introduced by the Directorate of Animal Health in 1969 with the purpose of eradicating the disease from the country. The KNP is, however, now virtually surrounded by a buffalo deterrent fence which is seldom transgressed. This was not the case in earlier years when BTB was transmitted from cattle to buffalo. With no communal grazing areas between livestock and buffalo, and considering that transmission between buffalo is almost exclusively aerogenously, the risk of transmission from buffalo to livestock must be considered very low. It is therefore possible and practical to isolate the disease within the confines of the Greater KNP. Isolation, or quarantine procedures will, however, affect transport of BTB susceptible animals, especially buffalo, out of the KNP to "clean" areas. It is also expected that BTB will eventually spread to all areas of the Greater KNP where buffaloes are found, such as the western private nature reserves. It has already been found in buffaloes and lions from the Sabie Sand Private Nature Reserve (Fig. 1). This also poses a risk to other areas which are designated to join up with the KNP, such as the envisaged trans-frontier park developments (South African Ministry of Environmental Affairs and Tourism 1999).

Bovine tuberculosis, being chronic and progressive in buffalo, is expected to directly or indirectly lead to the death of its host. If assumed that the 1.2% animals, which had generalized tuberculosis or extensive caseating tuberculous pneumonia would, within reasonable time limits, have died from the effects of the disease, then it also provides a rough indication of expected mortality from BTB. A further analysis of the data indicates that the higher the prevalence rate within a herd, the more advanced cases, or the higher the mortality, is to be expected. Available data suggests a potential mortality reaching up to 10% in buffalo herds having a BTB prevalence of 50% or higher. Considering that BTB in buffalo also spreads progressively within herds, then it must be assumed that most infected herds will eventually reach high prevalence levels and elevated mortality rates.

These observations correspond closely with findings on wildlife elsewhere. Woodford (1982a) gave a very rough estimate of 1% mortality from BTB for buffalo in the Ruwenzori National Park, which does not differ markedly from findings in the KNP. Gallagher *et al.* (1972), on the other hand, estimated a BTB prevalence rate of 36% and mortality rate of 20% per annum for lechwe from the Lochinvar National Park,

Zambia. Fuller (1966) reported a BTB prevalence rate of 39% and a corresponding mortality rate of 6% in bison from the Wood Buffalo National Park in Canada, and blamed it partially for a decline in their numbers, from 12 to 15 thousand in the late 1940s to approximately 5000 in 1986 (Tessaro, Forbes & Turcotte 1990). There can therefore be no doubt that the disease has potential decimating properties.

With such a formidable reservoir of infection, other species in the vicinity of these infected buffalo herds are at high risk of contracting BTB. This has already occurred, with spillover infection having been diagnosed in five other species. Although relatively few cases have been reported, and infection appears to be incidental at this stage, the potential of some of these species to become maintenance hosts, and the decimating effect that BTB may have on these animals, should not be ignored. Indications are that lions in the KNP, being in the top position and on the receiving end of the food chain, are already severely affected (Keet *et al.* 1998).

Considering the KNP's relatively high biodiversity, including 147 mammal species, of which 65% can be classified as "small mammals" (Pienaar *et al.* 1987), the spread to other gregarious and known susceptible species, *inter alia* warthog, impala, blue wildebeest (*Connochaetes taurinus*), African elephant, black rhinoceros and square-lipped rhinoceros (*Ceratotherium simum*), must be considered a distinct possibility. What is encouraging to note however, is that the culling of 400 elephant south of the Sabie River (high BTB prevalence area), since 1992, provided a clean bill of health to them. Surprisingly, the disease has also not yet been found in warthog, a species which, in Queen Elizabeth National Park, Uganda, has been identified as highly vulnerable to BTB (Woodford 1982a & b). In addition "small mammals" have not yet been incriminated in the Kruger National Park. However, *M. bovis* was isolated from one large spotted genet (*Genetta tigrina*) in 1998 in a conservation area neighbouring the Kruger National Park (unpublished data). If experiences in New Zealand with brushtail possums (*Trichosurus vulpecula*) (Coleman, Jackson, Cooke & Grueber 1994), in Britain with badgers (*Meles meles*) (Nolan & Wilesmith 1994) and South Africa with hares (Paine & Martinaglia) are taken into account, then a possibility of BTB finding a niche amongst the 96 "small mammal" species in the KNP should not be overlooked.

Humans are as susceptible to *M. bovis* as to *M. tuberculosis* (Huchzermeyer *et al.* 1994). There is, however, very little opportunity for transmission to take place. The only risk to humans would be through close contact with infected animals. Veterinary and game capture staff are at risk at acquiring the disease by close contact in confined spaces such as crates and pens and by handling infected carcasses.

Slaughtered animals are processed in an abattoir, using standard abattoir procedures (De Vos *et al.* 1983). Products that are destined for human consumption are therefore considered safe. With no direct contact with buffalo, the risk to tourists to the KNP is considered virtually non-existent.

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