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## PREVALENCE OF BOVINE TUBERCULOSIS IN AFRICAN BUFFALO AT KRUGER NATIONAL PARK

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**ABSTRACT:** Bovine tuberculosis (BTB) was first detected in Kruger National Park (KNP) in a single African buffalo (*Syncerus caffer*) in 1990. In 1991/1992, 2,071 African buffalo were examined for BTB as part of a culling program that removed animals from all known herds in KNP. The prevalence of BTB in 1991/1992 was estimated to be 0%, 4.4% ( $\pm 0.6\%$ ), and 27.1% ( $\pm 1.4\%$ ), in the north, central, and south zones of KNP, respectively. In 1998, a stratified, two-stage cluster sampling method was used to estimate that the prevalence of BTB was 1.5% ( $\pm 2.5\%$ ), 16% ( $\pm 5.3\%$ ), and 38.2% ( $\pm 6.3\%$ ), in the north, central, and south zones, respectively. This represented a significant increase in prevalence ( $P \leq 0.05$ ) in the south and central zones, but not in the north zone. Continued monitoring of BTB in KNP is important for understanding disease transmission risks, potential population effects, and the efficacy of disease management strategies. The methodology and sample sizes used in 1998 are appropriate for future BTB monitoring in KNP.

**Key words:** African buffalo, bovine tuberculosis, cluster sampling, disease monitoring, *Mycobacterium bovis*, *Syncerus caffer*.

### INTRODUCTION

Bovine tuberculosis (BTB) is caused by the pathogen *Mycobacterium bovis*, which can cause chronic wasting in a wide range of mammalian hosts, including humans (Morris et al., 1994). Many wildlife populations around the world have been infected with this pathogen (Barlow, 1993; White and Harris, 1995; Schmitt et al., 1997), but there is little empirical data regarding how the prevalence of infection changes over time in large, free-ranging mammals. At present, there are no practical methods for treating wildlife populations infected with *M. bovis*, and there is no convincing evidence that individual wild animals are able to resolve the disease naturally (O'Reilley and Daborn, 1995). However, pressure to monitor and manage BTB in wildlife is increasing as a result of concerns for the health of infected wildlife populations (Joly et al., 1998), and because of the risks these populations pose to domestic animals and to humans (Brown et al., 1994; Krebs et al., 1998).

Bovine tuberculosis was apparently first introduced into African buffalo (*Syncerus caffer*) in the southern region of Kruger National Park (KNP, Republic of South Africa) in the 1960's or 1980's from domestic cattle (Bengis et al., 1996). By the late 1980's, BTB was largely eradicated from the domestic animal populations surrounding KNP (Klocek, 1998), but inside KNP it apparently persisted undetected until 1990, when it was diagnosed opportunistically in a single African buffalo (Bengis, 1999). In 1991 and 1992, African buffalo culled in the annual KNP culling program (De Vos et al., 1983) were examined for BTB. Culling was terminated in 1992 following massive drought-related mortalities and systematic BTB surveillance was discontinued. In 1998, BTB monitoring was resumed, and at this time a sampling design was utilized that provided precise prevalence estimates with a minimum sample size. This report summarizes the results of the 1991/1992 and 1998 BTB surveys, and outlines the use of stratified, two-stage cluster sampling for

the design and analysis of BTB prevalence surveys in KNP. We also demonstrate a significant increase of BTB prevalence during this time, and outline considerations for long-term monitoring of this disease in KNP.

## MATERIALS AND METHODS

With an area of about 20,000 km<sup>2</sup>, KNP is the largest wildlife refuge in South Africa, and one of the largest protected environments in Africa. Kruger National Park lies between latitudes 22°19' and 25°32'S, and longitudes 30°52' and 32°03'E. For the purposes of this study, the KNP African buffalo population was divided into three geographic zones, and a BTB prevalence estimate was calculated for each zone. The south zone lies between the south KNP border and Sabie River, the central zone lies between the Sabie and Olifants rivers, and the north zone lies between the Olifants River and the north boundary of KNP. These perennial rivers largely restrict permanent African buffalo movements, and the effectiveness of these boundaries is also supported by evidence from radio telemetry studies of animal movements (Whyte, 1996).

In 1991 and 1992, 2,071 of the 2,716 African buffalo culled in the annual KNP culling program were examined for BTB. Approximately 20 to 30 individuals were culled from each KNP herd, and necropsy and histopathology were used to detect BTB positive animals. These data were retrospectively stratified into zones, and a single 1991/1992 estimate of prevalence was calculated using the same analytical methods described below for the 1998 sample. A different sampling scheme was used in 1998 since the culling program was terminated after 1992. Our goal in 1998 was to sample the minimum number of African buffalo needed to obtain a precise BTB prevalence estimate. We used stratified, two-stage cluster sampling (Thrusfield, 1995) to estimate BTB prevalence, where the first stage of the sample was the herd, and the second stage was the individual in that herd. Prevalence was calculated for each zone (stratum) independently, and approximate 95% confidence intervals were calculated using the normal approximation, adjusted for a finite population size (Thrusfield, 1995). A difference in prevalence was considered significant (95% confidence level) if the confidence intervals from two prevalence estimates did not overlap.

The expected precision for a prevalence estimate calculated using two-stage cluster sampling is based on the expected disease prevalence, total sample size, and the number of

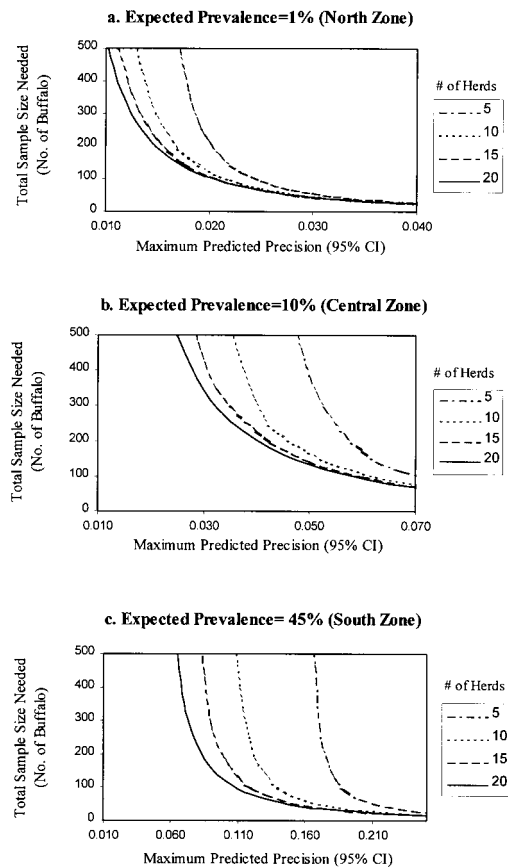


FIGURE 1. Power curves used for maximizing precision and minimizing sample size for the 1998 bovine tuberculosis (BTB) prevalence estimates in Kruger National Park (Republic of South Africa). Graphs represent expected precision for the BTB prevalence estimate for buffalo in the (a) north zone, (b) central zone, and (c) south zone. Curves were calculated based on equations in Thrusfield (1995) with inter-herd variance from 1991/1992 samples, and expected 1998 BTB prevalence in each zone.

clusters (herds) sampled. We examined the trade-off between sample size, number of herds, and the expected precision, with power curves (Fig. 1) based on sample size equations in Thrusfield (1995). Between-herd variance for these equations was derived from 1991/1992 prevalence data in the south and central zones. In the north zone where no between-herd variance was available (no herds were positive for BTB in 1991/1992), the central zone variance (adjusted with a lower expected prevalence of 1%) was used. We chose a sample of 200 African buffalo from 10 herds (20 animals from each herd) in each zone to maximize the precision of the prevalence estimate at both the

TABLE 1. Estimates of bovine tuberculosis prevalence in African buffalo from Kruger National Park (Republic of South Africa) in 1991/1992 and 1998. Prevalence estimates are based on gross necropsy and histopathology.

Year	Zone	Total number buffalo	Total herds	Number herds sampled	Number buffalo sampled	Percent herds positive	Number buffalo positive	Zone prevalence (%)	95% C.I. <sup>c</sup> (%)
91/92	North <sup>a</sup>	11,890	124	21	594	0	0	0.0	0.0–0.0
	Central <sup>b</sup>	7,392	21	20	850	45	37	4.4	3.7–5.0
	South <sup>b</sup>	3,849	20	19	627	89	170	27.1	25.7–28.5
98	North	9,310	39	10	203	10	3	1.5	0.4–4.0
	Central	6,680	15	10	206	80	33	16.0	10.8–21.3
	South	5,277	21	10	207	100	79	38.2	31.8–44.5

<sup>a</sup> Buffalo culled in 1992.<sup>b</sup> Buffalo culled in 1991.<sup>c</sup> Prevalence estimates with 95% confidence intervals (95% C.I.) that do not overlap are significantly different.

herd and population level, while keeping mortalities to a minimum. A total of 600 African buffalo from 30 different herds was chosen from the population. The expected precision of our estimates was <13%, 5%, and 2%, in the south zone, central zone, and north zone, respectively. We could have increased the precision of the estimate for each zone by selecting fewer animals from more herds (Fig. 1). However, a minimum of 20 animals per herd (200 animals/10 herds) was desirable to formulate precise estimates of prevalence at the herd level and to collect essential demographic data (Rodwell et al., 2001).

Sampled herds were chosen at random from the breeding herds counted in the 1998 total KNP census. Since African buffalo herds may have internal social structures associated with them and may not be homogenous (Prins, 1989), a random quadrant of each chosen herd was pre-selected, and approximately 20 animals were collected from that quadrant of the herd, without regard to age or sex. Animals were culled using the standard KNP culling technique outlined in De Vos et al. (1983).

Bovine tuberculosis was diagnosed by necropsy of the entire animal, with detailed macroscopic inspection of intestinal and thoracic organs and lymph node sections. Animals were determined to be positive for BTB if they had lesions that were consistent with *M. bovis* infection (Corner, 1994). Gross lesions that were positive or suspicious (possibly not caused by *M. bovis*), were confirmed with histopathology. Specimens taken for histopathology were preserved in 10% buffered formalin and later embedded in paraffin wax. Sections of 4–6 µm were cut from the specimen and stained with haematoxylin, eosin, and Ziehl-Neelsen stains for visualization with light microscopy (Bengis et al., 1996).

Bacterial cultures were also prepared from lymph node sections of all animals sampled in 1998, except for the pathology-positive African buffalo from the south zone, which were omitted by an oversight. Culture and identification of *M. bovis* was performed by the Onderstepoort Veterinary Institute, Republic of South Africa, in the following manner. Bacterial lesions were excised, homogenized, and decontaminated with 2% HCL or 4% NaOH. The sample was then centrifuged and used to inoculate 3 separate tubes containing Lowenstein-Jensen medium with glycerine, without glycerine, or with 0.5% pyruvate. Tubes were incubated at 37 C and acid-fast colonies were identified using standard techniques (Bengis et al., 1996). Comparisons of prevalence estimates from 1991/1992 and 1998 were based only on necropsy and histopathology results since bacterial culture was not performed in 1991/1992.

## RESULTS

In 1991/1992, approximately 10% (207/2,071) of the animals, and 43% of the herds (26/60), were positive for BTB (Table 1). The prevalence of BTB within herds ranged from 55.6% in the south zone, to non-detectable levels in the north zone (Fig. 2). In 1998, 18.6% (115/616) of the animals, and 63.3% (10/30) of the herds sampled showed lesions consistent with BTB infection (Table 1, Fig. 2). The prevalence of BTB differed significantly among all zones in both 1991/1992 and 1998 (Table 1). There was a significant increase in prevalence ( $P \leq 0.05$ ) in the south and central zones from 1991/1992 to

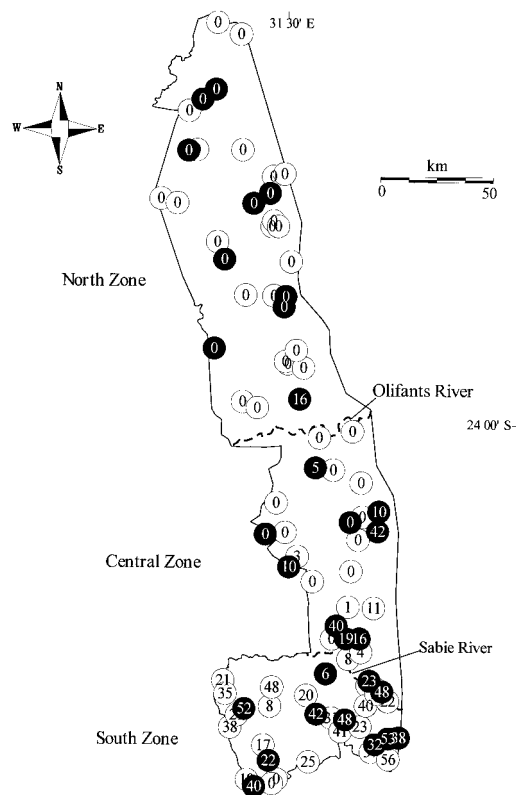


FIGURE 2. Map of Kruger National Park (Republic of South Africa) with estimates of bovine tuberculosis prevalence in all herds sampled in 1991/1992 and 1998. Open circles represent buffalo herds sampled in 1991/1992, and filled circles represent buffalo herds sampled in 1998.

1998, but there was no significant change in the north zone (Table 1). The overall change in prevalence from 1991/1992 to 1998 was equivalent to an average annual increase of 1.6% in the south zone and 1.7% in the central zone.

Bacterial culture was more sensitive than pathology for detecting *M. bovis* in the central zone (40 positive cases were detected by culture and 33 were detected by pathology). In the north zone where the prevalence was very low, three cases were detected with pathology and only one case was detected with culture. In the south zone only pathology-negative samples were cultured, and it was thus not possible to conclude which method of detection was more sensitive. Combined use of both

culture and pathology results (the sample was considered positive if either method detected a positive case) was the most sensitive method of detecting BTB. Combined use of pathology and culture yielded 3, 43, and 83 positive cases in the north, central, and south zones, respectively; whereas pathology alone detected 3, 33, and 79 positive cases in the north, central, and south zones.

## DISCUSSION

As of 1998, African buffalo herds in all zones of KNP should be considered exposed to BTB, with a decreasing probability of actually being infected the further north the herd is located (Table 1, Fig. 2). The prevalence of BTB increased significantly in the south and central zones from 1991/1992 to 1998, due to increases in both the average herd prevalence and the total number of herds infected with BTB (Table 1). These results indicate that both intra-herd and inter-herd transmission of BTB are likely to be important factors in the ecology of this disease in KNP. None of the 21 north zone African buffalo herds examined in 1991/1992 were positive for BTB, but our sample size was insufficient to say that the north zone was free from disease (Cameron and Baldock, 1998). Based on the 1998 results, the prevalence in the north zone was estimated to be  $>0$  (three positive animals were detected) and  $\leq 4\%$  (table 1).

Outside of KNP, BTB has only been reported in African buffalo in Queen Elizabeth and Ruwenzori National Parks in Uganda (Guilbride et al., 1963; Woodford, 1982). There are no published reports of long-term trends in prevalence in any large mammal population. Based on theoretical calculations, McCarty and Miller (1998) predicted a 19% increase in BTB prevalence in white-tailed deer (*Odocoileus virginianus*) over 25 yr. Present data are not sufficient to accurately predict trends in KNP, but if conditions remain as they are now, it appears that the prevalence of BTB will continue to increase in



the south and central zones. We did not detect a significant increase in BTB in the north zone. However, given the biology of BTB and the similar density of African buffalo in the north zone (0.9 animals/km<sup>2</sup>) compared with the other zones (central zone = 1.2 animals/km<sup>2</sup>, south zone = 1.4 animals/km<sup>2</sup>), it is likely that the prevalence in the north zone will increase significantly in the future.

There is convincing evidence that BTB was introduced into the KNP African buffalo from domestic cattle in the southeast corner of KNP (Bengis et al., 1996; Kloeck, 1998). The current distribution of the disease (Fig. 2) is likely the consequence of that initial infection event, followed by the subsequent northward transmission of the disease from herd to herd. Each of the three zones is thought to contain largely independent sub-populations of buffalo. There are limited African buffalo movements between zones which appear to be temporary in nature (Whyte, 1996), but severe environmental changes could change herd dynamics in an unpredictable manner.

Prior to collecting data in 1998, we did not know how BTB prevalence had changed in each zone, thus it was not possible to determine what precision we would need to detect a significant difference between 1991/1992 and 1998 (with 95% confidence). Based on the 1991/1992 BTB results, we estimated that prevalence could have changed by approximately 10%, 5%, and 1% in the south, central, and north zones, respectively, and we aimed for precision in this range in each zone. However, the number of animals needed for this expected level of precision in the north and south zones ( $n > 500$  animals per zone) (Fig. 1) was an unacceptably high proportion of the population in those zones. We therefore adjusted our precision requirements and sampled at least 200 animals from each zone, which yielded an expected precision of <13%, 5%, and 2% for our prevalence estimates

for the south, central, and north zones (Fig. 1).

Bacterial culture was useful for detecting BTB infections that were not detected by pathology in both the central and south zones. The enhanced sensitivity provided by bacterial culture was probably due to the detection of early infections where lesions were not yet visible on gross or microscopic examination. Bacterial culture failed to identify some animals that were clearly positive by necropsy and histopathology, probably because the culture procedure is sensitive to contamination by other microorganisms which can overgrow the *M. bovis* culture. Combining pathology and bacterial culture results was the most sensitive method for detecting BTB in all zones. However, the differences among the BTB prevalence estimates based on pathology or culture, or pathology and culture combined, were not substantial. If only prevalence information is sought, and resources are limited, either detection method could be used in the future. However, it may be prudent to continue using both pathology and culture as future diagnostic tools, because bacterial culture may provide useful information on early infections in the population (cases where lesions are not yet visible), and pathology is the method that has been used consistently since 1991/1992.

The prevalence of BTB in KNP should be monitored consistently and precisely if meaningful predictions about future transmission risks and potential disease effects on different host populations are to be made. At the same time, if surveys are conducted more frequently than survey precision merits, African buffalo will be killed unnecessarily. If stratified, two-stage cluster sampling of 600 animals (200 animals per zone, from 10 herds) is used to estimate the prevalence of BTB in the future, we can expect similar precision to that obtained in the 1998 survey, that is, approximately 2.5%, 5.3%, and 6.3%, in the north, central, and south zones, respectively (Table 1). Furthermore, based

on the average annual increase in BTB prevalence in KNP from 1991/1992 to 1998, we would probably not be able to detect a significant change in prevalence if we conducted another prevalence survey sooner than about 2003.

*Mycobacterium bovis* is an introduced pathogen in KNP and thus is considered an alien species. Kruger National Park has an obligation to remove alien organisms, but it also has an obligation to protect the host species. Bovine tuberculosis does not appear to affect the fertility or lactation rates of female African buffalo in KNP, but there are indications that adult African buffalo were underrepresented as an age class in infected herds (Rodwell et al., 2001). Until the explicit mortality risk of bovine tuberculosis in African buffalo is determined, we can only speculate about future effects. However, as the prevalence of BTB in African buffalo increases, there is an increased risk of spillover into other species (Mfairfin et al., 1998). Therefore, even if BTB does not have an appreciable effect on the African buffalo population, concerns regarding BTB transmission to other species dictate that monitoring should be continued.

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