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The gamma-interferon test: its usefulness in a bovine tuberculosis survey in African buffaloes (*Syncerus caffer*) in the Kruger National Park

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ABSTRACT

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A survey to determine the bovine tuberculosis status of buffalo herds north of the Olifants River in the Kruger National Park was conducted, using a new diagnostic approach. Diagnosis of *Mycobacterium bovis* infection was accomplished using the gamma-interferon assay technique in 608 adult buffaloes out of a total of 29 discreet herds. The animals were immobilized in groups of 10–15, bled, individually marked and then revived and released on site. As soon as test results were available (after 26–36 h), the same buffalo herd was relocated by tracking the frequency of a radio-collar previously fitted to one adult cow per group during the initial operation. Bovine reactors were identified, darted and euthanased from the helicopter. Necropsy and culture findings of all culled buffaloes showed excellent correlation with the results of the ante-mortem gamma-interferon test. The survey revealed that over and above the two positive herds that had been identified during a previous survey carried out in 1996, there were three additional, but previously unidentified, infected herds in the region north of the Olifants River.

Keywords: African buffalo, bovine tuberculosis, gamma-interferon test, Kruger National Park, *Mycobacterium bovis*

INTRODUCTION

In the absence of suitable control measures, bovine tuberculosis (BTB) can progressively infect increasing numbers of cattle in a given population, resulting in significant economic losses as well as a zoonotic risk. Infection by the causative agent, *Mycobacterium bovis*, is by no means restricted to cattle, and spillover into a wide range of domestic and wild species, as well as humans, has been reported (Collins 1995; O'Reilly & Daborn 1995).

There is strong circumstantial evidence, confirmed by molecular typing of Kruger National Park (KNP) *M. bovis* isolates, that BTB was introduced into the Park by cattle-to-buffalo *(Syncerus caffer)* transmission across the southern Crocodile River boundary near Hectorspruit during the late 1950s (Kloeck 1998; De Vos, Bengis, Kriek, Michel, Keet, Raath & Huchzermeyer 2001). Once BTB had established itself in the buffaloes, spatio-temporal spread occurred within and between buffalo herds resulting in a gradient of infection, with prevalence rates ranging from 1.5% (northern herds) to 55% (southern herds).

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"Spillover" of infection by direct and indirect transmission occurred in a number of other wildlife species (Keet, Kriek, Penrith, Michel & Huchzermeyer 1996; Keet, Kriek, Penrith & Michel 1998; Bengis, Keet, Michel & Kriek 2001) in this national park. Buffaloes have proved to be true maintenance hosts of the disease and today, more than half of the buffalo herds in the KNP are infected.

In order to contain BTB in the KNP and reduce further spatial spread, it is of crucial importance to possess a sensitive, specific and practical antemortem test to diagnose *M. bovis* infection under field conditions and with minimal manipulation of the buffalo herds.

During the 20th century many countries worldwide successfully eradicated BTB from their cattle populations using control (test-and-slaughter) measures based mainly on the intradermal tuberculin (IDT) skin test (Collins 1995). Although the IDT has also proved both sensitive and specific in free-ranging buffaloes (J.P. Raath, unpublished data 1996), this technique is costly and impractical, and has inherently more risk owing to the necessity of repeated chemical immobilization and animal holding facilities required for it. During a comparative field evaluation of the commercial gamma-interferon test (Bovigam™, CSL, Australia) and the skin test in cattle and buffaloes in South Africa, a non-specific reactor problem caused by cross-reactions with environmental mycobacteria was identified. It was found that the specificity of the test could be increased considerably when the test was modified in such a way that animals whose immune response was stimulated by environmental mycobacteria could be differentiated from true bovine reactors (A.L. Michel, unpublished data 2000). In the same evaluation the sensitivity of the IFNg assay in buffaloes was found to be 84.6 %. Based on this study it was decided to use this technique to determine the BTB status of all buffalo herds in the northern part of the KNP. Pending the outcome of this project the test could form an integral part of the future BTB management strategy in the KNP.

MATERIALS AND METHODS

Identifying buffalo herds

The 1999 aerial census results (Whyte 1999) were used as a basis to identify all the buffalo herds in the area north of the Olifants River. A Eurocopter Colibri EC120 was used for all the aeronautical requirements, including aerial darting. Some of the herds in the far north had been fitted with radio-collars in 1999 before the present study commenced and the remaining herds were marked with radio-collars (MOD-600 transmitter, Telonics, 932 E. Impala Av., Mesa, Arizona, 85204-6699, USA) transmitting a specific unique frequency, during the study.

Capture

Once a buffalo herd was located, a group of about 25–40 animals was selected and cut out of the herd. Target animals in the group to be sampled were then darted. Only adult animals were selected for the study and, depending on various factors, such as the terrain and the workability of the group, 10–15 animals were immobilized together. The KNP-developed aluminium dart system (4 mℓ darts each fitted with a 45 mm collared needle), fired from a modified 20 gauge shotgun was used to deliver the anaesthetic "cocktails" at the following dosage rates and composition:

Adult bull: 8 mg etorphine hydrochloride (M99; Logos Agvet) + 100 mg azaperone (Stresnil; Janssen Pharmaceutica)

Adult cow: 7 mg etorphine hydrochloride + 80 mg azaperone

Down times (the time for the animal to become immobilized after being darted) ranged from 5–8 min. After the samples had been collected and the animals suitably marked for future identification (see below), anaesthesia was reversed by administrating 20 mg (bulls) or 18 mg (cows) diprenorphine hydrochloride (M5050; Logos Agvet) intravenously into an ear vein. All the animals were observed until they were mobile, a process that generally took about 2–5 min.

Collection of samples

Blood samples were collected by venopuncture of the jugular vein using a 38 mm 18 G Vacutainer needle. Ten millilitres of blood was collected from each animal into clearly marked tubes containing heparin for the purposes of the gamma-interferon test.

Marking of individual buffaloes

The allocated identification number of each immobilized buffalo was painted on its back using aluminium paint. These numbers were large enough (25 cm) to be visible from the air, making it possible to identify positive animals after the test results had

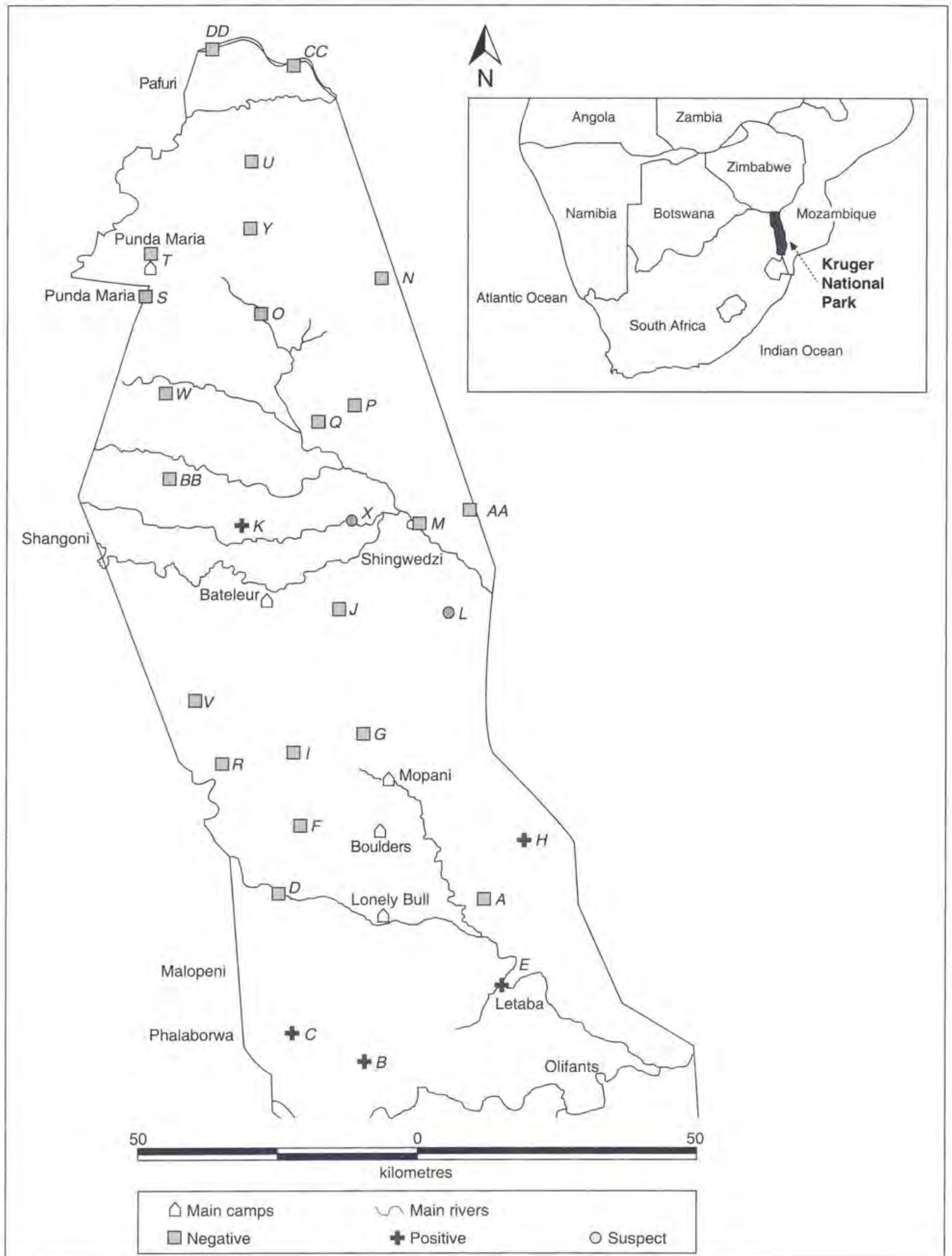

FIG. 1 Locations and BTB results of buffalo herds in the northern Kruger National Park

become available. In some cases the numbers were still legible after 1 week. Each herd was allocated an alphabetical letter that was boldly painted on the right shoulder of each buffalo sampled from that herd. In addition, a hot "Z" brand was used to brand all buffaloes permanently on the right rump as a retrospective means of linking them to the BTB 2000 survey.

Gamma-interferon test

The commercial Bovigam kit was used in a modified form (Michel, Nel, Cooper & Morobane 2000) to detect buffaloes infected with *M. bovis*. Briefly, between 1 h and 4 h after collection the heparinized blood samples from buffaloes were stimulated with bovine and avian tuberculin PPD (ID-Lelystad, The Netherlands) as well as a crude protein extract of *M. fortuitum* to assist with the differentiation of specific and non-specific reactions. Incubation and detection of the gamma-interferon assay were carried out according to the manufacturer's instructions. All handling and testing of blood samples were performed in a mobile laboratory near the capture sites.

Necropsies and culture

Buffaloes that tested positive on gamma-interferon assay were then traced by helicopter using the herd radio-collar frequency and identified from the air by their herd designation and specific individual numbers. These positive reactors were then removed from the herds by darting with an overdose of succinyl dicholine. Necropsies were performed on these animals in the field and samples from all the lymph nodes of the head and thorax, as well as from suspect lesions were taken for histopathology and bacteriological culture. The samples for culture were stored at –20 °C until transferred and processed at the Tuberculosis Laboratory of the ARC-Onderstepoort Veterinary Institute according to standard procedures (Bengis, Kriek, Keet, Raath, De Vos & Huchzermeyer 1996).

RESULTS

All results are summarized in Table 1 and the locations of herds in Fig. 1.

Interpretation of the gamma-interferon assay

During extensive studies in buffaloes it was previously shown that cross-reactivity with environmental mycobacteria could be detected by the additional stimulation of blood cultures with a crude protein extract from *M. fortuitum* which modified the commercial Bovigam into a triple comparative gamma-interferon assay (Michel, Nel, Cooper & Morobane 2000)

In brief, a test result was considered positive for infection with *M. bovis* infection if the following criteria were met:

$OD_{bovine} - OD_{avian} > 0.20$ and if $OD_{fortuitum} - OD_{nil} < 0.15$, provided that $OD_{nil} < 0.25$.

In cases where $OD_{fortuitum} - OD_{nil} > 0.15$ the buffalo was classified as multiple reactor (MR). In our previous studies we found this pattern of multiple reactions in infected as well as in uninfected buffaloes. For the purpose of this survey it was decided to exclude this group of reactors from culling.

Gamma-interferon tests (IFNg)

Out of a total of 29 buffalo herds comprising approximately 8 390 animals, 608 were tested using the IFNg assay. A total of nine test-positive buffaloes were identified (Tables 1 and 2). Seven out of eight bovine reactors were shown to have macroscopic lesions of tuberculosis in the lymph nodes associated with the head and the respiratory tract or the lungs and *M. bovis* was isolated on culture. One test-positive animal could not be retrieved for culling, as it had not joined up with its parent herd. Necropsy of another test positive buffalo failed to reveal macroscopic lesions of tuberculosis and culture of the lymph nodes collected was negative. A total of three multiple reactors and 26 avian reactors were detected but not identified for culling.

DISCUSSION

In previous BTB surveys in the KNP, the TB status of selected buffaloes was determined either by necropsy or by the intradermal tuberculin test that required the test animals to be contained in a holding facility (boma) for 72 h. BTB-monitoring practices based on culling might be acceptable in high prevalence herds such as in the southern part of the KNP. It has, however, met with growing opposition in low prevalence herds because of the large sample sizes needed to detect infection, the possible adverse effects on the genetic diversity of the herds, and other ecological and ethical considerations.

The intradermal tuberculin test is associated with high costs and a high level of handling stress to animals due to the double immobilization and contain-

TABLE 1 IFNg test results obtained in the survey on bovine tuberculosis in buffaloes in KNP

Herd name	Herd symbol	Herd size n	No. of buffaloes tested	Herd % tested	No. of test positives	No. of culture-positive animals
Letaba	A	145	14	9.65	3	2
Macetse	B	320	23	7.18	3	2*
Masorini	C	550	27	4.90	1	1
Blach Heron dam	D	180	23	12.77	0	N/A
Maloponyane	E	290	26	8.96	0	N/A
Stapelkop dam	F	310	21	6.77	0	N/A
Grysbok	G	650	20	3.07	0	N/A
Shawu	H	430	22	5.11	1	1
Stamp en stoot	I	240	20	8.33	0	N/A
Nkokodzi	J	250	19	8.63	0	N/A
Tussen-in	K	230	20	8.69	1	1
Mahlati	L	800	28	3.50	0	N/A
Shingwedzi	M	240	21	8.75	0	N/A
Shirombi pan	N	300	20	6.66	0	N/A
Magamba	O	190	21	11.05	0	N/A
Nkulumbeni	P	400	18	4.50	0	N/A
Boyela	Q	200	17	8.50	0	N/A
Klein Letaba	R	190	19	10.00	0	N/A
Punda Maria	S	210	19	9.04	0	N/A
Mahonie loop	T	260	18	6.92	0	N/A
Nkovakulu	U	120	21	17.50	0	N/A
Shangoni koppies	V	140	19	13.57	0	N/A
Malahlapanga	W	345	32	9.27	0	N/A
Shipande	X	280	25	8.92	0	N/A
Klopperfontein	Y	210	24	11.42	0	N/A
Gadzingwe	AA	280	26	9.28	0	N/A
Mooigesig dam	BB	400	19	4.75	0	N/A
Gwalali	CC	110	13	11.81	0	N/A
Makwadzi	DD	120	13	10.83	0	N/A
Total			608		9	

N/A Not applicable
* One test-positive buffalo could not be retrieved for necropsy

TABLE 2 OD values detected in the IFNg assay in 15 buffaloes with various diagnostic results

Animal no.	OD_{bov}	OD_{av}	OD_{fort}	OD_{nil}	Result
A5	0.54	0.18	0.23	0.12	Positive
A7	1.43	0.33	0.13	0.09	Positive
A8	0.83	0.23	0.11	0.08	Positive
B3	0.46	0.22	0.13	0.08	Positive
B6	2.86	0.38	0.22	0.09	Positive
B22	0.43	0.14	0.07	0.07	Positive
C22	0.93	0.20	0.06	0.05	Positive
H12	0.79	0.11	0.10	0.09	Positive
K6	0.89	0.13	0.09	0.09	Positive
B16	0.19	0.16	0.07	0.05	Negative
D15	0.13	0.18	0.10	0.09	Negative
B7	1.36	0.37	0.55	0.20	MR
Q1	0.80	0.39	0.31	0.09	MR
H14	0.88	1.40	0.51	0.08	AV

MR Multiple reactor
AV Avian reactor

ment in the boma. Animals often refuse to drink after capture leading to dehydration and occasionally death. The interpretation of the skin test is inevitably compromised in dehydrated animals (Raath, Bengis, Bush, Huchzermeyer, Keet, Kernes, Kriek & Michel 1993).

The gamma-interferon technique has been used and evaluated in buffaloes in South Africa mostly to overcome the problems associated with the skin test and to avoid unnecessary culling of healthy buffaloes. It was previously found that in buffaloes, the IFNg assay had similar sensitivity and specificity ranges to the comparative intradermal skin test (Raath *et al.* 1993; Michel, personal communication 2000). In the present survey the diagnosis of bovine tuberculosis in buffalo herds was, for the first time, based exclusively on the IFNg assay. The strong correlation between test-positive and culture-positive buffaloes confirms the high specificity of the IFNg test (99.3 %) found in the comparative field evaluation (Michel, Nel, Cooper & Morobane 2000), although cross-reactivity with environmental mycobacteria did not seem to be a major complicating factor in this study, since only three buffaloes (0.5%) showed a "multiple reaction". In comparison, the previous field evaluation revealed a multiple reactor rate of 4 %, meaning that under standard test conditions, which lack the use of *fortuitum* protein, those buffaloes would have been falsely classified as bovine reactors (Table 2). These buffaloes' blood samples were collected in different geographical and climatic areas in South Africa throughout the year. The animals either roamed free or semi-free or were kept in a boma for varying periods of time. It is possible that any of these factors may have influenced the non-specific reactor rate in the present survey which was carried out in KNP during the dry winter season. In conclusion, the sensitivity could not be determined for this study as no gold standard method was included in the study design. However, *M. bovis* infection was confirmed in the Macetse and Letaba herds, previously known to be affected. In addition, three new infected herds, namely Masorini (Phalaborwa), Shawu (Mopanie) and Tussen-in (Woodlands) were identified. This indicates an increase in the overall BTB prevalence in the northern region of the KNP compared to the findings of a survey conducted in 1998 in which 1.45 % of buffaloes were found to be infected (Rodwell 2000). In retrospect, in January 1999 during routine buffalo capture operations, a young cow from the Nkokodzi herd was found to be positive on both the gamma-interferon and the skin tests. On necropsy a tubercular lesion (20 mm) was found in the lung and *M. bovis* isolated from it. This was the first recorded and confirmed case north of the Letaba River. However, during the 2000 survey, no positive reactors were found in the sample from this herd.

Based on these observations as well as on the data provided by the previous validation (Michel *et al.* 2000; A.L Michel, unpublished data 2000) the results of this study are believed to demonstrate a satisfactory sensitivity of the IFNg test under field conditions. Our data further show that the use of *fortuitum* protein in a triple comparative IFNg test is of distinct advantage in our situation where pressing ethical and economic considerations do not allow an "overkill" of buffaloes due to reduced specificity of the bovine tuberculosis control measure.

In the KNP, the average buffalo herd comprises 200–300 individuals (Whyte 1999). The sampling technique employed in the survey described here allowed for the capture and testing of 5–10 % of each herd in the study area. This correlates with the required sample size for detecting infection in herds with an infection prevalence of between 10 % and 15 %, at a confidence level of 95 % and using total random sampling (Thrusfield 1995). Although the expected prevalence of BTB in the northern part of KNP is below 5 % the selection of adult buffaloes could help to increase the probability of disease detection at this sample size as previous investigations have revealed a positive correlation between age and likelihood of infection (De Vos *et al.* 2001).

The BTB lesions that were found were suggestive of "early" infections as the lesions were small and found mainly in the lymph nodes of the head and lungs. Discrete focal tubercular lesions were also found in the lungs of four animals. These results indicate active infection, probably with temporo-spatial spreading of the disease. In addition, a single cow in both the Shipande and Mahlati herds tested positive on gamma-interferon, but could not be recovered for necropsy purposes.

CONCLUSION

In conclusion, we believe that the results of this survey are encouraging for detecting BTB-infected herds in a geographical area, bearing in mind the limitations of sample size. The use of the gamma-interferon test as described in this paper may be an important tool for future "test and remove" actions to control bovine tuberculosis in free-ranging buffalo

populations. The modified gamma-interferon test has significant advantages over the skin test and culling methods with regard to financial, conservation and ethical considerations.

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REFERENCES

BENGIS, R.G., KRIEK, N.P.J., KEET, D.F., RAATH, J.P., DE VOS, V. & HUCHZEMEYER, H.F.A.K. 1996. An outbreak of bovine tuberculosis in a free-living buffalo population in the Kruger National Park. *Onderstepoort Journal of Veterinary Research*, 63:15–18.

BENGIS, R.G., KEET, D.F., MICHEL, A.L. & KRIEK, N.P.J. 2001. Tuberculosis, caused by *Mycobacterium bovis*, in a kudu (*Tragelaphus strepsiceros*) from a commercial game farm in the Malelane area of the Mpumalanga Province, South Africa. *Onderstepoort Journal of Veterinary Research*, 68:239–241.

COLLINS, J.D. 1995. Ireland, in *Mycobacterium bovis infections in animals and humans*, edited by C.O. Thoen & J.H. Steele, 1st ed. Ames, Iowa: Iowa State University Press: 224–238.

DE VOS, V., BENGIS, R.G., KRIEK, N.P.J., MICHEL, A., KEET, D.F., RAATH, J.P. & HUCHZERMEYER, H.F.K.A. 2001. The epidemiology of tuberculosis in free-ranging African buffalo (*Syncerus caffer)* in the Kruger National Park, South Africa. *Onderstepoort Journal of Veterinary Research*, 68: 119–130.

GRIFFIN, J.F.T., CROSS, J.P., CHINN, D.N., RODGERS, C.R. & BUCHAN, G.S. 1994. Diagnosis of tuberculosis due to *Mycobacterium bovis* in New Zealand red deer (*Cervus elaphus*) using a composite blood test and antibody assays. Offprint from the *New Zealand Veterinary Journal*, 42:173–179

KEET, D.F., KRIEK, N.P.J., PENRITH, M.-L., MICHEL, A. & HUCHZERMEYER, H. 1996. Tuberculosis in buffaloes (*Syncerus caffer*) in the Kruger National Park: spread of the disease to other species. *Onderstepoort Journal of Veterinary Research*, 63:239–244.

KEET, D.F., KRIEK, N.P.J., PENRITH, M.-L. & MICHEL, A. 1998. Tuberculosis in free-ranging lions in the Kruger National Park. *Proceedings of the ARC-Onderstepoort OIE International Congress with WHO-Cosponsorship on Anthrax, Brucellosis, CBPP, Clostridial and Mycobacterial Diseases*, 9–15 August 1998, Berg-en-Dal, Kruger National Park: 444– 453.

KLOECK, P.E. 1998. Tuberculosis of domestic animals in areas surrounding the Kruger National Park. *Proceedings of a Colloquium on the Challenges of Managing Tuberculosis in Wildlife in Southern Africa*, 30–31 July 1998, Mpumalanga Parks Board, Nelspruit, South Africa.

MICHEL, A.L., NEL, M., COOPER, D. & MOROBANE, R.N. 2000. Field evaluation of a modified "gamma interferon" assay in African buffalo (*Syncerus caffer*) and cattle in South Africa. *Proceedings of the 3rd International Conference on Mycobacterium bovis*, 14–16 August 2000, Cambridge, United Kingdom.

O'REILLY, L.M. & DABORN, C.J. 1995. The epidemiology of *Mycobacterium bovis* infections in animals and man: a review. *Tubercle Lung Disease* (Supplement 1), 76:1–46.

RAATH, J.P., BENGIS, R.G., BUSH, M., HUCHZERMEYER, H., KEET, D.F., KERNES, D.J., KRIEK, N.P.J. & MICHEL, A. 1993. Diagnosis of tuberculosis due to *Mycobacterium bovis* in the African buffalo (*Syncerus caffer*) in the Kruger National Park, in *Tuberculosis in wildlife and animals*, edited by F. Griffin & G. de Lisle. Otago Conference Series, University of Otago Press, Dunedin: 313–315.

RODWELL, T.C. 2000. The epidemiology of bovine tuberculosis in African buffalo (*Syncerus caffer).* Ph.D. dissertation, University of California, Davis, USA.

THRUSFIELD, M. 1995. *Veterinary epidemiology*, 2nd ed. Oxford, Halden, Massachusetts: Blackwell Science.

WHYTE, I.J. 1999. *Elephant and buffalo census report for 1999.* Scientific Services, Kruger National Park, Skukuza.