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BUFFALO IN THE NORTHERN NATAL GAME PARKS SHOW NO SEROLOGICAL EVIDENCE OF INFECTION WITH FOOT-AND-MOUTH DISEASE VIRUS

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ABSTRACT

ESTERHUYSEN, J. J., THOMSON, G. R., FLAMMAND, J. R. B. & BENGIS, R. G., 1985. Buffalo in the northern Natal game parks show no serological evidence of infection with foot-and-mouth disease virus. *Onderstepoort Journal of Veterinary Research*, 52, 63–66 (1985).

A total of 594 sera collected from buffalo *(Syncerus caffer)* in the Hluhluwe/Umfolozi Game Reserve complex, Ndumu Game Reserve and the eastern shores of Lake St Lucia were examined for antibody to SAT 1, 2 and 3 types of foot-and-mouth disease (FMD) virus in neutralization tests. No neutralization of SAT 2 or 3 viruses was exhibited by any of the sera tested at final dilutions $>10^{0.9}$. A small proportion (2,9 %) of sera neutralized SAT 1 virus at dilutions up to $10^{1.7}$, but these were considered to be due to non-specific reactions. This, together with the absence of clinical FMD in both cattle and game in this region over at least a 45-year period and the failure to isolate FMD virus from pharyngeal scrapings of buffalo sampled in the area, leads to the conclusion that FMD does not occur in these buffalo populations.

INTRODUCTION

Infection with the SAT types of foot-and-mouth disease (FMD) virus is widespread in buffalo *(Syncerus caffer)* populations in southern and eastern Africa (Hedger, 1976a & b). As an exception, those in the Addo National Park (Fig. 1) have no serological evidence of infection (R. S. Hedger, personal communication, 1977).

In the context of wildlife conservation and utilization, buffalo in southern Africa present a problem because in a number of game reserves their recruitment rate is such that programmes to control buffalo numbers have had to be applied. Due to the danger of FMD virus escaping into domestic livestock, these programmes, in most instances, do not allow live animal translocation or the sale of unprocessed products from "culled" buffalo.

In northern Natal the situation is confusing. FMD has only once been diagnosed in either domestic stock or wildlife in that region, namely in 1938 in cattle in the Vryheid district (J. P. van der Merwe, personal communication, 1984). On the other hand, a majority of 68 sera, obtained from buffalo in the Hluhluwe/Umfolozi Game Reserve complex and examined at the World Reference Laboratory for FMD (WRL)*, were found to have neutralizing antibody to SAT 1, 2 and 3 viruses (Table 1). Probang (pharyngeal scraping) specimens from 20 of the 68 animals were also examined at the WRL with negative results and it was therefor felt that the low levels of serum antibody were probably due to heterophile responses (R. S. Hedger, personal communication, 1981).

The recent commissioning of the FMD Laboratory at Onderstepoort has enabled us to re-examine this problem.

MATERIALS AND METHODS

Study area

The locations of the game reserves in Natal from which sera were collected are shown in Fig. 1. The number of buffalo estimated to be present in each reserve at the last count (1983) is shown in brackets adjacent to each reserve in Fig. 1.

Collection of sera

Between 1978 and 1984 a total of 594 buffalo sera were collected in the northern Natal game parks, mostly during routine culling operations. The numbers obtained from each reserve were: Hluhluwe/Umfolozi complex (HUC) 570, which included the 68 sera previously tested by the WRL, Ndumu Game Reserve (NGR) 13 and the eastern shores of St Lucia (ES) 11. The 37 sera collected in the Kruger National Park (KNP) in 1984 were included for comparative purposes, since infection with the SAT types is known to be widespread in buffalo in this National Park (Hedger, 1976a & b).

Following separation of the serum from the blood clots by centrifugation, the sera were stored at −20 °C until tested.

Virus neutralization (VN) tests

Constant virus/varying antiserum tests were performed in flat bottomed microtitre plates containing 100 µl of cell suspension (3–5 × 10^5 IB-RS-2 cells in a Hank's/Earle's mixture supplemented with lactalbumin hydrolysate and containing 5 % bovine serum free of antibody to FMD virus and antibiotics), 50 µl of virus suspension containing $10^{2.0\pm0.4}TCD_{50}$ and 50 µl of inactivated (56 °C for 30 min) serum dilution per well. Sera were initially screened at final serum dilutions between 1:8 and 1:64 in duplicate wells. Sera that showed 50 % end-points ≥1:32 were tritrated out between 1:32 and 1:1024, using 4 replicate wells per doubling serum dilution, the latter being performed in the microplates using a multipipette diluter. Serum/virus mixtures were incubated at 37 °C for 1 hour in an atmosphere containing 5 % CO_2. After addition of the cell suspension, the plates were sealed with transparant adhesive tape and incubated for 72 hours at 37 °C, when the results were read directly with an inverted microscope. Serum titres were expressed as the logarithm of the reciprocal of the final serum dilution present in the serum/virus mixture at the 50 % end-point. The latter was calculated by the method of Kärber (1931).

The virus strains used in VN tests were: SAR 17/80 (SAT 1), PHALAB/2-6/83/2 (SAT 2) and SAR 1/80 (SAT 3). These were all isolated from recent outbreaks of FMD in cattle within the boundaries of the RSA.

RESULTS

Fig. 2 shows that all 594 buffalo sera which originated from the northern Natal game parks had VN titres $<10^{1.0}$ against SAT 2 and 3 viruses, while against SAT 1 577/594 sera (97,1 %) failed to exhibit demonstrable neutralization. The remaining 17 sera had titres between $10^{1.0}$ and $10^{1.7}$.

By contrast, the 37 sera derived from KNP buffalo had a different titre distribution, >65 % having titres $>10^{1.8}$ against SAT 1. The distribution of titres against SAT 2 and 3 were similar (Fig. 2). Only 1 of the KNP sera failed to neutralize any of the 3 SAT viruses.

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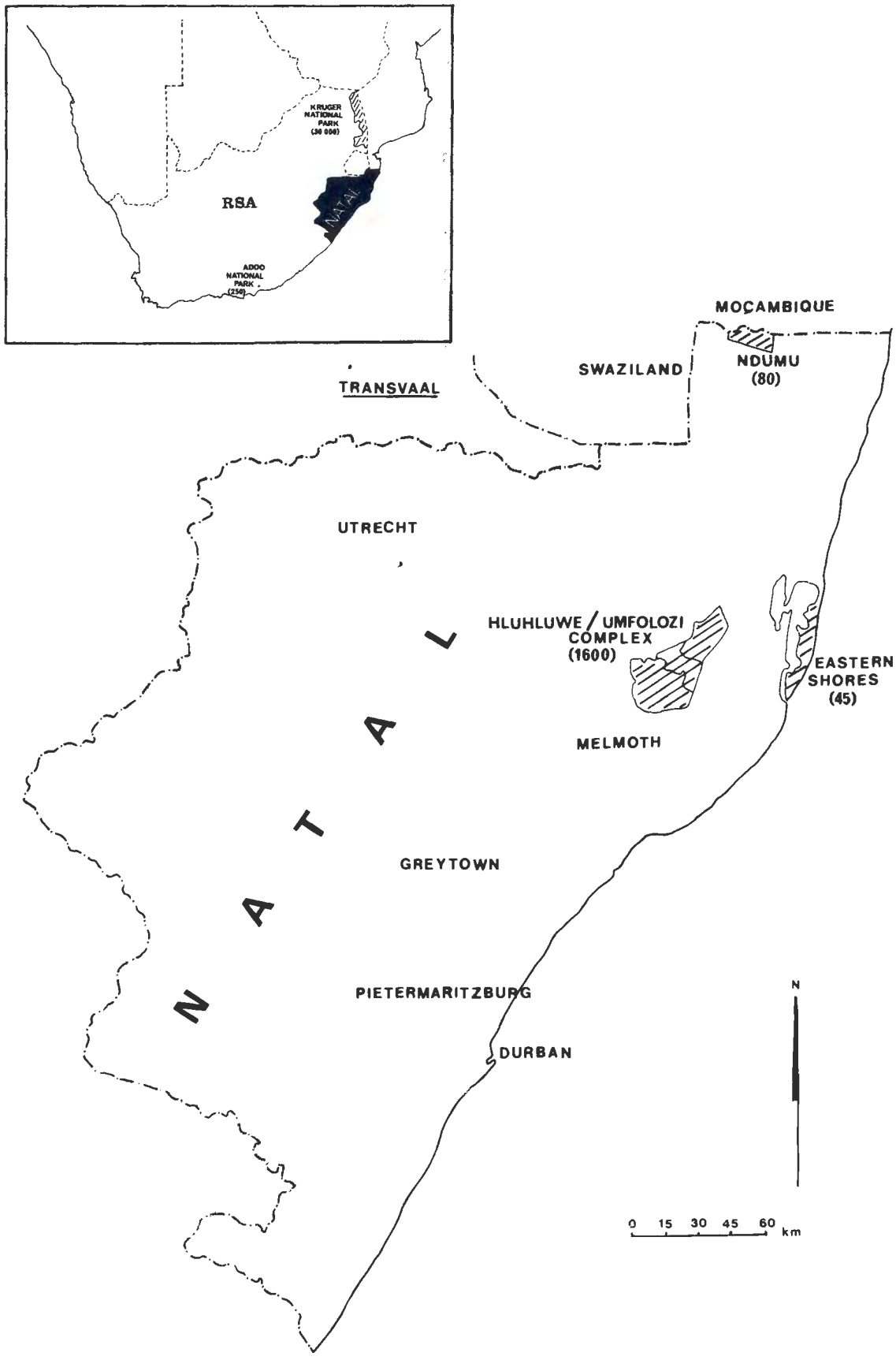

FIG. 1 The distribution of buffalo in South African game parks. Numbers of buffalo in individual parks are shown in brackets

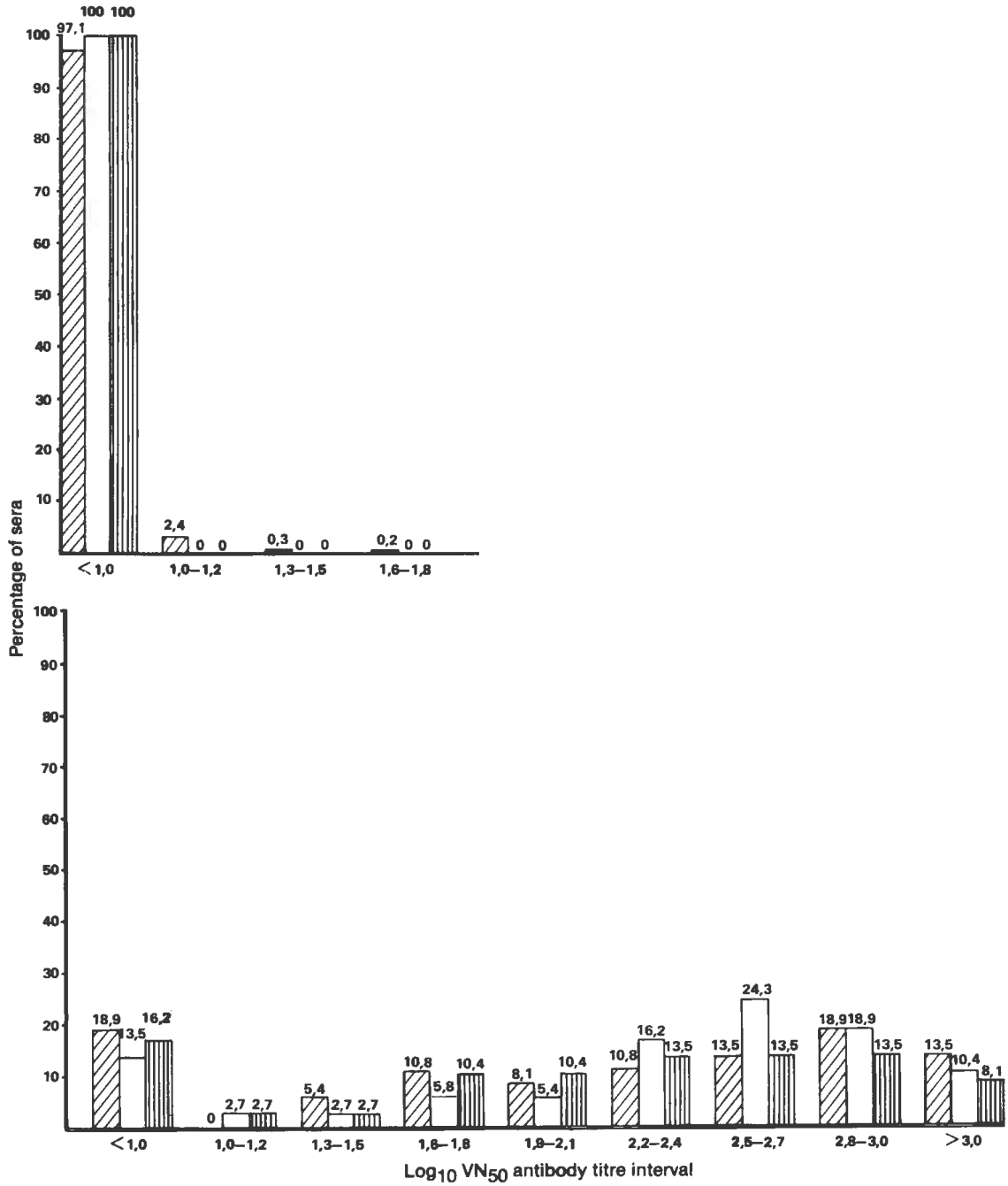

FIG. 2 The distribution of VN_{50} antibody titres to SAT 1 (oblique hatching), SAT 2 (open columns) and SAT 3 (perpendicular hatching) viruses in 594 buffalo sera from the Natal game parks (above) and 37 from the Kruger National Park (below)

Discussion

The only free-living population of African buffalo previously found to be free of FMD virus infection was in the Addo National Park (Fig. 1). The 21 animals examined there (approximately 10 % of the population) all had VN titres $<10^{0.8}$ against all 3 SAT types (R. S. Hedger, personal communication, 1977).

Buffalo have been found to develop higher VN titres to SAT 1 and 2 viruses than do cattle following contact exposure and homologous titres $>10^{4.0}$ have been regularly recorded (Esterhuysen, Thomson, Gainaru & Bengis, unpublished results, 1983). In addition, a small group of infected buffalo (<100 animals) was found to maintain high VN titres when isolated from other buffalo for more than 10 years and individual animals kept in small groups maintained high VN titres for >4 years (R. S. Hedger, personal communications, 1981; 1984). Thus the high titres ($\geqslant 10^{3.0} VN_{50}$) in 10 % or more of sera from the KNP against all 3 SAT types (Fig. 2) is typical of endemicity in buffalo.

The finding of VN titres between $10^{1.0}$ and $10^{2.0}$ in a proportion of the 68 sera from the HUC examined at the WRL (Table 1) was a surprise because, firstly, this area has, at least since 1938, not been associated with FMD outbreaks in cattle or game animals and secondly, the titres were lower than those previously found in infected buffalo (Hedger, 1976b). The question arose therefore as to whether these titres were due to heterophile reactions similar to those which have been found in cattle sera from areas known to be free of FMD (Andersen 1975, 1978) or to active infection. We therefore retested the original 68 HUC sera in addition to a further 502 from

TABLE 1 The distribution of titres to SAT 1, 2 & 3 viruses determined by the World Reference Laboratory in 68 buffalo sera from the Hluhluwe/Umfolozi Game Reserve Complex[1]

50 % End-point dilution interval	SAT 1	SAT 2	SAT 3
<1,0[2]	57(84)[3]	37(54)	16(24)
1,0–1,2	9(13)	19(25)	20(29)
1,3–1,5	2(3)	7(10)	15(22)
1,6–1,8	—	4(6)	13(19)
1,9–2,1	—	1(2)	4(6)
≥2,2	—	—	—

[1]Information extracted from R. S. Hedger, personal communication, 1981

[2]Reciprocal of the $\log_{10}$ VN_{50} titre

[3]The number of sera with 50 % end-points within that dilution interval (%)

the same locality, as well as a few sera from the small buffalo populations present in the Ndumu Game Reserve and on the eastern shores of Lake St Lucia (Fig. 1). There are no buffalo present in other game reserves in Natal. In this instance, the viruses used in the VN tests were local and more recent in origin that those employed by the WRL.

Whereas the WRL found that a high proportion of the original 68 sera had VN titres $\geq 10^{1.0}$ when tested with SAT 2 and 3 viruses (46 % and 76 % respectively, Table 1), we could find no such titres amongst the 570 sera tested from the HUC or the 24 from the other 2 localities (Fig. 2). The latter results are in agreement with the findings of Guinet, Falconer, Guillemin & Lombard (1984), although they sampled only 26 buffalo. Conversely, all 3 laboratories detected a proportion of sera from the HUC with titres between $10^{1.0}$ and $10^{1.7}$ against SAT 1 (Table 1; Fig. 2) and the same was also true of sera obtained from Ndumu Game Reserve and the eastern shores of Lake St Lucia.

It is unlikely that such low SAT 1 titres in the Natal buffalo sera were due to active infection with FMD virus because (i) the titre distribution is typical of a "tail-off" and differs from that of a population known to be infected (Fig. 2), (ii) no virus was isolated from pharyngeal scrapings from 20 of the 68 buffalo tested at the WRL (R. S. Hedger, personal communication, 1981), whereas FMD virus was isolated by the WRL from 80 % of probang samples obtained from KNP buffalo over 3 years of age (D. G. Gradwell, personal communication, 1976) and (iii) there has been no clinical evidence of FMD virus infection in that area in either cattle or game for at least 45 years.

Andersen (1978) found specific plaque reduction titres up to $10^{2.5}$ against a SAT 1 virus in the sera of steers which had had no experience of FMD infection and had been experimentally infected with a bovine enterovirus. Hence, heterophile reactions between SAT 1 viruses and enterovirus have been demonstrated. At present we do not have access to buffalo enteroviruses to test this possibility further.

We conclude that the buffalo in the northern Natal game parks, like those in the Addo National Park, are free of infection with FMD virus.

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