

Natural transmission of foot-and-mouth disease virus between African buffalo (*Syncerus caffer*) and impala (*Aepyceros melampus*) in the Kruger National Park, South Africa

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SUMMARY

VP1 gene sequences of SAT-2 type foot-and-mouth disease (FMD) viruses recovered from impala and African buffalo in the Kruger National Park (KNP) were used to determine intra- and interspecies relationships of viruses circulating in these wildlife populations. On this basis five distinct lineages of SAT-2 virus were identified in routine sampling of oesophageo-pharyngeal epithelium from buffalo between 1988 and 1996. Different lineages were associated with discrete geographic sampling localities. Over the period 1985–95, four unrelated epizootics occurred in impala in defined localities within the KNP. Evidence for natural transmission of FMD between buffalo and impala is presented for the most recent 1995 outbreak, with data linking the 1985 and 1988/9 impala epizootics to viruses associated with specific buffalo herds.

INTRODUCTION

The Kruger National Park (KNP) is situated in the north eastern corner of South Africa (between 22° 25' and 25° 32' latitude south and 30° 50' and 32° 02' longitude east) and is bordered by Zimbabwe and Mozambique, to the north and east respectively. The KNP and surrounding area is the only region in South Africa where foot-and-mouth disease (FMD) occurs, with the remainder of the country being recognized as free of the disease by the O.I.E. (Office International des Epizooties). Since the last recorded outbreak of FMD in livestock in South Africa in 1983, the disease has been restricted to wildlife within the FMD control area (Records of the Directorate of Animal Health). Clinical disease has primarily involved impala (*Aepyceros melampus*), a medium sized antelope, but it is well recognized that infection with SAT 1, 2 and 3 viruses is endemic to African buffalo (*Syncerus caffer*) herds within the KNP.

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Of the 18 southern African wildlife species found to have antibodies to FMD [1, 2], only the African buffalo has been shown to be an efficient maintenance host of the virus [3] and is considered the primary source of infection for domestic and wild ungulates. This is due to its ability to both maintain and transmit the disease [4], with transmission from carrier buffalo to cattle being demonstrated under both experimental and natural conditions [5–8]. In contrast, evidence of field transmission between buffalo and other cloven-hoofed wildlife species is lacking despite the co-occurrence of buffalo and a variety of antelope in game parks throughout southern Africa and the known susceptibility of some antelope species to FMD virus (FMDV) infection [9, 10].

In South Africa, the diagnosis of clinical FMD in impala has been regularly recorded since 1944 [2, 11–13]. Over 90% of these outbreaks have occurred in the months of June to November within the southern half of the KNP. This area, south of the Olifants river (Fig. 1) coincides with the region of the

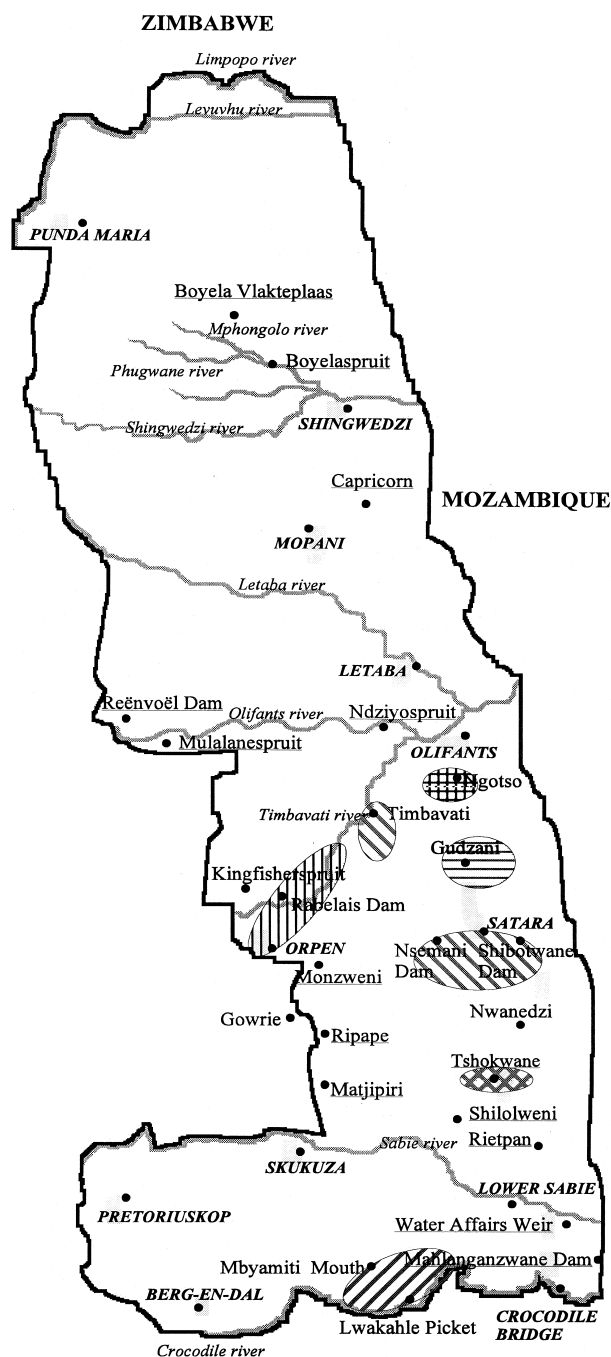


Fig. 1. Map of the Kruger National Park indicating the geographical origin of impala viruses isolated from epizootics occurring between 1985 and 1995. ▨, 1985; ▤, 1988; ▩, 1989; ▧, 1992; ▦, 1993; ▨, 1995.

KNP that is most densely populated by both impala and buffalo [14, 15]. In addition, the months of June to August are generally rain-free and therefore a period of water scarcity, which leads to animals congregating at permanent water points [2]. This is

believed to facilitate disease transmission and is supported by the observation that some outbreaks in impala have spread along water courses [13]. In addition to shared drinking water, mutual grazing of at least three grass species by impala and buffalo [16] is another factor promoting selection of common environs.

Despite the high incidence of outbreaks in impala [2] and the active collection and characterization of viruses obtained from buffalo in recent years [17], direct evidence of natural transmission between these species is lacking. VP1 gene nucleotide sequence analysis has, however, been instrumental in determining the relatedness of successive epizootics in impala [2, 13]. In a study of FMD outbreaks in impala in 1983–9, Vosloo and co-workers [13] showed that viruses isolated 6 months apart were derived from the same epizootic. A later study focussing on the 1992/3 epizootic in the KNP revealed that related impala viruses were collected 12·8 months apart [2]. Although these results infer the possibility of viral persistence in impala populations, other studies have shown that most experimentally infected impala do not become carriers [9]. This was confirmed in a separate study where virus could not be isolated from any tissues of infected impala, 7 or more days after inoculation [10]. Given the low antibody levels of impala during inter-epizootic periods and their apparent inability to act as long-term carriers, it is unlikely that these antelope are capable of maintaining the disease independently and therefore African buffalo are presumed to be the usual source of infection for impala [2].

In order to determine whether buffalo are indeed the source of infection for impala, a 12-year study period (1985–96) was identified in which clinical FMD was diagnosed on six occasions in impala in the KNP (Fig. 1). The availability of impala isolates from these SAT-2 epizootics and buffalo field strains collected within the same time period, together with improved SAT-type genetic characterization methods [18], prompted an investigation to determine whether inter-species transmission could be demonstrated.

METHODS

Viruses used in this study

Impala viruses used in this study originate from the southern and central region of the Kruger National Park, South Africa (Fig. 1) and were isolated from

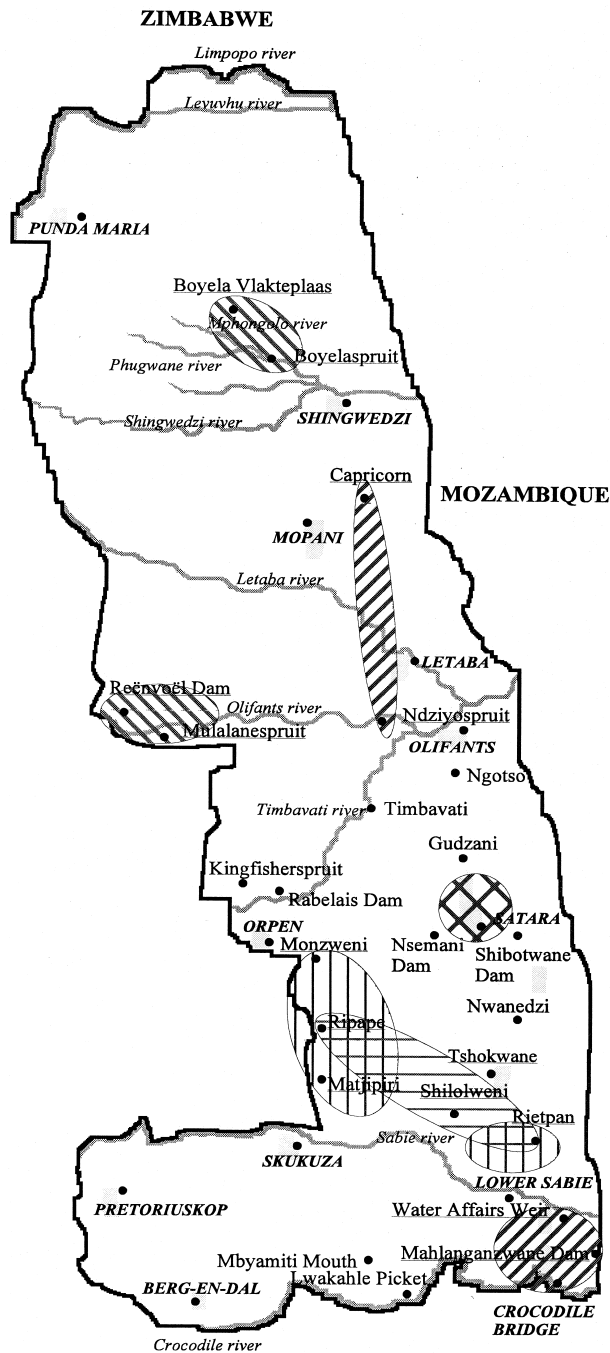


Fig. 2. Map of the Kruger National Park indicating the geographical origin of buffalo virus genotypes. ▨, Genotype C; ▤, Genotype A; ▥, Genotype D; ▦, Genotype E; ▧, Genotype B.

clinical FMD lesion material. Oesophageo-pharyngeal specimens (probangs) obtained from buffalo during routine surveys in the KNP in 1988–96 (Fig. 2), were used as a source of viruses circulating in buffalo. The geographical and species origin of all viruses used in this study is summarized in Table 1.

Genetic characterization

Viral RNA was extracted from cell culture isolates of buffalo and impala viruses by a modified guanidinium thiocyanate /silica method [19]. A 500 bp fragment corresponding to the C-terminus half of the VP1 gene was amplified, purified and sequenced as previously described [18]. All reactions were performed in duplicate and independently of each other. Nucleotide sequences were aligned [20] and further analyses were conducted on an homologous 414 nt sequence corresponding to the carboxyl terminal 131 amino acids (position 85–216) of the VP1 gene and the adjacent 7 amino acids of 2A. Pairwise comparisons of amino acid and nucleotide sequences of impala outbreak strains and buffalo isolates were performed using the MEGA programme [21]. Distance methods prescribed by Martin and co-workers [22] for the construction of gene trees using partial VP1 gene sequences of FMD viruses were applied. In addition, UPGMA and parsimony methods were used in infer phylogeny [21] so that the reliability of the results could be assessed [23]. All nucleotide sequences used in this study have been submitted to Genbank under the accession numbers indicated in Table 1.

RESULTS

Genetic relationships of buffalo viruses

VP1 gene trees of SAT-2 type FMDV were used to identify the major buffalo virus lineages within the Kruger National Park. Both distance (UPGMA and neighbor-joining) and character-based phylogenetic inference methods produced trees with identical topology (results not shown). The neighbor-joining tree depicted in Figure 3 indicates that there are five distinct buffalo virus lineages (coded A–E) south of the Olifants river, which cluster according to geographical origin in the following manner:

- (A) Monzweni (1995), Ripape (1989), Rietpan (1988) and Matjipiri (1989) viruses
- (B) Water Affairs Weir (1991) and Mahlanganzwane Dam (1996) viruses
- (C) Satara (1991) buffalo virus
- (D) Rietpan (1988), Ripape (1988 & 1989) & Shilolweni (1988) viruses
- (E) Mulalanespruit (1996) buffalo virus

The results (Fig. 3) show the co-circulation of two different SAT-2 genotypes within a single buffalo

Table 1. List of Kruger National Park viruses of buffalo and impala origin (1985–96)

Strain	Sampling locality	Grid reference	Species of origin	Sampling date	Genbank acc. no
KNP 1/85	Gudzani	31° 50' E 24° 15' S	Impala	21 Nov 1985	AF136986
KNP 7/88	Rietpan	31° 58' E 24° 54' S	Buffalo	8 Jul 1988	AF137000
KNP 8/88	Rietpan	31° 58' E 24° 54' S	Buffalo	8 Jul 1988	AF137001
KNP 9/88	Shilolweni	31° 50' E 24° 48' S	Buffalo	27 May 1988	AF137002
KNP 10/88	Shilolweni	31° 50' E 24° 48' S	Buffalo	27 May 1988	AF137003
KNP 14/88	Ripape	31° 37' E 24° 44' S	Buffalo	19 Oct 1988	AF137004
KNP 16/88	Kingfisherspruit	31° 26' E 24° 27' S	Impala	10 Oct 1988	AF136987
KNP 17/88	Orpen	31° 24' E 24° 28' S	Impala	7 Oct 1988	AF136988
KNP 19/88	Rabelais Dam	31° 30' E 24° 27' S	Impala	11 Oct 1988	AF136989
KNP 20/88	Timbavati River	31° 28' E 24° 26' S	Impala	25 Oct 1988	AF136990
KNP 2/89	Ngotso	31° 43' E 24° 13' S	Impala	30 Apr 1989	AF136991
KNP 19/89	Ripape	31° 37' E 24° 44' S	Buffalo	25 Oct 1989	AF137005
KNP 25/89	Matjipiri	31° 36' E 24° 47' S	Buffalo	24 Oct 1989	AF137006
KNP 40/89	Ripape	31° 37' E 24° 44' S	Buffalo	25 Oct 1989	AF137007
KNP 5/91	Satara	31° 47' E 24° 24' S	Buffalo	20 Jun 1991	AF137008
KNP 143/91	Reënvoël Dam	31° 20' E 23° 58' S	Buffalo	13 Jul 1991	AF137009
KNP 147/91	Reënvoël Dam	31° 20' E 23° 58' S	Buffalo	13 Jul 1991	AF137010
KNP 160/91	Nd ziyospruit	31° 34' E 23° 59' S	Buffalo	18 Jul 1991	AF137011
KNP 183/91	Water Affairs Weir	31° 56' E 25° 08' S	Buffalo	24 Jul 1991	AF137012
KNP 1/92	Nsemani Dam	31° 43' E 24° 23' S	Impala	3 Aug 1992	AF136992
KNP 5/92	Timbavati	31° 38' E 24° 16' S	Impala	3 Aug 1992	AF136993
KNP 8/92	Shibotwana Dam	31° 50' E 24° 24' S	Impala	3 Aug 1992	AF136994
KNP 32/92	Boyela Vlakteplaas	31° 17' E 22° 54' S	Buffalo	22 Jun 1992	AF137013
KNP 9/93	Boyelaspruit	31° 20' E 23° 01' S	Buffalo	17 Aug 1992	AF137014
KNP 16/93	Capricorn	31° 26' E 23° 29' S	Buffalo	6 Aug 1992	AF137015
KNP 51/93	Tshokwane	31° 51' E 24° 47' S	Impala	26 Aug 1993	AF136995
KNP 18/95	Monzweni	31° 38' E 24° 34' S	Buffalo	16 Nov 1995	AF137016
KNP 31/95	Monzweni	31° 38' E 24° 34' S	Buffalo	16 Nov 1995	AF137017
KNP 43/95	Mbyamiti Mouth	31° 46' E 25° 18' S	Impala	29 Nov 1995	AF136996
KNP 44/95	Lwakahle Picket	31° 41' E 25° 23' S	Impala	2 Dec 1995	AF136997
KNP 49/95	Mbyamiti Mouth	31° 46' E 25° 18' S	Impala	2 Dec 1995	AF136998
KNP 52/95	Lwakahle Picket	31° 41' E 25° 23' S	Impala	2 Dec 1995	AF136999
KNP 6/96	Mahlanganzwane Dam	32° 01' E 25° 14' S	Buffalo	17 Apr 1996	AF137018
KNP 24/96	Mulalanespruit	31° 18' E 23° 57' S	Buffalo	29 Jun 1996	AF137019

herd. This is demonstrated by the grouping of KNP/7/88 and KNP/8/88 with the 'A' and 'D' buffalo genotypes respectively, despite having been sampled on the same day and from the same herd. Two similarly sampled buffalo viruses (KNP/19/89 and KNP/40/89) from Ripape also fall within the 'A' and 'D' clusters. Furthermore, the geographical distribution of buffalo genotypes (Fig. 2) reveals that there are shared genotypes across the Olifants river. This is illustrated by the clustering of the extreme southern viruses in the Crocodile Bridge area (KNP/183/91 and KNP/6/96) with the Capricorn and Nd ziyospruit viruses (KNP/160/91 and KNP/16/93) to the north. In addition, two Reënvoël Dam viruses (KNP/143/91 and KNP/147/91) sampled north of the Olifants river, group with a Mulalanespruit virus

(KNP/24/96), sampled south of the river and form part of a larger cluster (E) containing Boyela viruses (KNP/32/92 and KNP/9/93) sampled in the far north. The five buffalo virus clusters identified in Figure 3 confirm the spatial rather than temporal grouping of FMD viruses, as exemplified by Cluster A (1988–1995) and Cluster B (1991–6). Overall sequence identity for the 20 buffalo viruses used in this study was 59.9% across the 414 nt regions specified in this study. Amino acid sequence identity for the same region was 74.6%.

Genetic relationships of impala viruses

Four genetically unrelated impala epizootics were identified by VP1 gene sequence analysis (Fig. 3), each

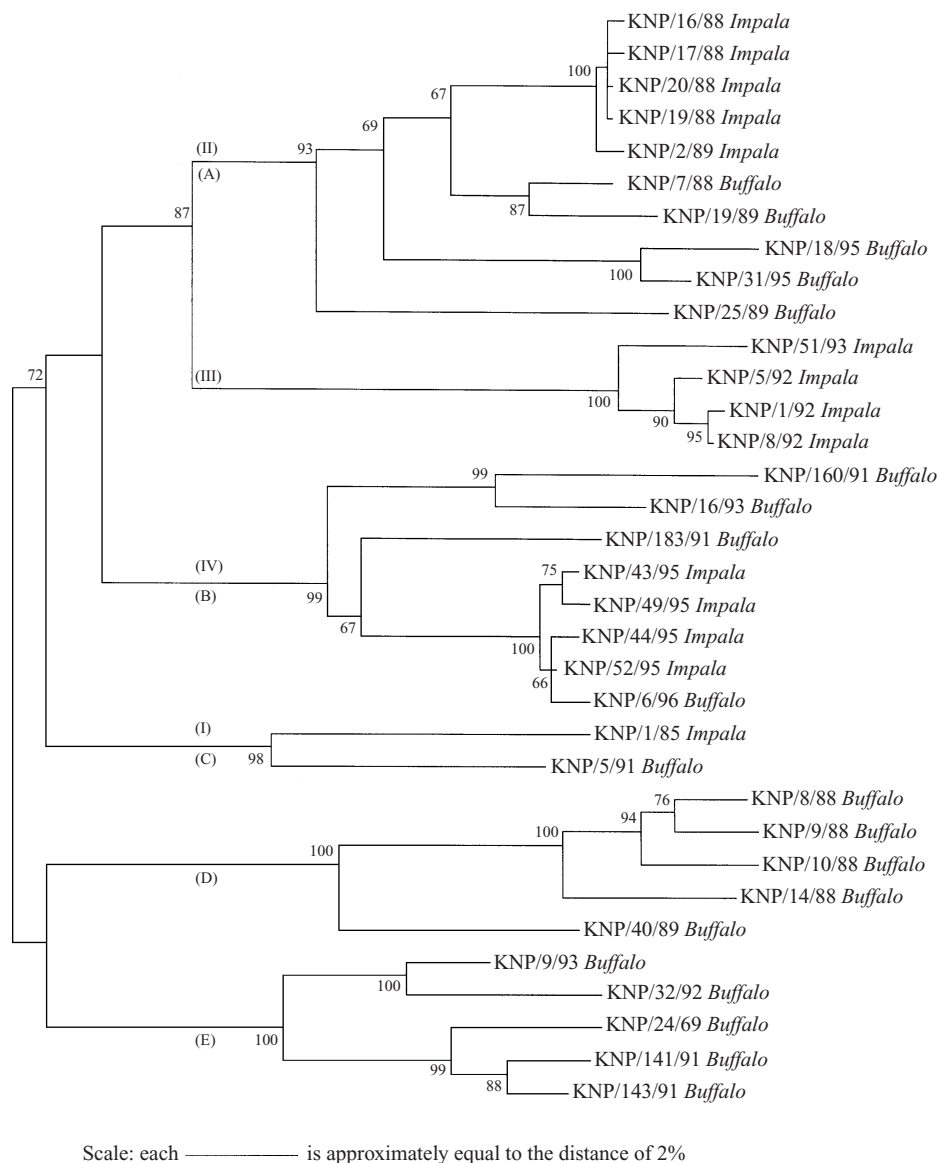


Fig. 3. Neighbor-joining tree (Jukes and Cantor correction) depicting VP1 gene relationships of buffalo and impala viruses from the Kruger National Park (1985–96). Bootstrap values ≥ 65 and based on 1000 replications are shown, with statistically significant bootstrap values (≥ 95) being indicated in bold. The scale can be used to determine percentage nucleotide sequence divergence between any two strains by obtaining the sum of the distances along the abscissas to the connecting node.

from a distinct geographical area indicated in the figure as follows:

- (I) Gudzani Area (1985 epizootic)
- (II) Orpen Area (1988/9 epizootic)
- (III) Satara/Tshokwane Area (1992/3 epizootic)
- (IV) Crocodile Bridge Area (1995 epizootic)

Pairwise comparisons of nucleotide and amino acid sequence data (results not shown) reveal that viruses of the same epizootic, sampled within a month of each other are usually identical or differ by less than 1%.

The 1988/9 and 1992/3 impala viruses, show marked differences on amino acid level over time. In the 6 months separating the 1989 virus from the 1988 viruses, there was a 1–2% accumulation in mutations, whilst the 1993 impala virus differed by between 3 and 4% from the 1992 viruses in the > 12 months between sampling. In the most recent SAT-2 epizootic, the 1995 impala viruses were shown to differ by 1–4% on amino acid level, despite being sampled within a 5-day period. This finding strongly suggests that clinically affected animals were probably only detected some time after the start of this outbreak and is supported

by field observations of animals with 3-month-old hoof lesions at the time this outbreak was recorded (Records of the State Veterinarian, KNP). Overall nucleotide sequence identity for the 14 impala viruses used in this study was 70.5% across the 414 nt specified in this study. Amino acid sequence identity for the same region was 80.4%.

Inter-species relationships of impala and buffalo viruses

The combined buffalo and impala data-set was used to determine inter-species relationships of viruses (Fig. 3). Statistically supported phylogenetic clustering of three of the four impala epizootics with specific buffalo lineages, is clearly demonstrated. The 1985 impala strain groups with buffalo virus KNP 5/91 (98% bootstrap support) and the 1988/9 outbreak strains cluster with 1988, 1989 and 1995 buffalo viruses from Rietpan, Ripape and Mondzweni (93% bootstrap support). The 1995 impala viruses from the Crocodile Bridge region are almost identical to a 1996 buffalo virus obtained 4 months later from the same locality (100% bootstrap support). The impala epizootic of 1992/93 did not group with any of the buffalo viruses sampled between 1985 and 1996. Overall nucleotide sequence identity for the 34 Kruger National Park viruses used in this study was 57.2% across the 414 nt region specified, with amino acid sequence identity being 69.6%.

DISCUSSION

The nucleotide sequence data indicates that four unrelated SAT-2 epizootics occurred in impala in the KNP from 1985–95, each with a different origin. The similarity in nucleotide sequence (>99%) between impala viruses from the 1995 epizootic and a virus of buffalo origin obtained 4 months later within the area of the outbreak provides the first evidence for natural transmission between these two wildlife species (Fig. 3). Similarly, evidence linking the impala epizootics of 1985 and 1988/9 to distinct buffalo genotypes was obtained. The 1992/3 impala outbreak could not, however, be linked to any buffalo field strains characterized thus far.

The high nucleotide sequence identity (>99%) between the 1995 impala viruses and the 1996 buffalo virus is significant, especially in view of the extent of the genetic variation in the maintenance host species. Amino acid sequence identity of the 20 buffalo field

strains used in this study was found to be 74.6%. Similar intratypic sequence identity determinations for European serotype viruses from diverse geographical origins were 73–83% [24]. Clearly the corresponding level of sequence variation (25.4%) within buffalo herds from a single game park and country exemplifies the high level of intratypic variation found in the SAT types as it surpasses or almost equals that determined for serotypes A, O and C.

The superimposition of the geographical localities of impala (Fig. 1) and buffalo viruses (Fig. 2) indicates that shared habitats between these species are likely to be important in transmission, as the origin of impala outbreaks overlap with the geographical range of the buffalo herds to which they can be linked. Although the sampling date of the buffalo viruses did not always coincide with year of the impala outbreak to which they were linked, it was shown that buffalo viruses group on the basis of geographic origin, rather than year of isolation, viz. relationships are spatial rather than temporal. Studies of buffalo behaviour have determined that buffalo herds have defined home ranges to which they confine themselves and which they use continuously for several years [25, 26]. This explains the close genetic relationship between temporally unrelated buffalo viruses from a specific locality. Thus, the clustering of the 1991 buffalo virus with the 1985 impala outbreak strain is not surprising as this 1991 strain is probably representative of the buffalo herd from which the impala outbreak strain probably originated.

It is known that buffalo home ranges can increase significantly in response to ecological pressures [26] and may vary from approx. 40–1000 km² [25, 26], with home range size being dictated by game park boundaries and other buffalo herds. In addition, bulls are known to move between buffalo herds [25]. This buffalo behaviour explains the genetic relatedness of buffalo viruses originating from the southern and northern regions of the game park. It also has implications for other buffalo infections of economic importance because pathogens, although generally confined to a specific herd within a small localized area, have the potential to spread widely.

The occurrence of over 90% of outbreaks of FMD in impala within the months of June to November, is significant in that it coincides with the time at which buffalo calves are likely to become infected for the first time. Strict seasonal breeding of buffalo [27] results in synchronized calving, with the majority of births

being recorded in February in the KNP [28]. These buffalo calves, when infected for the first time probably excrete virus in approximately the same quantities and by the same routes as do cattle [29]. Studies have shown that this first acute infection is likely to occur at approx. 3–8 months of age when maternally derived antibodies have waned sufficiently to make them susceptible to infection [30]. On this basis it follows that buffalo calves in the KNP may become infected for the first time from the month of May onwards. It is during this period that they are most likely to be a source of infection for other species within the KNP and therefore notable that most outbreaks in impala have occurred at a slightly later, but overlapping time period.

Factors facilitating inter-species transmission are poorly understood, but as buffalo and impala do not come into direct contact in natural circumstances, it can be assumed that elements driving them to share habitats are important. Shared drinking and grazing localities together with the high population density of both species in the southern part of the KNP are likely to facilitate transmission. It is interesting to note that of the five grazing routes identified by Meeser [12] to be paths of FMD infection, three overlap with the origin of recent impala epizootics, discussed here. This lends credence to the possibility that shared grazing may play a role in transmission and is further supported by the observation that impala grazing (as opposed to browsing) peaks at approx. 85% in November [31], and includes two grass species of preference for the African buffalo, viz. *Panicum maximum* and *Digitaria eriantha* [31, 32].

The role of the impala in the epidemiology of FMD remains unclear, although the historical use of this antelope as an indicator species for predicting both the course of an epizootic and outbreaks in livestock [12], would seem to imply that they may be intermediaries in the transmission of FMD from buffalo to cattle. Conflicting evidence exists in the literature, however, regarding the susceptibility of impala to infection and their ability to transmit the disease. Evidence for transmission of virus from buffalo to impala, where shared drinking and feeding was experimentally enforced, could not be demonstrated [29]. Conversely, under experimental conditions where no close contact between the species occurred, it was shown that carrier buffalo inadvertently infected impala [9] and that acutely infected impala transmitted SAT 1 virus to buffalo persistently infected with SAT 2 virus [8]. Clearly, a special set of circumstances is

required to effect indirect interspecies viral transfer and the role of impala as intermediaries in disease transmission remains to be clarified. Circumstantial evidence does however indicate that infected antelope provided the most likely link between buffalo and cattle in a recent outbreak of FMD in cattle in Zimbabwe (unpublished observations). In that instance, buffalo were indisputably the primary source of infection.

As more southern African countries attempt to obtain and retain FMD free zones, it becomes critical to elucidate all aspects pertaining to the epidemiology of the disease so that adequate disease control measures are implemented. Confirmation of field transmission between buffalo and impala, and the circumstantial evidence pointing to the role of impala as intermediaries in disease transmission make it essential to determine the epidemiological significance of this antelope species. In addition, environmental factors facilitating inter-species transmission need to be investigated in order to permit more accurate risk assessment.

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