Chapter 6

Tracing the origin and course of foot-and-mouth epizootics in southern Africa

Summary

Genetic characterization of 54 FMD viruses involved in SAT-type outbreaks in livestock and wildlife were compared to 140 buffalo viruses representative of different southern African buffalo populations in order to determine the origin and course of epizootics in southern Africa. Isolates of historical importance, such as the immunologically distinct regional viruses first identified in 1948, and those from recent outbreaks of the disease were included in this study. The results confirm that African buffalo in close proximity to livestock and wildlife are the most likely source of infection for these cloven-hoofed species. On rare occasions, transboundary movement of animal and animal products have precipitated outbreaks. Further evidence for the role of antelope as intermediaries in disease transmission between buffalo and cattle is presented and the threat to disease security posed by the illegal trade in FMD-infected buffalo is also highlighted.

6.1 Introduction

Following the rinderpest pandemic of 1889-1897, FMDV disappeared from southern Africa until 1931, when a SAT-2 outbreak occurred in cattle in Zimbabwe. In the decades following this outbreak, epizootics were regularly recorded along South Africa's borders with Zimbabwe and Botswana, up until 1960 (reviewed by Thomson 1994). The drop in the number of outbreaks in SADC countries from the 1980's onwards is attributed in part to improved disease control through vaccination and fencing. Vaccination campaigns commenced early in most southern African countries. Initially, aphthisation (the infection of cattle with FMD positive vesicular fluid or ground tissue suspensions) was practiced from the 1930's until the 1960's, after which attenuated vaccines were used, followed by vaccination with inactivated adjuvant vaccines from the 1970's onwards. Recognition of the role of wildlife in disease transmission was also recognized and addressed through the erection of game proof fences. The efficacy of these disease control measures is reflected in the low number of FMD outbreaks relative to other regions in Africa (see Table 1.2, Chapter 1).

Efforts to control the disease were driven by the need to establish disease-free areas in countries where FMD-infected buffalo occur in order to permit trade. A number of SADC countries were successful in their endeavours to obtain international recognition of FMD zoning within their borders by the OIE. These countries include Botswana, South Africa, Namibia and Zimbabwe. The disease-free status which permitted trade in animals and animal products, has recently come under threat due to the increased numbers of outbreaks both within and outside of the FMD-control areas. Prior to 2000, Namibia, South Africa, Botswana and Swaziland had been free of the disease in livestock since 1994, 1983, 1981 and 1980, respectively (Table 6.1). However in 2000, Namibia, South Africa and Swaziland reported FMD outbreaks. South Africa and Namibia both reported outbreaks within their FMD control areas in August 2000. As these outbreaks were effectively dealt with and contained, recognition by the OIE of FMD-free zones in both countries was maintained. However, following two unrelated FMD outbreaks in the FMD-free zones in South Africa, recognition of disease free status was suspended for this country. Botswana and Namibia are currently the only southern African countries to retain recognition by the OIE of their FMD free zones (OIE list of FMD free countries, January 2001; http://www.oie.int).

The economic implications of an outbreak are far-reaching due both to the immediate cost of controlling the outbreak and the loss of revenue resulting from suspension in trade. Following an

outbreak of FMD certain conditions apply before freedom from the disease can be accepted. If widespread vaccination was instituted, a 2-year period without further outbreaks is required from the cessation of vaccination. However, if vaccination to control an outbreak was avoided, or restricted to ring vaccination, the interval to declaration of freedom from the disease is only 3 months. This shorter time period is however dependent on the absence of further outbreaks, the slaughter of all infected animals and those in close contact, and extensive serological surveys demonstrating freedom from antibody-positive animals.

TABLE 6.1 Summary of FMD outbreaks in livestock in southern African countries prior to 2000

Country	**Last recorded outbreak prior to 2000**	**Serotype/s involved**
¥Angola	1978	A, O, C, SAT -1, SAT-2
Botswana	1981	SAT-2
Lesotho	--	--
†Mozambique	1984	SAT-2
*Malawi	1998	O
§ Namibia	1994	SAT-3
§ South Africa	1983	SAT-2
Swaziland	1969	SAT-2
Zambia	1999	SAT-1
Zimbabwe	§1999	SAT-1 & SAT-3

* Based on data provided by the World Reference Laboratory (WRL), Pirbright, UK
† Based on data provided by the Botswana Vaccine Institute (BVI)
¥ Based on data in Thomson 1994
§ Outbreaks occurring within the FMD control areas

It should be noted that following the outbreak of civil war in Angola and Mozambique that reporting of FMD ceased and that the data for these countries is therefore unreliable. It is likely that the serotypes recorded prior to cessation of reporting are still prevalent in these southern African countries.

6.1.1 Outbreaks in South Africa in 2000

Three separate outbreaks occurred in three different provinces in South Africa in 2000. The first in the Northern Province involved a SAT-1 virus, the second in KwaZulu-Natal was caused by an imported type O strain and the third outbreak in Mpumulanga province was due to a SAT-1 virus.

6.1.1.1 SAT-1 in the FMD-control area

In August 2000, 45 serum samples were collected from buffalo and cattle forming part of an experimental project to breed buffalo free of FMD and corridor disease (CD). The experimental farm housing 67 adult buffalo cows, 26 buffalo calves and 25 Jersey cows adjoins the KNP and falls within the FMD-control area. All sera tested positive for SAT-1. Probang and nasal swabs were subsequently collected from 9 buffalo calves and from 29 jersey cows that were acting as foster mothers to the buffalo. SAT-1 type FMDV was isolated from at least four animals, one dairy cow and three buffalo.

6.1.1.2 Type O in Kwazulu-Natal

In September 2000, serotype O FMD was diagnosed in Kwazulu-Natal province for the first time. The virus initially only affected pigs causing 30 % mortalities in these animals. The source of the infection was believed to have been swill obtained from a shipping carrier in Durban harbour, which was fed to pigs. The farm where the index case was identified was placed under quarantine, as was the zone within a 10km radius around this farm. A surveillance zone of 20km radius was declared around the 10km restriction zone. The disease was subsequently spread to cattle, pigs and goats in neighbouring areas. Stamping out procedures were initially carried out, prior to vaccination within a 15km radius with type O_1 Manisa, a 1969 field strain from Turkey, shown to be antigenically related to the outbreak strain. These viruses, although genetically characterized (Sangare *et al.* 2001) are not included in the present study which specifically focussed on the molecular epidemiology of SAT-type viruses.

6.1.1.3 SAT-1 outside the FMD control area, Mpumulanga province

On 23 November SAT-1 type FMD was diagnosed in 8 of the 110 cattle imported from Mpumulanga province in South Africa into Swaziland at an abattoir in Matsapha (26°31'S

- 31°18'E). Subsequent investigations at the feedlot from which these cattle were imported in Middelburg, South Africa, revealed that 30 animals had clinical FMD lesions and were positive for SAT-1 type virus. Quarantine and surveillance zones were identified and emergency vaccination with a trivalent (SAT 1-3) vaccine, supplied by the Onderstepoort Veterinary Institute was carried out.

6.1.2 FMD in Namibia in 2000

In Namibia an outbreak of SAT-1 (typing done by BVI) was reported in August 2000 in the Eastern Caprivi district (25°07' E - 17°48' S) which lies within the foot-and-mouth disease infected zone. Approximately 30,000 cattle around the focus of infection were vaccinated with trivalent (SAT 1-3) vaccine. By 4 October 2000, 138,542 cattle in the area had been vaccinated and an additional 18 000 head of cattle were revaccinated in the Kasika area (http://www.oie.int).

6.1.3 FMD in Zambia in 2000

In Zambia, four FMD outbreaks were reported in three different provinces in 2000. The first was at Mpulungu, Northern Province (31°30'E - 9°24'S) where the infection is estimated to have occurred on 21 February 2000. The affected cattle herds were 10km from the border and the source of infection is believed to have been from a neighbouring country. The serotype involved was not identified. In May 2000 a second outbreak was reported in Chapalonga village, Lundazi district, Eastern Province (11°50'S-33°18'E). In August 2000 SAT-1 outbreaks were reported in two localities in the Western Province, the first was in the Mwandi area, Sesheke (17°32'S - 24°54'E) whilst the second was at Sikuzu crushpen, Sesheke (17°32'S - 24°51'E; http://www.oie.int).

Reports of FMD outbreaks were made by 6 southern African countries at the 14th Conference of the Regional Commission for Africa of the OIE held in Arusha (Tanzania), 23-26 January 2001. In addition to the outbreaks in South Africa, Swaziland, Zambia and Namibia, detailed above, officials from Malawi and Angola reported outbreaks in 2000 (OIE press release, 26 January, 2000, Paris). It is noteworthy that although no official reports of FMD in Angola were made to the OIE in 2000,

the delegate from this country confirmed the presence of the disease there during the year under review confirming the view that there is under reporting to the OIE.

6.1.4 FMD in Zambia in 1999

A SAT-1 virus isolated by the Botswana Vaccine Institute (BVI) from an outbreak reported in August 1999 at Kazungula, Southern Province (25°15'E -17°38'S) was supplied to the Onderstepoort Veterinary Institute. The affected population was a transhumant herd of cattle located on a river island. The affected herd was suspected to have been in contact with young buffaloes from a nearby national game park (http://www.oie.int).

6.1.5 FMD in Zimbabwe 1999

On the 25th of June 1999 samples from an FMD outbreak at Mapanza Estate (20° 55' S - 31° 47' E), Chiredzi, Masvingo Provine were received by OVI where the virus was identified as SAT-3. On 21 July 1999, samples from Mkwasine Ranch (20° 50' S - 32°00' E), also within the Chiredzi district of Masvingo province (http://www.oie.int), were submitted to OVI by Zimbabwean authorities. The virus was identified in the latter outbreak was SAT-1 and therefore unrelated to the outbreak occurring 2 weeks earlier. The source of the infection was believed to be wildlife as the areas adjoining the ranches where the outbreaks occurred were mainly stocked with buffalo (*Syncerus caffer*), impala (*Aepyceros melampus*), kudu (*Tragelaphus strepisiceros*) and eland (*Taurotragus oryx*). Transmission from buffalo to cattle is unlikely as buffalo-proof fences separate wildlife and livestock. It is believed that infected antelope crossed the FMD control fences from wildlife areas to cattle areas and facilitated transmission. The SAT-1 and SAT-3 outbreaks occurred despite the fact that both Mapanza Estate and Mkwasine Ranch fall within the FMD vaccination control zone where vaccination of cattle with trivalent vaccine occurs regularly. No new cases of FMD have been reported from Zimbabwe since 28 July 1999.

6.1.6 FMD in South Africa in 1998

Infection with SAT-3 virus was detected in buffalo in August 1998 in the Potgietersrus district in the Northern Province of South Africa. This area occurs within the zone recognized by the OIE as free from FMD. The 'disease-free' buffalo were purchased from a farm in the Free State Province, which is outside of the FMD control area. Because of the origin of these buffalo, intensive serological surveys in the area surrounding the source farm were conducted in order to ensure that the disease had not spread from the buffalo. Sera from 1295 bovine, 784 ovine and one buffalo were screened, all of which were negative for the three SAT-types. In the interim, the buffalo which had tested positive for FMD were quarantined and later destroyed. SAT-3 type virus was isolated from three of the 7 buffalo purchased.

6.1.7 FMD in Zimbabwe in 1997

In 1997 a SAT-2 outbreak occurred in cattle in the Chiredzi district in southern Zimbabwe. Kudu and impala in adjoining wildlife areas were shown to have antibodies to SAT-2 virus. These results together with the fact that the cattle were separated from buffalo by game proof fences which were of an insufficient height to contain the former antelope species, provides circumstantial evidence for the role of antelope in disease transmission in this case.

In order to preclude future outbreaks of the disease in the southern African region it is essential to establish the origin of recent outbreaks so that appropriate disease control measures can be put in place. In this chapter, both historical and contemporary outbreaks of FMD will be investigated through molecular epidemiological studies. The role of the African buffalo as the ultimate source of infection for both wildlife and cattle will also be addressed by including viruses representative of as many southern African maintenance host populations as possible.

6.2 Materials and Methods

6.2.1 SAT-1 viruses used in this study

A total of 60 buffalo viruses were selected for this study, of which 30 were genetically characterized in Chapter 3. The remaining 30 buffalo viruses were selected to compliment the existing SAT-1 buffalo database (Chapter 3). Twenty-six outbreak viruses recovered from wildlife and cattle, from 1948 to 2000 were selected for genetic characterization. Included in the outbreak virus group is the first SAT-1 virus typed, BEC/1/48, as well as SAT-1 viruses recovered from clinical cases in cattle in Swaziland and South Africa in 2000. Eighty-six viruses were ultimately included in the phylogenetic analysis, the details of which are summarized in Table 5.1.

6.2.2 SAT-2 viruses used in this study

Seventy viruses were used to determine the genetic relationships of SAT-2 type field strains recovered from buffalo, impala and cattle sampled between 1948 and 2000. Due to the extensive characterization of SAT-2 viruses recovered from impala outbreaks in the Kruger National Park (Chapter 3), impala viruses isolated from the 1988-89, 1992-93 and the 1995 epizootics (N=13) were not included here. Instead isolates recovered from recent outbreaks of FMD in Kenya, Eritrea, Saudi Arabia and Zimbabwe were selected for this study and as were outbreaks of historical interest. The latter include RHO/1/48, a historical SAT-2 virus and PAL/5/83, the last SAT-2 type virus causing an outbreak of FMD in cattle in South Africa prior to 2001. The geographical and species origin of previously uncharacterized SAT-2 type viruses are detailed in Table 5.2. They include 20 cattle strains, 4 impala viruses and 8 new buffalo isolates. Details of the remaining 42 buffalo viruses are available in Chapters 3 and 5, Tables 3.2 and 5.3, respectively.

6.2.3 SAT-3 viruses used in this study

In addition to the 30 buffalo viruses previously characterized in Chapter 5, an additional 8 outbreak strains were characterized here. Seven of the viruses are from clinical cases of FMD in cattle and one virus was obtained from a FMD-infected buffalo sourced from a farm outside the FMD control area.

6.2.4 Genetic characterization and analysis

Genomic amplification of the VP1 gene and nucleotide sequencing of the purified product was performed as described in previous chapters. Phylogenetic reconstruction was performed using the neighbor-joining algorithm and Jukes & Cantor correction included in MEGA (Kumar *et al.* 1993), with support for each of the virus clusters being assessed by 1000 bootstrap replications. Gene regions used for the analyses correspond to those previously outlined in Chapter 5, for each of the SAT serotypes.

TABLE 6.2: Summary of previously uncharacterized SAT-1 viruses used in this study

Virus name	Country of origin	Year of sampling	Place of origin	Grid reference	Species of origin
BEC/1/48	Botswana	1948	NA	NA	Cattle
SAR/13/61	South Africa	1961	Potgietersrus	29°01'E-24°11'S	Cattle
BOT/1/68	Botswana	1968	Satau	24°25'E-18°02'S	Cattle
SAR/10/71	South Africa	1971	Sugar Station, Komatipoort	31°00'E-24°00'S	Impala
SAR/11/71	South Africa	1971	Sugar Station, Komatipoort	31°00'E-24°00'S	Impala
SAR/1/73	South Africa	1973	Area 3, Kruger NP	31°16'E-24°15'S	Impala
SAR/5/75	South Africa	1975	Grootdraai, Hectorspruit	31°00'E-25°20'S	Cattle
BOT/1/77	Botswana	1977	Nonaneng	22°11'E-19°40'S	Cattle
BOT/17/77	Botswana	1977	30km SW of Habu	NA	Kudu
BOT/24/77	Botswana	1977	Habu	NA	Cattle
MOZ/3/77	Mozambique	1977	Choque	33°00'E-24°15'S	Cattle
SAR/9/81	South Africa	1981	Pafuri, Kruger NP	Pafuri	Impala
KNP/3/86	South Africa	1986	Tshokwane, Kruger NP	31°51'E-24°47'S	Buffalo
KNP/6/86	South Africa	1986	Tshokwane, Kruger NP	31°51'E-24°47'S	Buffalo
KNP/1/87	South Africa	1987	Mooiplaas, Kruger NP	31°27'E-24°34'S	Buffalo
KNP/4/89	South Africa	1989	Mala Mala, Kruger NP	31°30'E-24°55'S	Buffalo
KNP/8/89	South Africa	1989	Meseldam, Kruger NP	31°13'E-25°07'S	Buffalo
KNP/20/89	South Africa	1989	Kwa Mfamebto, Kruger NP	31°12'E-25°06'S	Buffalo
NAM/2/89	Namibia	1989	W. of Kwando river, Caprivi	23°20'E-17°50'S	Buffalo
ZIM/HV11/90	Zimbabwe	1990	Hippo Valley	31°35'E-21°10'S	Buffalo
ZIM/HV28/90	Zimbabwe	1990	Hippo Valley	31°35'E-21°10'S	Buffalo
ZIM/HV29/90	Zimbabwe	1990	Hippo Valley	31°35'E-21°10'S	Buffalo
ZIM/14/90	Zimbabwe	1990	Bumi Hills	28°22'E-16°49'S	Buffalo
ZIM/16/90	Zimbabwe	1990	Doma SA	30°15'E-16°20'S	Buffalo
ZIM/25/90	Zimbabwe	1990	Chirisa SA	28°15'E-18°00'S	Buffalo
ZIM/26/90	Zimbabwe	1990	Dande SA	30°20'E-15°55'S	Buffalo
ZIM/38/90	Zimbabwe	1990	Chirisa SA	28°15'E-18°00'S	Buffalo
ZIM/47/90	Zimbabwe	1990	Dande SA	30°20'E-15°55'S	Buffalo
KNP/148/91	South Africa	1991	Renosterkoppies, Kruger NP	31°36'E-25°07'S	Buffalo
ZIM/Gn16/91	Zimbabwe	1991	Gonarezhou NP	32°00'E-21°30'S	Buffalo
ZIM/GN34/91	Zimbabwe	1991	Gonarezhou NP	32°00'E-21°30'S	Buffalo
ZIM/1B/91	Zimbabwe	1991	Urungwe SA	28°55'E-16°30'S	Buffalo

Virus name	Country of origin	Year of sampling	Place of origin	Grid reference	Species of origin
ZIM/5/91	Zimbabwe	1991	Larry Cummings GR, Matetsi	25°44'E-10°03'S	Buffalo
ZIM/8/91	Zimbabwe	1991	Urungwe SA	28°55'E-16°30'S	Buffalo
ZIM/6/94	Zimbabwe	1994	Hwange NP	27°00'E-19°00'S	Buffalo
ZIM/3/95	Zimbabwe	1995	Kariba	28°20'E-16°47'S	Buffalo
ZIM/12/95	Zimbabwe	1995	Kariba	28°20'E-16°47'S	Buffalo
UGA/1/97	Uganda	1997	Queen Elizabeth NP	30°00'E-00°00'S	Buffalo
KNP/131/98	South Africa	1998	Phelwane Camp, Kruger NP	31°34'E-24°26'S	Impala
KNP/143/98	South Africa	1998	Kempiana, Kruger NP	NA	Impala
KNP/144/98	South Africa	1998	Kempiana, Kruger NP	NA	Impala
NAM/288/98	Namibia	1998	West Caprivi GR	23°00'E-18°00S	Buffalo
TAN/1/99	Tanzania	1999	Menganyi Range, Matara	NA	Cattle
TAN/2/99	Tanzania	1999	Menganyi Range, Matara	NA	Cattle
ZAM/1/99	Zambia	1999	Kazungula, Southern Province	25°15'E-17°18'S	Cattle
ZIM/5/99	Zimbabwe	1999	Mkwasine Range, Chiredzi	32°00'E-20°50'S	Cattle
ZIM/6/99	Zimbabwe	1999	Mkwasine Range, Chiredzi	32°00'E-20°50'S	Cattle
ZIM/7/99	Zimbabwe	1999	Mkwasine Range, Chiredzi	32°00'E-20°50'S	Cattle
SAR/1/00	South Africa	2000	Phalaborwa District, Northern Province	31°00'E-24°06'S	Buffalo
SAR/2/00	South Africa	2000	Phalaborwa District, Northern Province	31°00'E-24°06'S	Buffalo
SAR/3/00	South Africa	2000	Phalaborwa District, Northern Province	31°00'E-24°06'S	Buffalo
SAR/4/00	South Africa	2000	Phalaborwa District, Northern Province	31°00'E-24°06'S	Cattle
SAR/8/00	South Africa	2000	Phalaborwa Distirct, Northern Province	31°00'E-24°06'S	Buffalo
SAR/32/00	South Africa	2000	Middleburg District, Mpumulanga Province	29°34'E-25°54'S	Cattle
SWL/1/00	Swaziland	2000	Middleburg District, Mpumulanga Province	29°34'E-25°54'S	Cattle
SWL/4/00	Swaziland	2000	Middleburg District, Mpumulanga Province	29°34'E-25°54'S	Cattle
SAR/34/00	South Africa	2000	Middleburg District, Mpumulanga Province	29°34'E-25°54'S	Cattle

GR: Game Reserve; NA: Not available; NP: National Park; SA: Safari Area

TABLE 6.3 Summary of previously uncharacterized SAT-2 viruses used in this study

Virus name	Country of origin	Year of sampling	Place of origin	Grid reference	Species of origin
RHO/1/48	Zambia	1948	NA	NA	Cattle
KEN/3/57	Kenya	1957	Wamba	NA	Cattle
ANG/4/74	Angola	1974	Quibala Raspaguil de Asias da Bola	11°00'E-15°00'S	Cattle
BOT/3/77	Botswana	1977	NA	NA	Cattle
RHO/2/79	Zimbabwe	1979	Chiredzi	31°50'E-21°10'S	Cattle
ZAI/1/82	DRC	1982	Bibatama, Nord Kivu	NA	NA
MOZ/4/83	Mozambique	1983	NA	NA	Cattle
PAL/5/83	South Africa	1983	Phalaborwa	31°00'E-23°58'S	Cattle
SAR/16/83	South Africa	1983	Leeupan	31°48'E-24°50'S	Impala
ZIM/7/83	Zimbabwe	1983	Nyamandhlovu	28°05'E-19°45'S	Cattle
KNP/1/85	South Africa	1985	Gudzane, Kruger NP	31°50'E-24°15'S	Impala
SWA/4/89	Namibia	1989	Sigwe village, East Caprivi	25°01'E-17°44'S	Cattle
BUN/1/91	Burundi	1991	Bujumbura province	NA	Cattle
NAM/1/91	Namibia	1991	East Caprivi	NA	Cattle
NAM/1/92	Namibia	1992	80km E of Rundu, Kavango	NA	Cattle
ZAM/7/96	Zambia	1996	Mulanga	NA	Buffalo
ZIM/1/97	Zimbabwe	1997	Chiredzi	31°48'E-20°50'S	Cattle
ZIM/4/97	Zimbabwe	1997	Chiredzi	31°48'E-20°50'S	Cattle
ZIM/44/97	Zimbabwe	1997	Mukazi Ranch, Chiredzi	31°48'E-20°50'S	Buffalo
ZIM/48/97	Zimbabwe	1997	Mukazi Ranch, Chiredzi	31°48'E-20°50'S	Buffalo
BOT/1/98	Botswana	1998	Nxaraga	23°15'E-19°40'S	Buffalo
BOT/18/98	Botswana	1998	Nxaraga	23°15'E-19°40'S	Buffalo
NAM/304/98	Namibia	1998	Mahango, W Caprivi GR	21°50'E-18°15'S	Buffalo
ERI/12/98	Eritrea	1998	Erythrea	NA	Cattle
KEN/5/99	Kenya	1999	Athi river, Machakos	NA	Cattle
KEN/7/99	Kenya	1999	Kikuya, Kiambu	NA	Cattle
KEN/8/99	Kenya	1999	Jkia, Nairobi	NA	Cattle
KEN/9/99	Kenya	1999	Kaloleni, Kilifi	NA	Cattle
RWA/1/00	Rwanda	2000	Gishwati district	NA	Buffalo
SAU/6/00	Saudi Arabia	2000	Al Kahrj, Riyadh	NA	Cattle
ZIM/1/00	Zimbabwe	2000	Elephant Walk, Tengwe Farm	29°30'E-17°12'S	Buffalo

GR: Game reserve, NA: Not available; NP: National Park; SA: Safari Area

TABLE 6.4 Summary of previously uncharacterized SAT-3 viruses used in this study

Virus name	Country of origin	Year of sampling	Place of origin	Grid reference	Species of origin
BEC/1/65	Botswana	1965	Yaoyaga	NA	Cattle
RHO/3/78	Zimbabwe	1978	Humani Ranch, Fort Victoria	NA	Cattle
NAM/1/94	Namibia	1994	Kasika, Caprivi	25°07'E-17°48'S	Cattle
NAM/5/94	Namibia	1994	Kasika, Caprivi	25°07'E-17°48'S	Cattle
POT/336/98	South Africa	1998	Potgietersrus district	29°03'E-24°10'S	Buffalo
ZIM/1/99	Zimbabwe	1999	Mapanza Estate, Chiredzi	31°47'E-20°55'S	Cattle
ZIM/3/99	Zimbabwe	1999	Mapanza Estate, Chiredzi	31°47'E-20°55'S	Cattle
ZIM/4/99	Zimbabwe	1999	Mapanza Estate, Chiredzi	31°47'E-20°55'S	Cattle

NA: Not available

6.3 Results

Genetic relationships of SAT-type viruses recovered from wildlife and domestic livestock in the southern African region were determined by phylogenetic analysis of nucleotide sequences of the major immunogenic protein of FMDV, the VP1 protein. The interpretation of VP1 data generated for the purpose of molecular epidemiological studies follows the rationale established in previous chapters (Bastos 1998; Bastos *et al.* 2000; Bastos *et al.* 2001) and by others (Saiz *et al.* 1993; O. Marquardt, pers comm 2000; Sangare *et al.* 2001), where the C-terminus half of VP1 (> 380 nt) has been characterized. Briefly, the genetic relationship of any two field strains of FMDV is reflected by their genetic distance and by phylogenetic resolution in the following manner :

- Contemporary isolates of an epizootic differ by 1 % or less
- Isolates from the same epizootic but different years differ by 2- 7 % from each other
- Viruses of the same genotype differ by no more than 14 % from each other
- Viruses from separate evolutionary lineages / topotypes differ by 20 % or more
- Viruses representative of different serotypes differ by > 35 %

6.3.1 Genetic relationships of SAT-1 viruses in southern Africa (1948-2000)

Four distinct evolutionary lineages were recovered by phylogenetic analysis of partial VP1 gene data (Fig. 6.1). The three southern Africa buffalo topotype distributions (I-III) previously identified (Chapter 5) were unaffected by the inclusion of a further 30 buffalo viruses. Inclusion of a buffalo virus from Queen Elizabeth National Park in Uganda (UGA/1/97) revealed that buffalo viruses circulating in this east African region are genetically unrelated to the southern African topotypes. An additional topotype was therefore allocated (topotype IV).

Within topotype I, seven genetically distinct outbreaks occurred in wildlife and cattle between 1971 and 2000. Three of the outbreaks can be directly linked to genotypes associated with specific buffalo herds. These include the link between the 1999 cattle viruses (ZIM 5-7/99) in southern Zimbabwe and buffalo viruses from Hippo Valley and Gonarezhou National Park (99 % bootstrap support), the grouping of impala viruses recovered from the 1998 outbreak in the Orpen area of the Kruger National Park and a buffalo virus, KNP/75/98 (91 % bootstrap support), and the link between cattle viruses from Swaziland and South Africa with a buffalo virus from Lower Sabie, KNP/22/96 (99 % bootstrap support). The viruses recovered from the latter Mpumulanga outbreak are clearly unrelated to the virus involved in clinical FMD in buffalo and cattle, 3 months earlier in the Northern Province. In that case a direct link between cattle (SAR/4/00) and buffalo calves, occurring at a private game farm within the red-line area (100 % bootstrap support), was clearly demonstrated, although the geographical area in the KNP from which this virus originated is not clear. The apparently prolonged circulation of FMDV in impala from 1971 (SAR/10/71 and SAR/11/71) to 1973 (SAR/1/73), is of interest as this aspect of the disease in this antelope species has only recently been recognized by genetic characterization of contemporary viruses (Vosloo *et al.* 1992; Keet *et al.* 1996). The 1971 and 1973 impala viruses, which were sampled 31 months apart differ from each other by 8 % on nucleotide level. Given the rapid rate of accumulation of mutations in the VP1 gene of SAT-type viruses (Vosloo *et al.* 1996) an 8 % difference on nucleotide level is possible for viruses of common origin over this time period. Thus the 1971 and 1973 impala viruses appear to be part of the same epizootic and point to the possibility of a >2.5 year circulation period of FMDV in impala populations, which is longer than that previously identified (Chapter 3) for SAT-2 type virus causing clinical disease in impala (Keet *et al.* 1996; Bastos *et al.* 2000). The possibility of prolonged circulation of a virus strain in the field is also provided by the grouping of MOZ/3/77 and SAR/9/81, where there is 100 % bootstrap support for the grouping of these viruses. The former is of cattle origin, whilst the latter was recovered from impala in the Pafuri area of the Kruger National Park.

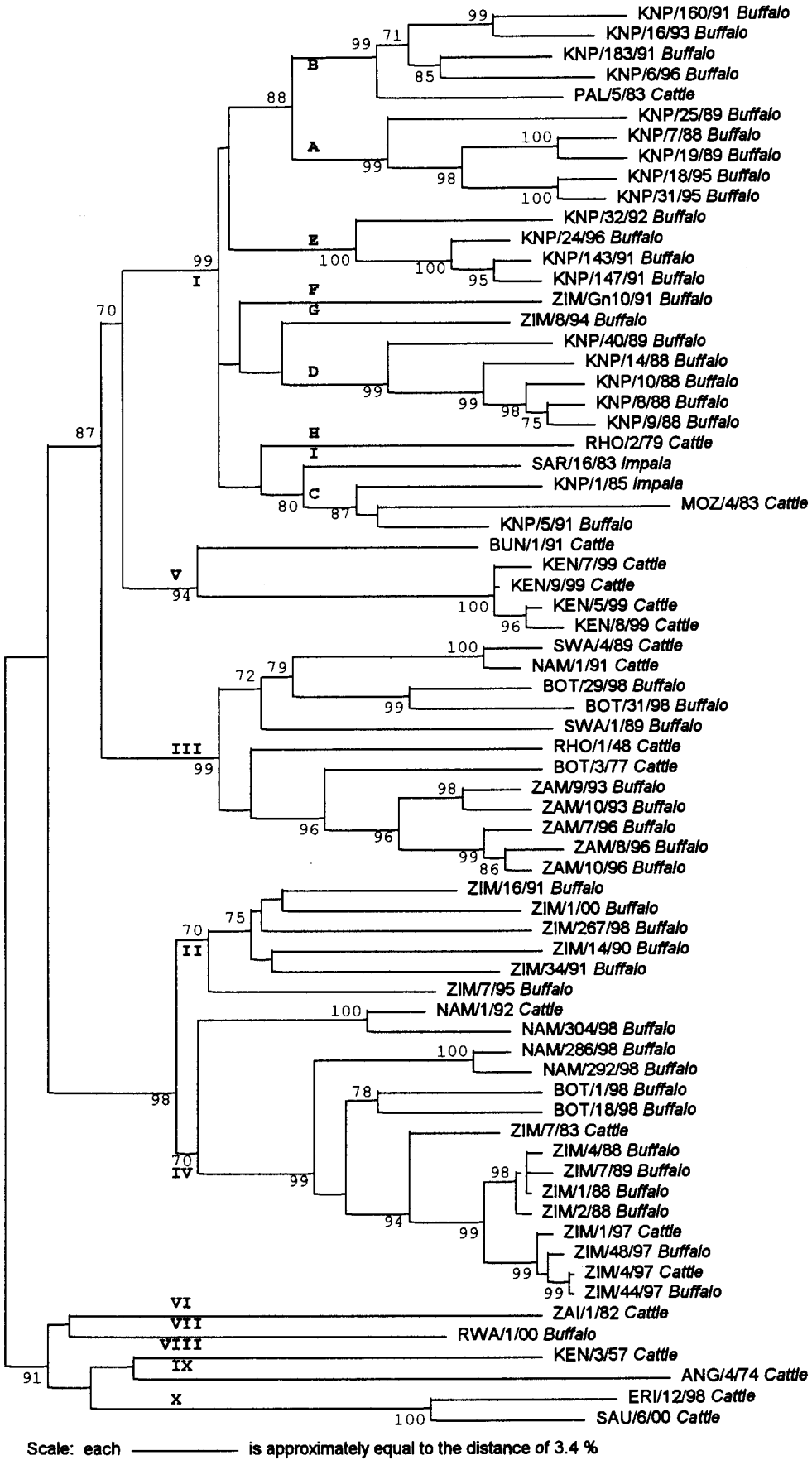

Fig. 6.2 Neighbor-joining tree depicting VP1 gene relationships of SAT-2 type viruses from buffalo, cattle and impala in southern, central and eastern Africa (1948-2000) and in Saudi Arabia (2000)

Five genetically unrelated outbreaks of FMDV are represented within the topotype II buffalo virus cluster, namely the 1948, 1968 and 1977 outbreaks in Botswana, the 1961 outbreak in South Africa and the 1999 outbreak in Zambia. The three 1977 outbreak strains are part of the same epizootic. BOT/1/77 from cattle and BOT/17/77 share more than 99 % nucleotide sequence identity and 100 % bootstrap support, providing additional evidence for inter-species transmission. The BOT/1/68 and SAR/13/61 cannot be directly linked to any contemporary buffalo viruses and the exact origin of these viruses remains unclear, although they do fall within a cluster comprising viruses from Namibia and Botswana (60 % bootstrap support). The direct link between ZAM/1/99 and ZIM/2/94 (94 % bootstrap support) provides evidence for transboundary transmission. Similarly the grouping of a SAR virus within topotype II (Botswana, Namibia and western Zimbabwe region) is also suggestive of virus introduction into South Africa from one of the afore-mentioned topotype II countries. The historical BEC/1/48 strain is unrelated to any of the buffalo virus lineages, but is clearly a topotype II virus (99 % bootstrap support).

In topotype III, all viruses are of buffalo origin with the exception of TAN 1-2/99. Phylogenetic resolution reveals that these Tanzanian viruses are most closely related to Zambian buffalo strains. More accurate determination of the origin was not possible due to the unavailability of SAT virus sequence data from Tanzanian buffalo populations.

6.3.2 Genetic relationships of SAT-2 viruses (1948-2000)

Ten distinct evolutionary lineages (I-X) were identified by phylogenetic analysis of SAT-2 viruses from the Middle East (Saudi Arabia) and from southern, central and eastern Africa (Fig. 6.2). Eight of the lineages correspond to geographically discrete localities, with the remaining two lineages (III and IV) displaying some overlap in distribution. Lineages VI-IX are represented by viruses, from the Democratic Republic of the Congo, Rwanda, Kenya and Angola, respectively. These viruses differ from each other and from all other viruses included in the analysis to such an extent (> 21 % in uncorrected pairwise comparisons), that four independent genetic lineages could be assigned, one for each of the viruses. Angola, represented by ANG/4/74 is the only southern African country which has a distinct evolutionary lineage (IX) within a virus grouping comprising primarily east and central African topotypes (IV-X; 89 % bootstrap support).

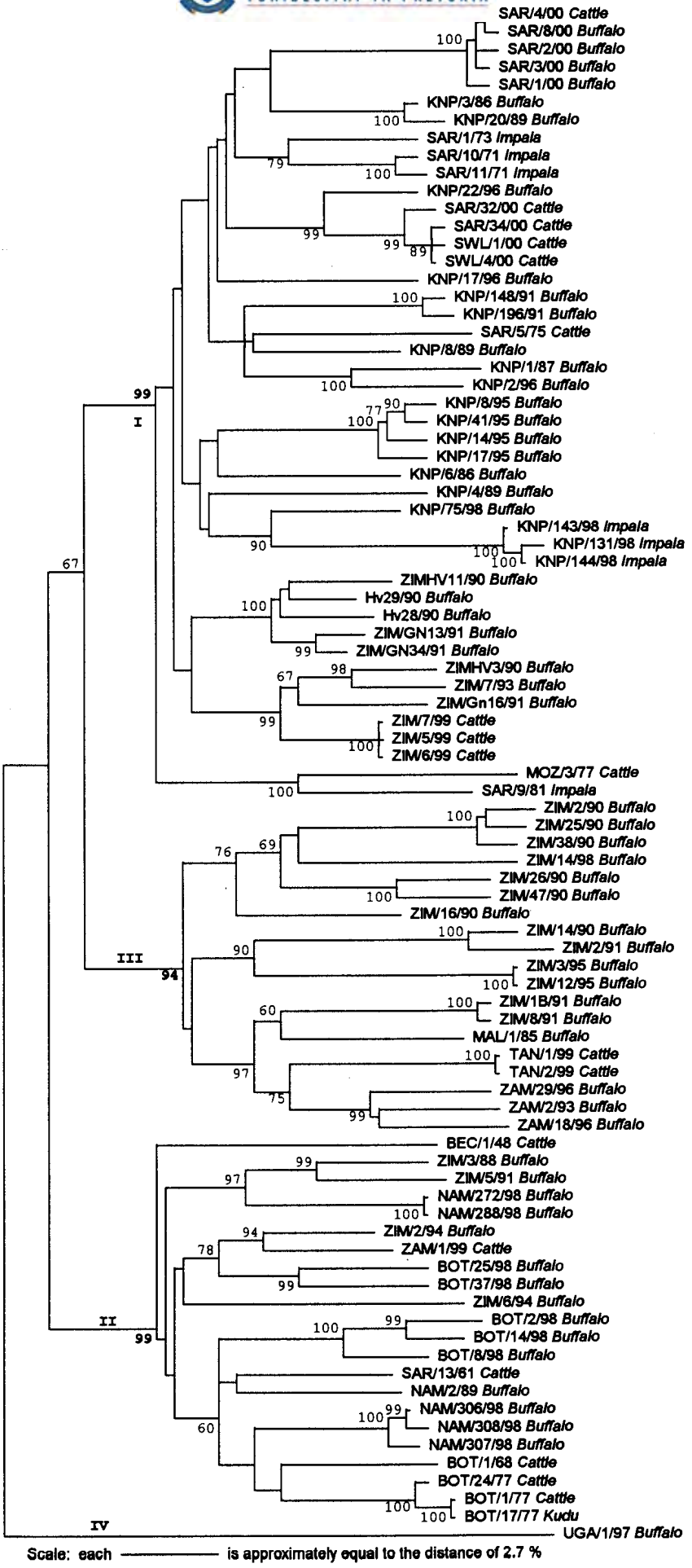

Fig.6.1: Neighbor-joining tree depicting VP1 gene relationships of SAT-1 viruses (1948-2000) recovered from African buffalo (*Syncerus caffer*), impala (*Aepyceros melampus*), kudu (*Tragelaphus strepsiceros*) and cattle

6.3.2.1 VP1 gene relationships of SAT-2 viruses in southern Africa

Within topotype I, nine distinct viral genotypes (A-I) occur, six of which (A-F) were previously identified in Chapter 3 (Bastos *et al.* 2000). Based on genetic distance, the five outbreak strains which fall within this topotype are not part of the same epizootic (differ by > 10 % or more) and cluster within four distinct genotypes (B, C, F and G). PAL/5/83 the last recorded SAT-2 virus causing FMD in cattle in South Africa (prior to the 2001 outbreak in the Northern Province) falls within a group of KNP buffalo viruses (99 % bootstrap support) from Ndziyospruit (KNP160/91), Water Affairs Weir (KNP183/91), Capricorn (KNP/16/93) and Malangangwane Dam (KNP/6/96). These four buffalo viruses were previously shown (Chapter 3) to comprise a single KNP buffalo genotype (B) which occurs both north and south of the Olifants river. RHO/2/79 and SAR/16/83 are genetically distinct from other topotype I viruses, whilst KNP/1/85 and MOZ/4/83 although both representative of genotype C, differ from each other by 14 % on nucleotide level and are therefore not part of the same epizootic.

Within topotype II no outbreak strains are represented, whilst in topotype III, two cattle viruses sampled 2 years apart, SWA/4/89 and NAM/1/91, are clearly part of the same epizootic (differ by less than 2.6 % from each other). Three unrelated outbreaks are presented in topotype IV. ZIM/7/83 and NAM/1/92 can be linked directly to buffalo viruses circulating in Hwange National Park (94 % bootstrap support) and Mahango (100 % bootstrap support), respectively. Viruses recovered from the 1997 outbreak in cattle in the Chiredzi district in southern Zimbabwe were more closely related to buffalo viruses from Hwange National Park (collected in 1988 and 1989) in western Zimbabwe, than to Gonarezhou buffalo viruses in the south. Subsequent field investigations revealed that buffalo from Hwange NP purchased two years prior to outbreak were on a private conservancy in close proximity to where the outbreak occurred. Sampling of these buffalo (ZIM/44/97 and ZIM/48/97) unequivocally showed that they were the source of infection for the affected cattle (99 % bootstrap support).

6.3.2.2 VP1 gene relationships of SAT-2 viruses in eastern Africa

The four Kenyan viruses (KEN/5/99 and KEN/7-9/99) are clearly part of the same epizootic as they differ by 3 % or less from each other. These viruses from Kenya together with an isolate from Burundi comprise Topotype V. Although part of the same topotype (92 %

bootstrap support) the viruses from these two countries represent two distinct genotypes (> 16 % difference). Of interest is the close genetic relationship of topotype V, a virus cluster comprising Kenyan viruses, with southern African topotypes (I-IV; 89 % bootstrap support). Viruses from other east African countries such as Rwanda and Kenya form distinct evolutionary lineages, represented by single viruses, separate from the southern African topotypes. Similarly, a virus from central Africa (ZAI/1/82; topotype VI) is genetically distinct from all other viral lineages. Of note is the link (100 % bootstrap support) between an Eritrean virus (1998) and the SAT-2 strain that was introduced into Saudi Arabia, SAU/6/00 (> 90 % sequence identity) which points to the role of this and perhaps other east African countries in virus introductions into the Middle East.

6.3.3 Genetic relationships of SAT-3 type viruses in southern Africa

Within the SAT-3 serotype, four unrelated FMD epizootics occurred in cattle between 1965 and 1999. Two of the outbreak lineages fall within topotype I and the remaining two within topotype III. In the former topotype, the 1999 outbreak which occurred in cattle in southern Zimbabwe could be directly linked to buffalo viruses circulating in the Gonarezhou National Park (97 % bootstrap support). The historical RHO/3/78 virus is not directly related to any of the buffalo viruses characterized here and is representative of a distinct topotype I genotype. Similarly, within topotype III, BEC/1/65 is unrelated to other viruses within this topotype, as are the 1994 cattle viruses recovered from the outbreak in Namibia. The FMD-infected buffalo (POT/336/98) originating from a farm in the Free State province, is most closely related to KNP buffalo viruses from the Langtoon Dam area in the northern part of the Park (67 % bootstrap support). Seropositivity of these buffalo to both FMD and *Theileria parva lawrencei*, the organism causing corridor disease (CD) in cattle, indicates that their true origin is from a region in South Africa where both diseases are prevalent. The Kruger National Park is the only region in South Africa where buffalo are infected with both FMD and CD. The genetic characterization of the FMD virus presented here confirms that KNP is indeed the origin and further identifies the northern region of the game park as being the most likely origin of these buffalo. This part of the game park is coincidentally an area in which TB infection rates in buffalo are relatively low and the only region from which buffalo translocations to adjoining private game farms in the FMD control area have been permitted in recent years.

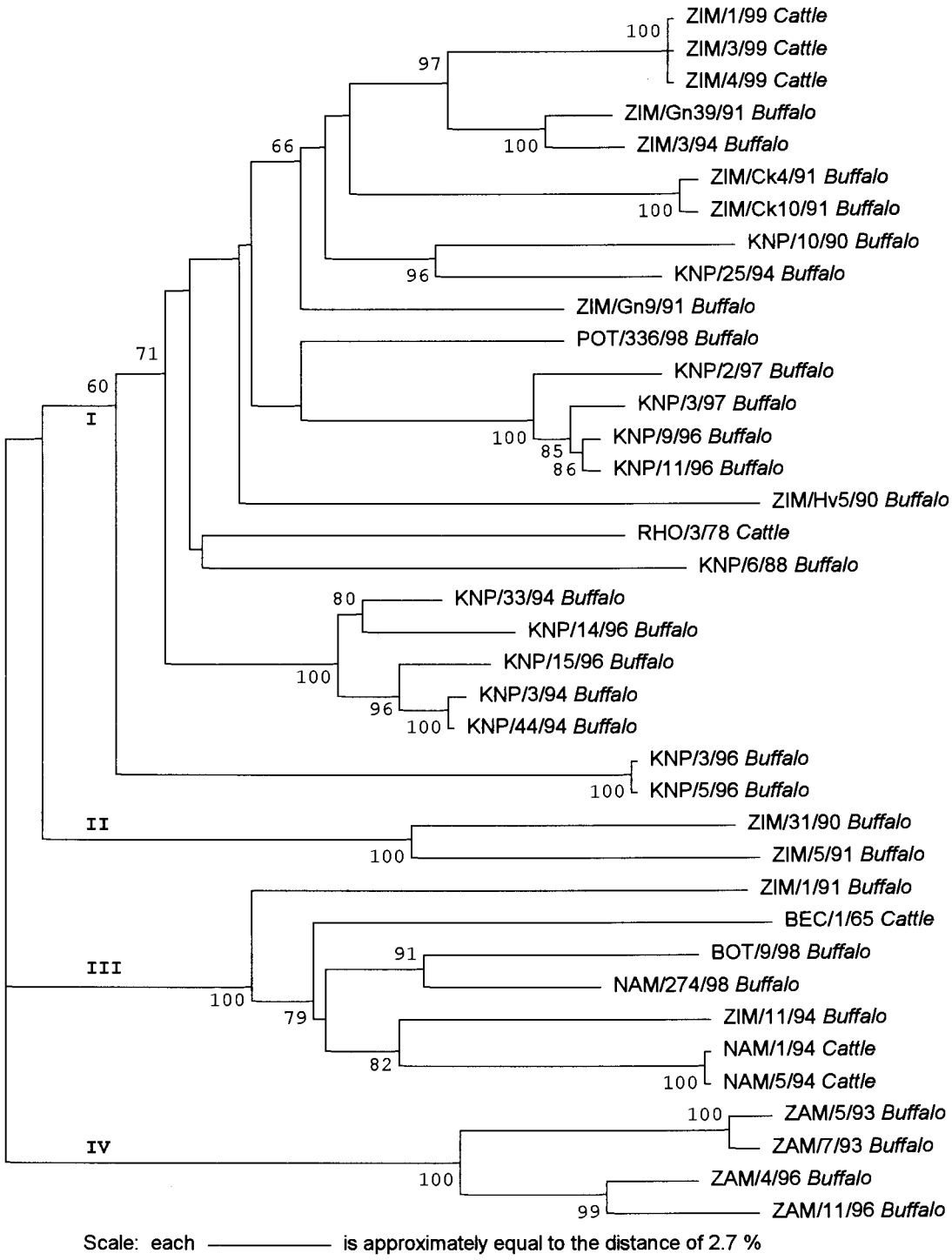

Fig. 6.3 Neighbor-joining tree depicting VP1 gene relationships of SAT-3 type viruses from buffalo and livestock in southern Africa (1965-1999)

6.3.4 Overall levels of genetic variation in the C-terminal half of the VP1 gene

Levels of variation per serotype are expressed as the percentage of sites that are not completely conserved across all viruses used in the analysis. In contrast to results obtained when 30 buffalo viruses were characterized per serotype (Chapter 5), the levels of genetic diversity within the VP1 gene of SAT-type viruses is highest here for SAT-1 and lowest for SAT-3 on both amino acid and nucleotide level (Table 6.5). This is likely due to the higher number of viruses analysed for the former serotype, compared to that analysed for the latter.

TABLE 6.5 Genetic variation in C-terminal VP1 gene sequences of SAT-type viruses of diverse geographical and species origin

Serotype	No of OTUs	Overall nucleotide variation	Overall amino acid variation
SAT-1	87	64 %	57 %
SAT-2	72	63 %	52 %
SAT-3	38	56 %	47 %

6.4 Discussion

By phylogenetic resolution of VP1 gene relationships of SAT-type viruses, it was shown that the geographical origin of outbreak strains generally coincide with the topotype assignment of buffalo viruses from the same localities. This indicates that most outbreaks occur following direct or indirect transmission of virus between animals in close proximity to each other rather than being due to importation of the disease or through the movement of infected animals between countries within the different topotype regions. However, importation of disease has occurred on occasion and is best illustrated by molecular epidemiological studies of SAT-1 type viruses. Prior to the erection of fences between South Africa and neighbouring countries transboundary transmission was implicated in outbreaks along borders. This was confirmed in this study by the finding that the SAT-1 virus causing an outbreak in cattle in the Potgietersrus area in South Africa in 1961 (SAR/13/61) originates from the buffalo topotype II region comprising viruses from Namibia, Botswana and western Zimbabwe. Other evidence of cross-border movement is provided by the link between the cattle outbreak in Mozambique (1977) and the widespread SAT-1 outbreak affecting numerous species of wildlife 1981

(SAR/9/81) in the Kruger National Park (Keet *et al.* 1996). In this case, the affected areas were in close proximity to each other. Records further indicate that movement of wildlife between South Africa and Mozambique was common along the eastern border of the Kruger National Park (Stevenson-Hamilton 1937), prior to the erection of game-proof fences, making it likely that common SAT-1 genotypes occur in these neighbouring countries. Although both cases refer to historical outbreaks, evidence from recent outbreaks suggests that transboundary movement of animals and animal products remains a threat. This is exemplified by the recent importation of SAT-1 virus into Swaziland from South Africa. Prior to this, Swaziland had been free of the disease for 31 years. Phylogenetic results also confirm that the outbreak occurring in Zambia in 1999 (ZAM/1/99) was most likely due to importation of the disease from western Zimbabwe as the outbreak strain groups with a buffalo virus from Hwange National Park (94 % bootstrap support). Of even greater concern was the type O outbreak occurring in KwaZulu-Natal in 2000, which arose after pigs were fed swill obtained from ships in Durban Harbour (Sangare *et al.* 2001). Type O had never been recorded in South Africa prior to this and vaccines against this serotype were not as readily available as those against the endemic SAT-type viruses. These three recent examples stress the need for greater vigilance by veterinary authorities, strict adherence to disease control measures (including the immediate decontamination of waste coming into a country via different means of transportation) and the availability of vaccines and diagnostic methods capable of addressing all FMD virus serotypes.

Molecular epidemiological studies are particularly useful for discerning whether outbreaks occurring in the same year are part of the same epizootic or not. In this study it was shown that the three SAT-2 outbreaks which occurred in 1983 (two in South Africa and one in neighbouring Mozambique) were not part of the same epizootic as the viruses represent three distinct virus genotypes (B,C and I). Similarly, SAT-1 outbreaks occurring in Botswana and Mozambique were shown to be unrelated as were the SAT-1 outbreaks in Tanzania, Zambia and Zimbabwe in 1999 and the two SAT-1 outbreaks in cattle in South Africa in 2000. Genetic characterization of viruses is also useful for identifying viral persistence in the field. In this study, prolonged circulation of viruses was shown to occur in livestock as well as in impala antelope. Two SAT-2 viruses recovered from cattle sampled two years apart, SWA/4/89 and NAM/1/91 were shown to be part of the same epizootic (97.4 % sequence identity) pointing to a circulation period of at least two years in the field. A circulation period of more than 2.5 years was demonstrated for SAT-1 viruses recovered from the 1971 and 1973 impala epizootics. Prolonged circulation of FMDV in impala populations in the KNP has been demonstrated on three

separate occasions and has involved two of the three SAT serotypes circulating in the game park. This is significant as impala and kudu antelope are believed to be intermediaries in disease transmission between buffalo and impala and were historically used as indicator species to determine the rate and spread of the disease from wildlife to livestock (Meeser 1962). Circumstantial evidence indicating that a 1958 SAT-3 outbreak in impala subsequently spread to other species, including cattle, has been presented (Meeser 1962), however unequivocal evidence of a shared outbreak strain between cattle, buffalo and antelope species is presently lacking. The results provided here do however show that viruses recovered from cattle and kudu (SAT-1; Botswana 1977) and from cattle and impala (SAT-1; Mozambique 1977 and South Africa 1981) were part of the same epizootic and that transmission between these species does occur in the field. In Chapter 3, the transmission of FMDV between buffalo and impala was also demonstrated by nucleotide sequence characterization of field strains (Bastos *et al.* 2000). The link between SAT-2 cattle and buffalo strains sampled in 1997 in Zimbabwe and serological evidence of infection of antelope species is perhaps the strongest evidence thus far obtained for the role of antelope as intermediaries in disease transmission.

In summary, this chapter has confirmed the role of African buffalo as the primary source of infection for livestock in southern Africa as most outbreaks could be linked to buffalo in close proximity to where the outbreak occurred. In some cases it was possible to identify herds within specific game parks, from which virus most likely originated, but in others it was only possible to link epizootics to specific genotypes or topotypes. This clearly illustrates that the accuracy with which the origin of an outbreak can be determined is directly dependent on the extent to which the viruses from FMD-infected buffalo populations within a country or region have been genetically characterized. Further genetic studies on buffalo viruses should be encouraged, particularly in Namibia, Botswana, Mozambique, Angola and Malawi, where there is an under-representation or no representation of genetic diversity in the field. This would permit the establishment of a truly representative database for the southern African region and provide a powerful means of determining factors threatening the disease-free status of SADC countries. It would also act as a deterrent to the illegal trade in FMD-infected African buffalo as the geographic origin of buffalo that have been moved illegally was clearly shown by molecular epidemiological studies. The trade in wildlife and 10-fold discrepancy in prices between disease-free and infected buffalo poses a serious threat to disease security and should be addressed through legislation and prosecution, the success of which may be dependent on the availability of accurate and powerful genetic tracking tools.

Chapter 7

Concluding remarks and future prospects

The economic impact of FMD in a country previously free of the disease is significant both in terms of the initial cost of controlling the outbreak and the loss of revenue following the ban on exports. This is exemplified by the 1997 outbreak in Taiwan which cost that country an estimated US$ 2 billion (Yang *et al.* 1999). As an OIE list A disease, FMDV is a major barrier to international export and trade with other regions. Following the outbreaks occurring in livestock in KwaZulu-Natal, Mpumulanga and Northern provinces of South Africa in 2000 and 2001 and the subsequent ban on exports, it is estimated that the outbreak cost the country 2 billion rand in the first five months following the initial type O outbreak. These economic consequences will undoubtedly continue to be felt given the conditions and time constraints precluding international trade following an outbreak (see section 6.1). One of the best defenses a country can have in the event of an outbreak is the rapid diagnosis of the disease and effective containment through the combined efforts of veterinary services and laboratory personnel. Modern molecular epidemiological techniques can assist in pin-pointing the most likely source of infection. This information is critical for assisting field staff in containing the disease in all affected areas, and proved useful in the SAT-1 outbreak initially identified in a feedlot in Middelburg, South Africa in 2000. Nucleotide sequencing showed that the outbreak strain was most closely related to a buffalo virus from Lower Sabie in the Kruger National Park (KNP), which is approximately 200 km east of the index case. Surveillance and sampling was therefore extended to include this region south of the KNP and led to the identification of additional infected animals by veterinary health personnel.

The PCR methodology outlined in this study is not only useful for characterizing outbreak strains, but can also be used to detect virus in a clinical sample, within 6 hours of receipt. The VP1 PCR detailed in Chapter 2, was primarily designed to detect SAT-type viruses in southern Africa. Application of this method to SAT-type viruses from West Africa indicates that the VP1Ub primer does not detect these genetic variants as efficiently as it does those from southern Africa (Sangare & Bastos 2000, unpublished). This is likely due to the occurrence of genetically distinct viruses in different African regions as indicated by nucleotide sequence determination of types A (Knowles *et al.* 1998), O (Sangare *et al.* 2001), SAT-1 (Bastos *et al.* 2001) and SAT-2 (Chapter 5). Furthermore, it was clearly shown that outbreaks sometime result from the transboundary movement of animals or animal products. In light of these factors it is clear that the sole use of the VP1Ub + P1 primers (Chapter 2) for disease diagnosis could potentially lead to false negative results. It is therefore recommended that this PCR be used in combination with primers targeting a more conserved genome region, such as the replicase (3D) gene. Although published primers are available for the 3D genome region, results indicate that they do not amplify all field variants of FMDV occurring on the African continent (Bastos 1998b). For this reason, SAT-type replicase gene sequences were generated and new primers were developed in which the 3D genome region is amplified by a one-step nested PCR approach. This novel 3D PCR test detects all FMDV serotypes and SAT field variants, is more sensitive than the VP1-targeting PCR (Bastos 1998b) and eliminates the possibility of contamination usually associated with the conventional two-step nested PCR. For diagnostic purposes it is therefore imperative that both the 1D (Bastos 1998a) and the 3D PCR (Bastos 1998b) be run simultaneously. If both PCRs are positive, the former product can be purified and used for genetic characterization, whilst the latter provides independent confirmation of a positive result. If however the former is negative, whilst the latter is positive, this would be indicative of inefficient primer binding due to the inherent hypervariablility of the VP1 protein. In such a case, alternative VP1 targeting primers would need to be screened in order to proceed with genetic characterization of the outbreak strain. A double negative result would indicate absence of FMD virus from the sample being analysed.

When comparing PCR with other more conventional FMDV detection methods such as virus isolation and typing, PCR is superior to both methods in terms of sensitivity and speed (when a

light-cycler is being used). Although a PCR-typing method which claims to differentiate all seven serotypes has been published (Callens & de Clercq 1997), the data generated in this thesis indicate that the reported type-specific primers will not accurately detect nor differentiate between all SAT-type variants present in southern Africa. There is therefore no reliable PCR test presently available which simultaneously permits detection and typing of all FMDV serotypes in a clinical sample. Furthermore, the results presented in this thesis indicate that development of a VP1-based serotyping test for use throughout Africa is unlikely as the variation levels within and between genotypes of SAT-type viruses from different regions preclude this. It is however possible to develop accurate regional typing PCRs for SAT-viruses. Preliminary investigations indicate that SAT-1 and SAT-3 viruses from southern Africa are readily distinguished by PCR (Bastos 1996), but differentiation of SAT-2 type viruses is more problematic due to the lack of sufficiently long stretches of type-specific sequences, suitable for designing primers within the VP1-coding region.

It was shown here that genetic characterization of field strains assisted with an investigation into the possibility of sexual transmission of SAT-type viruses from persistently infected buffalo (Chapter 3). The recovery of a genetically unique FMD virus from the reproductive tract of a male buffalo provides the first indication that this may be alternative site of viral persistence, which unlike the oesophageo-pharyngeal area, is immunologically privileged. The results were however equivocal and are likely to remain so until more accurate methods are developed for differentiation between acute and persistently infected animals. The recovery of FMDV from buffalo semen does however indicate that viral transmission by the sexual route is a distinct possibility and has implications for buffalo breeding programmes in which the use of artificial insemination methods is intended.

Nucleotide sequencing of viruses recovered from impala epizootics and their relatedness to FMDV buffalo genotypes (Chapter 4) provided the first evidence of natural transmission between these wildlife species. These results are important not only because they demonstrate the usefulness of detailed genetic characterization of viruses of maintenance host populations, but also because they confirm the possible role of antelope as intermediaries in disease transmission. Further evidence for this was obtained from retrospective studies which showed that cattle and antelope outbreaks were caused by the same virus (Chapter 6), thereby confirming interspecies transmission. Conclusive

evidence, as would be provided by the recovery of an identical virus from the three major role players, buffalo antelope and cattle, in this transmission scenario is however still lacking. The results presented here do at least confirm that interspecies transmission between buffalo and impala and between kudu and cattle has occurred previously. The role of impala and kudu in the epidemiology of the disease should therefore be further investigated in order to allow for the implementation of appropriate disease control measures where these antelope species are implicated as intermediaries in disease transmission.

The cumulative results of this study and those from previous epidemiological studies indicate that the SAT-type FMD virus can be independently maintained within an isolated herd of buffalo for up to 24 years (Condy *et al.* 1985). Under field conditions, virus can be transmitted to cattle (Dawe *et al.* 1994a) and impala (Bastos *et al.* 2000) in close proximity to buffalo. Such transmission would most likely result from the shedding of virus by acutely infected buffalo calves (Gainaru *et al.* 1986). Disease transmission can however also be precipitated on rare occasions by persistently infected buffalo (Hedger & Condy 1985; Dawe *et al.* 1994b; Vosloo *et al.* 1996), possibly by the sexual route (Bastos *et al.* 1999). Circumstantial evidence indicates that interspecies transmission between buffalo and impala results from environmental contamination of shared environs when buffalo calves are simultaneously infected for the first time (Bastos *et al.* 2000). Results from experimental infections indicate that the reverse is also true and that acutely infected impala can act as a source of infection for buffalo in close proximity (Vosloo *et al.* 1996). This scenario presumably applies to any cloven-hoofed species shedding large amounts of virus in close proximity to other susceptible species. Interspecies transmission between livestock and antelope (Chapter 6) was unequivocally demonstrated through the recovery of a common virus in both species. The results of the epidemiological investigations detailed in Chapters 3, 4 and 6, and those from previous studies make it possible to summarize the likely routes of FMDV transmission and the role of different cloven-hoofed species in the southern African sub-region (Fig. 7.1). It should however be stressed that although these routes of transmission are now well-recognized, the mechanisms and conditions facilitating interspecies transmission remain to be clarified.

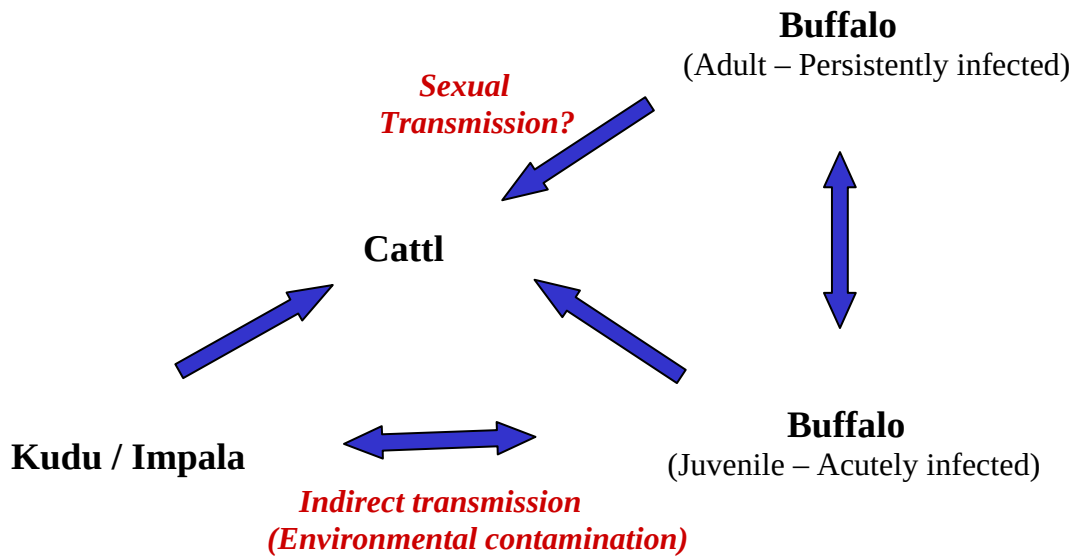

Fig 7.1. Diagrammatic representation of the possible routes of transmission of foot-and-mouth disease virus from African buffalo to other cloven-hoofed species

The identification of locality-specific buffalo virus genotypes (Chapter 5)was shown to be useful for more accurately determining the source of infections in susceptible cloven-hoofed species in close proximity (Chapter 6). The accuracy with which outbreaks could be traced was however dependent on the extent to which viruses from representative buffalo populations had been characterized. In South Africa and Zimbabwe sources of infection were readily established as the viral genotypes within these countries are well studied. In contrast, little or no data was available from Angola, Namibia, Botswana and Mozambique. Clearly the establishment of a regional database requires that more information on the genetic variation of FMD virus isolates within these SADC countries be obtained. This is particularly important in view of the imminent establishment of a 'peace park' incorporating KNP, southern ZIM and Mozambique. The geographical genetic variation of buffalo in Mozambique has not been assessed nor has the impact of buffalo movement from northern and western Zimbabwe into southern Zimbabwe been considered. Confirmation that the introduced topotypes occurring in other regions of Zimbabwe (topotypes II, III or IV) have become established within the southern topotype (I) region to be incorporated within the peace park, would clearly have

implications for disease control though vaccination. It is also likely to have major financial implications as the vaccine would need to contain viruses representative of all circulating topotypes in order to ensure adequate protection. In South Africa, the trivalent vaccine (SAT 1-3) presently in use is primarily inclusive of topotype I virus variants and would therefore not provide adequate protection, following the removal of border fences and likely establishment of new topotype viruses resulting from the peace-park initiative.

FMD, together with vesicular stomatitis, swine vesicular disease and rinderpest are OIE list A diseases and as such pose serious constraints to international trade of live animals and animal products. Many countries in the developing world are embarking on disease control and eradication programmes in order to obtain access to the lucrative foreign markets. For many countries FMD is the only remaining obstacle to free trade and there is therefore always the temptation to delay reporting and eradicate the disease before it halts trade. This delay often results in the disease spreading even further and recent evidence suggests that illegal cattle trade has played a major role in the spread and introduction of FMD (Kitching 1999). In South Africa and Zimbabwe there is an additional threat to disease security posed by the trade in illegal buffalo, as these are the only southern African countries that produce a surplus of buffalo for translocation purposes. The demand for disease-free buffalo for eco-tourism and hunting purposes is high due to their 'big-five' status. Buffalo prices vary according to the disease-status of the animals. In 1998 foot-and-mouth disease (FMD) infected buffalo were valued at between US$500 and US$1000, those infected with Corridor Disease (CD) alone cost between US$4300 and US$7200, whilst disease-free (clean) buffalo fetched upwards of US$16500 (Winterbach 1998). The difference in price paid for diseased versus disease-free buffalo together with limited number of disease-free buffalo and increased demand for animals of this status, are a major driving force in the illegal trade in these animals. The effects of illegal buffalo trade are potentially detrimental and should be curbed by whatever means possible. Extensive genetic characterization of KNP buffalo viruses may act as an effective deterrent as similar molecular epidemiological studies of human viral disease have successfully been used in legal prosecution (Albert *et al.* 1994; Ross *et al.* 1999). To be an affective means of combating illegal movement of buffalo in South Africa, all populations within the KNP would need to be characterized. Although adequate representation of southern KNP populations is available, the

northern KNP buffalo are under-represented in the genetic database. This short-coming and the importance of tracing buffalo is currently being addressed through a project funded by the Red Meat Board of South Africa.

In conclusion, this study has provided numerous epidemiological insights into SAT-type FMD in southern Africa. In particular, the role of the African buffalo and antelope in disease transmission has been addressed and initiation of a regionally representative buffalo virus database has permitted more accurate determination of the source of outbreaks in the southern African region. In addition, methodologies for the detection and characterization of viruses detailed here not only assist with rapid and accurate disease diagnosis, but can potentially be used for the successful legal prosecution of parties transgressing buffalo movement regulations. The results further identify a need for re-evaluating disease control measures, such as the height of perimeter fences intended to be 'game-proof', and for stricter regulation on the importation and incineration of animal products and waste, in order to ensure disease security.